MolBioCon2014

III. International Congress of the Molecular Biology Association

Abstract Book

Topics:

Biochemistry	Cell Biology	Immunology	Proteomics
Bioinformatics	Developmental Biology	Microbiology	Signal Transduction
Biotechnology	Gene Regulation	Neuroscience	Stem Cell Research
Cancer Biology	Genomics	Plant Molecular Biology	Systems Biology

10-12 September 2014

İzmir Institute of Technology

http://mbd2014.iyte.edu.tr

ABSTRACT BOOK

CONTENT

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Assoc. Prof. Turgay Ünver (Çankırı Karatekin University)

PROGRAM

10 Sep 2014 (Wednesday)

13:00 - Registration (MBG Building)

16:00 - Welcome Reception (MBG Building)

17:00 - Opening Ceremony (Prof. Dr. Erdal Saygın Conference Hall)
17:00 - Prof. Ahmet Koç (Organization Committee)
17:10 - Assoc. Prof. Nesrin Özören (President, Molecular Biology Association)
17:20 - Prof. Mustafa Güden (President, İzmir Institute of Technology)

17:30 - Opening Lecture by Prof. Vadim N. Gladyshev (Harvard Medical School)
"Comparative Genomics of Aging"

18:30 – Session-I (Bioinformatics & OMICS) (Session Chairs: Yeşim Aydın Son, Özlen Konu)

Selected Oral Presentation-1- The evolutionary history of synchronous urothelial tumors revealed by exome sequencing (Mehmet Somel)

Selected Oral Presentation-2- Role of thiol peroxidases in the redox control of genome stability and cell fitness at a single nucleotide resolution (Alaattin Kaya)

Oral Presentation-3- PHISTO: a web-based platform for studying infection mechanisms through pathogen-human interactions (Saliha Durmuş Tekir)

19:30 - Dinner (Main Cafeteria)

11 Sep 2014 (Thursday)

09:00 - Keynote Lecture by Prof. Stefan Dmitrov (CNRS, Grenoble)
"Histone Variants : Key Epigenetic Factors Involved in Cancer"

10:00 – Session-II (Gene Regulation) (Session Chairs: Sezai Türkel, Özden Yalçın Özuysal)

Invited Talk by Dr. Sophie Jarriault (Institute of Genetics and Molecular and Cellular Biology, France)
"Sequential partitioning of histone methylation and demethylation activities ensures the robustness of natural transdifferentiation"

Selected Oral Presentation-4- Sp1 regulates URG-4 promoter in hepatoma and osteosarcoma cells (Esra Tokay)

Selected Oral Presentation-5- Glucose signaling controls the programmed frameshift efficiency of the EST3 gene in *S. cerevisiae* (Saliha Elif Yıldızhan)

Selected Oral Presentation-6- Genome-wide localization analysis for IRF4 target gene identification in melanoma cell lines (Ulduz Sobhiafshar)

11:15 - Coffee Break

11:45 – Session-III (Cancer Biology-I) (Session Chairs: Mesut Muyan, Engin Özçivici)

Invited Talk by Prof. Sabine Werner (Institute of Molecular Health Sciences, ETH Zurich, Switzerland)
"Parallels between wound healing and cancer"

Selected Oral Presentation-7- Alternative polyadenylation dependent 3'-UTR shortening in triple negative breast cancer (H. Begum Akman)

Selected Oral Presentation-8- The effect of HL-60 myeloid leukemia cells treated with ATRA, vitamin D3, IFN-γ, or LPS on T cell co-stimulation (Diğdem Yöyen-Ermiş)

Selected Oral Presentation-9- A conditional knockout mouse model uncovers essential roles of Cdk1 in the mammalian cell cycle and tumorigenesis (M. Kasım Diril)

13:00 - Lunch Break (Main Cafeteria)

14:00 – Session-IV (Immunology) (Session Chairs: Ayten Nalbant, Batu Erman)

Invited talk by Assist. Prof. Melanie Blokesch, (Global Health Institute Ecole Polytechnique Fédérale de Lausanne, Switzerland)
"Visualizing horizontal gene transfer in the human pathogen *Vibrio cholerae*"

Invited Talk by Assoc. Prof. Hüseyin Sarıbaşak (Şifa Üniversity, Turkey)
"ATAD5 deficiency decreases B-cell division and Igh recombination"

Selected Oral Presentation-10- Mutation of the NF-kB binding site in the IL-7Ralpha gene enhancer region using TALEN proteins (Canan Sayitoğlu)

Selected Oral Presentation-11- NLRC3 protein is a novel inhibitor of inflammasome (Elif Eren)

15:30 - Coffee Break

16:00 – Session-V (Biotechnology & Plant Molecular Biology) (Session Chairs: Nedim Mutlu, H. Çağlar Karakaya)

Invited Talk by Prof. Tarlan Mammedov (Akdeniz University, Turkey)
“Plant-based transient expression technology provides an inexpensive and convenient system for the production of functional active recombinant proteins”

Selected Oral Presentation-12- Genetic modification of Cadmium determinant 1 (Cdm1) enhances fortification of *Arabidopsis seeds* with essential minerals but not Cadmium (Emre Aksoy)

Selected Oral Presentation-13- Pulsed electromagnetic field (PEMF) effect on biochemical pathways (Mehmet Gümüşay)

Selected Oral Presentation-14- Electrochemical detection of cancer biomarker microRNAs based on p19 protein on cell lysate (Mehmet Ozsoz)

Oral Presentation-15- Determination, function, and subcellular localization of plant fungal pathogen effectors (Mahinur Akkaya)

17:30 - Poster Display Session-I

19:30 - Dinner (Main Cafeteria)

12 Sep 2014 (Friday)

09:00 – Session-VI (Cell and Developmental Biology & Stem Cell Research) (Session Chairs: Gülistan Meşe Özçivici, Ersin Akıncı)

Invited Talk by Dr. Aynur Kaya-Copur (Max-Planck-Institute of Biochemistry, Germany)
“An essential role for neuron-muscle cross-talk during flight muscle morphogenesis in *Drosophila*”

Invited Talk by Prof. Fisun Hamaratoğlu (Center for Integrative Genomics University of Lausanne, Switzerland)
“A quantitative approach to size and shape regulation”

Selected Oral Presentation-16- Moving beyond the parts list: protein proximity mapping at the origin of centriole duplication (Elif N. Firat-Karalar)

Selected Oral Presentation-17- HOX and TALE gene expression profile of bone marrow derived mesenchymal stem/stromal sells from Fanconi anemia patients and donors (Ilgın Çağnan)

Selected Oral Presentation-18- Mechanical vibrations normalize molecular and ultrastructural properties of mesenchymal stem cells during adipogenesis (Öznur Baskan)

10:45 - Coffee Break

11:15 – Session-VII (Biochemistry) (Session Chairs: Alaattin Şen, Mustafa Köksal)

Invited Talk by Assist. Prof. Nuri Öztürk (Gebze Institute of Technology, Turkey)
“Wake up call: photosignaling by cryptochrome to reset the biological clock”

Invited Talk by Assist. Prof. Güneş Özhan (Dokuz Eylül University, Turkey)
“Fine tuning of Wnt/β-catenin signaling: welcoming Wnt ligands at the plasma membrane”

Selected Oral Presentation-19- An enzyme of a novel clock component: Cystathionine-B-Synthase (CBS) (Eylem Külköylüoğlu)

Selected Oral Presentation-20- Deletion of mitochondrial metabolism genes PPA2, DSS1 and AFG3 affects mitochondrial morphology and functions that harbor life span prolongation (Khandaker Ashfaqul Muid)

Selected Oral Presentation-21- Identifying novel physical and functional interaction patterns in mammalian ER-associated degradation (Petek Ballar Kırmızıbayrak)

13:00 - Lunch Break (Main Cafeteria)

14:00 – Session-VIII (Cancer Biology-II) (Session Chairs: Mehmet Öztürk, Nesrin Özören)

Invited Talk by Assist. Prof. Özgür Şahin (İhsan Doğramacı Bilkent University, Turkey)
“Biomarker-guided sequential targeted therapy: a novel approach to overcome resistance in rapidly evolving tumors”

Selected Oral Presentation-22- Transcriptomic and proteomic analyses identify DEK oncogene as a target of the RNA binding factor CUGBP1 in melanoma (Metehan Çifdalöz)

Selected Oral Presentation-23- Epigenetic mechanisms underlying the dynamic expression of cancer-testis genes, PAGE2, -2B and SPANX-B, during mesenchymal-to-epithelial transition (Sinem Yılmaz-Özcan)

Selected Oral Presentation-24- Natural killer cell based immunotherapy of cancer (Tolga Sütlü)

Invited Talk by Prof. Mehmet Öztürk (Dokuz Eylül University, Turkey)
“Life sciences in Turkey: from past to future”
Please note: this talk will be in Turkish.

15:45 - Poster Display Session-II

17:45 - Closing Ceremony and Poster Awards

18:15 - Molecular Biology Association Meeting

20:00 - Conference Dinner (TBA)

13 Sep 2014 (Saturday)

Social Event: Çeşme boat cruise

WORKSH0PS

Flow Cytometry Workshop

By Drs. Ayten Nalbant and Batu Erman

10 Sep 2014 (Wednesday)

13:00 – 16:00 Lectures given by Batu Erman and Ayten Nalbant (Location TBA)
13:00 – 13:45 Fluorescence, antibodies, fluorescent proteins
14:00 – 14:45 Flow cytometry theory
15:00 – 15:45 Compensation theory

11 Sep 2014 (Thursday)

16:00 – 18:30 Practical Session I
Group 1: BD Accuri (12 students) Conference Hall
Group 2: Attune Acoustic Focusing Cytometer (12 students) Laboratory

11 Sep 2014 (Thursday)
16:00 – 18:30 Practical Session II
Group 1: Attune Acoustic Focusing Cytometer (12 students) Laboratory
Group 2: BD Accuri (12 students) Conference Hall

How to Write a Scientific Paper Workshop

By Dr. Batu Erman

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11 Sep 2014 (Thursday)
21:00 – 22:00 Lectures given by Batu Erman (Location TBA)

INVITED SPEAKERS ABSTRACTS

Comparative Genomics of Aging

Vadim N. Gladyshev

Brigham and Women's Hospital, Harvard Medical School, Boston, USA

E-mail: vgladyshev@rics.bwh.harvard.edu

Abstract

Understanding the mechanisms that control lifespan is among the most challenging biological problems. Many complex human diseases are associated with aging, which is both the most significant risk factor and the process that drives the development of these diseases. It is clear that the aging process and the maximum lifespan of species can be regulated and adjusted. For instance, mammals are characterized by >100-fold difference in lifespan, which can both increase and decrease during evolution. We employ this diversity in mammalian lifespan and the associated life-history traits to shed light on the mechanisms that regulate species lifespan. For this, we utilize methods of comparative genomics to examine the genomes of exceptionally long-lived species and carry out analysis of lifespan across a panel of mammals. We sequenced the genomes of several mammals with exceptional lifespan, including the naked mole rat and the Brandt's bat, and identified genes that may contribute to their longevity. We further apply transcriptomics approaches to analyze the molecular basis for adaptations associated longevity across mammals. It is our hope that a better understanding of molecular mechanisms of mammalian lifespan control will lead to new therapeutic approaches to diseases of aging.

Biography

Vadim Gladyshev received his B.S./M.S. degree in 1988 and Ph.D. degree in 1992 from Moscow State University, Russia, followed by postdoc training at NIH, and a faculty position at University of Nebraska. Since 2009, he has been a Professor of Medicine and Director of the Center for Redox Medicine at Brigham and Women's Hospital, Harvard Medical School, and an Associate Member of the Broad Institute. Dr. Gladyshev has been working in the areas of selenium and redox biology as applied to aging and cancer, in which he has made contributions, such as characterization of the human selenoproteome (an entire set of selenium-containing proteins), methods to predict catalytic redox-active cysteines in proteins, and sequencing the genomes of exceptionally long-lived mammals. He has a long-term interest in the understanding of aging, functions of trace elements and mechanisms of redox control. Dr. Gladyshev has published >250 articles, edited books on selenium and redox biochemistry, and was elected as an AAAS fellow. He is a recent recipient of the NIH Director's Pioneer Award.

Histone Variants: Key Epigenetic Factors Involved in Cancer

Stefan Dimitrov

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Abstract

Histone variants are nonallelic isoforms of the conventional histones. Incorporation of histone variants confers novel structural and functional properties of the nucleosome. Histone variants are key epigenetic players and their role in cancer is just beginning to emerge. For example, mice deficient for both the histone variant H2AX and p53, developed rapidly immature T and B lymphomas, sarcomas, leukemia and solid tumors. The histone variant H2A.Z appears to be the main player in the development of breast cancer and mutations in the histone variant H3.3 were associated with the development of pediatric glioblastoma. The histone variant macroH2A expression was linked to the prediction of cancer recurrence.

My talk will be focused on CENP-A. CENP-A replaces conventional histone H3 at the centromeres and is a universal epigenetic marker of the centromeres. CENP-A is overexpressed in numerous cancers. It is involved in genomic instability and plays an important role in aneuploidy and cancer development. However, how does CENP-A function in both normal and pathological conditions remains elusive. We have dissected the mechanism of function of CENP-A by using a unique combination of molecular and cell biology techniques and structural approaches. Our data reveal how the cell uses the distinct CENP-A nucleosome conformations and CENP-A post-translational modifications to control mitosis and cytokinesis and allow to shed light on the mechanism of CENP-A function in tumorogenesis.

Biography

1996-present First Class Director of Research (DR1), National Center of Scientific Research (CNRS) and Head of Laboratory "Chromatin and Epigenetics" Institute Albert Bonniot, Grenoble
Head of team "Chromatin Structure and Function"
Ecole Normale Supérieure de Lyon, Lyon (2004-2009)
1992-1995: Visiting Scientist, Laboratory of Molecular Embryology, NICHD, NIH, Bethesda, USA. Laboratory Chief: Dr. *A. P. Wolffe*.
1991-1992: Visiting Professor at The Cancer Research Center, Laval University, Quebec, Canada.
1990: Visiting Fellow at The Institute for Experimental Cancer Research, Chemin des Boveresses 155, CH-1066 Epalinges, S./Lausanne. Laboratory Chief: *P. Wellauer*
1989-1992: Director of Research at the Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria.
1983-1988: Assistant Professor at the Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria.
1978-1982: Ph. D., Molecular Biology, Institute of Molecular Biology, Sofia, Bulgaria and the Institute of Molecular Biology (Head of Department: *M.Volkenstein*), Moscow, Russia
1974-1976: B. S., Biophysics, Sofia University, Department of Solid State Physics, Sofia, Bulgaria.
1970-1974: Undergraduate student, Department of Solid State Physics, Sofia University, Sofia, Bulgaria

Sequential Partitioning of Histone Methylation and Demethylation Activities Ensures the Robustness of Natural Transdifferentiation

Sophie Jarriault

Institute of Genetics and Molecular and Cellular Biology, Strasbourg, France

E-mail: sophie@igbmc.fr

Abstract

Whereas postmitotic somatic cellular identity is generally a stable feature of multicellular organisms, natural interconversions between functionally distinct somatic cell types (aka transdifferentiation or Td) have been reported in species as diverse as jellyfish and mice. In some cases, Td events occur with remarkable precision and efficiency. For example, our laboratory has recently shown that a rectal cell suddenly looses its differentiated identity and is reprogrammed into a motoneuron with invariant precision, in 100% of the wild type *Caenorhabditis elegans* animals (n > 2 200). This fascinating Td event proceeds through discrete steps: dedifferentiation and then re-differentiation into the new cell type, similarly to vertebrate examples of Td, such as newt lens regeneration. As the molecular events that ensure such invariance are poorly understood, the efficiency and reproducibility observed in this defined reprogramming event represent a powerful and as yet unexploited avenue in which to probe mechanisms ensuring robust cell conversion. Focusing on the players identified in an unbiased genetic screen targeting this Td event, I will present our latest results on the dynamic events that underlie this exceptional efficiency *in vivo*.

Biography

After a PhD at the Pasteur Institute, where she proposed what has become the classical model for Notch signal transduction, directly from the membrane to nuclear gene modulation, and a postdoc at Columbia University on organogenesis of the egg-laying apparatus in *C. elegans*, Sophie Jarriault started her lab at the IGBMC, Strasbourg, on a new endeavour: deciphering cell identity maintenance and reprogramming. Taking advantage of the power of *C. elegans*, her work established the worm as a novel model to study natural transdifferentiation events at the single cell level *in vivo*. Work in her lab has unravelled steps, key molecular complexes and the relative contribution of genetic and epigenetic players in this process.

Parallels between Wound Healing and Cancer

Sabine Werner

Institute of Molecular Health Sciences, ETH Zurich, Switzerland

E-mail: sabine.werner@biol.ethz.ch

Abstract

Recent studies revealed remarkable parallels between wound healing and tumor development, and it has been suggested that tumors make use of the wound healing program of the host to promote their own growth. Based on these parallels it is of major interest to identify and characterize the genes that are regulated by skin injury and also in malignant skin tumors. One of these genes encodes the growth and differentiation factor activin, which we identified as a major regulator of wound healing and scar formation. Interestingly, overexpression of activin also promoted the development of malignant skin cancers through induction of a pro-tumorigenic microenvironment. This is important for the human situation, since activin is strongly overexpressed in malignant epithelial human skin cancers. These results demonstrate that the identification and characterization of wound-regulated genes provides insight into the mechanisms of cancer development and progression.

Biography

Sabine Werner has been Professor of Cell Biology at the ETH Zurich since February 1999.
She is from Reutlingen, Germany, and she studied Biochemistry at the Universities of Tubingen and Munich. In 1989 she earned her Ph.D. at the University of Munich, after having completed her dissertation at the Max-Planck-Institute of Biochemistry in Martinsried in the department of Prof. Peter Hans Hofschneider. After a short postdoctoral period at the same institute, she moved to the University of California San Francisco, where she started to work on the molecular mechanisms of growth factor action and tissue repair as a postdoctoral scientist in the laboratory of Prof. Lewis.T. Williams. From 1993-1999 she was a group leader at the Max-Planck-Institute of Biochemistry in Martinsried, Germany. In 1996 she obtained a Hermann-and-Lilly Schilling professorship of Medical Research at the same institute and from 1995-1999 she was also Associate Professor of Biochemistry at the Ludwig-Maximilians-University of Munich.

Selected Honors and awards:

- Kékulé-Fellowship of the "Verband der Chemischen Industrie", 1987
- Otto-Hahn-Medal of the Max-Planck-Society, 1990
- Postdoctoral Fellowship, Max-Planck-Society, 1991
- Young Investigator Award of the Wound Healing Society, 1994
- Hermann-und-Lilly-Schilling Professorship of Medical Research, 1995
- Pfizer Academic Award, 1998
- Wound Healing Award of the German Surgical Society, 2002
- Research Award of the AETAS Foundation, 2003
- Elected to the Research Council of the Swiss National Science Foundation, 2005
- Cloëtta Award (Foundation Prof. Dr. Max Cloëtta), 2008
- CE.R.I.E.S. Research Award for Achievements in Dermatological Research, 2009
- 30th Alfred Marchionini Memorial Lecture, Hamburg, Germany, 2010
- Elected Member of the Leopoldina (German Academy of Sciences), 2011
- Elected as EMBO Member, 2012

Visualizing Horizontal Gene Transfer in the Human Pathogen *Vibrio cholerae*

Melanie Blokesch

Global Health Institute, School of Life Sciences, Swiss Federal Institute of Technology Lausanne (EPFL), CH-1015 Lausanne, Switzerland

E-mail: melanie.blokesch@epfl.ch

Abstract

The human pathogen *Vibrio cholerae* resides in aquatic habitats and is often found associated with zooplankton and their chitinous exoskeleton molts. Upon growth on such chitinous surfaces, *V. cholerae* enters the developmental state of natural competence for transformation, which is an important mode of horizontal gene transfer. Natural competence enables the bacterium to take up free DNA from the environment and to incorporate the imported DNA into its own genome. How exactly the DNA enters the bacterial cell and the mechanistic aspects of this DNA uptake event are unknown.

In this study, we aimed at investigating the DNA uptake machinery of *V. cholerae* using a cellular microbiology-based approach. This allowed us to localize proteins that are involved in the DNA translocation event over time and space. Our initial results indicated that *V. cholerae* produces a type IV pilus structure as part of the DNA uptake complex. Furthermore, by using time-lapse microscopy imaging, we followed the localization of the competence protein ComEA throughout the DNA uptake process. These data led us to propose a model in which DNA uptake across the outer membrane of *V. cholerae* is driven by ratcheting and entropic forces associated with ComEA's binding to the DNA. Finally, we inspected the DNA translocation event across the inner membrane by visualizing the accepting DNA-binding and recombination protein RecA within the cytoplasm of the bacterium. We conclude with a new model and propose that both steps of the DNA uptake process are spatially coupled, suggesting the presence of a macromolecular complex, but that the translocation of the DNA across the two membranes is temporally uncoupled.

Biography

Melanie Blokesch holds a PhD degree from the Ludwig-Maximilians-Universität in Munich, Germany. After a postdoctoral stay at Stanford University (USA; Department of Microbiology and Immunology) she joined EPFL as a tenure-track assistant professor in 2009. Melanie Blokesch has been honored with many scholarships and prizes, for example, the Prize for Junior Scientists of the German National Academy of Sciences Leopoldina and an ERC Starting Grant in 2012.

ATAD5 deficiency decreases B-cell division and Igh recombination

HUSEYIN SARIBASAK

Department of Basic Medical Sciences, School of Medicine, Sifa University, Izmir, Turkey
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Abstract:

Mammalian ATAD5 and its yeast homolog Elg1 are responsible for the unloading of PCNA from newly synthesized DNA. Prior work in HeLa and yeast cells showed that a decrease in ATAD5 protein levels resulted in an accumulation of chromatin-bound PCNA and slowed cell division. We used B cells from heterozygous (Atad5+/m) mice to examine the effects of PCNA buildup and decreased cell proliferation on the immune response. An accumulation of cells in Sphase occurred in heterozygous samples and resulted in decreased cell proliferation. ATAD5 haploinsufficiency did not affect the frequency or spectrum of somatic hypermutation, indicating that PCNA accumulation did not alter base excision repair and mismatch repair activity in the Igh locus. Importantly, the Atad5+/m cells exhibited a significant decline in double-strand breaks, class switch recombination, and translocations in the switch region without affecting microhomology-mediated end joining. These results provide another description of a defect in DNA replication that affects Igh recombination via reduced cell division.

Biography

Jan. 2012 – present Assistant Professor, Sifa University, School of Medicine, Department of Basic Medical Sciences, Izmir, TURKEY

2006 – 2011 Postdoctoral Fellow**,** National Institutes of Health (NIH), National Institute on Aging (NIA), Laboratory of Molecular Gerontology & Laboratory of Molecular Biology and Immunology Baltimore, MD; USA

2006 Ph.D., Technical University of Munich (TUM), Center of Life & Food Sciences Weihenstephan; Munich / GERMANY

2002 M.Sc., Fatih University, Department of Biology; Istanbul / TURKEY

1999 B.Sc., Middle East Technical University (METU), Department of Biology; Ankara /TURKEY

Plant-Based Transient Expression Technology Provides an Inexpensive and Convenient System for the Production of Functional Active Recombinant Proteins

Tarlan Mammedov

Akdeniz University, Department of Agricultural Biotechnology, Antalya, Turkey

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Abstract

At present, several eukaryotic expression systems, including yeast, insect and mammalian cells and plants, are used for the production of recombinant proteins. Plant-based transient expression systems, on the other hand, are emerging as fast and inexpensive approaches for producing vaccine antigens and therapeutic proteins. Plants offer several advantages compared to other recombinant protein expression systems, including simple, highly scalable, cost-effective manufacturing, relative product safety due to the lack of any harbored mammalian pathogens, the presence of the eukaryotic post-translational modification machinery, and the ability to fold and assemble complex proteins accurately and accumulate hundreds of milligram quantities of target protein per kilogram of biomass in less than a week. Here, we discuss a plant-based transient expression system as an inexpensive and convenient platform for the ***production of valuable recombinant proteins*** including ***antibodies***, ***vaccines***, other ***pharmaceuticals*** and industrial ***proteins.***

Biography

Apr 2014–present Professor & FounderAkdeniz University · Department of Agricultural Biotechnology Turkey
May 2009–Apr 2014 Senior Scientist/Group Leader Fraunhofer USA Center for Molecular Biotechnology · Innovation & Technology United States
Jun 2004–Apr 2009 Research Assistant Professor University of Nebraska at Lincoln Department of Chemical and Biomolecular Engineering United States Lincoln
Oct 2002–Jun 2004 Senior Research Associate University of Nebraska at Lincoln Department of Biochemistry United States · Lincoln
Oct 2001–Sep 2002 Visiting Scientist Japan International Research Center for Agricultural Sciences Japan Ishigaki, Okinawa
Jul 1998–May 2001 STA Fellow National Agricultural Research Center for Tohoku Region Plant Phisiology lab Japan · Morioka-shi, Iwate
Dec 1990–May 1998 Chief Scientist Institute of Botany, Azerbaijan National Academy of Sciences Azerbaijan Baku
Awards & achievements
Jun 2014 Award: Correspondent member of the National Academy of Sciences of Azerbaijan
Apr 2010 Award: PROFESSOR, 2010
Jun 2008 Award: DOCTOR OF SCIENCE, 2008
Jun 2001 Award: JIRCAS Visiting Fellow, 2001-2002.
May 1999 Award: STA Fellow, 1999-2001
Jul 1998 Award: STA Fellow (Japan Science and Technology Agency), 1998

Tension and force-resistant attachment are essential for myofibrillogenesis in Drosophila flight muscle

Aynur Kaya-Çopur, Manuela Weitkunat, Stephan Grill, Frank Schnorrer

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Abstract

Muscles allow mobility to all higher animals. A better understanding of how muscles form, connect to tendons and assemble their contractile machineries is essential for understanding the causes of many skeletal and cardiac myopathies. Drosophila is an excellent model organism for muscle research. Similar to vertebrates adult Drosophila possess various muscles such as cardiac muscles, striated body muscles, and specialized indirect flight muscles enabling fast wing oscillations. Moreover, most structural components important for formation or function of sarcomeres in vertebrate muscles are conserved in Drosophila. To build a functional network, developing muscles establish stable connections with tendons and assemble their contractile machineries. Using Drosophila indirect flight muscles we investigate how muscle-tendon attachment and myofibrillogenesis are coordinated during development. Here we report that initiation and maturation of flight muscles-tendon attachment precedes the assembly of myofibrils throughout the entire muscle fiber. Laser cutting experiments show that concomitantly with the maturation of muscle-tendon attachments, mechanical tension increases and integrin signalling at myotendinous junctions is activated. If tension buildup within the myotendinous is perturbed by either genetically perturbing muscle-tendon attachment and integrin recruitment, or by severing the tendons using laser cutting, myofibrillogenesis is strongly defective. Moreover, we show that myosin motor activity is required for both tension formation and myofibril assembly, suggesting that myofibril assembly itself contributes to tension buildup. We suggest that force-resistant attachment allows strong tension increase in the myotendinous system that results in simultaneous myofibril self-assembly throughout the entire muscle fiber. Due to evolutionarily conservation of the components and structure of muscles, we believe that such a tension-based system to initiate myofibrillogenesis can also be utilized in vertebrates as well.

Biography:

I did my undergrad studies in Bilkent University, in Department of Molecular Biology and Genetics. After graduation, I moved to Heidelberg to do my PhD in EMBL, in the lab of Dr Damian Brunner. There, I worked on how forces created by different tissues are coordinated during Drosophila embryonic development.

For my postdoctoral studies, I moved to Munich to work in the lab of Dr Frank Schnorrer in Max-Planck Institute of Biochemistry. Here, I work on flight muscle development, with a focus on myofibrillogenesis. As the next step, I would like to have my own research group focusing on developmental problems using Drosophila as a model organism.

A Quantitative Approach to Size and Shape Regulation

Oguz Kanca[1], Romain Guiet[2], Olivier Burri[2], Arne Seitz[2], Markus Affolter[1], Fisun Hamaratoglu[3*]

[1] Growth & Development, Biozentrum, University of Basel, Basel, Switzerland

[2] Bioimaging and Optics Platform, EPFL, Lausanne, Switzerland

[3] Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland

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Abstract

Growth and patterning occur concurrently in the developing organs of all multi-cellular organisms from flies to humans. Their coordination is of paramount importance to yield functional organs of proper size and shape. How this coordination is achieved is a long-standing question. We aim to understand, with quantitative resolution, how two central signalling cascades, namely the Hippo and the Bone Morphogenetic Protein (BMP) pathways, work together in achieving reproducible body forms.

I will present a new tool, called Raeppli after the confetti used in the Basel Carnival, that will help us greatly in achieving this goal. Raeppli is a lineage tracing method that permanently marks a cell with one of 4 different fluorescent markers at the desired time point. Importantly, all progeny of the first cell inherits the color-code forming a "clone". Cell division rates then can be inferred from clone sizes. Since all clones are induced simultaneously, differences in clone sizes reflect differential growth rates. Hence, Raeppli provides a reliable and quantitative measure of growth rates in live and fixed samples.

Using multiple copies of the construct increases the spatial resolution, but simultaneously makes the image analysis more challenging. Our progress in obtaining quantitative information from images with multiple copies of Raeppli will be presented.

Biography

Fisun Hamaratoglu received her PhD in 2007 from the Baylor College of Medicine for her work in the group of Georg Halder at the MD Anderson Cancer Center in Houston, USA. Her thesis work identified several upstream regulators, including the first receptor, of the Hippo tumor suppressor pathway. In 2008, she joined Markus Affolter's laboratory at the Biozentrum, University of Basel, for her post-doctoral training, funded by HFSP, Marie Curie and Roche postdoctoral fellowships. In Basel, as part of the WingX.ch initiative, she established the fly wing as a model system to study scaling quantitatively and at a molecular level.

She joined the CIG in October 2013 as a Maître Assistant suppléant and will be a Swiss National Science Foundation Professor in April 2014.

Wake Up Call: Photosignaling by Cryptochrome to Reset the Biological Clock

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Abstract

The circadian clock regulates many physiological events in synchrony with the geological clock. Dysfunction of the circadian clock is associated with diseases such as cancer, diabetes, and neurological disorders. Sunlight entrains the circadian clock to a 24 hr period through photoreceptors located in the eyes. In this seminar I will discuss light-induced reactions in the fruit fly (*Drosophila*) photoreceptor Cryptochrome. I will describe the light-induced biochemical (conformational and photoreduction) changes of Cryptochrome and the light-dependent clock resetting through degradation of Cryptochrome by a novel E3 ubiquitin ligase complex [1-3]. These light-induced reactions ensure that the circadian clock is reset by Cryptochrome in the early morning only once a day.

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Biography

BSc in Biology, Istanbul University, 1998
MSc in Molecular Biology and Genetics, 2000, Bilkent University (Supervisor: Prof. Dr. Mehmet Ozturk)
PhD in Molecular Biology and Genetics, 2006, Bilkent University (Supervisor: Prof. Dr. Mehmet Ozturk, Thesis: HETEROGENEITY OF HEPATOCELLULAR MALIGNANT PHENOTYPE)
Postdoctoral study in the laboratory of Prof. Dr. Aziz Sancar at the University of North Carolina at Chapel Hill, USA between 2006-2012. Subject: 1- The relationship between the circadian clock and cancer 2- The biochemical mechanism of the circadian clock resetting by light.
Research Assistant Professor at the Department of Biochemistry and Biophysics at the University of North Carolina at Chapel Hill, USA between 2012-2014 Subject 1- Biochemical regulation of the core clock transcription factors in mammals and Drosophila. 2- Magnetoreception and Cryptochrome (as a part of a DARPA project).
Assistant Professor at Gebze Institute of Technology 2014-continues. Research area: 1- The circadian clock and aging 2- Light-controllable gene expression systems.

Fine Tuning of Wnt/B-Catenin Signaling: Welcoming Wnt Ligands at the Plasma Membrane

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Abstract

Wnt/β-catenin signaling plays critical roles during embryogenesis, tissue homeostasis, and regeneration. Due to these essential roles of Wnt signaling, misregulation of the Wnt pathway causes cancer, hereditary defects and many other human diseases. Considering the deleterious consequences of pathway misregulation, it is probably not surprising that the Wnt/β-catenin signaling pathway is complex and that a large number of positive and negative modifiers of β-catenin signaling have evolved. We have recently characterized new pathway modifiers that are critical for the activity of Wnt-receptor complex. Here, we identify the Ly6 family protein LY6/PLAUR domain-containing 6 (Lypd6) as a positive feedback regulator of Wnt/β-catenin signaling. *lypd6* enhances Wnt signaling in zebrafish and Xenopus embryos and in mammalian cells, and it is required for wnt8-mediated patterning of the mesoderm and neuroectoderm during zebrafish gastrulation. Lypd6 is GPI anchored to the plasma membrane and physically interacts with the Wnt receptor Frizzled8 and the coreceptor Lrp6. Biophysical and biochemical evidence indicates that Lypd6 preferentially localizes to raft membrane domains, where Lrp6 is phosphorylated upon Wnt stimulation. *lypd6* knockdown or mislocalization of the Lypd6 protein to non-raft membrane domains shifts Lrp6 phosphorylation to these domains and inhibits Wnt signaling. Thus, Lypd6 appears to control Lrp6 activation specifically in membrane rafts, which is essential for downstream signaling. Furthermore, new data suggest that raft-specific LRP6 phosphorylation is a consequence of preferential Wnt-receptor complex formation in raft domains and that this initial process also involves Lypd6.

Biography

2003 B.Sc., Molecular Biology and Genetics, Middle East Technical University, Ankara, Turkey
2005 M.Sc., Molecular Biology, International Max Planck Research School for Molecular Biology, Gottingen, Germany
2009 Ph.D., Evolutionary Developmental Biology, Max Planck Institute for Developmental Biology, Tubingen, Germany
2009-2013 Postdoctoral fellow, Biotechnology Center & Center for Regenerative Therapies, University of Technology Dresden, Dresden, Germany
2014- Assistant Professor, Advanced Biomedical Research Center, Dokuz Eylul University, Izmir, Turkey

Biomarker-Guided Sequential Targeted Therapy: A Novel Approach to Overcome Resistance in Rapidly Evolving Tumors

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Abstract

Combinatorial targeted therapies are more effective in treating cancer by blocking by-pass mechanisms or inducing synthetic lethality. However, their clinical application is hampered by resistance and toxicity. To meet this important challenge, we developed and tested a novel concept of biomarker-guided sequential applications of various targeted therapies using ErbB2-overexpressing/PTEN-low, highly aggressive breast cancer as our model. We found that sustained activation of ErbB2 and downstream pathways drives trastuzumab resistance in both PTEN low/trastuzumab resistant breast cancers from patients and mammary tumors from genetically engineered mice. Although lapatinib initially inhibited trastuzumab resistant mouse tumors, tumors by-passed the inhibition by activating the PI3K/mTOR signaling network which was also observed in neo-adjuvant lapatinib-treated patients manifesting lapatinib-resistance. Trastuzumab+lapatinib resistance was effectively overcome by sequential application of a PI3K/mTOR dual kinase inhibitor (BEZ235) with no significant toxicity: however, BEZ235 treatment led to increased ErbB2 expression and phosphorylation in mouse tumors and in 3-D culture leading to BEZ235 resistance. Mechanistically, we identified ErbB2 protein stabilization and activation as a novel mechanism of BEZ235 resistance which was reversed by subsequent lapatinib+BEZ235 combination. Remarkably, this sequential application of targeted therapies guided by biomarker changes in the rapidly evolving resistant tumors doubled the life-span of mice bearing exceedingly aggressive tumors. This fundamentally novel approach of using targeted therapies in a sequential order can effectively target and reprogram the evolving resistant cancer signaling networks during treatment.

Biography

Dr. Özgür Şahin graduated from the Department of Molecular Biology and Genetics at Middle East Technical University in 2003. He then earned his Ph.D. degree in cancer systems biology at German Cancer Research Center (DKFZ) and the University of Heidelberg in 2008. He held positions as a group leader in the Division of Molecular Genome Analysis at DKFZ and as research faculty in the Department of Molecular and Cellular Oncology at the University of Texas MD Anderson Cancer Center (MDACC). He is a recipient of the MDACC Specialized Program of Research Excellence from the USA, and the Wilhelm Sander Foundation Grant in Cancer Research from Germany. Since September 2013, he is an assistant professor in the Department of Molecular Biology and Genetics at Bilkent University. There, is projects are supported by EMBO Installation Grant and Marie Curie Career Integration Grant. He is also a recipient of TUBA-GEBIP and BAGEP awards in 2014.

Life sciences in Turkey: from past to future

Mehmet Ozturk

Advanced Biomedical Research Center, Dokuz Eylul University, Izmir, Turkey

Biography

Mehmet Ozturk, Professor of Medical Biology, is currently the Director of Dokuz Eylul University Advanced Medical Biology Research Center. He previously served as the head of Bilgen Genetics and Biotechnology Research Center and Professor of Molecular Biology at Bilkent University. After graduating from Gazi University Faculty of Pharmacy, he obtained a PhD degree in biochemistry from Paris XI University in 1985. He worked at Harvard Medical School and Massachusetts General Hospital in Boston (USA) between 1985-1992, as a post-doctoral fellow and assistant professor of biochemistry. Dr. Ozturk created the Molecular Oncology Unit of Leon Berard Cancer Center in Lyon (France) in 1992 and worked as an INSERM Research Director at this institution between 1992-1995. He, then, moved to Ankara (Turkey) to create the Department of Molecular Biology and Genetics of Bilkent University in 1995. This department received Koc Award in 2004 for its outstanding contributions to life sciences in Turkey. Prior joining his current position, he worked as INSERM Research Director at CRI INSERM_UJF U823 (Institut Albert Bonniot, Grenoble) jointly with his position at Bilkent University.

Dr. Ozturk is a molecular biologist working on genetic and molecular mechanisms of cancer. He published more than 100 research papers that received more than 7000 citations (h-index: 40). He received several awards including TUBITAK-TWAS Science Award. He is a member of EMBO, European Molecular Biology Organization and TWAS, The Academy of Sciences for the Developing World. Turkish Academy of Sciences. He served as a UNESCO International Bioethics Committee Member between 1998-2001.

ABSTRACTS SELECTED AS ORAL PRESENTATIONS

Session: Bioinformatics/Omics

The evolutionary history of synchronous urothelial tumors revealed by exome sequencing

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Abstract

Next-generation sequencing has opened up new avenues for understanding the mechanisms of carcinogenesis. One long-standing contention concerns the origin of multiple synchronous tumors in bladder cancers: are the separate tumors monoclonal, or do they represent independent clones that developed due to carcinogenic exposure? [1-7] Here we used high coverage exome-sequencing data from two separate synchronous tumor samples and neighboring healthy tissue. Evolutionary analysis of single nucleotide variants supported a single origin for the two tumors in this individual. Moreover, by studying the spectrum of mutations and comparing with known driver genes, we predicted the evolution of tumorigenesis in this individual: (1) the process initiated with rapid accumulation of TC->TT mutations across the genome driven by APOBEC overactivity, (2) at least two driver genes were hit by TC->TT missense mutations, (3) once tumorigenesis started, one clone migrated to neighboring tissue and the second tumor developed. Our results demonstrate the power of genomic analysis in uncovering details of tumorigenesis.

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Role of Thiol Peroxidases in the Redox Control of Genome Stability and Cell Fitness at a Single Nucleotide Resolution

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Abstract

Thiol peroxidases are critical enzymes in the redox control of cellular processes that function by reducing low levels of hydroperoxides and regulating redox signaling. These proteins were also shown to regulate genome stability, but how their dysfunction affects the actual mutations in the genome is not known. *Saccharomyces cerevisiae* has 8 thiol peroxidases of glutathione peroxidase and peroxiredoxin families, and the mutant lacking all these genes (Δ8) is viable. In this study, we employed two independent Δ8 isolates to analyze the genome-wide mutation spectrum that results from deficiency in these enzymes. Deletion of these genes was accompanied by a dramatic increase in point mutations and mutation clusters, consistent with oxidative DNA damage. We also subjected multiple lines of wild-type and Δ8 cells to long-term mutation accumulation, followed by genome sequencing and phenotypic characterization. Δ8 lines showed a significant increase in non-recurrent point mutations and indels. The original Δ8 cells exhibited reduced growth rate and decreased lifespan, which were further reduced in all Δ8 mutation accumulation lines. The original Δ8 isolates showed similar patterns of gene expression, suggesting the role of thiol peroxidases in the observed phenotypes. This study shows how deficiency in non-essential, yet critical and conserved oxidoreductase function, leads to increased mutational load and decreased fitness.

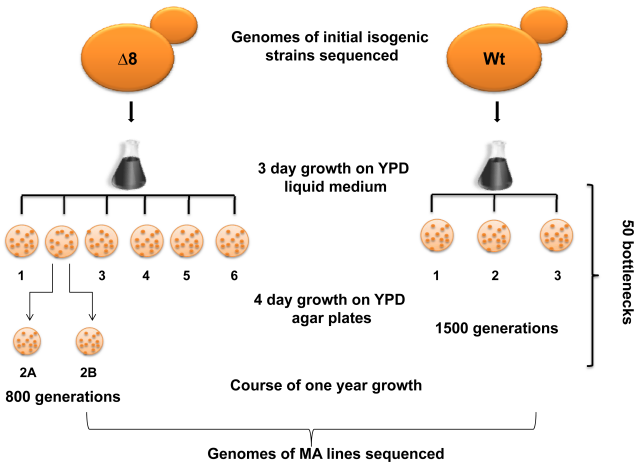

Figure 1. Experimental scheme.

***Keywords*:** Thiol peroxidase, mutation, genome stability, *Saccharomyces cerevisiae*

Phisto: A Web-Based Platform for Studying Infection Mechanisms through Pathogen-Human Interactions

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Infectious diseases are one of the leading causes of death and disability worldwide. The molecular interactions between the infectious microorganisms, pathogens and their human hosts allow the microorganisms to manipulate human cellular mechanisms to their own advantage, resulting in infection. A thorough understanding of these pathogen-host interactions (PHIs) will elucidate the mechanisms involved in infections, and allow novel therapeutic solutions to be devised. In the post-genomic era, following the advances in genomics, proteomics, and then interactomics, PHI data can be produced in large-scale within the last decade. We have developed a Web-based platform, PHISTO (Pathogen Host Interaction Search Tool) to make PHI data available from one single source for systems pharmacology research. It enables access to the most up-to-date PHI data for all pathogen types whose experimentally-verified protein interactions with human are available, via a user-friendly and functional Web interface at www.phisto.org. Our goal is to facilitate the efforts focusing on enlightening infection mechanisms through PHIs. The platform also offers integrated tools for visualization of PHI networks, graph theoretical analysis of human proteins targeted by pathogens and BLAST search. Recently, we have implemented additional bioinformatics tools into PHISTO to enable users to analyze the functional properties of human proteins targeted by pathogens during infections. This type of analysis may give crucial insights on infection strategies used by pathogens, in terms of their attacking behavior. Such bioinformatics tools increase the potential of PHISTO to serve as a network analysis platform to investigate the interspecies protein interaction networks between pathogens and human to elaborate on infection mechanisms.

Session: Gene Regulation

Sp1 Regulates URG-4 Promoter in Hepatoma and Osteosarcoma cells

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URG-4 (Up-regulated Gene 4) Gene that located on choromosome 7, was firstly found in Hepatitis B-infected liver cells [1]. It was suggested that URG-4 gene was natural effector of HBxAG protein encoded by Hepatit B virus [2]. Then, it was clarified that URG-4 may be associated with the onset of tumorigenesis and accelerated tumor development in nude mice. Our results also showed that this gene was expressed nearly all cancer cell types such as osteosarcoma, breast, prostate and colon carcinoma [3]. However, there is no information on the transcriptional regulation of the gene. Therefore, in order to to elucidate the transcriptional regulation of the URG-4 gene, 530bp URG-4 promoter site (-482/+63 pMet) and different truncated promoter constructs (-109/+63 pMet, -261/+63 pMet and -344/+63 pMet) were amplified by PCR based approach and cloned into pMetLuc lusiferase vector. -109/+63 pMet URG-4 promoter construct has the highest basal activity. Promoter constructs were then transient transfected into Hepatoma cells. Sp1 and USF transcription factors were cotransfected with promoter constructs and Sp1 and USF transcription factors upregulated promoter activity. EMSA analysis were also carried out with promoter sites containing putative Sp1 and USF sites. EMSA showed that SP1 transcription Factor is able to bind to URG-4 promoter sites, -121/-159 both Hep3B and Saos-2 nuclear extracts.

***Keywords*:** URG-4, transcriptional regulation, Sp1, promoter

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Glucose Signaling Controls the Programmed frameshift Efficiency of the *EST3* Gene in *S. cerevisiae*

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Abstract

The expression of the *EST3* gene, which encodes the regulatory subunit of telomerase, is regulated by a programmed ribosomal frameshift mechanism at the translation level. [1] The full length Est3p is synthesized by a +1 ribosomal frameshift. In the absence of the programmed ribosomal frameshift, the translation of *EST3* mRNA is terminated at an internal stop codon and a 93 amino acid long truncated Est3 peptide with no known functions is produced. [2] In this study, we have shown that the translation of the full length Est3p is regulated by glucose signaling in *S. cerevisiae*. When the yeast cells were grown in glucose medium, the frameshift rate of *EST3* was determined to be 9% of the translation events in the BY strain of *S. cerevisiae*. Unexpectedly, growth of the yeast strain in glycerol or ethanol media resulted with an 8 to 10-fold decrease in the programmed ribosomal frameshift rate in *EST3* mRNA. Shifting yeast cells from nonfermentable carbon source media to glucose medium increased the frameshift rate at least 15-fold, indicating that the expression of the EST3 gene at the translation level is regulated by glucose signaling. We have also tested the effects of Stm1p, which is located at the mRNA binding site on the ribosome. Our results have shown that the frameshift rate in the *stm1* mutant yeast strain is decreased 2-fold. In addition, we have also tested the effects of Asc1p, which is involved in glucose sensing and signaling. Asc1p is also a ribosomal protein. Frameshift rate of *EST3* decreased 4-fold in the *asc1* mutant yeast strain. These results showed that glucose signaling regulates the expression of the *EST3* gene in *S. cerevisiae*. Stm1p and Asc1p are also involved in the regulation of the frameshift rate in *EST3* in response to glucose signaling.

Keywords: *S. cerevisiae*, Ribosomal frameshift, Glucose signaling, Telomerase, Translational control.

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Genome-wide Localisation Analysis for IRF4 Target Gene Identification in Melanoma Cell lines

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Abstract

With increasing rate of diagnosis worldwide, melanoma is one of the most lethal and aggressive form of skin cancer. Melanoma originates from accumulation of genetic alterations and transformation of melanocytes which gives them abnormal proliferation, invasion and metastasis abilities. [1] Interferon Regulatory Factor 4 (IRF4), is a transcriptional regulator, with functional roles in differentiation and function of immune cells. Previous studies have demonstrated that IRF4 expression levels are elevated in some lymphoid cancers and IRF4 is one of the key transcription regulators for the survival of multiple myeloma and ABC-type diffuse large B-cell lymphomas (ABC-DLBCL) cells. [2, 3] Recent studies from our lab and elsewhere demonstrated high expression levels of IRF4 in melanocytes and melanoma cells, [4, 5, 6] which encouraged us to investigate the functional roles of IRF4 in melanoma. In this study, we investigate the genome-wide localisation of IRF4 via high-throughput sequencing of immunoprecipitated chromatin (ChIP-seq) in melanoma cell lines, to help identify IRF4-regulated genes and pathways in melanoma. Initial results from ChIP-qPCR experiments in our lab demonstrated IRF4 binding on Tyrosinase gene which is one of key developmental genes in melanocytes.

***Keywords*:** IRF4, Melanoma, ChIP-seq

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Session: Cancer Biology

Alternative Polyadenylation Dependent 3'-UTR Shortening in Triple Negative Breast Cancer

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Abstract

Triple Negative Breast Cancers (TNBCs) are aggressive and highly proliferative tumors characterized by lack of estrogen (ER) and progesterone (PR) and human epidermal growth factor 2 (HER2) receptors [1]. Due to high proliferative indices of TNBCs; we hypothesized alternative polyadenylation (APA) to play a role in TNBC. APA has newly emerging roles in gene expression regulation by causing shortening or lengthening of 3'-UTRs (Untranslated Regions) [2]. To address 3'-UTR shortening events in TNBCs, a probe based meta-analysis tool (APADetect) was used. When we compared 520 TNBC and 82 normal breast tissue samples with APADetect, we detected 113 3'-UTR shortening events. After a detailed *in silico* analysis, we focused on candidate genes showing significant 3'-UTR shortening events. For further validation, first we confirmed the existence of short 3'-UTR isoforms by RACE. Later, we used RT-qPCR to analyze 8 TNBC cell lines and a panel of breast cancer patient cDNAs. Increased protein levels due to mRNA shortening in TNBC cell lines were shown by Western blotting. Our results, for the first time, show a possible role of APA and a significant shift to shorter 3'-UTR usage in TNBCs. In addition to gene expression analysis, identification of post-transcriptional regulations such as APA could improve current knowledge on the molecular etiology of TNBCs.

Keywords: Alternative polyadenylation, Triple negative breast cancer, 3'-UTR

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The effect of HL-60 myeloid leukemia cells treated with ATRA, vitamin D3, IFN-γ, or LPS on T cell co-stimulation

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All-trans retinoic acid (ATRA) and 1α,25-dihidroxyvitamin D3 (D3) can reduce proliferation and promote maturation of acute myeloid leukemia (AML) cells [1]. In this study, HL-60, a representative AML cell line, was treated with ATRA or D3 and/or IFN-γ or LPS. These derivatives of HL-60 were evaluated for expression of co-stimulatory molecules and their effect on T cell proliferation. Optimal concentrations and incubation periods inducing HL-60 maturation were determined. Myeloid marker CD11b and B7 family co-stimulatory molecules (CD80, CD86, PD-L1, PD-L2, B7-H2) were determined by flow cytometry or RT-PCR. CD11b(+) mature AML cells and CD4(+) helper or CD8(+) cytotoxic T cells were sorted by fluorescence-activated cell sorting (FACS). AML cells and T cells were co-cultured with anti-CD3 antibody and T cells' proliferation was determined with CFSE dilution. ATRA or D3 increased the cells' sensitivity to LPS or IFN-γ. The level of co-activator B7-H2 was decreased whereas CD86 and co-inhibitors PD-L1 and PD-L2 were increased especially on CD11b(+) cells in the presence of IFN-γ. These cells significantly reduced both helper and cytotoxic T cell proliferation. T cell proliferation was least promoted with vitamin D3 and IFN-γ-treated HL-60 cells. Thus, CD11b positivity in AML may correlate with increased expression of co-inhibitory PD-L1 and PD-L2 molecules reducing anti-leukemic T cell responses.

Key words: Leukemia, ATRA, D3, co-stimulation, CD11b, tumor immunology

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A conditional knockout mouse model uncovers essential roles of Cdk1 in the mammalian cell cycle and tumorigenesis

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Abstract

The mammalian cell cycle is regulated by concerted actions of cyclin-dependent kinases (Cdk) and their partner cyclins. To this date, all Cdks (1, 2, 4 and 6) have been targeted *in vivo* using mouse models. Cdk1 was found to be the only member of the Cdk family that is essential for cell division. Loss of Cdk1 was reported to be lethal prior to embryonic day 3.5. To analyze the effects of Cdk1 loss in different organs and tissue types, we have generated a conditional knockout mouse model that utilizes Cre/LoxP recombination system. Deletion of Cdk1 from mouse embryonic fibroblasts (MEF) results in halt of cell divisions and proliferation. Cdk1 ^{NULL} cells do not die but enter cellular senescence prematurely. Cdk1^{NULL} blastocysts are viable and have fewer but larger nuclei. Similarly, liver specific Cdk1 knockout mice are viable and have fewer hepatocytes with enlarged nuclei. After induction of liver regeneration by partial hepatectomy, Cdk1^{NULL} hepatocytes can re-enter cell cycle as efficiently as WT controls. However, Cdk1^{NULL} livers are immune to liver tumorigenesis induced by a combination of Sleeping Beauty transposase, activated Ras oncogene and inactivated p53 pathways. Liver tumor cells lines with Cdk1^{FLOX} genotype can be induced to become Cdk1^{NULL} which abolishes cell proliferation and growth *in vitro.*

***Keywords*:** Cyclin-dependent kinase, cell cycle, tumorigenesis, transgenic mouse models

Session: Immunology

Mutation of the NF-kB binding site in the IL-7Ralpha gene enhancer region using TALEN proteins

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Abstract

Nuclear factor kappa B (NF-kB) is one of the most important transcription factors in cell signaling. Interleukin 7 receptor (IL-7R) is an essential element in T lymphopoiesis and IL-7 is the key signaling factor in early thymocytes. IL-7R is composed of an alpha chain and IL-2R gamma chain. An NF-kB binding motif was identified in the evolutionary conserved region 3 (ECR3) of the IL-7Ralpha containing a putative transcriptional enhancer. To identify the functional significance of this NF-kB binding motif, we mutated it in murine T lymphocyte cell lines using transcription activator-like effector nuclease (TALEN) genome editing technology. TALENs consist of a sequence-specific TALE DNA binding domain fused to a FokI nuclease domain, which generates DNA double stranded breaks in targeted gene loci. We hypothesized that mutation of this NF-kB motif would downregulate IL-7R expression in the murine RLM11 CD4+ thymoma cell line that is naturally IL-7Rhigh. We used restriction fragment length polymorphism (RFLP) assay to detect the presence of mutations in the targeted region and we analyzed IL-7R expression by flow cytometry. We sequenced PCR amplicons of mutated, IL-7Rlow RLM11 cells and identified TALEN-induced mutations resulting in 8bp and 16bp deletions near or containing the NF-kB binding site. We cloned these mutant sites into luciferase reporter constructs and identified their transcription activation properties that were significantly lower than WT enhancers. Our studies so far have shown that NF-kB is a regulator of IL-7Ralpha expression and TALEN-induced genome mutagenesis is a reliable, target-specific method that can be used to manipulate gene expression.

***Keywords*:** Immunity, T Lymphocyte, TALEN, genome editing, IL-7R, NF-kB

NLRC3 Protein is a Novel Inhibitor of Inflammasome

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Abstract

Inflammasomes are protein complexes which recognize pathogen and activate inflammation. The Cryopyrin inflammasome has been widely studied due to its implication in the physiopathology of several auto-inflammatory diseases. To avoid accidental induction of the inflammation, tight regulation of the Cryopyrin inflammasome activation is necessary. NLRC3, another member of the NLR family, was found to be a negative regulator of T-cell activation [1], NFκB pathway [1, 2] and STING-dependent cytokine secretion [3]. However, the effect of NLRC3 on the Cryopyrin inflammasome is still unknown. We proposed that NLRC3 could be a novel regulator of the Cryopyrin inflammasome. Co-expression of NLRC3 with Cryopyrin protein in HEK293FT cells resulted in a significant decrease in both IL-1β secretion and ASC speck formation, which are the hallmarks of the Cryopyrin inflammasome activation. Similarly, IL-1β secretion and cleavage in response to several known Cryopyrin inflammasome activators was significantly higher in THP-1 NLRC3 KD stable lines compared to control cells expressing NLRC3. Moreover, ectopic expression of NLRC3 in inflamed rat eye decreased IL-1β levels. In conclusion, we have found that NLRC3 interferes with ASC speck formation and represses IL-1β secretion and cleavage in all systems tested. Thus, NLRC3 is a novel negative regulator of the Cryopyrin inflammasome.

***Keywords*:** NLRC3, Cryopyrin, Inflammasome, IL-1β, Inhibition

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Session: Biotechnology/Plant Molecular Biology/Signal Transduction

Electrochemical Detection of Cancer Biomarker microRNAs Based on p19 Protein on Cell Lysate

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Abstract

MicroRNAs (miRNAs) are small (~22 nucleotides) non-coding RNAs that regulate gene expression post-transcriptionally. Deregulation of miRNAs (up or down regulation) in cancer cells could be thought as an indication for the cancer, detection of miRNAs is very crucial for early cancer diagnosis and treatment of most of the cancer types. Due to low content inside the cell and high similarity between other miRNAs, detection of specific miRNAs is very important. Enzymatic detection of mir 21 has been investigated by our group[1]. In this work, a novel electrochemical biosensor was designed for the detection of mir21 from cell lysates based on the oxidation signal of tryptophan amino acid inside the protein , p19. Due to size specific recognition property of p19, target miRNA is detected in a direct, label-free, rapid and inexpensive way upon hybridization with its complementary target followed by p19 interaction[2]. The altered tryptophan oxidation signals after and before the interaction with hybrid miRNA, is the indication of the detection and the conformational change of p19.

Similarly the detection of mir 122 electronic detection of miRNA-122a at attomolar level with high specificity have been done via protein p19[3].

***Keywords*:** microRNA , p19 protein, Biosensor

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Genetic Modificaiton of *Cadmium determinant* (CDM1) Enhances Fortificaiton of Arabidopsis Seeds with Essentıal Minerals but not Cadmium

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Abstract

Cadmium (Cd) is a toxic heavy metal contaminant in arable lands resulting from anthropogenic activities. Due to its similarity to essential metals such as iron (Fe), Cd is readily absorbed by plants, and causes severe health risks both in plants and in humans even in minute concentrations. Therefore, understanding novel molecular mechanisms that are necessary for accumulation of Fe in edible parts of plants, and tolerance against Cd toxicity will be the first step for the development of crops that are better Fe sources yet do not accumulate Cd. Here we present CADMIUM DETERMINANT1 (CDM1) as a novel metal transporter that is highly induced upon Cd treatment. Transgenic Arabidopsis expressing *GFP-CDM1* showed specific fluorescence in plastids, indicating role of plastids in Cd accumulation mediated by CDM1. The *cdm1* mutants were more tolerant to Cd than wildtype, and accumulated less Cd, Fe and Zn both in shoots and roots. In contrast, overexpression of *CDM1* caused hyperaccumulation of Cd specifically in roots, and Cd hypersensitivity in plant. Interestingly, overexpression of *CDM1* in plants enhances the accumulation of essential metals (Fe,Zn,Mn,Co,Cu) while inhibiting the accumulation of Cd in seeds. Overall, CDM1 can be utilized for bio-fortification of crop species in future.

***Keywords*:** cadmium, iron, Arabidopsis, biofortification, seed

Pulsed Electromagnetic Field (PEMF) Effect on Biochemical Pathways

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Abstract

Pulsed generations and antenna-applicator systems are used in wound healing applications. PEMF is a non-invasive method which causes time-varying current formation in tissues and has a potential of increasing tissue regeneration. Cell culture studies have represented effects of electromagnetic fields on both signal transduction pathways and growth factor synthesis. We investigated the effects of our design applicator and different signal types on wound healing and Ca^{+2} fluxes with *In Vitro* assays. Earlier studies have determined the effect of a PEMF signal modulates Ca^{+2} binding to calmodulin (CaM) so the signaling molecule nitric oxide (NO) activity may increase. [1] L929 cells were seeded in a 24-well plates then a small area was scratched using 200 µL pipette tip, and treated with non-invasive PEMF signals. Two different signals were used targeting the calcium/calmodulin pathway which is early step in the anti-inflammatory cascade and growth factor stages of tissue repair. PEMF has been shown to increase the binding of calcium to calmodulin. After 1 week, 75 Hz sinus signal had a better effect on wound repair, but statistically not a significant increase in wound tensile strength compared to control.

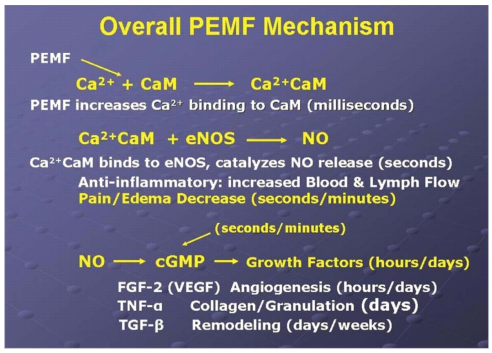

Figure 1. A proposed model for PEMF transduction mechanism.

***Keywords*:** Electromagnetic field, wound healing, PEMF signal, tissue regeneration.

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Determination, Function and Subcellular Localization of Plant Fungal pathogen Effectors

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Understanding plant-pathogen interactions is critical in designing strategies for protecting the plants, most importantly crops, against diseases. Plants defend themselves by encompassing specific recognition particles, elicitors of pathogen associated molecular patterns (PAMPs) with PAMP-triggered immunity (PRRs). This defense layer induces specific immune response mechanisms in a very swift and transient manner while ensuring host cell survival in most cases. Successful pathogens may evade this defense trap by suppressing pathogen triggered immunity (PTI) with the help of pathogen effectors. However, plants may counteract by detecting and interacting with these pathogen effectors through specific R proteins. This second layer of immunity is known as effector triggered immunity (ETI). It recognizes effectors in race specific or gene-for-gene manner. It is robust and prolonged and it stimulates hypersensitive response and systemic acquired resistance upon activation.

We are investigating the function of the candidate effectors obtained by transcriptome sequencing. The candidate effectors of *Puccinia striiformis* f. sp. *tritici* (Pst) known to be expressed either during germination of spores or in the host are cloned and preliminary experiments of their subcellular localizations were conducted. The other major focus of our study is to identify microRNA like small RNAs of Pst, *Blumeria graminis* f. sp. *hordei* and *Priformaspora indica* targeting host gene messages to promote or suppress disease formation, respectively. Number of known and miRNA like novel RNAs are (mirLN) detected based on small RNA sequencing data we obtained. Current data will be presented.

Session: Cell Biology/ Developmental Biology / Stem Cell Research

Moving beyond the parts list: Protein proximity mapping at the origin of centriole duplication

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Abstract

The centrosome is the main microtubule-organizing center of animal cells and is composed of two centrioles associated with pericentriolar material. Centriole duplication happens once and only once during cell cycle and is analogous to DNA duplication. Defects in centriole duplication are associated with a variety of human diseases including cancer and microcephaly. Centriole duplication occurs once per cell cycle and is effected by a set of proteins including PLK4, CEP192, CEP152 and CEP63. Information on the relationships between these components is limited due to the difficulty in assaying interactions in the context of the centrosome. We use proximity-dependent biotin identification (BioID) combined with centrosome enrichments to identify proximity interactions among centrosome duplication proteins [1]. Surprisingly, BioID for CEP63 and its paralog CCDC67 revealed extensive proximity interactions with centriolar satellite proteins, an array of granules around the centrosome. Focusing on these satellite proteins identified two new regulators of centriole duplication, CCDC14 and KIAA0753. Both proteins co-localize with CEP63 to satellites, bind to CEP63 and identify other satellite proteins by BioID. KIAA0753 positively regulates centriole duplication and CEP63 centrosome localization, whereas CCDC14 negatively regulates both processes. These results suggest that centriolar satellites have a previously unappreciated function in regulating centriole duplication.

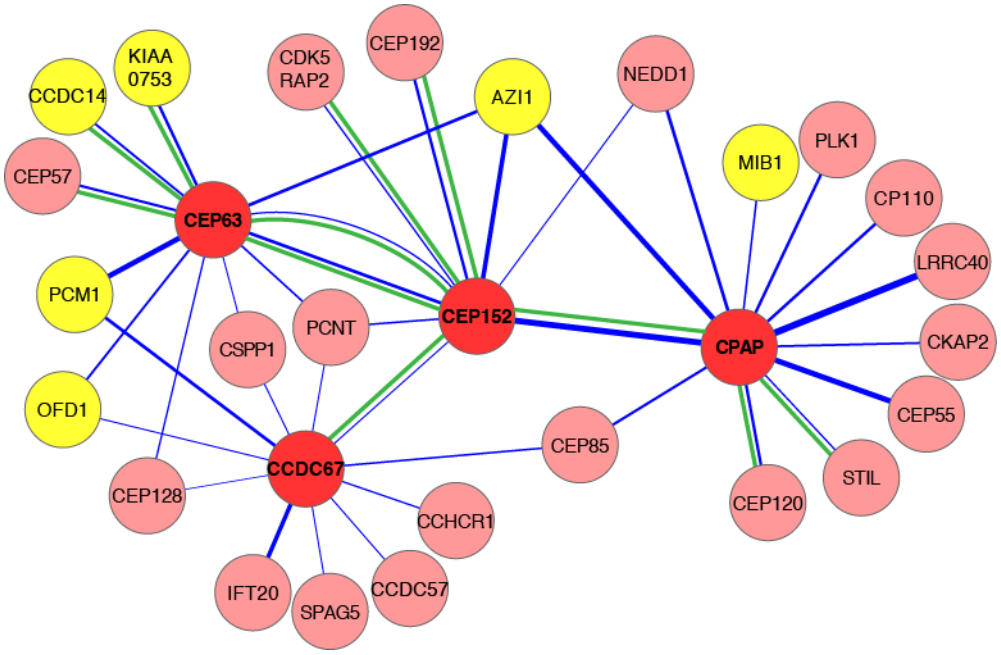

Figure 1. Proximity interaction map at the origin of centriole duplication

***Keywords*:** centrosome, centriole duplication, BioID, centriolar satellites

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HOX and TALE Gene Expression Profile of Bone Marrow Derived Mesenchymal Stem/Stromal Cells from Fanconi Anemia Patients and Donors

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Abstract

HOX/TALE proteins regulate embryonic development and tissue homeostasis in adults [1,2,3]. HOX/TALE proteins maintain correct hematopoiesis and their misexpression disregulate self-renewal and differentiation of hematopoietic stem cells (HSCs) [3]. Fanconi anemia (FA) patients are characterized with congenital anomalies and profound bone marrow (BM) failure [4]. Given the intense crosstalk between HSCs and mesenchymal stem/stromal cells (MSCs), *HOX*/TALE expression may change in BM of FA patients and have a role in the progression of anemia. Herein, 39 *HOX* and 8 TALE (*MEIS1*, *MEIS2*, *PREP1*, *PREP2*, *PBX1*, *PBX2*, *PBX3*, *PBX4*) gene expression of FA pre- (n=12)/post- (n=6) BM transplant (BMT) and donor (n=17) MSCs were determined using hydrolysis probes and LightCycler 480-II RT-qPCR (Roche). One-way ANOVA was used to test for statistical significance among groups in terms of relative gene expression. Random Forest (RF) and support Vector Machines (SVMs) algorithms were applied to predict genes that may distinguish groups. *HOXA13*, *HOXC13* and *PREP2* expression of preBMT MSCs were significantly lower than donors ($p<0.05$). Both RF and SVMs algorithms also predicted *HOXA13* and *PREP2* genes as discriminative genes. Our results indicate *HOXA13*, *HOXC13* and *PREP2* expression dysregulation of BM stroma may be a cause of bone marrow failure in FA.

Keywords: *HOX*/TALE gene expression, mesenchymal stem/stromal cells, Fanconi anemia, bone marrow failure

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P.S. This study was previously presented at '*1. Uluslararası Katılımlı Kök Hücre ve Hücresel Tedaviler Kongresi*' in Kocaeli, Turkey and at COST Action BM0805 HOX and TALE Meeting in Egmond aan Zee, The Netherlands.

Mechanical Vibrations Normalize Molecular and Ultrastructural Properties of Mesenchymal Stem Cells during Adipogenesis

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Obesity is associated to cardiovascular and metabolic diseases. Prevalence of obesity have increased across the years based on technological developments that supported nutritional availability and sedentary lifestyles [1]. Restoring mechanical activity with physical exercise suppresses obesity, but lack of compliance limits the integration of those benefits to the majority of the population [2,3]. Mechanical loading can also be delivered passively with whole body vibrations with high frequency and low magnitude [4], but how stem cells responsible for adipogenesis sense this mode of loading is currently unknown. Here, we tested whether vibratory signals (0.15g, 90Hz, 15min/day for 7 days) affect the ultrastructure and molecular profile of mesenchymal stem cells *in vitro* during quiescence and adipogenesis. Adipogenesis increased actin content 2-fold compared to quiescent cells, and vibrations reduced that increase by 22% ($p<0.05$). Adipogenesis also increased cellular circularity by 50%, and similar to actin content, vibrations reduced that increase by 20% ($p<0.05$). Moreover, daily application of mechanical vibrations decreased the expression of genes responsible for adipogenesis such as adipsin, c-EBP/α and PPARγ up to 87%. In conclusion, results suggest that mesenchymal stem cells can directly sense and adapt to mechanical loads during adipogenesis, expressing a phenotype that is closer to quiescent cells.

***Keywords*:** cell ultrastructure**,** mechanical loads, mesenchymal stem cells, obesity

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Session: Biochemistry

An Enzyme of A Novel Clock Component: Cystathionine-B-Synthase (CBS)

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The circadian clock is a biological timing system that oscillates with a period of 24 hours and is coordinated with the day-night cycle. This timing machinery is necessary for the body to anticipate environmental changes, to react external changes and adapt molecular, physiological and behavioural processes to maintain metabolic homeostasis but of course, disruption of circadian rhythmicity generally leads to diseases. Therefore, understanding the molecular clockwork help us develop treatments against various disease and disorders such as diabetes, cardiovascular diseases, obesity, jetlag, insomnia, and psychological disorders. Mammalian circadian clock is composed of transcriptional-translational feedback loop which allows the generation of daily rhythms. There is a set of core clock genes identified so far that constitute molecular feedback loop involving BMAL1, Clock, Npas2, Period1,2,3, Cryptochrome 1,2 genes.

Besides known core clock components, more gene loci were shown that they change wild type oscillation pattern of core clock genes. These additional clock relevant genes which were identified by recent genetic analyses indicate that, circadian clock contains multiple and complex genetic interactions. By taking this fact into account, it is likely that many more genes underlying circadian rhythmicity will be discovered in near future.

Recently, by applying high throughput genetic and proteomics techniques in combination with computer - assisted screening, candidate clock components were identified. The ones that show highest similar characteristics to known clock genes were selected. Among them, CBS (Cystathionine-β-Synthase) is one of the most prominent genes with its clear interaction with mCry1. Besides its role on circadian activity, CBS is an important enzyme for cardiovascular function of the body and cysteine synthesis pathway. To understand physiological role of CBS in circadian clock mechanism, we used cell biology, molecular biology and biochemical approaches.

Here, we demonstrate with mammalian two hybrids that CBS interacts with last twenty amino acids of C-terminal of mCry1. This physical interaction is confirmed with coimmunoprecipitation. Then, transcriptional repression activity of CBS was tested by high throughput luciferase assay in N2A and NIH3T3 cell lines in the absence and presence of mCry1. It is clearly seen that CBS enhances repression activity of mCry1 on E-box promoter. Along with repression activity, we also tested how CBS enzymatic activity is affected with its interaction with mCry1. To do this, we performed hydrogen sulphur assay with mouse liver containing highly expressed endogenous CBS in the presence of mCry1 and mCry1 truncated at the last 20 aminoacid of C-termini. CBS is a prominent enzyme which has high probability of being a core or modifier component of the circadian clock. We conclude that CBS may be a functional clock component providing a new layer of control on circadian molecular dynamics.

Keywords: Cystathionine-B-Synthase, Circadian Rhythm, Biological Clock

Deletion of mitochondrial metabolism genes PPA2, DSS1 and AFG3 affects mitochondrial morphology and functions that harbor life span prolongation

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As time goes by, an organism or tissue or a post mitotic cell ages and start losing energy, becoming weak and ultimately fall in death; which implies the central role of mitochondria in the aging process. We addressed the question by asking whether manipulations to mitochondrial metabolism genes can extend life span in yeast. In this study, we have been screening strains derived from the yeast open reading frame (ORF) deletion collection to identify single deleted mitochondrial genes of *S.cerevisiae* (yeast) that increase life span. This has resulted in the isolation of three long-lived mutants' Δppa2 (28% extended), Δdss1(20% extended) and Δafg3 (40% extended) that are chosen for this study. These long lived cells comprised relatively less amount of mtDNA at the young stage with effective proliferation rate while mtDNA was completely lost in old compared to wild type, indicating mtDNA content play role on longevity. Complete absence of Endogenous reactive oxygen species (ROS) level was observed both in long lived young and old cells, suggested that they may get benefit from this no-ROS state, which in turn may facilitate longevity. Compared to serpentine nature of wild type mitochondria, a different dynamics and disrtibution pattern of long lived cell's mitochondria was shown as mitochondrial aggregation and colonization that helps to retain the mitochondrial density within cells. In addition, the elevation of the mitochondrial membrane potential ($\Delta\Psi^{mito}$) was found to predominate the relative degree of longevity. Both young and old long lived cells produced relatively low and a certain level of ATP and observed as sufficient for cellular activity. Thus this investigation reveals the longevity role of these genes through the remodeling of mitochondrial morphology and function.

***Keywords*:** Cellular aging and longevity, Mitochondrial DNA abundance, ROS, Mitochondrial dynamics, Cellular ATP and Mitochondrial membrane potential

Identifying novel physical and functional interaction patterns in mammalian ER-associated degradation

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Many proteins that play critical roles in cellular physiology transit through the secretory pathway. Although most secreted proteins fold properly, around 30% fold inefficiently or slowly, particularly due to mutations or cellular stress. Since unfolded proteins can compromise cellular function and in most cases trigger cell death, their recognition and selectively degradation via ER associated degradation (ERAD) is vital. ERAD has been linked to a growing number of human diseases and with its complexity and selectivity, it is possible to modulate the expression of disease-related proteins; it is thus very attractive targets for therapeutic intervention. Many critical players of ERAD have been characterized using a variety of model systems. However, it is likely that there are several ERAD machineries with different protein combinations functioning in substrate–specific manner. Our studies are mainly focused on two major ERAD ubiquitin ligases (Hrd1, gp78) and p97/VCP, a key protein of retrotranslocation. We have revealed that Hrd1 negatively regulates gp78–mediated ERAD by targeting its degradation. We also identified two retrotranslocation machineries; p97/VCP-Ufd1-Npl4 complex and p97/VCP-Npl4 complex specific for Hrd1- and gp78-mediated ERAD, respectively. In a recent study, ERAD substrate accumulation, solubility profile, subcellular localization and functional partners of disease-related mutants of p97/VCP have been investigated. The identified differential impairments in p97/VCP interactions with its functional partners should help our understanding of the molecular pathogenesis of IBMPFD.

Session: Cancer Biology

Transcriptomic and proteomic analyses identify DEK oncogene as a target of the RNA binding factor CUGBP1 in melanoma

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Despite great progress in identifying genetic and epigenetic defects accumulated during melanoma progression, the molecular bases underlying the aggressive behavior of this tumor type are not completely understood. For example, thousands of mRNAs have been found deregulated in malignant melanocytic cells. Intriguingly, although number of oncogenes and tumor suppressors can be regulated by alternative splicing, the expression and regulation of spliceosome proteins remains unexplored in melanoma. In this study we focused on the role CUGBP1 -an RNA binding protein that can influence RNA splicing, translation and stability- in melanoma cells. We found that CUGBP1 is overexpressed in melanoma cells and tissue specimens; and is required to sustain melanoma cell proliferation, being rather dispensable for normal melanocytes. Gene Set Enrichment Analyses revealed that CUGBP1 controls a series of genes involved in cell cycle and mitosis. Proteomic studies revealed pleiotropic functions of CUGBP1 in DNA replication, cell division and chromatin architecture. Here we also show that DEK oncogene, is a target of CUGBP1, and restoring DEK levels in CUGBP1 depleted melanoma cells reverts some of the phenotypes observed upon CUGBP1 knock-down. Our results illustrate the power of comprehensive analyses of RNA regulators in the identification of novel malignant features of melanoma cells.

***Keywords*:** Melanoma, Alternative Splicing, DEK Oncogene

Epigenetic mechanisms underlying the dynamic expression of cancer-testis genes, *PAGE2*, *-2B* and *SPANX-B*, during mesenchymal-to-epithelial transition

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CT genes show restricted expression in germ cells, among normal tissues, but are frequently expressed in various cancers. Demethylation of promoter CpGs is considered to be the primary epigenetic mechanism in CT gene expression. To further study mechanism leading to CT gene expression, we used the Caco-2 spontaneous differentiation model. We showed PAGE2,-2B and SPANX-B were induced significantly as the cells differentiated. The differentiation was characterized as mesenchymal to epithelial transition as the mesenchymal markers (Fibronectin1, Vimentin and Transgelin) decreased and the epithelial markers (E-cadherin, Claudin 4 and Cdx2) increased concomitantly. Surprisingly, we found no difference in promoter DNA methylation of CT genes during CT upregulation. Instead, we found increased DNA hydroxymethylation within the gene body and promoters of these CT genes with a concordant increase in TET enzyme levels. TET2 protein was co-expressed with CT proteins in the same cells. Besides, we found that that the increase in hydroxymetylation happened in parallel to changes in EZH2, H3K27me3 and HP1 promoter-occupation. Reversal of differentiation resulted with the loss of CT gene expression and EMT induction. For the first time, we describe dynamic expression of CT genes linked to DNA hydroxymethylation in mesenchymal to epithelial transition.

Natural Killer Cell Based Immunotherapy of Cancer

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The development of any malignancy is under close surveillance by NK cells as well as other members of the immune system. Nevertheless, malignant cells find ways to escape the immune system, resulting in defective immunity secondary to tumor development. Therefore, to use autologous NK cells effectively for tumor immunotherapy, a reversal of phenotypic and functional defects is of paramount importance.

This research primarily investigates the feasibility and potential of *ex vivo* expanded NK cells in cancer immunotherapy, producing a system that has the capacity to expand polyclonal and highly cytotoxic NK cells showing selective anti-tumor activity.

Furthermore, both as a basic method to identify these interactions, and as part of further plans to use genetically retargeted NK cells in cancer immunotherapy, we have investigated methods for efficient lentiviral genetic modification of NK cells. Our studies have revealed an optimized stimulation and genetic modification process that greatly enhances lentiviral gene delivery.

The work presented here aims to bring us closer to optimal *ex vivo* manipulation of NK cells for cancer immunotherapy. Clinical trials with expanded NK cells as well as further preclinical development of NK cell genetic modification processes are warranted.

Bioinformatics / Systems Biology

P25. Cpipe - A computational pipeline for structure based evaluations of Cys reactivity in proteins.

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Abstract

Depending on their function, reactive Cysteine (Cys) residues can be roughly divided in (i) structural (disulfide, metal binding), (ii) catalytic, and (iii) regulatory Cys (i.e. post-translational modifications that may be involved in signaling). Identifying reactive Cys is not a trivial job. Although various methods were proposed, none is single-handedly enough to efficiently separate reactive Cys from nonfunctional residues. It was shown that environmental properties, such as pKa and solvent accessibility, can help researchers identifying most reactive Cys; these properties are tightly linked with protein flexibility, which therefore needs to be accounted for. Meanwhile, alternative approaches achieved some success by comparing structural similarities of a target Cys with known cases. Herein we present an algorithm (Cpipe) that fuses both strategies (i.e. both energy and knowledge-based) to address the problem. By applying wide-range filters for known cases and integrating molecular dynamics support for energetic evaluations, Cpipe computes all relevant properties for Cys residues in any input protein structure; ultimately it processes all computed properties and ranks/scores each Cys based on their reactivity (results are accessible via a user-friendly interface). Cpipe provides researchers in the field with a new powerful tool for the identification of novel reactive sites in protein datasets.

Keywords: Bioinformatics, Cysteine, Protein Flexibility, Functional prediction

P26. miRNA-TF-mRNA Circuits in Breast Cancer

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Abstract

Breast cancer (BC) is the most common cancer among women and it is not completely understood what plays main roles in the BC regulation. Over the past few years, miRNAs have emerged as important regulators of gene expression like transcription factors (TFs). To understand biological processes and diseases such as BC, it is crucial to unravel the close relation of TFs, miRNAs and their targets within regulatory networks. In our study, mRNA and miRNA microarray studies including breast tumors and normal samples searched in GEO microarray database. 2 independent mRNA studies and a miRNA study were selected to be analyzed. Differentially expressed mRNAs and miRNAs between breast tumor and normal samples were extracted ($p \leq 0.05$, 2 fold change). 187 downregulated and 77 upregulated genes were found to be common for tumor vs. normal comparison with 18 transcription factors including SOX10. 23 miRNAs were also found to be differentially expressed (11 upregulated and 12 downregulated). CircuitsDB2 analysis resulted with 3 significant circuits that are SOX10 and has-miR-301a dependent, (SOX10→hsa-miR-301a→HOXA3, SOX10→hsa-miR-301a→KIT, SOX10→hsa-miR-301a→NFIB). This study showed that regulatory motifs involving miRNAs and TFs may be useful for understanding the breast cancer regulation, and for predicting new biomarkers.

***Keywords*:** Breast cancer, miRNA, mRNA, Transcription factor, Circuits

P27. Bioinformatic Analysis of Parkinson's Disease, Huntington's Disease and Multiple Sclerosis to Reveal Disease-Specific Metabolic Patterns

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Abstract

Neurodegenerative diseases (ND) are chronic and often fatal illnesses which comprise death and/or dysfunction of nerve cells in Central Nervous System as a result of molecular, biochemical and physiological changes in neurons. ND share some common malfunctions such as functional loss (ataxia) and sensory dysfunction (dementia) due to neuron degeneration. Parkinson's Disease (PD), Huntington's Disesase (HD) and Multiple Sclerosis (MS) are the three of ND which are frequently encountered around the World. Changes in gene expression profiles and metabolism are difficult to analyze due to complex molecular and biochemical pathways in all diseases including PD, HD and MS. Computational Systems Biology is a newly emerged and multi-disciplinary study field to reveal these integrated and interacted networks of genes, proteins and biochemical reactions [1]. Here, we focused on determining the genes and biochemical reactions that can affect the metabolisms of PD, HD and MS by the use of computational biology. For this purpose, transcriptome datasets (GSE26927) [2] for HD, PD and MS were either analyzed by using statistical (t-test) and dimension reduction (PCA) techniques to elicit significantly changed disease-specific metabolic genes. Then, the transcriptome data were integrated with a recently reconstructed genome scale brain metabolic model, iMS570 [3] to generate disease-specific metabolic models for each disorder. This enabled the calculation of metabolic fluxes and identification of active metabolic pathways for each disease.

***Keywords*:** *Neurodegenerative diseases, systems biology, metabolic modelling, PCA, Flux Balance Analysis, MOMA.*

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P28. How The Chimpanzee Testis Grows Three Times the Size of Human Testis?

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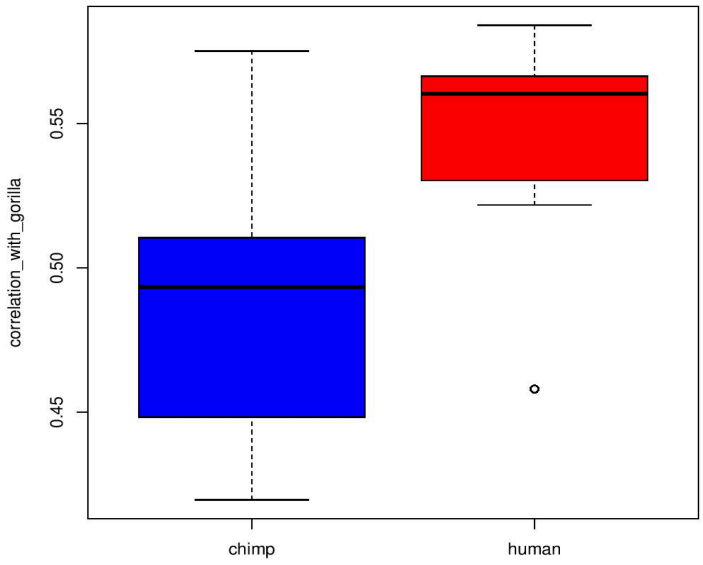
Abstract

Testis size differs considerably among hominoids with different levels of sperm competition. Chimpanzees, a promiscuous species, have ~3 times larger testes than humans, who tend toward monogamy [1]. Gorillas, with harem systems, also have small testes [1]. At transcriptome level, humans and gorillas have similar testis expression profiles, distinct from those of chimpanzees [2,3]. Here, we ask whether this testis expression divergence among hominoids is driven by independent evolutionary changes across hundreds of genes, or by shifts in developmental timing. When we analyze testes transcriptome of macaques and mice at different ages, we found that chimpanzee are closer to those of adult macaques and mice, while human and gorilla are both closer to those of younger animals, implying neoteny in the latter. Thus, an explanation for these distinct testis phenotypes and molecular profiles can be a simple regulatory change during developmental processes leading variation in cell type proportions among species. We are currently studying the adaptive and regulatory mechanisms underlying this developmental divergence. For this, we are estimating ancestral mating type and testis size among primate species, as well as common transcription factor and microRNA regulators of differential gene expression in testis transcriptomes.



***Keywords*:** Sperm competition, testis size, transcriptome, neoteny

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P29. Workflow of Microarray Data Analysis for Identifying Genes Related with Hutchinson-Gilford Progeria Syndrome

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Abstract

Hutchinson-Gilford Progeria Syndrome is a rare genetic disorder with premature aging symptoms [1]. In this study, gene expression profile associated with Hutchinson-Gilford Progeria Syndrome was investigated using freely available bioinformatics tools. Fibroblasts and induced pluripotent stem cells (iPSCs) which originated from a diseased individual were compared with wild type fibroblasts and iPSCs. A wide variety of genes were found to be differentially expressed between diseased and wild type phenotypes. Reprogramming fibroblasts to iPSCs narrowed down the variety of differentially expressed genes. Some of these genes might be particularly important in disease progression.

***Keywords*:** Hutchinson-Gilford Progeria Syndrome, induced pluripotent stem cells, gene expression profile

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P30.Identifying the Cold Stress Specific Protein-Protein Interaction Network in *Arabidopsis thalina*

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Abstract

Cold stress is one of the important obstacles of crop production as it causes loss in crop production every year. Thus and understanding interactions behind this phenomenon is essential to increase the annual production. Here we tried to construct a cold stress specific protein-protein interaction network by using a computational systems biology approach. Two different microarray datasets (GSE 3326 and GSE 37130) obtained from Gene Expression Omnibus database were used to analyze transcriptomic changes in cold stress conditions. It was found that the expressions of 521 genes were significantly changed in both datasets, and 39 of them were annotated with transcription factor (TF) activity. Using BioGRID (Database of Protein and Genetic Interactions) database we constructed protein interaction network model for *A. thaliana* in cold stress conditions and identified a network with 485 nodes and 608 edges (Fig 1). Graph theoretical properties (degree and betweeness centrality) were calculated for these TFs to rank them in terms of their importance in the constructed protein-protein interaction network. The network was further statistically analyzed to identify cold-stress associated TFs which showed a coordinated transcriptional change in the connected genes. The computational systems-based analyses could led to the identification of candidate TFs for further experimental analysis.

***Keywords*:** Arabidopsis, cold, stress, systems biology, microarray

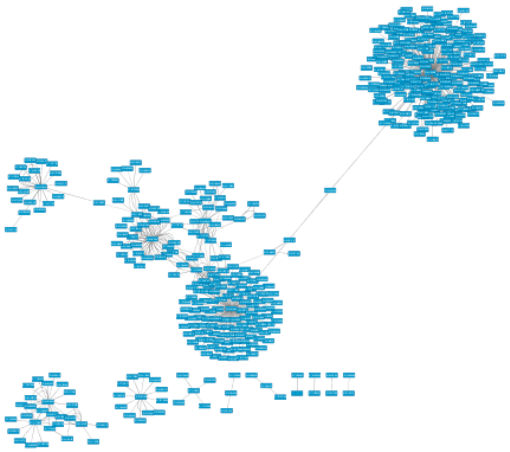

Figure 1. Cold stress responsive protein-protein interaction network constructed with the interactions of the detected 39 transcription factors

P31. Zebrafish Gene Expression Exhibit Individual and Gender Difference Independent of Conditions

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Abstract

Zebrafish is an ideal model organism to study human diseases. Here, we analyzed multiple microarray data performed in zebrafish brain. We aimed to find any effects of pooling versus individual usage of samples and also investigated high and low variability genes in each study alone, or in combinations. We included data [1] for age and gender differences, data [2] for behavior, and data [3] for strain variability. We identified the high- and low-variance gene sets in R. We utilized BioMart and WebGestalt to annotate and group genes into GO and KEGG pathways. We found that pooling of the samples [4] reduced variability among samples when compared to non-pooled samples. Low- and high-variability gene lists were subject to change when data was analyzed within the same gender or in combination. When individual array results were combined, we obtained a significant set of genes that were highly variable and stable, independent of the strain or conditions. We consider the results of this study to be valuable in analyzing further gene expression data. A gene with stable expression may be used as a housekeeping gene and one with a high individual variation may be considered unsuitable as a biomarker for a specific disease.

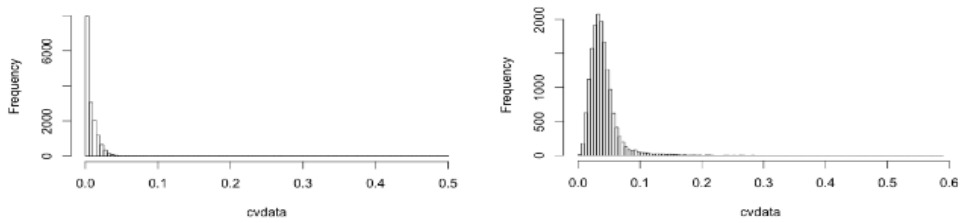

Figure 1. Coefficient of variation histograms of pooled [4] (left) and nonpooled [2] (right) data.

***Keywords*:** Zebrafish, Microarray, Gene Ontology

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P32. Notable decrease in transcriptome conservation during mammalian aging

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Abstract

Aging is nearly universal across metazoa, but its mechanisms have remained elusive. "Mutation accumulation" is among the keystone aging theories postulated to date [1]. It states that mutational load of genes expressed late in life is higher than of genes expressed earlier in life, due to reduced *negative selection with age [2]. The idea was previously tested using phenotypic data, with mixed results [3,4], but to our knowledge, not using transcriptomic/genomic data. We had previously indentified a trend toward age-related decrease in transcriptome conservation in the human brain, measured by diminishing* correlations between gene expression levels and protein conservation levels during aging [5]. This is consistent with the mutation accumulation theory. In this study, we investigated the universality of this phenomenon in humans, chimpanzees, macaques using independent transcriptome datasets from distinct brain regions [5,6,7,8], as well as human skeletal muscle datasets [7]. Across all tested tissues and species, we detected the same trend of decreasing transcriptome conservation. The signal was not explainable by changes in total mRNA expression during aging. To our knowledge, our findings provide the first genomic evidence corroborating the idea that mutation accumulation might contribute to aging.

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***Keywords*:** aging, transcriptome, evolution

P33. A computational strategy for structure based evaluations of Cys reactivity in proteins

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Abstract

Cysteine (Cys) residues often play critical roles in proteins, where, relative to other amino acids, they are frequently found in functional sites. However, precise determinants behind Cys activation are unclear: what activates specific Cys in proteins? The answer to this question, and in general the prediction of Cys reactivity from first principles is very challenging. We recently demonstrated that even small, but localized, protein movements (e.g. due to natural flexibility) can significantly affect Cys reactivity, occasionally to the point of turning ON/OFF the reactivity. This aspect cannot be overlooked while developing computational protocols. Herein we present a novel, first of its kind, computational tool that allows a mobility-comprehensive and fully automated screening of all Cys residues in any given input protein submitted to the pipeline. This tool is expected to positively impact and benefit a broad range of research areas, from redox bioloy and thiol regulation to protein engineering and rational design.

Keywords: Bioinformatics, Cysteine, Protein Flexibility, Functional prediction

P34. Network Analysis of Gene Expression Profiles in Down Syndrome Brain Tissue

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Abstract

The characteristic Down syndrome occur about 1 per 1000 newborns of each year. This common genetic disorder is known to be strongly associated with congenital heart disease, some immune system deficiencies, and even with Alzheimer and leukemia [1, 2, 3]. In this study we have further analyzed the microarray data on Down syndrome brain tissue obtained from Gene Expression Omnibus (GEO) [4] with systems biology approach, inspecting the gene co-expression networks and enriched biological pathways. After identifying differentially expressed genes and their functionalities, biological pathway and disease associations are extracted. HLA-DPA1, HLA-DRA, HLA-DRB1, and HLA-DRB4 genes, which are shown to be significantly overexpressed in Down syndrome brain tissue, have been found to be also observed in viral myocarditis and asthma pathways, suggesting possible molecular etiology for the genetic predisposition to heart and immune system diseases. Furthermore, some signs of association with Alzheimer and leukemia have been detected through the up-regulation of the APOE and NCAM1 genes.

Keywords: Gene Expression Analysis, Down Syndrome, Heart and Immune System Diseases, Alzheimer, Leukemia

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P35. Combinatory Strategies to Maximize The Number of Proteins Identified In HeLa Whole-cell Extracts Using nano-LC MS/MS

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Abstract

Mass spectrometry based proteomic analysis allows an unbiased and large-scale analysis of proteins in cell mixtures. In this study we aimed to optimize the number of proteins identified using our proteomic setup equipped with nano-Liquid Chromatography Q-Exactive Orbitrap Mass Spectroscopy (nLC-MS/MS) analytical system supported with Mascot, Sequest and MS Amanda search engines. Hela whole cell lystae were used for the optimizatin experiments. Trypsin based in solution digested of Hela cell samples were run on 90 min gradient by varying MS parameters. The resulting spectra were searched using Thermo Scientific Proteome Discoverer Programme. The data analysis was done using different combination of search engines (Mascot, Sequest, Sequest HT and MS Amanda) using different work-flows and search parameters. Optimized MS parameters and search combinations of our proteomics analysis on the nano-lc ms/ms will be presented.

Keywords: Maximum proteins and peptides numbers, nano-LC MS/MS, MS parameters, in solution digest

P36. Quantitative Phosphoproteomic Analysis of "Cytokinesis"

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Abstract

Cell division is an essential cell process by which all living things propagate and maintain their lives. One of the main drivers of the cell division and cell cycle is phosphorylation-dephosphorylation events through the activities of kinases and phosphatases. For instance, CDK kinase is the master regulator of the mitosis which phosphorylates 100-1000 of its substrate during mitosis. [1] Phosphorylation-dephosphorylation events also heavily involved in the last step of the cell division, "cytokinesis" albeit their substrates are less clear. In the previous studies it was shown that phosphorylation by the Aurora kinases promote microtubule binding of many proteins in cytokinesis. [2]. With this project, we aimed a global analysis of phosphorylation-dephosphorylation events during cytokinesis by LC/MS. This will bring a better understanding to the organization and regulatory mechanism of cytokinesis.

Phosphopeptides have dynamic nature and low abundance, which makes quantification essential. Thus, phosphoproteomics is highly challenging. We compared the phosphorylation events in three cell cycle stages, mitosis-cytokinesis-interphase. We used stable isotope dimethyl labeling, targeting the primary amine groups of peptides for quantification. To increase the detection of phosphopeptides, we combined TiO_2 phospho-enrichment with a prior strong cation exchange chromatography. Currently, we obtained and quantified around 10000 phoshopeptides from all stages. We will reveal selectively regulated phosphopeptides during cytokinesis.

***Keywords**:* Phosphoproteomics, TiO_2 enrichment, cytokinesis, ion exchange chromatography, LC-MS

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P37. A novel fixation bias for extended homopolymers in the human genome

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Abstract

Depending on genomic positions and bases involved, mutations occur at different rates across genome, and also spread at different rates within populations. For instance, A or T to G or C mutations spread and fix rapidly due to GC-biased gene conversion [1, 2]. The effect of the neighboring sequences on such fixation biases remains unknown. By using human data from the 1000 Genomes Project [3], we investigated possible fixation biases in the genome at the level of quintuplets: 5 bp sequences where the middle base is mutated. Intriguingly, we detected a fixation bias for mutations that extend homopolymers (same base sequences, e.g. GGGG) across the genome. As an example, the C to G mutation, AGCGG to AGGGG, has fixed in the human genome ~2.5 times more frequently when compared to its symmetric "disruptive" mutation, AGGGG to AGCGG. We also observed that frequencies of derived alleles within population are higher, which supports fixation bias in favor of extended homopolymers. Interestingly, the bias is absent in genic sites or in CpG islands. Currently, we are studying possible mechanisms underlying this novel fixation bias and try to explain this phenomenon on a functional / adaptive basis.

Keywords: Population genomics, SNP, homopolymers, fixation bias

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P38. Apolipoprotein E Isoforms in Coronary Artery Disease

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Abstract

Apolipoprotein E (APOE) is a protein, which plays a role in carrying excess cholesterol to the liver from the blood for further lipid metabolism. APOE has three isoforms; ε2, ε3, ε4 and these isoforms can be risk factors for many different conditions. Some APOE alleles can cause coronary artery disease. Therefore, we chose patients (n=40) in coronary artery disease group are ones diagnosed with coronary angiography and directed to bypass operation. As a control group (n=40), we chose patients in non-coronary artery disease are ones undergoing non-coronary bypass open heart surgery and diagnosed as not having coronary artery disease by coronary angiography. DNA isolation was done with phenol/chloroform method and APOE was amplified by PCR and products were analyzed according to their restriction profiles. We found that ε3 is the most frequent. While ε3 lowers total cholesterol levels, ε4 raises them which means, ε4 may has an affect on coronary artery disease. Accordingly, we found that ε4 seen more frequently in patients than control (Figure 1). As a result, APOE can be a major risk factor for coronary artery disease and studies of screening isoforms contribute to the genotype-drug interaction that might influence the course of disease.

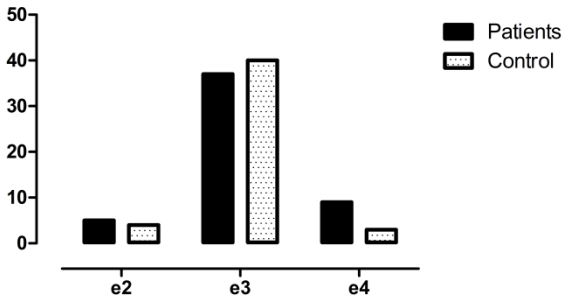

Figure 1. The distrubiton of APOE isoforms.

Keywords: Coronary artery disease, APOE, polymorphism

P39. Investigation of difference in genotype frequencies of *APOE* polymorphism between BPH patients and prostat cancer patients

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Abstract

Aim: Prostate cancer is the most common cancer diagnosed in men and the second lead of death due to malignancy, but the progression of the disease is still unpredictable. Apolipoprotein E (APOE), which is a central regulatory protein in cholesterol metabolism and a potent inhibitor of angiogenesis and tumour cell growth, might be a risk factor for prostate cancer as well. It was also known that the most important identified genetic risk factor for the neurodegeneration in Alzheimer disease is the *APOE* alleles. We aimed to investigate 158 prostate cancer patients and 94 benign prostate hyperplasia (BPH) patients as control group were included the study **Methods:** The genotypes of the APOE polymorphisms were determined with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results:** We did not find any difference in genotype frequencies of *APOE* polymorphism between control group and prostat cancer patients ($p>0.05$). There was no substantial difference between control and the patient groups in terms of ε4 allele carrier ($p>0.05$). **Conclusions:** Our findings suggest no direct association of *APOE* genotype with prostate cancer in Turkish patients. In conclusion, our data do not support the hypothesis that the ε4-allele is a risk factor for prostate cancer.

Keywords: Prostate cancer, benign prostate hyperplasia, polymorphism, *APOE*

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P40. Establishing Alternative Strategies for Cell Surface Isolation

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Abstract

Cell surface is an important cellular component which functions in multiple processes including communication, homeostasis, signaling, and tissue development. Thus, understanding the changes in the cell surface has great importance in understanding of these cellular events. In our group, we aim to investigate the cell surface changes using mass spectrometry (MS) based quantitative proteomic to understand their dynamic reorganization during cellular events. For the MS-based proteomic analysis one of the most critical steps is to obtain highly pure, homogenous samples therefore efficient isolation of the cell surface is the main challenge for analyzing the protein composition of the cell surface using quantitative proteomics. The most commonly used method for cell surface isolation is amine reactive biotinylation of cell surface proteins. This method is based on the modification of exposed primary amine groups of lysine and arginine residues using a succinimidyl (NHS) ester of biotin. However, modification of these residues may block the digestion sites for proteolytic enzymes such as LysC and Trypsin and thus, limits the efficiency and sensitivity of proteomic analysis. Therefore we aim to develop an orthogonal approach to overcome such problems and establish a new method for cell surface labeling that can increase the efficiency and sensitivity of proteomic analysis.

***Keywords*:** Cell surface, isolation, biotinylation, proteomics

P41. Effect of Small Nettle' (*Urtica urens*) Ethyl Acetate Extract on Whole Genome Transcriptome in Human Prostate Cancer Cell Line

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Urtica urens L. (Small Stinging Nettle) is one of the most commonly used plant in alternative and complementary treatment of cancer patients in our country. However, studies in literature showed that there are limited studies investigating the biologic activities of this plant. In this regard, the present study was aimed to investigate potential effects of ethyl acetate extract of small nettle in different cellular pathways by whole-genome transcriptome. For this purpose, firstly, extract of dried stinging nettle leaves was prepared by using ethyl acetate. Extract were dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 0.1% and was applied different concentration to the LNCaP (human prostate cancer cell line) cells ($1x10^3$ cells/well) for 48 hours. At the end of 48 hours, the survival rate of cell was determined by WST reagent and LD50 value was calculated. It was found that 36 µg/ml was 50% cytotoxic dose for these cells. This dose was applied to cell and cRNA samples were prepared. For whole genome transcriptome analysis, Human HT-12 V4 BeadChip with more than 47.000 probes was used in Illumina iScan plarform. Transcriptome analysis revealed that 1320 different probes were significantly changed ($p<0.05$) by ethyl acetate extract. This data showed that extract altered a group of genes involved in DNA replication, repair and division, senescence and autophagy, TAK1 activates NFkB by phosphorylation and activation of IKKs complex, metabolism of nitric oxide, regulation of apoptosis, degradation of beta-catenin by the destruction complex, APC-C mediated degradation of cell cycle proteins, androgen receptor signaling pathway, Wnt signaling pathway and pluripotency. These results provide a comprehensive view of the changes in gene expression when prostate cancer cells was exposed to urtica extract, and shed light on its complicated effects on these cells.

This work is supported by TUBITAK 111T515.

Key words: whole genome transcriptome, Small Stinging Nettle, Human Prostate Cancer Cell Line (LNCaP), pathway analysis.

P42. Neandertal Introgression into Western Asian Human Populations

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Abstract

It has recently been shown that present-day humans from Eurasia carry in their genomes a small percentage of Neandertal DNA (1.5 to 2.1 %). These were supposedly induced by two independent admixture events: one occurred in the Middle East about 60,000 years ago, and the other occurred more recently in Asia [1-2]. In this study, we aim to test the hypothesis that modern humans in Western Asia interacted with Neandertals for longer periods of time than European and East Asian populations. Based on this hypothesis, we predict that present-day Western Asian populations have stronger signatures of Neandertal introgression than do Eastern and Western Eurasian populations. In addition, we predict that these signals should gradually decrease with distance from Western Asia. To test these hypotheses, we are currently estimating and comparing Neandertal introgression rates in present-day Turkish population in comparision to present-day European populations. For that purpose, we are applying a newly developed population genetics technique called D-statistics to Turkish and European genomic data compiled by Turkish Genome Project [3] and the 1000 Genomes Project [4], respectively. Our study will greatly improve the understanding of the extent and the nature of Neandertal introgression into modern human populations.

Keywords*:* genomics, Neanderthal, introgression, Turkey

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[3] Alkan et al, BMC Genomics, under review.

[4] The 1000 Genomes Project Consortium, *Nature,* **2012**, 491, 56-65.

P43. Transcriptome Profiling Analysis Reveals Modulatory Effects of Small Nettle' (*Urtica urens*) Hexane Extract in Colon Cancer Cells

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ABSTRACT

Urtica urens L. (Small Stinging Nettle) has been used for medical purposes for centuries in Turkey. It has been used in the treatment of cancer as an alternative and complementary therapy. However, studies in literature showed that there are limited studies investigating anti-carcinogenic activity of this plant. In this regard, the present study was aimed to investigate potential effects of hexane extract of small nettle not only for anti-carcinogenic activity but also other cellular pathways. To test our proposal, we used whole genome transcriptome strategy, which are now widely used in functional genomic research and has become necessary to better understand the molecular mechanisms of herbal medicine. For this purpose, firstly, extract of dried stinging nettle leaves was prepared by hexane. The cytotoxicity of this extract in CaCo-2 (human colon cancer cell line) cells was investigated by WST-1 assay. It was found that 35 mg/ml extract was 50% cytotoxic dose for these cells. This dose was applied to cell for 48 hour and cells were harvested. cRNA samples was prepared and whole genome transcriptome analysis was performed by using Human HT-12 V4 BeadChip with over 47.000 probes in Illumina iScan platform. Total 223 different probes were significantly changed ($p<0.05$) by hexane extract. Transcriptome profiling analysis showed that several genes were modulated by hexane extract in Caco-2 cells, including those involved in insulin signaling, glutathione metabolism, hydroxyproline metabolism, iron uptake and transport. Notably, a number of genes coding for proteins involved in Apoptosis were also changed. This showed that hexane extract has possible anti-carcinogenic activity. In conclusion, using transcriptome profiling analysis, we demonstrated the molecular basis for the anti-carcinogenic effects of hexane extract of *Urtica urens.* Moreover, these results provide a comprehensive view of the changes in gene expression in CaCo-2 cells. This study supplied new clues for other pharmacological and toxicological studies of urtica species.

This work is supported by TUBITAK 111T515.

Key words: Whole genome transcriptome, Small stinging nettle, Human Colon Cancer Cell Line (CaCo-2), pathway analysis.

P44. Optimization of extracellular tyrosinase enzyme production from native *Bacillus sp*. isolate

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ABSTRACTS

Bacterial tyrosinase enzyme with a high oxidizing capacity could make their applications in phenolic biotransformation, food processing, cosmetics, and textile industry. In this research a melanogenic soil bacterium was isolated by differential screening medium from different regions of turkey. According to biochemical and morphological characterization the native isolate determined as a bacillus sp. M26. Culture conditions for extracellular enzyme production were optimized and the produced enzyme was characterized. 37^{O}C as optimum temperature, pH 7.0 as optimum pH, 4 days as optimum incubation time and 190 rpm as optimum agitation speed was determined for the enzyme production.
Tyrosinase activity was studied using L-tyrosine and L-DOPA as substrates. The optimum pH and temperature for activity of the enzyme were determined as about pH 7.5 and 70^{O}C. The enzyme conserved 100% of maximum activity at 5-45 ^{O}C, at 60^{O}C stability decreased to 50% and at 80^{O}C stability fall down to zero. In addition, the tyrosinase enzyme activity was increased by SDS below the critical micelle concentration and was inhibited by 1mM EDTA. Conversion of L-tyrosine to L-DOPA by the enzyme was observed in TLC and HPLC analysis.

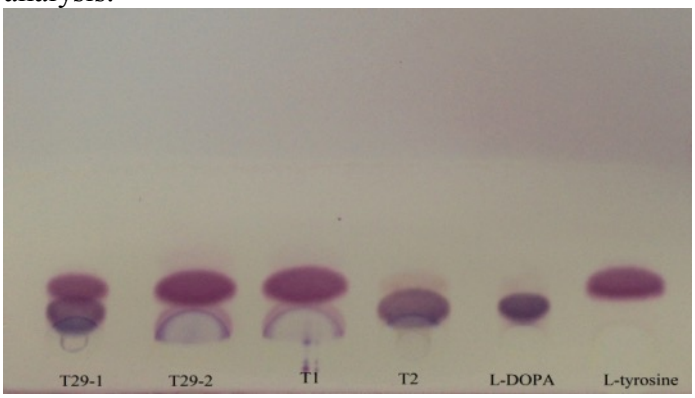

Figure1. TLC analyses of L-DOPA production

Key word: tyrosinase, L-DOPA, melanogenic, *Bacillus* sp.

Key word: tyrosinase, L-DOPA, melanogenic, *Bacillus* sp.

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P45. Optimization and Strain improvement by UV and EMS for Enhanced Production of Keratinase in *Bacillus sp*. C-11

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Nutritional parameter optimization of C-11 *Bacillus sp*. isolate was carried out for keratinase enzyme production. By this optimization the yield of production increased to 520 U/mL. The optimum pH and temperature for enzyme production of the wild strain were determined as pH 10.0 and 55ºC. The maximum keratinase yield of C-11 was achieved after 96 h. Also, the wild C-11 strain was mutated by UV and EMS (Ethyl Methyl Sulphonate) to increase and improvement of enzyme production. Among 248 mutant strains of *Bacillus sp*. C-11, the mutant CUV8-11, UV mutant, gave the highest value of keratinase activity (1120 U/ml) in feather medium for 20 mintues incubation. zymogram analysis of the partially purified enzyme revealed a single active band as 40.9 kDa. The optimum pH for activity of the partially purified enzyme was determined as 11.0 and its optimum temperature as 60°C. The enzyme conserved 93% of its original activity at temperature ranging from 30 °C to 80 °C. Also, it was stable at pH from 7.0 to 10.0 with about 97% activity. TLC analyses of the feather hydrolysis by the enzyme revealed the presence of proline, systeine, systine, tyrosin and etc.

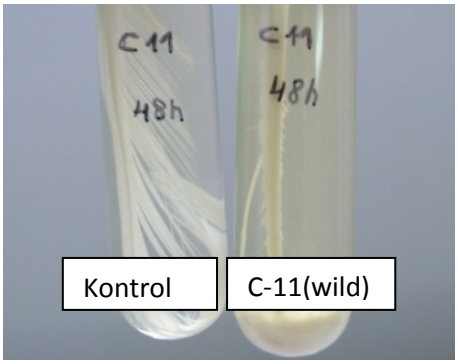

Figure 1. Chicken Feather hydrolysis of Keratinase C-11

Keywords: *Bacillus sp., Keratinase, UV, EMS, Chicken Feather*

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P46. Cloning and expression of phytase gene in *Escherichia coli* Bl21DE3 from native isolate *Bacillus subtilis*

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Phytases catalyze the hydrolysis of phosphomonoester bonds of phytate (*myo*-inositol hexakisphosphate), thereby creating lower forms of myo-inositol phosphates and inorganic phosphate. Phosphorus is one of the necessary mineral nutrients for animals during their growth, reproduction and calcification of the bones. In this research, one native *Bacillus sp*. isolate containing phytase activity was identified as a *bacillus subtilis* by biochemical and 16srDNA sequence analysis. Primer pairs were designed according to phytase gene sequence of *Bacillus subtilis* present in NCBI web site. After amplification by PCR the gene was ligated to pET29b vector as expression vector and transformed to *E.coli Bl21DE3* by heat shock method. In preparation of PCR product and vector for ligation two restriction enzymes (*Hind III* and *BamH1*) were used. Consequently high level expression and production of the related enzyme was obtained in this research.

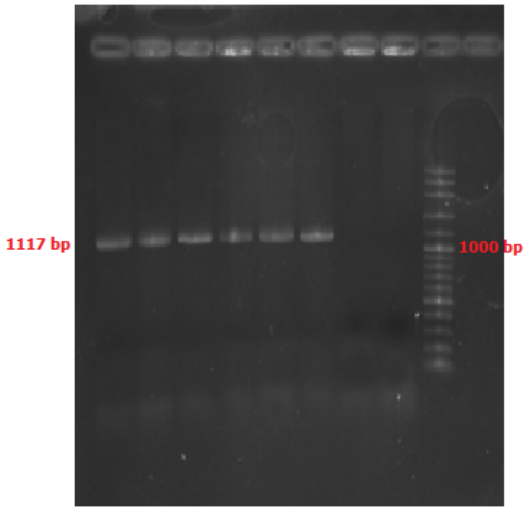

Figure 1. Analysis of recombinant colonies by PCR method

***Keywords*:** phytase, pET29b, *Bacillus subtilis,* cloning

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P47. A NOVEL CELLULASE FROM *Anoxybacillus gonensis* A4 ISOLATED FROM GEOTHERMAL AREA IN TURKEY

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Abstract

Thermophilic organisms exhibit high thermal stability and have resistance towards the denaturation impact of acidic and alkaline environments. The most important feature of thermophilic organisms is production of enzymes that catalyze biochemical reactions at higher levels of temperatures. In recent years, many enzymes from these bacteria have been successfully purified, characterized [1]. Among these enzymes, cellulases have a wide range of applications such as textile, wine, food processing, animal feed, olive oil extraction [2] and especially in detergent, leather and paper industries because these applications demand highly stable and active enzymes at extreme pH or temperature. [3]. In this study, identification of A4 strain which was isolated from Agri Diyadin Hotspring, the extracellularly cellulase production ability of *A. gonensis* A4 and purification, characterization and examination the kinetics of cellulase produced by this strain was studied. It was concluded that the catalytic activities of the cellulase purified from *A. gonensis* A4 were rather high, highly stable against the temperature, different pH values and resistant to the metal ions. Thus, purified cellulase could be suitable in different industrial areas

***Keywords*:** *Anoxybacillus gonensis*, 16S rRNA sequencing, cellulase, characterization

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P48. Purification and Characterization of Thermostable Alkaline Protease Enzyme from *Bacillus licheniformis* A10

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Proteases (EC.3.4) are enzymes that hydrolyze proteins to produce small peptides and catalyze peptide synthesis in the presence of organic solvents [1,2]. Proteases have diverse applications in various industrial products and processes [3,4]. Thus, characterization and purification of novel proteases is the most important for the enzyme industry. In this study, purification and characterization of a new alkaline protease produced by *Bacillus licheniformis* A10 isolated from Ayder Hot Spring was investigated. The enzyme was purified from the culture supernatant to homogeneity using ammonium sulfate precipitation and ion-exchange chromatography with 1.38-fold and 9.44% recovery. The molecular weight of the purified enzyme was estimated to be 40.55 kDa by SDS-PAGE. Optimal pH and temperature were determined for the enzyme as 9.0 and 70ºC, respectively. The enzyme was extremely stable in the pH range of 7.5–9.5 and between 60°C and 70°C temperatures. The enzyme was completely inhibited by PMSF which is a general serine protease inhibitör. The activity of the enzyme increased in the presence of Mg^{2+}, Mn^{2+}, K^{+}. It is also observed that the enzyme lost its activity in the presence of surfactants, oxidants and organic solvents. The V_{max} and K_M for the enzyme was 0.033 mg/ml and 8.17 µmol. $ml^{-1}.dk^{-1}$ respectively.

***Keywords*:** Thermophilic bacteria, Alkaline protease, *Bacillus licheniformis*

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Acknowledgments.**This work was financially supported** by Atatürk University Scientific Research Projects Coordination Commission (ATAUNI-BAP) with project number 2013/297.

P49. Fabrication of 3D Controlled *in vitro* Microenvironments

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Abstract

Microfluidics-based lab-on-a-chips have many advantages, one of which is to provide physiologically relevant settings for cell biology experiments. Thus there is an ever increasing interest in their fabrication. Our goal is to construct three dimensional (3D) Controlled in vitro Microenvironments (C*iv*Ms) that mimic the in vivo microenvironments. Here, we present our optimized fabrication method that works for various lab-on-a-chip designs with a wide range of dimensions. The most crucial points are: (1) While using one type of SU-8 photoresist (SU-2075) fine tuning of ramp, dwell time, spin speed, durations of soft bake, UV exposure and development allows fabrication of SU-8 masters with various heights from 40 to 600 mm. (2) Molding PDMS (polydimethylsiloxane) at room temperature for at least two days instead of baking at higher temperatures prevents not only tears and bubbles in PDMS stamps but also cracks in the SU-8 master. (3) 3D nature of the C*iv*Ms is ensured by keeping the devices inverted during gel polymerization [1].

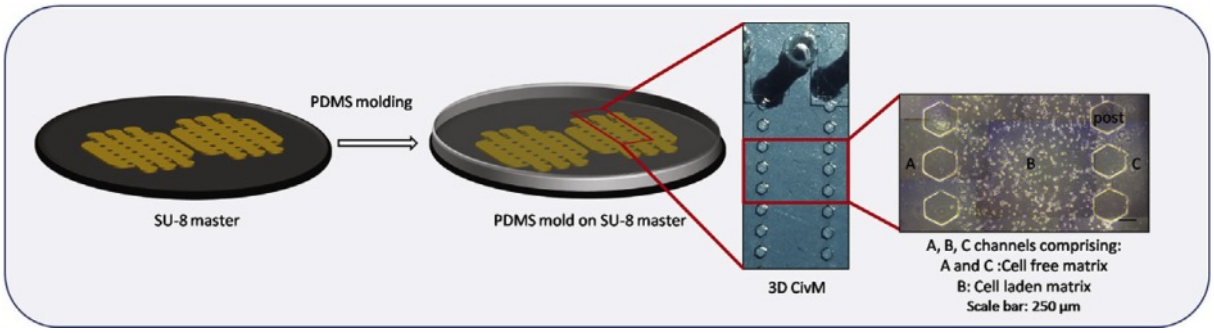

Figure 1. Experimental Method for Fabrication of 3D C*iv*Ms.

***Keywords*:** UV Lithography, Polydimethylsiloxane, Microfluidic device, Lab-on-a-chip, Microenvironment, Hydrogel, Matrigel

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P50. Spinal Muscular Atrophy Gene Therapy by in vitro naked-DNA Gene Delivery

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Abstract

Spinal muscular atrophy (SMA) is a hereditary autosomal recessive neurodegenerative disorder characterized by degeneration of alpha motor neurons of the anterior horn of the spinal cord, leading to progressive paralysis with symmetrical muscle weakness[1]. Gene therapy is an attractive approach for this incurable disease[2]. In the present study, the specific DNA fragment including SMN1 cDNA and homolog upstream and downstream sequences was subcloned into a pGH vector and Transformed to *E. coli TOP10* bakteria aimed to amplify the SMN cDNA sequence. Isolated plasmids from bacteria were cutted by BamHI restriction enzym and DNA fragments were extracted from agorose gel. SMA typeI fibroblast cell line (Coriel institue, GM03813) was transfected with DNA fragments using Lipofectamine LTX Plus Reagent (invitrogen, 15338). PCR analysis was performed to confirm the occurrence of homologous recombination in transfected cells. Protein expression of SMN gene was confirmed by RT-PCR, Western Blot and Fluorescent microscopy analysis. Protein expression of SMN gene 48 hours after gene delivery, indicated that gene replacement was achieved in one step using naked DNA transfection. In vivo delivery of non-viral naked DNA fragments into patient's stem cells and trasplantation of corrected cells to patients could be more investigated by animal model for achiving effective treatment of SMA disease.

***Keywords*:** Gene therapy, Survival motor neuron gene, spinal muscular atrophy, naked DNA delivery

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P51. Isolation, Partial Purification and Characterization of a Cellulase from *Bacillus* sp. NSC10

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Abstract

The cellulase producing *Bacillus* sp. NSC10 strain was isolated from soil samples. The enzyme was produced, partially purified and characterized. For this aim, bacterial growth and enzyme production capacities were investigated at temperatures between 30ºC-60ºC and pH 4.0-10.0. This strain showed an optimum growth at pH 5.0 and 40ºC on CMC agar plate. Molecular weight and cellulase activity of enzyme were determined by CMC containing SDS-PAGE which revealed four independent bands (67; 40,3; 30,4 and 26,8 kDa).

The optimum activity was obtained at pH 6.0 and 60ºC. The enzyme presented up to 95% and 42% residual activity after pre-incubating at 30-40°C and 50-100°C for 60 min, respectively. After pretreatment at 5.0-7.0 pH for 24 h, it showed up to 100% activity, although, the enzyme retained up to 31% of its maximum activity at 3.0-10.0 pH ranges.

The enzyme showed up to 78% activity in 3-30% NaCl concentration. Enzyme activity was enhanced in the presence of $CaCl_2$ (21%), $MnCl_2$ (80%), Triton X-100 (19%), Tween 80 (10%) and β-mercaptoethanol (47%). The enzyme was not effected by $MgSO_4$, EDTA, Urea and Tween 20. On the other hand, $ZnCl_2$, $FeSO_4$, $CuSO_4$ and SDS decreased the original activity to 59%, 60%, 33% and 76%, respectively.

***Keywords*:** *Bacillus* sp., Cellulase, Halotolerant, Surfactant resistant

P52. The Investigation of Alteration Soluble Endothelial Protein C Receptor (EPCR) Level in Vitro Hyperhomocysteinemia by Sandwich ELİSA Method

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Abstract

Hyperhomocysteinemia is a disorder characterized by the increased level of homoctesine in plasma samples. (2) Endothelial protein C receptor (EPCR) has an important role in protein C anticogulant pathway. There are two forms of EPCR which are transmembrane and soluble forms. In constrast to transmembrane EPCR form, soluble EPCR inhibits anticougulant activity of activated protein C (APC) and functions as procoagulant activity (1) In this study, the effect of hyperhomocysteinemia on soluble EPCR secreted by endothelial cells was investigated. Human umbilical vein endothelial cell (HUVEC) were cultured with different concentration of homocystein in DMEM medium. The supernants of this medium were collected and the soluble EPCR levels were measured by home-made Sandwich ELİSA method. it was observed that there was a change in soluble EPCR levels of the cells treated with homocystein. The examination of the relationship between hyperhomocysteinemia and sEPCR levels can give information about diagnose of several cardiovascular diseases.

Keywords**:** Hyperhomocysteinemia, soluble endothelial protein C receptor, Sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA)

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P53. An alkaline, thermostable and thermophile serine protease from *Bacillus* sp.AB12

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Abstract

An alkaline and thermostable protease producing *Bacillus sp*. AB12 was isolated from Van Soda Lake in Turkey. The AB12 protease was partially purified and characterized. The enzyme showed a 85 kDa active band in zymogram analysis. The enzyme exhibited maximal activity at pH 9.5 and 90ºC. It was highly active at pH 8.5-11.5. More than 94% of its original activity was conserved after pre-incubation at pH 9.5 and 100ºC for 15 min. The enzyme was kept more than 76% of its stability at pH 9.5 for 21 h. In the presence of 0.4% TritonX-100 and 0.5% SDS, the enzyme remained 92% and 75% of its original activity, respectively. It was not inhibited by EDTA, whereas the activity of the enzyme was increased with 1,10-phenanthroline. On the other hand, the enzyme activity was reduced to 30% and 23% by 1mM and 3 mM PMSF, respectively. Also, the enzyme showed dose depent inhibition in the presence of Ca^{2+} and Zn^{2+}. According to these results, AB12 protease is a serin protease, alkaline, thermostable and resistant to chelators and detergents. It is useful for laundry detergent formulations and leather applications [1,2].

Keywords: *Serine protease, Alkaline, Thermostable*

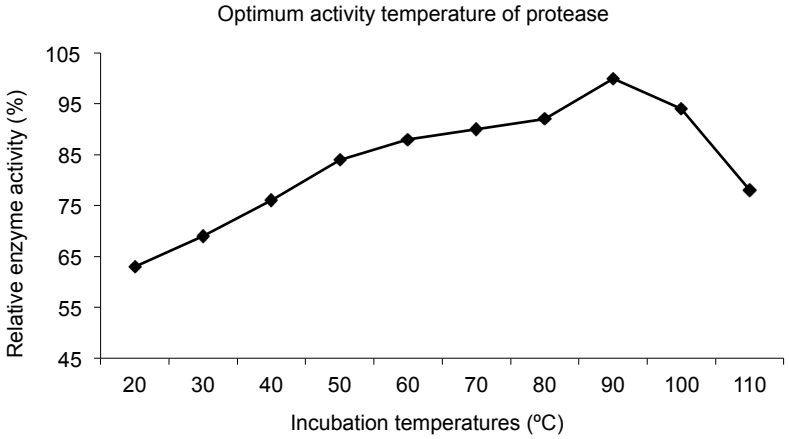

Figure 1. Effect of temperature on the activity of protease

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P54.Potential Kinase Inhibitors: Isatin and Isatin Derivatives

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Abstract

Isatin, is found in plants that had *Isatis* genus like *Calanthe discolor LINDL., Couroupita guianensis Aubl.*[1], in paratid glans of *Bufo* frog, in humans as a metabolic derivative of adrenaline and in mammal tissues and fluids as an endogenous indole (Fig. 1) [2, 3].

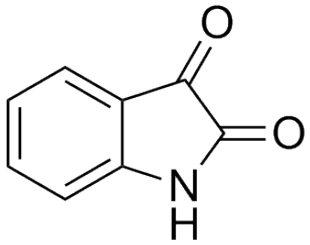

Figure 1. Isatin (indoledione 2,3).

The use of isatins can be listed as raw materials of drug synthesis, precursors of synthesis of heterocyclic compounds like quinolones, β-lactams or indoles. Isatin and its derivatives have been reported to have bioactivity as anti-protozoal, anti-convulsant, anti-viral, anti-HIV, anti-inflammatory, anti-bacterial, anti-fungal, anti-oxidant, anti-neoplastic, herbicidal, and sedative-hypnothic [4-6]. In literature, it is reported that especially, Schiff and Mannich bases of isatin and isatin derivatives show significant pharmaceutical effects [7, 8].

Derivatives of substituted isatins have been investigated using -SH, -SMe, =NH, and =S substituents. Conformational analysis and optimizations have been carried out at B3LYP/6-31++G**, B3LYP/aug-cc-PVDZ, B3LYP/cc-PVTZ levels. Same calculations have been repeated with wB97XD and PBE0PBE functionals using the same basis sets.

***Keywords*:** Isatin, Kinase Inhibitor, Conformer Analysis, Density Functional Theory

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P55.Recombinant Protein of Immunogen Sequence of Human ASCT2 and ASCT2 Polyclonal Antibody Production

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Abstract

Recombinant protein technology is the expression of proteins that have been produced by recombinant DNA techniques. It is vital process in therapeutic and biotechnological studies. Antigen, antibody, hormone, and enzyme could be manufactured by this technology [1]. Neutral amino acid transporter, ASCT2 is thought to be important in the glutamine release from cells [2]. In this study, immunogen sequence site of human ASCT2 was produced as a recombinant protein in *E.coli*. The produced recombinant ASCT2 protein was purified by nickel affinity chromatography. In order to get polyclonal antibody of ASCT2 antigen, BALB/c mice immunized with the recombinant protein. From serum samples, anti ASCT2 polyclonal antibody was tested by using tissue and cell samples. Although there was much nonspecific binding, it was observed that produced anti ASCT2 polyclonal recognized the target antigen. In the following step, polyclonal ASCT2 antibody purification method will be optimized. After antibody purification step, it is aimed to use produced ASCT2 polyclonal antibody in our experiments, western blotting and immunofluorescent staining as a marker. Finally, we plan to develop ELISA kits.

***Keywords*:** ASCT2, Recombinant Protein, Polyclonal Antibody

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P56.The effect of plant growth regulators on adventitious shoot regeneration of *Silene bolanthoides*

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Abstract

In this study, we investigated the effect of plant growth regulators in order to establish efficient adventitious shoot regeneration system for *Silene bolanthoides* Quézel, Contandriopoulos et Pamukçuoğlu, an endemic plant of Kazdagi Mountain Turkey. Hypocotyl and leaf explants of *in vitro* growing seedlings were embedded in solid MS medium supplemented with 4,43 µM BAP and in combination with 2,65 µM NAA at a density of ten explants per plate. All cultures were maintained in the growth chambers at 25 ±2°C under 16/8 h photoperiod with 72 µmol m^{-2} s^{-1}. The mean number of shoots per explants and percent of explants forming shoots were recorded after 4 weeks in culture. We found that the induction of callus and shoot regeneration were significantly affected by explants type and the concentration of PGRs. Hypocotyl explants are more effective for adventitious shoot regeneration. In addition, MS medium supplemented with 4,43 µM BAP without NAA gave the highest number of explants per explants. Our results showed that hypocotyl explants can be cultured in MS medium with 4,43 µM BAP for plant regeneration of *Silene bolanthoides.*

***Keywords*:** *Silene bolanthoides, in vitro*, regeneration, tissue culture, adventitious shoot.

P57.The Effect of Inital pH on Extracellular Tannase Production by *Aspergillus niger* OZ-3

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Abstract

Tannase is an enzyme that cleaves ester linkages in hydrolysable tannins, producing glucose and gallic acid. Tannase has several applications in the food, juice, beer, cosmetic, pharmaceutical and chemical industries [1]. The main purpose of this study was to investigate the effect of initial pH on tannase production by local isolate *Aspergillus niger* OZ-3. Effect of pH was studied by adjusting the production media containing 1% tannic acid at various levels of pH ranging from 5.0 to 9.0. Production medium was inoculated with 2% (v/v) spor suspensions and incubated at 30 °C and 150 rpm for 48 h. The culture supernatants were used for assay of tannase activity by colorimetric method of Mondal *et al.* [2]. Results demonstated that more enzyme production was achieved at acidic pHs and the production decreased as the pH approached the alkaline range. Maximum tannase production was recorded at pH 5.0 as 0.95 U/mL in fermented medium containing 33.2 mg/ml total protein. The enzyme had the size of 99.77 kDa in SDS-PAGE. This work showed a view of using a novel *A. niger* isolate as a microbial source for the cost effective production of tannase which has applications in the food, industrial and pharmaceutical industry.

Keywords: *Aspergillus niger* OZ-3*,* Tannase, Microbial Production, pH

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P58.Lipase Production by *Yarrowia lipolytica* B9 Using Various Plant Oils

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Abstract

Lipases which can catalyze numerous different reactions are used in the production of detergents, cosmetics, pharmaceuticals, flavour enhancers and foods was investigated [1,2]. This study was performed to produce extracellular lipase by *Yarrowia lipolytica* B9. A yeast starter culture was prepared by adding one loopfull of a 48-h-old slant culture into erlenmayer flasks containing inoculum medium and incubating at 20°C for 24 h on a rotary shaker at 150 rpm. One ml of the yeast starter was then transferred to the different production media containing 2% various plant oils containing olive, lavander, rosemary and coriander oil as the sole carbon source. The inoculated media were incubated at 20°C and 150 rpm for 48 h. The culture supernatants were assayed for lipase activity by a spectrophotometric assay method [3]. According to the results, maximum lipase activity determined as 32.1 U/l in fermented medium containing rosemary oil and the following activity values observed with coriander, olive and lavander oil, respectively. The results showed that lipase could be produced by *Y. lipolytica* B9 using renewable low-cost substrates such as plant oils and also the least biomass amount obtained lavander oil was thought inhibition of the yeast cells.

Keywords: Lipase, *Yarrowia lipolytica* B9, Plant Oils

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P59. Effect of pH on Production of Protease by the Immobilized Cells of *Gliocladium roseum* F2

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Abstract

Alkaline proteases are particularly suitable for industrial applications due to their high stability and activity under harsh conditions such as high temperature, alkaline pH and in the presence of surfactants or oxidizing agents [1]. Alkaline protease producer moulds were isolated on agar medium (pH 8.0). Whole cell immobilization for the best isolate *Gliocladium roseum* F2 was performed by using sodium alginate. Protease production with immobilized cells was performed in production medium containing (g/L) 20 glucose, 3 skim milk powder, 1.5 KH_2PO_4, 1 $MgSO_4$, 0.3 $CaCl_2$ and 0.03 $FeSO_4$ (pH 8.0). Effect of initial pH on protease production was studied in the range of 5.0 to 10.0 at 30 °C and 150 rpm for 72 h. The culture supernatants were used for determination of protease activity by modified from Takami *et al.*[2] and the total protein content was determined by Bradford method [3]. Maximum protease activity (38.5 U/mL) was achieved at pH 9.0 in fermented medium containing 8.6 mg/ml total protein. SDS-PAGE analysis illustrated that extracellular protease enzyme had the size of 111.94 kDa. It was thought that this result was important, since proteases with high activity and stability in high alkaline range are interesting in bioengineering and biotechnological applications.

Keywords: *Gliocladium roseum* F2, Protease, Immobilization, pH

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P60. Invertase Production by Cold-Adapted Local Isolate *Cladosporium* sp. MT2 Using Sugar Beet Molasses

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Abstract

Invertase is an enzyme which catalyzes the hidrolysis of sucrose to glucose and fructose. It is widely used in food and beverage industrial production of liquid sugars, artificial honey and noncrystallizable creams[1]. In this study, production of extracellular invertase by locally isolated cold-adapted mould *Cladosporium* sp. was investigated. Preliminary experiments were focused on the isolation and screening of invertase producer moulds. For the best invertase producer isolate, different temperatures (5-35 °C), initial pHs (5-9) and incubation times (with 12-h intervals up to 108 h) were optimized, respectively. The screening and production experiments were conducted in 250 ml flasks containing 100 ml of basal medium (%4 molasses-containing medium). The culture supernatants were used for determination of invertase activity by the colorimetric method of Miller [2]. Among the total 25 isolate, the isolate MT2 was found to be the best invertase producer, and it was identified as *Cladosporium* sp. Optimal conditions for the maximum invertase production (32.76 U/mL) with this isolate were initial pH 6.0, temperature 20 °C and cultivation time of 60 h. The results exhibite that this enzyme is important due to acidic and cold-adapted property in the production of non-crystallizable invert sugars.

Keywords: *Cladosporium* sp. MT2, Cold-adapted, Invertase, Molasses, pH

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P61. Expression levels of the laccase gene in lichen *Pseudevernia furfuraceae* subjected to Pb^{+2} heavy metal stress

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Abstract

Environmental pollution is one of the most important problems that the world is facing today. Heavy metal contamination in the wastewaters can lead to toxic, carcinogenic or mutagenic effects for the human health. Classical methods of removing and recovering heavy metals from wastewaters are generally expensive and have some disadvantages. Lichens are natural, easily available and low cost biomass for dissolved heavy metal ions and are thought to be used as a alternative biological organism for removing heavy metals from wastewaters. In recent years there are many efforts to improve their removing capacity by using molecular methods. For this purpose, we investigated mRNA levels of laccase gene using qRT-PCR in *Pseudevernia furfuraceae* subjected to Pb^{+2} heavy metal to see the possible roles of that gene in removing Pb^{+2} heavy metal from wastewater. The effect of initial metal concentration, initial pH, stirring speed and contact time on biosorption efficiency on mRNA levels of laccase gene were also studied. We determined that different biosorption efficiency parameters were lead to changes in mRNA levels of laccase gene. As a result, laccase gene derived from *P. furfuraceae c*an be used for a alternative biotechnological tool in removing heavy metals from wastewater.

Keywords: *Pseudevernia furfuraceae,* laccase gene, qRT-PCR, Pb^{+2}

P62. Inductions of alternative electron sinks and the water-water cycle in chloroplasts protect *Thellungiella parvula* from salinity induced oxidative stress

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Abstract

The main aim of this study was to reveal how an extreme halophyte *Thellungiella parvula* tolerates salinity in the manner of reactive oxygen species (ROS) production, antioxidant system and redox regulation of electron transport system in chloroplasts. *T. parvula* were grown for 30 d and then treated with 50-200-300 mM NaCl. Activities of antioxidant enzymes were measured in whole leaf and isolated chloroplasts. In addition, the expressions of chloroplastic redox components such as ferrodoxin thioredoxin reductases (FTR), NADPH thioredoxin reductases (NTRC), thioredoxins (TRXs) and peroxiredoxins (PRXs) were measured. Gradually increased salt treatment affected water relations negatively and the accumulation of osmolyte proline was increased by salinity. *T. parvula* could cope with 300 mM NaCl in long term as evident by H_2O_2 content and lipid peroxidation. While Ca^{+2} and K^+ contents decreased by salinity, Na^+ and Cl^-contents were enhanced. Efficient induction of water-water cycle enzymes superoxide dismutase (SOD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) prevented accumulation of excess ROS in chloroplasts and therefore the photosynthetic machinery could be protected in T. parvula. The redox homeostasis in chloroplasts was achieved by efficient induction of expressions of redox regulatory enzymes such as FTR, NTRC, TRXs, PRXs under salinity.

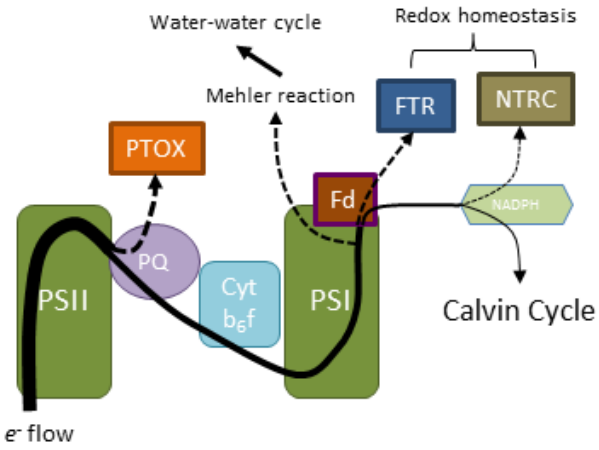

Figure 1. Scheme summarizing the alternative electron sinks and mechanisms that can relax the electron load on chloroplastic electron transport chain.

***Keywords*:** Alternative electron sink, antioxidant enzymes, chloroplastic redox, halophyte, water-water cycle

P63.The Use of RAPDs Technique for the Detection of Genetic Stability of *Hypericum perforatum* L. Derived from Micropropagated Plantlets

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Abstract

Hypericum perforatum L. is a representative of the Clusiaceae family with confirmed therapeutic effects on burns, bruises, swelling, anxiety, mild to moderate depression and its antidepressant, antiviral, wound-healing, analgesic, hepatoprotective, antioxidant and antimicrobial properties [1]. Plant tissue culture is an effective method for the production of medicinal and economically important plants. *Hypericum perforatum* nodal explants on MS medium supplemented with 0,5 mg/l BAP and 0,5 mg/l Kin. callus was obtained after 8 weeks and adventitious shoots formation was achieved when callus transferred to MS medium supplemented with 2mg/l BAP. Shoots rooted on MS medium supplemented with 1mg/l IBA. Plantlets were acclimatized and successfully transferred to soil. RAPD – PCR analysis using seven universal primers were performed on DNA extracted from the fresh healthy leaves of the randomly selected micropropagated plantlets derived from tissue culture. Reproducible RAPD patterns were obtained using seven primers, six primers showed completely monomorphic bands in all samples tested of the progeny. RAPD appears to be an efficient technique and a simple fast DNA marker for the early detection of DNA marker for the early detection of genetic variations in plants propagated by tissue culture technique.

***Keywords*:** BAP, DNA, *Hypericum perforatum* L., IBA, Kin., RAPD.

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P64. Genetic Stability Assessment of Micropropagated Marjoram (*Origanum majorana* L.) through RAPD

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Abstract

The genus *Origanum majorana* L. is an aromatic, perennial, herbaceous plant belonging to the family Lamiaceae [1]. Its essential oil and alcoholic extracts are applied in production of pharmaceuticals, perfumes and cosmetics [2]. In this study, randomly amplified polymorphic DNA (RAPD) markers were used to determine the genetic stability of micropropagated *Origanum majorana*. Eigth randomly selected micropropagated plants were used. Genomic DNA from the leaves of the selected micropropagated plants before the hardening stage were isolated with GeneJET plant Genomic DNA Purification Mini Kit according to the manufacturer's protocol. DNA fingerprinting by RAPD was performed by polimerase chain reaction (PCR) amplification of genomic DNA with 6, 10-mer random oligonucleotides as primers (F16,015,011,N12,K6,T16,P6,M6). RAPD analyze revealed two primer showed polymorphic bands and six primers showed monomorphic bands.

***Keywords*:** Micropropagation, *Origanum majorana* L.,RAPD-PCR markers.

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P65. Gluten allele frequencies and Glu-1 quality scores in local wheat populations originated from Turkey

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Abstract

Turkey is located in an interception between the gene centers Of Near East and Mediterranean. Numerous wild forms and local varieties of wheat could easily be found in Turkey. The endosperm of hexaploid wheat contains gluten proteins which are recognized as important components affecting the processing quality of wheat flour. Glutens have characteristic low and high molecular weight subunits [1, 2]. 129 Turkish landraces and 15 standard varieties were obtained from "Ankara and İzmir Agricultural Research Institutes" and the modified method of Gao *et al.,* [5] were used for gluten extraction. Glu-1 quality score was estimated based on distribution of individual HMW subunits in the SDS-PAGE, and scaled using the individual subunit scores [8, 6, 7]. Frequency of alleles encoded by Glu-1 region (*Glu-A1, Glu-B1* ve *Glu-D1*) was calculated using the method of Gupta et al., [8]. Allelic combination frequencies were estimated by dividing observed number of each allele to total number of populations. Average Glu-1 quality score of populations across Turkey was estimated to be 6.07. Frequencies of Axnull, Ax2*, Ax1, Bx7+By8, Bx20+By20, Dx2+Dy12, Dx5+Dy10 were found to be 30, 26, 9, 54, 12, 60, 28%, respectively. Total 22 different alleles were observed in 50 different combinations in 129 populations. It is concluded that among allelic combinations Bx7+By8-Dx2+Dy12 has the highest frequency with a value of 23%, and the highest Glu-1 quality score (7.18) was observed in Marmara region.

Keywords: Gluten, Glu-1 quality score, Allele frequency

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P66. DEVELOPMENT OF SUBNILS FOR FINE MAPPING OF SALT TOLERANCE IN TOMATO (*SOLANUM LYCOPERSICUM*)

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Abstract

Salt tolerance is a complex trait that is not easily bred into plants. Salt tolerance can be manifested in several ways such as the ability to increase shoot and/or root mass despite salt stress, a greater than normal accumulation of antioxidants like phenolic compounds and flavonoids, and an improved ability to exclude sodium ions. In previous work, *Solanum lycopersicum* M82, *Solanum pennellii* LA716 and a *S. pennellii* IL population were evaluated to identify QTLs responsible for controlling antioxidant parameters under both control and stress conditions. Salt tolerance characteristics were observed in IL11-1, IL6-1 and IL7-4-1. The aim of this project was the development of subNILs for fine mapping of salt tolerance related traits within these introgression regions. To this end, each *S. pennellii* IL was crossed with *S. lycopersicum* M82 to produce F2 populations of approximately 1600 individuals for IL6-1, 1600 individuals for IL7-4-1 and 3000 individuals for IL11-1. These individuals were screened with molecular markers that delimit the 30-40 cM introgressions contained in each line. Because of low number of recombinants in IL6-1 and IL7-4-1 populations, they were not selfed to produce subNILs for future analysis. Recombinant F2 plants in IL11-1 population were self-pollinated to generate F3 recombinant families. Each F3 recombinant plant was characterized with several codominant molecular markers in the introgression region. 228 homozygous recombinant F3 plants and 620 heterozygous recombinant plants were identified. At the end of this work, fine mapping populations were developed.

***Keywords*:** Fine mapping, Salinity, Tomato, subNILs

P67. Analysis of genetic diversity in Turkey originated local wheat populations

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Abstract

Turkey with a rich biodiversity and genetic resources is located in a point of intersection between two main gene centers Near East and Mediterranean region. Accordingly numerous wild forms and local varieties of wheat can be found in Turkey. Among five main groups of proteins in endosperm of hexaploid, glutens have characteristic alcohol-soluble low molecular weight subunits and alcohol-insoluble high molecular weight subunits regarding the quality [1,2]. The studies were carried out in populations of Turkey originated bread-wheat. Fifteen standard wheat varieties were obtained from "Ankara and İzmir Agricultural Research Institutes". Modified methods of Doyle and Doyle [3], McCarthy [4], and Gao *et al.,* [5] were used for DNA isolation from fresh tissues, seeds, and gluten extraction, respectively Statistical analyses were done using the POPGEN32 software program, version 1.31. Genetic variation and genetic diversity analyses in subpopulations were carried out according to Nei [6]. The average gene diversity (h) and the rate of genetic similarity (I) in Glu-1 locus were estimated to be 0.15 ± 0.17, and 0.24 ± 0.23, respectively. Genetic diversity in Glu-1 locus was largely believed to be due to variations within the population. The average genetic diversity in Glu-1 locus (Ht), within populations (Hs), and between populations (Gst) were determined to be 0.15 ± 0.03, 0.14 ± 0.02, and 0.08, respectively. Gene flow among populations (Nm) was calculated as 5.35. The highest average heterozygosity between regions was observed in Aegean with a value of 2.82. On the other hand, the lowest heterozygosity was observed in populations of Eastern Anatolia with a rate of 0.82.

Keywords: Gluten, Wheat, Genetic diversity

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P68. HMW Gluten polymorphism in Turkey originated local wheat populations

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Abstract

Turkey is an important gene center with a rich biodiversity in an intersection between Near East and Mediterranean region. Accordingly numerous wild forms and local varieties of wheat could be found easily. The endosperm of hexaploid wheat contains five groups of proteins mainly being albumins, globulins, gliadins (proamines), and glutenins (glubenins) [1]. Glutens have characteristic alcohol-soluble low molecular weight subunits and alcohol-insoluble high molecular weight subunits. Low and high molecular weight subunits are of the factors determining the quality [1,2]. The present study was carried out on bread wheat populations. 129 Turkish landraces and 15 standard varieties were obtained from "Ankara and İzmir Agricultural Research Institutes". The modified methods of Doyle and Doyle [3], McCarthy [4], and Gao *et al.,* [5] were used for DNA isolation from fresh plant tissues, seeds, and gluten protein extraction, respectively. Bx7, By8, Dx2, Dy12, Ax2*, Axnull, Bx20, Dx5 and Dy10 were found to be the most frequently encountered alleles in populations of Turkey. One new allele (Dy12*) has been identified among studied 129 bread wheat populations. This new allele was differentiated from previously characterized Dy12 in terms of its faster mobility in SDS-PAGE gel.

Keywords: Gluten, Glu-1 quality score, Allele frequency

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P69. Effects of copper on growth, chloroplast 2-cysteine peroxiredoxin (*2-Cys-Prx*) and photosystem II D1 protein (*psbA*) gene transcription in *Chlorella vulgaris* determinate using semi-quantitative RT-PCR

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Abstract

Copper is an essential micronutrient for growth, metabolism and enzyme activities of various algae, and other organisms; however, it is also a proven inhibitor of algal growth as well as photosynthetic and respiratory activities at high concentrations[1,2,3]. In this study the effects of short term copper toxicity in green algae *C. vulgaris* were investigated at physiological and transcriptional level. The results showed that algal growth, negatively affected by 2.5 µg/mL copper treatment for 72 and 96 h. The expression of *psbA* gene and chloroplast *2-cys-Prx* gene were quantified using semi-quantative RT-PCR. Increased *2-Cys-Prx* and *psbA* mRNA transcript levels were observed in the *C. vulgaris* that treated with different concentration of copper (0.16, 0.35, and 0.75 µg/mL) for 24, 48 and 72 h. The *psbA* gene transcription levels after treatment with 0.75 and 2.5 µg/mL concentration of copper for 96 h decreased by 1.5 and 1.8-fold. The reduction of *psbA* mRNA transcripts may decrease the activity of PSII and electron transfer rates, which was observed by a decrease in chlorophyll content. As a conclusion, our data demonstrated that *2-cys-prx* and *psbA* mRNA transcript levels could be play important roles in copper tolerance.

Keywords: copper, chloroplast peroxiredoxin, *psbA* gene, algal growth

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P70. DNA Fingerprinting of Some Olive Cultivars in Turkey Using Random Amplified Polymorphic DNA (RAPD) markers

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Abstract

Olive (*Olea europea* L.) is one of the oldest cultivated plants characteristic in the Mediterranean area, where it is the most important oil-producing crop. The cultivated olive (*O. europaea* L. var. *europaea*) is propagated by cutting or grafting, whereas wild olive (*O. europaea* L. var. *sylvestris*) is reproduced from seeds. These two olive types are interfertile and have led to a large number of varieties. Morphological descriptions are not entirely reliable, due to numerous synonyms and homonyms in designations, labelling mistakes, the presence of varietal clones, and the uncertain identification methods thus far applied. Molecular markers, as random amplified polymorphic DNA (RAPD) markers, are environment-independent and efficient to identify olive varieties and to detect synonymous and homonymous. In this study, fifteen selected RAPD markers are used for determination of relationships among fourty individuals belonging to eight important Turkish olive cultivars [Figure 1]. Our results showed that RAPD markers can be used to differentiate olive cultivars [1, 2, 3].

Keywords: DNA fingerprinting, Olive (*Olea europea* L.), RAPD Markers.

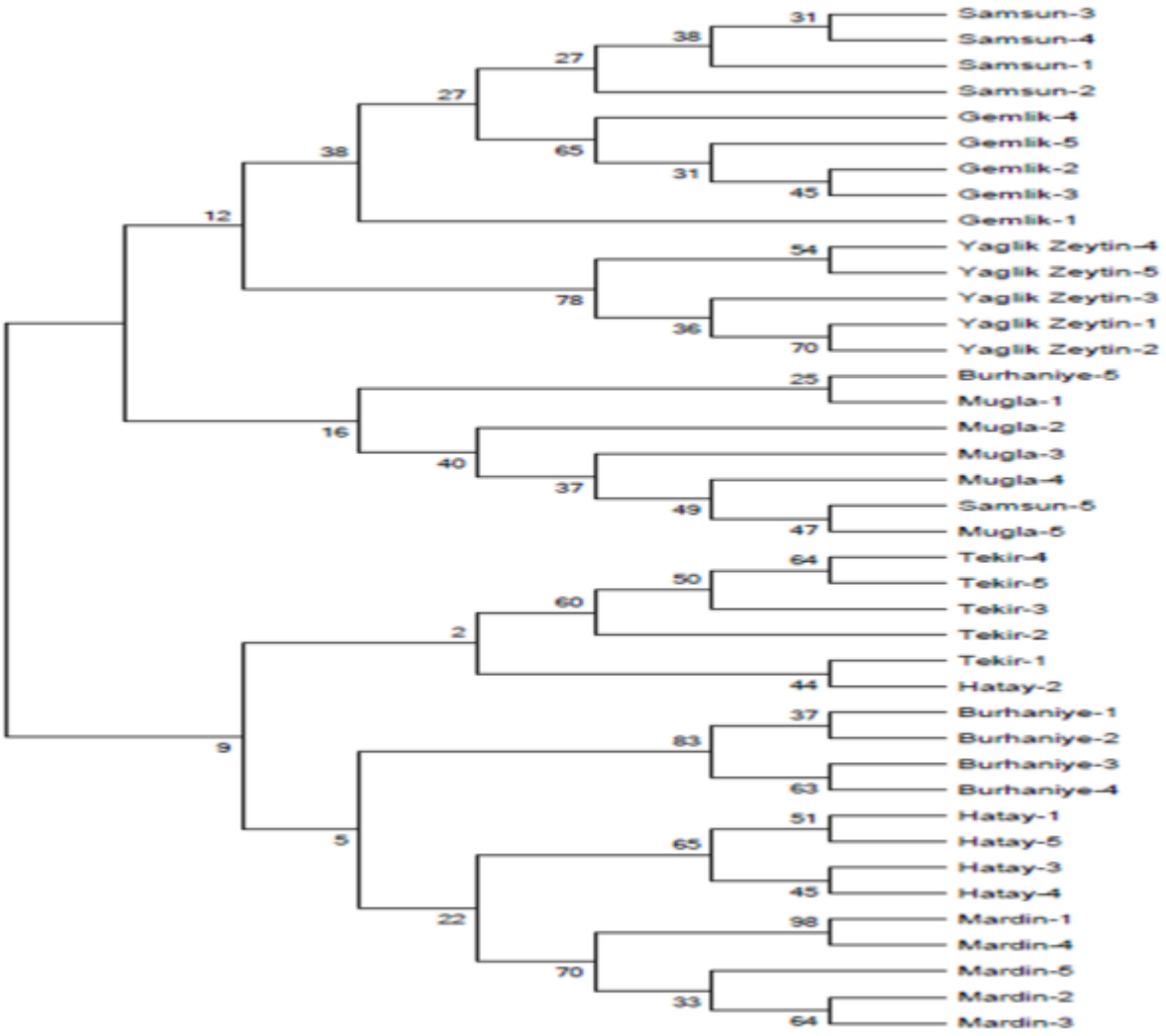

Figure 1. UPGMA dendrogram obtained from RAPD analysis data for eight important Turkish olive varieties.

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P71. The Effect of Polyamine on mRNA Level of Photosynthesis-Related and Antioxidant Enzymes Gene under High Light Stress

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Abstract

In this study the effects of short term high light condition and polyamine application on the green algae *Chlorella vulgaris* were investigated at physiological and transcriptional level. Our results suggest that exposed to high light leads to disruption of photochemical processes such as photosynthetic activity and physiological processes such as growth rate, chlorophyll a/b ratio and lipid peroxidation in an exposure time dependent manner. The results showed that high light affected algal growth, especially at 3 h and while Chl a/b ratio was significantly reduced; lipid peroxidation rate was significantly increased at this time. However, The results showed that the application of spermidine, putressine and spermine could mitigate high light-induced oxidative damage through an increase in mRNA level of antioxidant enzymes (superoxide dismutase and ascorbate peroxidise), and could stabilized *psb*A transcript levels.

Keywords: *psbA*, *psaB*, photosynthesis, polyamine, light stress

P72. Investigation of stress response of FAD2 gene to salt and drought stress in safflower at mRNA level with qRT-PCR

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Abstract

Safflower (*Carthamus tinctorius*) is one of the important oilseed crops which have a high adaptation to different conditions such as resistance to drought and salinity. Today, one of the main objectives of oil crop breeders is to provide the stability of oleic acid content in oil crops subjected to any types of environmental conditions. By this way it is important to identify the genes involved in stress response mechanism in plants. Hence we investigated mRNA levels of *FAD2* genes with qRT-PCR in response to salinity and drought stresses in safflower plant. *FAD2* genes play an important role in convertion of oleic acid to linoleic acid in safflower and are thought to may be included in stress response mechanism. For this purpose, we used two safflower species which have different origin and namely TRE-ASO12/08(Turkey) and BDYAS-4(ABD). Safflower species were stressed by application of 100mM NaCl and PEG at the equivalent osmotic potential and stressed plants were harvested at 3 and 27th hours of stress applications. In case of analyzing mRNA levels of *FAD2* gene, several alterations were observed at mRNA levels of the target gene and these results show that *FAD2* gene may be responsible for stress defense in safflower.

Keywords: *Safflower, FAD2 gene, stress response, drought, salinity*

P73. Identification of boron-related genes following boron-toxicity and supplementary boron-enriched fertilizer to deficiency condition in wheat cultivars differing in boron tolerance

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Abstract

Uptill now, a few studies have suggested integrative view of plant responses to boron (B) deficiency stress at the transcriptional level. However, too few genes induced by toxic B and no gene after application of B-enriched fertilizer to B deficiency condition have been reported to date. In this study, our aim was to assess the changes in expression profiling of two contrasting wheat cultivars by using Affymetrix wheat GeneChip in response to B toxicity, deficiency, and application of Tarımbor to deficiency condition. Another objective was to clarify the physiological differences in these cultivars such as growth, water status and B distribution under same conditions. The visual symptoms of B-toxicity, regarding chlorosis and necrosis, were only seen in leaf tips of Atay. Coordinately, B accumulates higher in Atay leaves than Bolal after high B. However, all B conditions did not cause a cessation of vegetative growth in both cultivars. More genes related protein degradation were induced under all B conditions in Atay than Bolal. These results considering programmed cell death in senescing leaves of Atay were confirmed by the visual phenotype of the plants. Our findings may introduce new targets for breeding researches aimed at improving B tolerance of crop plants.

Keywords: affymetrix, boron deficiency, boron toxicity, Tarımbor, wheat

P74. IDENTIFICATION OF CHROMOSOMAL SEGMENTS FOR ACYLSUGAR LEVELS DERIVED FROM *Solanum pennellii*

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Abstract

Tomato is attacked by a variety of insect pests, such as tomato leaf miner *Tuta absoluta* that cause losses through feeding on leaves and fruits resulting in reduction in yield and quality. Insecticides are becoming increasingly unacceptable for use as sole solution due to their inhibitevly high cost, health and environmental issues. Development of pest-resistant tomato is an attractive alternative strategy. Host / insect interactions for plant protection are classified as being due to antibiosis, antixenosis (nonpreferance), or tolerance, usually under complex inheritance. The acylsugar levels derived from *Solanum pennellii* LA 716 corralate well with resistance against Tuta absoluta. Thus, objectives of this study was to identify genes / QTL for acylsugar levels in 76 tomato introgression lines that represent the whole genome of resistant source *Solanum pennellii* LA 716. The second generation introgression lines (IL) derived from *Solanum pennellii* LA 716 constitute the resistance source. The IL lines were grown along with LA 716 and a susceptible tomato line that was crossed to the IL lines and used as low acylsugar parent. The homozygous and heterozygous IL lines and parents were grown in a greenhouse in a replicated manner. Because the acylsugar level of LA716 derived tomato lines was shown to be directly related with part of the resistance against the pest, the acylglucose and acylsucrose (total acylsugar) contents of the homozygous and heterozygous IL lines were determined. Based on the acylsugar content of homozygous and heterozygous IL lines, inheritance and the chromosomal segments associated with enhanced acylsugar level for resistance to *Tuta absoluta* were determined. The two segments on chromosomes 3 and 10 had the highest contribution for acylsugar level. The corresponding chromosome segments of IL lines are being screened for polymorphic markers that can expedite development of resistant tomato lines / cultivars against *Tuta absoluta* where they can be used to pyramid QTLs responsible for resistance in marker assisted backcross breeding programs (MAB). The outcome of the study holds the promise of significant economic implications through reducing loss of yield and quality, and lower pesticide use.

Biochemistry

P75. Modeling Histidine Chelates of Cu^{+} and Cu^{2+}

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The interactions between histidine and metals play essential roles in a wide range of important biological processes including enzyme catalysis and signal transduction. Copper-histidine complexes are used as a treatment for Menkes disease. Additionally, the discovery of the antibacterial, antifungal and anticancer activity of several copper complexes is subject of interest. It is also found that amino acid ligand based drugs are highly efficient. Study of the interaction between drugs and transition metals is an important and active research area in bioinorganic chemistry[1-3]. The present study investigates the histidine (His) complexes with Cu^{+} and Cu^{2+} by molecular modeling. Different His forms and Cu ions and complex formation between these were investigated by Density Functional Theory (DFT) methods. B3LYP/cc-PVTZ level optimizations were carried out on the previously obtained geometries. Most stable geometries were than selected to interact with Cu^{+} and Cu^{2+} ions at B3LYP/GEN level where GEN basis set was defined as cc-PVTZ for C, O, N, H and LANL2DZ for copper. Calculations have been performed in vacuum (gas phase) and in solution using the Polarizable Continuum Model (PCM). Our preliminary results indicate that Cu binding to His is selective in nature and the environment (solvent) strongly affects the complex formation.

Keywords: Histidine, Copper Ion, Molecular Modeling, Complex Formation, DFT

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P76.Computational Study of Tautomerism of Nucleobases and Their Interactions with Mono and Di Mg^{2+} Cations

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Abstract

Interactions between tautomers of nucleic acid nucleobases and Mg^{2+} have been investigated to understand the structural and functional properties of M-box riboswitch. Enviromental factors including water, chemical modification, metalic cation interaction, irradition, and excitation play an important role in tautomerization [1]. Presence of metal ions can affect the nucleobase's electron distribution and consequently tautomeric equlibria [2]. Thus, metal ions play role in formation of rare tautomers. This topic, especially tautomeric equilibria of nucleobases in vacuum and in a microhydrated environment has been studied frequently alongside of nucleobase-metal ion interactions [2]. Nucleobases are highly polar so metal ions and water can play important role for tautomers of nuclebases. The effects of metal ions on the stability and properties of the nucleobase tautomers have been observed by using molecular modeling tools. It has been shown that rare tautomers of DNA and RNA nucleobases have different stabilities in the presence of metal ions. In addition, two Mg^{2+} interactions between nucleobases have been calculated. These calculations can reveal the tautomerism mechanism and the stability of tautomeric nucleotides. Our results at B3LYP/cc-PVTZ level indicate that these rare tautomers form more stable complexes with Mg^{2+} and two Mg^{2+} compared to canonical nucleobase Mg^{2+} complexes.

***Keywords*:** Nucleobase, tautomerism, riboswitch, RNA, DNA, molecular modeling

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P77. Determination of DNA Damage Marker (8-OHdG) in Patients with Chronic Obstructive Pulmonary Disease (COPD)

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Chronic abstructive pulmonary disease (COPD) is a slowly progressive condition characterized by poorly reversible airflow limitation associated with an abnormal inflammatory response of the lung. The main causal factor of COPD are chronic axidative stress as a result of long-term smoking, use of biomass fuels and air pollution[1]. 8-Hydroxy-2-deoxyguanosine (8-OH-dG) is produced by the oxidative DNA damage by reactive oxigen and nitrogen species and serves as an established marker of oxidativeb stress. For this parameter used in DNA damage studies[2]. In this study we aimed to determine oxidative DNA damage marker (8-OH-dG) in patients with COPD. Levels of serum 8-OHdG which are marker of oxidative DNA damage were measured by enzyme-linked immunosorbent assay (ELISA). For this purpose oxidative DNA damage was studied in serum samples of 30 patients COPD and 30 healty control group. As a result, 8-OHdG levels were increased in COPD patients group when it compared healty control group.

***Keywords*:** Chronic obstructive pulmonary disease (COPD), 8-OHdG, ELISA

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P78. In vitro Inhibition of Some Dihydroxyanthraquinone Derivates as Antitumor Antibiotics on Paraoxonase1 Activity

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A dihydroxyanthraquinone is any of several isomeric organic compounds with formula $C_{14}H_8O_4$, formally derived from 9,10-anthraquinone by replacing two hydrogen atoms by hydroxyl groups. Dihyroxyantraquinones have been studied since the early 1900s, and include some compounds of historical and current importance.[1] The isomers differ in the position of the hydroxyl groups, and of the carbonyl oxygens (=O) of the underlying anthraquinone

The unqualified term "dihydroxyanthraquinone" usually means a hydroxyl derivative of 9,10-anthraquinone. The dihydroxy-9,10-anthraquinone functional group occurs widely in natural products, and is an important feature of the anthracycline antitumor antibiotics. [2] In particular, 1,8-Dihydroxy-9,10-anthraquinone is the precursor for the important topical antipsoriatic drug anthralin, 1,8-dihydroxy-9-antrone.[3]

Serum paraoxonase 1 (EC 3.1.8.1, PON1), a calcium-associated enzyme, has an ability to hydrolyze organophosphate compounds. Related to this property, PON1 has a critical role in antioxidant mechanisms. It is well-known that the enzyme protects LDL from oxidation. In this study we investigated the in vitro inhibitory effects of some antitumor antibiotics. These drugs are alizarin, quinizarin, purpurin, dantron. It was determined that all of the compounds inhibit the enzyme activity by different rate.

***Keywords*:** Antitumor antibiotis, paraoxonase, inhibition

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P79. Identification and Characterization of LeprotL1 protein in Mammalian Circadian Clock Mechanism

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The circadian clock is a biological timing system that oscillates with a period of 24 hours and is coordinated with the light cycle of the environment. Circadian clock affects many metabolic processes in body such as sleep/wake cycle, feeding, hormone secretion and many others. Most importantly it is known that dysfunction in circadian rhythmicity leads various metabolic and psychological diseases such as jet-lag, depression, obesity and even cancer.

At the physiological level it is thought that circadian rhythm makes organisms ready for daily activities. However, it is not very well defined what happens in the molecular level. Researchers identified several proteins that are responsible for the generation of rhythmicity. Some of these core clock components are cryptochrome1/2 (CRY1/2), period 1/2/3 (PER1/2/3), clock, Brain and muscle ARNTL-like protein 1 (BMAL1). Yet, the clock mechanism and the components of the machinery is not entirely solved. It is a big challenge to identify core clock components among researchers and one of the mostly commonly used methods on this purpose is the naive Bayesian strategy. With naïve Bayesian strategy, it becomes possible to select genes that show high correlation with circadian rhythm by defining some criteria which are essential for a protein to be a core clock component via computer based screening.

In this work, high probability clock genes identified by the naive Bayesian strategy were experimentally screened through mammalian two-hybrid assay system for likely interactions with the core clock components. The entire screen yielded a hit which is Leptin receptor overlapping transcript like-1 protein (LeprotL1). LeprotL1 is protein that has metabolic function within organisms. It is already shown that LeprotL1 decrease the hepatic growth hormone action in mice though its function in circadian rhythm is unknown. Here in, it is shown that LEPROTL1 interacts with both mCRY1 and mCRY2 according to the mammalian two hybrid assay. This interaction is also confirmed with co-immunoprecipitation assay which means there is a direct interaction in between. The involvement of *Leprotl1* in the circadian clockwork was investigated by analyzing the interacting domain of CRY1 protein. It was also shown that depletion of *Leprotl1* with siRNAs resulted in damped amplitude, long-period and phase advance phenotypes of the circadian rhythm's 24 hours of oscillation in U2OS and NIH3T3 cells, suggesting a role of *Leprotl1* in metabolism and circadian rhythm correlation.

Keywords: circadian rhythm, leprotl1, cryptochrome1/2, core clock components

P80. Endothelial Nitric Oxide Synthase Gene in the Development of Diabetic Nephropathy

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Abstract

Diabetic nephropathy (DN) is one of the most common microvascular complications of type 1 and type 2 diabetes mellitus, and is the leading cause of end-stage renal disease worldwide [1]. Intron 4 polymorphism may influence the risk of developing diabetic nephropathy in patients with type 2 diabetes mellitus, and especially type 1 diabetes mellitus [2]. The purpose of the present study was to investigate the polymorphism of the constitutive endothelial nitric oxide synthase gene in patients with diabetic nephropathy. This study is conducted with the blood specimens of 25 patients with diabetic nephropathy and 25 healthy control groups. When we examined the a-deletion/b-insertion in intron 4 of endothelial nitric oxide synthase gene, the genotype and allele frequencies were not significantly different between the patients with diabetic nephropathy and control group. A report was published that suggested on association between constitutive endothelial nitric oxide synthase gene polymorphism and diabetic nephropathy in patient with type 1 and type 2 diabetes mellitus [3]. The findings of our study shown that does not association between the constitutive endothelial nitric oxide synthase gene polymorphism and the development of diabetic nephropathy in patients with type 2 diabetes mellitus.

***Keywords*:** Diabetic nephropathy, diabetes mellitus, endothelial nitric oxide synthase gene, polymorphism

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P81.Oxidative Stress in Rat Cortex

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Abstract

Iron is an indispensible element for brain development, oxygen transport, neurotransmitter synhtesis as well as normal brain functions [1]. However, excess iron intake increases free radical formation. These reactive oxygen metabolites may be deleterious for nervous system, and it induces neurotoxicity. Biological systems have an endogenous defense mechanism to protect cells against oxidative damage via the antioxidant defense system [2]. The main purpose of this study was to examine the toxic effects of iron on the brain antioxidant system at the enzymatic and molecular level. Our results showed that brain antioxidant systems was affected by iron overload at the enzymatic and molecular level, but the actual effect of iron was observed at protein level. Reduced glutathione (GSH) level, either increased or decreased GSH level is an indicator of oxidative stress [3], was markedly reduced in the present of iron.

***Keywords*:** Iron, Rat, Cortex, Neurotoxicity, Glutathione System

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Acknowledgments. This work was financially supported by Atatürk University Scientific Research Projects Coordination Commission (ATAUNI-BAP) with project number 2010/177.

P82.The Effects of Boron on Apopitosis and DNA Damage in STZ Diabetics Rats

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Abstract

Diabetes is a chronic metabolic disorder and increasing rapidly worldwide. Many underlying mechanisms have been suggested in the pathogenesis and complications of diabetes. Free radicals that are main cause of the complications most accepted mechanisms. For this reason used drug for therapy, should support the antioxidant balance while providing the antidiabetic effect. DNA damage which is a contributing factor in diabetes as well as follow up of chronical degenerative diseases.Apoptosis (physiological cell death) also known as suicide of cells is physiological event. When cells are determined to die by apoptosis, they indicate some biochemical (DNA fragmentation, phosphatidylserine translocated to the outer leaflet of apoptotic cell membranes) and morphological changes (cell shrinkage, chromatin condensation, ap optotic bodies) after indicate for apoptosis. In this the purpose of study is to investigate the effect of boron on serum CK18 levels and DNA repair in rats with type 1 diabetes mellitus. Consequently; Experimental diabetes caused to DNA damage in mononuclear leukocytes and apoptosis. However, treatment of boron decreased severity of DNA damage and apopitosis in blood.

***Keywords*:** DNA, apoptosis, diabetes, boron

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P83.Effect of *Capparis ovata* Water Extract on the Lipid Metabolism Used as an Alternative and Complemenatary Treatment for Multiple Sclerosis

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Abstract

Combination of flowering buds, flowers and fruit of *Capparis ovata* have been traditionally used in the treatment of multiple sclerosis. This study evaluates the effect of *Capparis ovata* water extract (*MSCov*) (Turkish Patent Institute, PT 2012/04 093) on the experimental animal model of multiple sclerosis at the molecular level and the expression of lipid and cholestasis metabolism genes in mice. Caper has been widely used by the traditional medicine for healing effects in certain pathological conditions [1-3]. C57BL/6 mice, about 6-8 weeks old and weighing 18-22 g (n=16), received 500 mg extract per kg body weight intragastrically for 28 days while control subjects received only water. Animals were killed by decapitation and liver were removed. The alteration in expression mRNA level of 49 genes which are known to be involved in lipid and cholestasis metabolism including ACAT 1-2, CPT, ECHS1, ACOT 1-2, APOE, etc. were detected by qRT-PCR in liver tissues of control and *MSCov* treated animals. We have observed that the expression levels of mRNA were not changed significantly. These results indicated that *MSCov* did not significantly elicit any major lipid and cholestasis metabolism genes expression.

This work is supported by the TUBITAK with project number 112S187.

***Keywords*:** *Capparis ovata*, lipid, cholestasis metabolism, genes expression, Multiple scelerosis

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P84. An Enzyme of A Novel Clock Component: Cystathionine-B-Synthase (CBS)

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The circadian clock is a biological timing system that oscillates with a period of 24 hours and is coordinated with the day-night cycle. This timing machinery is necessary for the body to anticipate environmental changes, to react external changes and adapt molecular, physiological and behavioural processes to maintain metabolic homeostasis but of course, disruption of circadian rhythmicity generally leads to diseases. Therefore, understanding the molecular clockwork help us develop treatments against various disease and disorders such as diabetes, cardiovascular diseases, obesity, jetlag, insomnia, and psychological disorders. Mammalian circadian clock is composed of transcriptional-translational feedback loop which allows the generation of daily rhythms. There is a set of core clock genes identified so far that constitute molecular feedback loop involving BMAL1, Clock, Npas2, Period1,2,3, Cryptochrome 1,2 genes. Besides known core clock components, more gene loci were shown that they change wild type oscillation pattern of core clock genes. These additional clock relevant genes which were identified by recent genetic analyses indicate that, circadian clock contains multiple and complex genetic interactions. By taking this fact into account, it is likely that many more genes underlying circadian rhythmicity will be discovered in near future. Recently, by applying high throughput genetic and proteomics techniques in combination with computer -assisted screening, candidate clock components were identified. The ones that show highest similar characteristics to known clock genes were selected. Among them, CBS (Cystathionine-β-Synthase) is one of the most prominent genes with its clear interaction with mCry1. Besides its role on circadian activity, CBS is an important enzyme for cardiovascular function of the body and cysteine synthesis pathway. To understand physiological role of CBS in circadian clock mechanism, we used cell biology, molecular biology and biochemical approaches. Here, we demonstrate with mammalian two hybrids that CBS interacts with last twenty amino acids of C-terminal of mCry1. This physical interaction is confirmed with coimmunoprecipitation. Then, transcriptional repression activity of CBS was tested by high throughput luciferase assay in N2A and NIH3T3 cell lines in the absence and presence of mCry1. It is clearly seen that CBS enhances repression activity of mCry1 on E-box promoter. Along with repression activity, we also tested how CBS enzymatic activity is affected with its interaction with mCry1. To do this, we performed hydrogen sulphur assay with mouse liver containing highly expressed endogenous CBS in the presence of mCry1 and mCry1 truncated at the last 20 aminoacid of C-termini. CBS is a prominent enzyme which has high probability of being a core or modifier component of the circadian clock. We conclude that CBS may be a functional clock component providing a new layer of control on circadian molecular dynamics.

Keywords: Cystathionine-B-Synthase, Circadian Rhythm, Biological Clock

P85. Molecular Characterization and Peptidomic Analysis of *Protoiurus kraepelini* (Scorpiones:Iuridae) Venom

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Abstract

The remarkable potency and pharmacological diversity of animal venoms has made them an increasingly valuable source of lead molecules for drug discovery [1]. In this respect, scorpion venom is an important natural resource for the development of new drugs. *Protoiurus kraepelini* scorpion has a widespread distribution in the Central and Western Mediterranean region of Turkey. In this work, peptide profile of *P. kraepelini* crude venom was studied. The protein and peptide fraction of the crude venom were separated with size exclusion chromatography and peptide fraction further separated with reversed phase chromatography[2]. Peptide fraction of *P. kraepelini* was analyzed by MALDI-TOF MS mass spectrometry. Electrophoretic profile of the crude venom and peptide fraction were studied with Glycine (for crude venom) and Trycine (for peptide fraction) SDS-PAGE and microfluidic capillary electrophoresis (MCE) [3]. Peptide mass profile between 1000-5000 Da was recorded by MALDI-TOF MS. Presented work is the first biochemical characterization of this scorpion species.

***Keywords*:** Scorpion Venom, *Protoiurus kraepelini*, Peptidomics

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P86. Determination of total glutathione-S-transferase enzyme activity in *Pinus brutia* needles and their roles in *Thaumetopoea wilkinsoni* attack

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Abstract

Pinus brutia Ten. is a native forest tree species in Turkey, due to both its economic and ecological assets. One of the problems faced by *Pinus brutia* afforestation sites is the attacks by *Thaumetopoea wilkinsoni* Tams. Glutathione S-transferases (GSTs) are ubiquitous multifunctional enzymes, which play a key role in cellular detoxification. The enzymes protect cells against toxicants by conjugating them to glutathione, thereby neutralizing their electrophilic sites, and rendering the products more water-soluble. We have studied the changes in GST activities in *P. brutia* related to herbivore attack. For this purpose, we selected 20 trees from resistant and susceptible clones in a seed orchard located in Antalya-Çığlık. We determined that average cytosolic GST activities of March and July samples show statistically significant increase with respect to the values of November. Each month was compared to November considering November values as control since trees were not under stress in November. The results showed that the highest enzyme activity found in March can be explained by herbivore stress. The highest defoliation by herbivore on the trees occurs in early springs. Our results show that GST activities from *P. brutia* would be a valuable stress marker.

Keywords: GST, Turkish red pine, herbivore attack, biotic stress

Acknowledgment*:* This work is supported by TUBITAK (Project Number: 110T976). This work was also supported in part by a grant from the Pamukkale University Research Fund (PAUBAP-2012FBE068, PAUBAP-2010FBE023)

P87. Investigation of the Effects of Antiepileptic Drugs on PON1, GST and AChE activities in Serum of Adult Patients with Epilepsy

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Abstract

Antiepileptic drugs (AEDs) have been widely used in patients with epilepsy. This study evaluated the adverse effects of two commonly prescribed AED monotherapies with carbamazepine (CBZ) and valproic acid (VPA) in adult patients with epilepsy. The aim of this study was to evaluate the influence of antiepileptic drugs on paraoxonase-1 (PON-1), glutathione S-transferases (GST) and acetylcholinesterase (AChE) activities in serum of adult patients with epilepsy. For this purpose, PON1, GST, AChE activities and glutathione (GSH) levels in 19 healthy adults and 56 adult patients with epilepsy were measured. Of the 56 epileptic adults, 28 were given valproate, and the remaining 28 were given carbamazepine; GSH levels in epilepsy patients with antiepileptic drug-treated was higher when compared to controls. But it was not significant, statistically. GST activity in epilepsy patients with antiepileptic drug-treated was insignificantly lower when compared to controls. Whereas PON1 and AChE activities was also significantly lower when compared to controls. There were differences in serum of the patients treated with carbamazepine and valproic acid monotherapy. The activities of PON1 and AChE in serum of the patients treated with carbamazepine monotherapy was lower than in patients treated with valproic acid monotherapy. The results indicate that the antioxidant systems and AChE activity in adult patients with epilepsy is modified by antiepileptic therapy.

P88. Heat Shock Protein 70 Dependent Proteasomal Degradation in Stress Conditions

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Abstract

Adaptation to mild oxidative stress and protein oxidation includes increased proteasome activity and increased proteasome-dependent degradation of oxidatively modified proteins. However this rapid increase may be due to increased protein synthesis and proteasome expression. In addition, activation of proteasome includes several regulatory mechanisms. Regulatory subunits such as 19S and 11S may be one of the factors and heat shock proteins may also have important role in the activation of proteasomal degradation. In the study, proteasomal response has been tested in the condition of oxidative stress in several time points. In the immediate response, 20S proteasome activity (ATP and ubiquitin independent) was shown to be activated whereas 26S proteasome activity (ATP and ubiquitin dependent) was declined which was returned to pretreatment levels in 3h. This decrease in 26S proteasome activity in the immediate response was related to 19S regulators. These regulators was shown to be bound to HSP70 chaperones, whose cellular levels are greatly increased. On the other hand, HSP70 siRNA application was shown to prevent 26S recovery confirming the role of HSP70 in the reconstition of 26S proteasome. The ability to adapt of living organisms to stress conditions is quite important. Therefore the role of heat shock proteins in the reconstition of proteasome may highlight important mechanisms for the treatment of several diseases related to proteasomal decline.

***Keywords*:** Proteasome, Heat Shock Protein 70, Protein Oxidation

Supported by TUBITAK COST-CM1001-110S281

P89.Identification and Characterization of LeprotL1 protein in Mammalian Circadian Clock Mechanism

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The circadian clock is a biological timing system that oscillates with a period of 24 hours and is coordinated with the light cycle of the environment. Circadian clock affects many metabolic processes in body such as sleep/wake cycle, feeding, hormone secretion and many others. Most importantly it is known that dysfunction in circadian rhythmicity leads various metabolic and psychological diseases such as jet-lag, depression, obesity and even cancer. At the physiological level it is thought that circadian rhythm makes organisms ready for daily activities. However, it is not very well defined what happens in the molecular level. Researchers identified several proteins that are responsible for the generation of rhythmicity. Some of these core clock components are cryptochrome1/2 (CRY1/2), period 1/2/3 (PER1/2/3), clock, Brain and muscle ARNTL-like protein 1 (BMAL1). Yet, the clock mechanism and the components of the machinery is not entirely solved. It is a big challenge to identify core clock components among researchers and one of the mostly commonly used methods on this purpose is the naive Bayesian strategy. With naïve Bayesian strategy, it becomes possible to select genes that show high correlation with circadian rhythm by defining some criteria which are essential for a protein to be a core clock component via computer based screening. In this work, high probability clock genes identified by the naive Bayesian strategy were experimentally screened through mammalian two-hybrid assay system for likely interactions with the core clock components. The entire screen yielded a hit which is Leptin receptor overlapping transcript like-1 protein (LeprotL1). LeprotL1 is protein that has metabolic function within organisms. It is already shown that LeprotL1 decrease the hepatic growth hormone action in mice though its function in circadian rhythm is unknown. Here in, it is shown that LEPROTL1 interacts with both mCRY1 and mCRY2 according to the mammalian two hybrid assay. This interaction is also confirmed with co-immunoprecipitation assay which means there is a direct interaction in between. The involvement of *Leprotl1* in the circadian clockwork was investigated by analyzing the interacting domain of CRY1 protein. It was also shown that depletion of *Leprotl1* with siRNAs resulted in damped amplitude, long-period and phase advance phenotypes of the circadian rhythm's 24 hours of oscillation in U2OS and NIH3T3 cells, suggesting a role of *Leprotl1* in metabolism and circadian rhythm correlation.

Keywords: circadian rhythm, leprotl1, cryptochrome1/2, core clock components

P90.The Antioxidant Activity in Water Extract Of The *Eremurus spectabilis*

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Abstract

Nowadays, it is known that the herbal supplements have many advantage for our health. These food's area has been expanding day by day in the world market. Epidemiological and in vitro researches on therapeutic plants has shown that the antioxidant activity of the plants has been essential against the oxidative stress in biological systems. The *Eremurus* genus is inside the Liliaceae family and one of the that genus' habitats is Turkey [1]. We aimed to investigate the antioxidant activity of the *Eremurus spectabilis*. In this study, we observed the antioxidant activity of the water extracts of leaves and roots. The different concentrations were measured (200, 100, 50, 25, 12,5 µg/ml). The total phenolic contents (L: leaves, R: roots, L: 161,29; R: 561,994) total phenolic acids (L: 1383,33; R: 1766,66), total flavonoids (L: 387,04; R: 2148,29), total antioxidants activity (R>L), scavening of hydroxylradicals by DPPH (L: approximately 30%; R: approximately 35%, were compared BHT, BHA antioxidants) were observed. As conclusion, the plants have significant antioxidant scavening activity. All these assays' results were confirmed with each other about the water extracts of leaves and roots. The data supported that the genus could use as a natural antioxidant.

Keywords: Antioxidant, *Eremurus spectabilis,* DPPH

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P91. Purification And Characterization Of Beta-glucosidase From Aspergillus Niger Strain Thatisolated On Tree Barks

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Abstract

β-glucosidases catalyze the hydrolysis of the β-glycosidic bonds and effectively on hydrolysis process of cellulose. *Aspergillus niger* was isolated from tree barks. After pure culture, strains were inoculated into Petri dishes containing CYA (Czapeck Yeast Agar), CZA (Czapeck-dox agar), MEA (Malt Extract Agar). After 7 days of incubation, the microscopic and macroscopic characteristics were observed. The *Aspergillu niger* strain was incubated at 27°C with shaking at 180 rpm in the malt broth medium. After incubated for 216 h, the cells were removed by centrifugation and the supernatant was used in the enzyme purification procedure. β-glucosidase of *Aspergillus niger* was purified by salting out and using sepharose-4B-L-tyrosine-1-napthylamine hydrophobic interaction chromatography. The purification was 2.2 fold with an overall enzyme yield of 19.3%. The molecular mass of the protein was estimated as 70kDa. The purified β-glucosidase was effectively active on p-/o-nitrophenyl-β-d-glucopyranosides (p-/o-NPG) with Km values of 5.1 and 17.0mM and Vmax values of 2.7 and 52.6U/mg, respectively. Its optimal activity was detected at pH 5 and 70^{o}C. Fe^{3+} enhanced the enzyme activity but Pb^{2+}, Cu^{+2}, Ag^{+2}, Zn^{+2} inhibited beta-glucosidase of *Aspergillus niger.* The enzyme was inhibited by glucose against *p*-NPG as substrate. IC_{50} value of glucose is detected 17.8 mM.

Keywords: Aspergillus niger, β-glucosidase, purification, characterization

P92. A genetic approach for establishing Allatostatin Receptor/ Allatostatin System to inhibit tumor growth

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Abstract

PROJECT PROPOSAL: G-protein-coupled receptors (GPCRs) are the largest family of membrane-bound receptors that initiate several signal transduction pathways upon ligand binding, which in turn promote conformational changes leading to G-protein coupling, ultimately leading to cellular response. Recent studies successfully linked anti-proliferative and apoptotic properties of somatostatin receptors (SSTRs) with their possible use in cancer treatment, by using chemotherapeutic agents targeting SSTRs [1, 2]. Allatostatin receptor (AlstR) is a GPCR and was first found *Drosophila melanogaster* as a homolog of somatostatin receptor (SSTR) [3]. It is involved in the inhibition of juvenile hormone synthesis in insects and is activated by the insect peptide Allatostatin (AST). AlstR was shown to activate mammalian GIRK channels via $G_{i/o}$ proteins in Xenopus oocytes [3]. Considering the inhibitory role of AlstR activation with its structural and functional resemblance to SSTRs, we hypothesized that AlstR/AST system can be used as a novel method to control tumour growth. The aim of the present project is generating a hepatocellular carcinoma cell line (Huh7) stably expressing AlstR, and then measuring the effect of AST treatment on cancer cell growth, and xenograft tumour growth in SCID mice. The second goal of the project is real-time monitoring AST-induced AlstR activation and its downstream signaling in mammalian cell lines via a FRET (fluorescence resonance energy transfer)-based system [4].

***Keywords*:** Allatostatin, Allatostatin receptor, fluorescence resonance energy transfer (FRET), G-protein-coupled receptor (GPCR)

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P93. *Eremurus spectabilis* has antioxidant, anticancer activity and induces apoptosis in PC-3 cells

Gürkan Aykutoğlu[1], Ekrem Darendelioğlu[1], Zeynep Öztürk[1], Musa Tartık[1], Gökhan Dervişoğlu[1], Zeynep Tuzcu[1], Gülden Koçlar[1], Can Ali Ağca[1], Gıyasettin Baydaş[1], Bülent Kaya[1*]

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Abstract

Human prostate cancer is one of the most prevalent cancer types all over the World [1]. *E. spectabilis* is used as a therapeutic agent against hemorrhoids and diabetics in Turkey [2]. In this study, the aim was to evaluate the effects of water extracts of leaves and roots of *E. spectabilis* on human prostate cancer cell line (PC-3 cells). Cell proliferation assay (WST-1) was used to show the cytotoxic effect of the extracts in dose-dependent manner. It is revealed that *E. spectabilis* extracts have cytotoxic effect on PC-3 cells compared to control. Maximum cytotoxicity was obtained at 500 µg/ml concentration of water extracts of *E. spectabilis*. Apoptotic cells were determined by Tunnel assay, the apoptotic index of control group is zero and the apoptotic index of water extracts of *E. spectabilis* is 2,2 higher than control. *E. spectabilis* has cytotoxicity on PC-3 cells and induces apoptosis. The expression of genes related to apoptosis was assessed by real-time RT-PCR. Apoptotic signaling pathway with increased cytochrome *c* release was investigated. Lipid peroxidation of control and water extracts treated cells was measured by the level of thiobarbituric acid reactive species (TBARS). The antioxidant effect of the water extracts of leaves and roots were tested by SOD (Superoxide Dismutase), CAT (Catalase) and DPPH (total antioxidant) assays. The SOD, CAT and DPPH assays showed that the cells have enhancing antioxidant activity through the extracts.

KeyWords: Antioxidant, Apoptosis, Prostate Cancer, PC-3 cell line, Tunnel Assay

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P94. Ethanol Extract of Turkish Propolis Induced Apoptosis in Human Prostate Cancer Cells

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Abstract

Propolis is a natural resin-like product of honeybees that has been used for centuries in folk medicine all over the world. It has been shown to exert an extensive spectrum of biological activities such as antibacterial antifungal, antiviral and anti-inflammatory activities [1]. With the present study we investigated whether ethanol extract of propolis induces apoptosis in prostate cancer cell line (PC3). In apoptosis, Bcl-2 protein family regulates intrinsic programmed cell death [2] and caspase 3 is a member of cysteine-aspartic acid protease enzymes that functions in extrinsic and intrinsic apoptotic pathways [3]. Propolis was collected from Solhan in Bingol, Turkey. Individual extracts at 0, 10, 20, 30, 50, 100 and 250µg/ml were incubated with PC-3 cells during 1 day culture. The anti-proliferative activity was assayed using WST1. Apoptosis and the apoptosis-related activation in the PC-3 cell line were evaluated by Western blotting. Ethanol extract of propolis has a dose dependent inhibition in the proliferation of the PC-3 cell line. Propolis-induced apoptosis was confirmed by assays with Tunnel Assay. Our results show that propolis not only downregulated expression of the anti-apoptotic protein Bcl-2 but also induced the activation of caspase-3. The results suggest that propolis acts as an effective anti-cancer agent by inducing apoptosis and inhibiting cell proliferation via in PC-3 cells.

***Keywords*:**Apoptosis, PC-3 cell line, Propolis, Tunnel Assay, Western Blotting

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P95. An Enzyme of a Novel Clock Component: Cystathionine-β-Synthase (CBS)

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The circadian clock is a biological timing system that oscillates with a period of 24 hours and is coordinated with the day-night cycle. This timing machinery is necessary for the body to anticipate environmental changes, to react external changes and adapt molecular, physiological and behavioural processes to maintain metabolic homeostasis but of course, disruption of circadian rhythmicity generally leads to diseases. Therefore, understanding the molecular clockwork help us develop treatments against various disease and disorders such as diabetes, cardiovascular diseases, obesity, jetlag, insomnia, and psychological disorders. Mammalian circadian clock is composed of transcriptional-translational feedback loop which allows the generation of daily rhythms. There is a set of core clock genes identified so far that constitute molecular feedback loop involving BMAL1, Clock, Npas2, Period1,2,3, Cryptochrome 1,2 genes. Besides known core clock components, more gene loci were shown that they change wild type oscillation pattern of core clock genes. These additional clock relevant genes which were identified by recent genetic analyses indicate that, circadian clock contains multiple and complex genetic interactions. By taking this fact into account, it is likely that many more genes underlying circadian rhythmicity will be discovered in near future. Recently, by applying high throughput genetic and proteomics techniques in combination with computer -assisted screening, candidate clock components were identified. The ones that show highest similar characteristics to known clock genes were selected. Among them, CBS (Cystathionine-β-Synthase) is one of the most prominent genes with its clear interaction with mCry1. Besides its role on circadian activity, CBS is an important enzyme for cardiovascular function of the body and cysteine synthesis pathway. To understand physiological role of CBS in circadian clock mechanism, we used cell biology, molecular biology and biochemical approaches. Here, we demonstrate with mammalian two hybrids that CBS interacts with last twenty amino acids of C-terminal of mCry1. This physical interaction is confirmed with coimmunoprecipitation. Then,transcriptional repression activity of CBS was tested by high throughput luciferase assay in N2A and NIH3T3 cell lines in the absence and presence of mCry1. It is clearly seen that CBS enhances repression activity of mCry1 on E-box promoter. Along with repression activity, we also tested how CBS enzymatic activity is affected with its interaction with mCry1. To do this, we performed hydrogen sulphur assay with mouse liver containing highly expressed endogenous CBS in the presence of mCry1 and mCry1 truncated at the last 20 aminoacid of C-termini. CBS is a prominent enzyme which has high probability of being a core or modifier component of the circadian clock. We conclude that CBS may be a functional clock component providing a new layer of control on circadian molecular dynamics.

Keywords: Cystathionine-β-Synthase, Circadian Rhytmicity, Biological Clock.

P96.Investigation of Interactions between Doxorubicin and Nucleobases by Molecular Modeling

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Abstract

An antracycline antibiotic doxorubicin with anti-tumor activity is produced by the bacterium *Streptomyces peucetius*. The interaction between doxorubicin and genetic material and the details of the intercalation with DNA have controversial issues. In order to understand the mechanism of action of doxorubicin, we aimed to study the interactions of doxorubicin with nucleobases by quantum mechanical methods. For this purpose, conformational analyses of doxorubicin were performed with Spartan 08 software. Geometry optimizations and frequency analyses were performed for each conformer using density functional theory (DFT) at B3LYP/6-31G** level using Gaussian 09 software. In order to determine the dispersive effects, calculations have been repated with wB97XD method. The most stable conformers of doxorubicin and nucleobase tautomers were optimized at wB97XD/6-31G** level and their interactions were analysed. Discovery Studio 3.5 software was used to draw the initial and final structures of geometries. As a result of conformational analyses, 422 conformers were determined for doxorubicin. The interactions between molecules have been investigated by using the most stable doxorubicin conformer in terms of energy, frequency analysis. Nucleobases were placed to the most possible interaction points and optimization analyses were performed.

***Keywords*:** Doxorubicin, DNA intercalation, molecular modeling

P97.Cytotoxic, Anti-cytotoxic And Antiproliferative Effect Of Usnic Acid On AGS Cancer Cell Line

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Lichens are very important and useful material in folkloric medicine. They have product extraordinary seconder metabolites. Many phenolic substances isolated from lichens, as well as their derivatives obtained by structural modification, are known for their ample variety of pharmacological activities, including antigenotoxic, anticytotoxic and anticanser ones. Well known seconder metabolite usnic acid is using different disease treatment. In this study, we aimed to determine cytotoxic, anti-cytotoxic and antiproliferative effect of usnic acid on AGS cancer cell line. As an indicator of cytotoxicity, LDH and WST-1 are used to determine the potency of cytotoxic, anti-cytotoxic and antiproliferative effect of usnic acid [1]. In conclusion, it was observed that usnic acid was blocked the proliferation and induces apoptosis at 18 hours. 200 micromolar dose showed high effects. Plants have been used for many years as a source of traditional medicine to treat various diseases and conditions. Many of these medicinal plants are also excellent sources for phytochemicals, many of which contain potent antioxidant and anticancer. Previous and our study showed that usnic acid have no cytotoxic effects. Our results showed that usnic acid have anticytotoxic and antiproliferative effect on AGS cancer cell line.

Key words: Usnic acid, AGS cell line, anti-cytotoxic, antiproliferation

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P98.Determination of Genotoxic And Antigenotoxic Effect of New Synthesize Chemical Compounds

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In this century, researchers have developed new ways to struggle against to diseases. Especially, chemistry industy produced new drugs for tratment. Total exports of chemicals and products, pharmaceutical chemistry ranks 5th. Scientists are working on producing new drugs or modifying in our country. In this study, we aimed to determine genotoxic, antigenotoxic properties of new synthesize chemical compounds. We determined genotoxic, antigenotoxic effects of (2S,3R,4S,5S,6R)-2-(2-((Z)-(2-hydroxy-4-nitrophenylimino)methyl)phenoxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol and 3-hydroxy-N'-(2-hydroxy-4-nitrophenyl)-N-o-tolyl-2-naphthimidamide chemical compounds against sodium azide with the sister chomatid exchange (SCE) metod [1,2]. Our results showed that (2S,3R,4S,5S,6R)-2-(2-((Z)-(2-hydroxy-4-nitrophenylimino)methyl)phenoxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol and 3-hydroxy-N'-(2-hydroxy-4-nitrophenyl)-N-o-tolyl-2-naphthimidamide chemical compounds have no genotoxic effects. We used 4 different doses in whole human blood culture. High dose showed most efficient antigenotoxic effect. New drug synthesis have importance for struggle against to diseases. Therefore, lot of researches focused on new chemical drug synthesis or modifying. Fort his reason, this chemical compounds might be new or candidate drug against to genotoxicity. As a result, our chemical compounds have biological activity and antigenotoxic effect.

Key words: Antigenotoxicity, SCE, chemical compound

[1] Latt, S.A., and Schreck, R.R., 1980. American Journal of Human Genetics, 32(3), 297-313.
[2] Perry, P. and Evans, H.J., 1975. Nature, 258, 121-125.

P99.Genotoxic And Antigenotoxic Effect of New Synthesize Chemical Compounds

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For years, scientists produce lots of chemical compound for treatment. Total exports of chemicals and products, pharmaceutical chemistry ranks 5th. In our country, many researchers are working on developing new drugs in our country. In this study, we aimed to determine genotoxic, antigenotoxic properties of new synthesize chemical compounds. Di-(1-(2-((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yloxy)benzylidene))urea and (2R,3S,4S,5R,6S)-2-(hydroxymethyl)-6-(2-((Z)-(4-propoxyphenylimino)methyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triol chemical compounds genotoxic, antigenotoxic effects were analyzed with the sister chomatid exchange [1,2]. Di-(1-(2-((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yloxy)benzylidene))urea and (2R,3S,4S,5R,6S)-2-(hydroxymethyl)-6-(2-((Z)-(4-propoxyphenylimino)methyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triol chemical compounds showed no genotoxic effects. 4 different dose were used. High dose showed strong antigenotoxic effects. Pharmaceutical chemistry is important for new treatment. The synthesis of new substances in the treatment of diseases has led to the emergence of new approaches. Therefore, This compunds might be new approache for antigenotoxic studies.

Key words: SCE, chemical compound.

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[2] Perry, P. and Evans, H.J., 1975. Nature, 258, 121-125).

P100. A Detail Investigation of the *In Vitro* Anti-proliferative Property and Mode of Action of a Coordinated Gold Compound ($CdC_{10}H_{24}N_6O_2Au$) on Some Cancer Cell Lines

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Coordination compounds have been used in medicine for treatment of various diseases including cancer. The present study was designed to determine antiproliferative and apoptotic effect for newly synthesized cyano-bridged $\{Au^{I}(CN)_2\}$ coordination compound, coded as AK4b ($CdC_{10}H_{24}N_6O_2Au$), against on HeLa, C6 and HT29 cancer cell line. The new coordination compound containing $Au^{I}(CN)_2$ was synthesized using "*brick-mortar*" method [1]. The anti-proliferative and cytotoxic activities of AK4b on cancer cell lines were determined using BrdU Cell Proliferation Assay (BCPA), lactate dehydrogenase assay (LDH assay) and topoisomerase I assay respectively. DNA laddering assay and TUNEL assay were used to determine whether this compound induce cell apoptosis. According to BCPA and LDH test results, AK4b was significantly anti-proliferative and cytotoxic on the tumor cell lines compared to control anti-cancer drug, 5-fluorouracil (5-FU). The LDH test results revealed that AK4b was as low cytotoxic as 5-FU, suggesting that AK4b may be detrimental to the cell membrane. The compound AK4b caused laddering of genomic DNA, indicating that it may act through inducing apoptosis on the cells. The results of the study revealed that the AK4b is a promising potent anti-proliferative agent for cancer cell lines by inducing apoptosis.

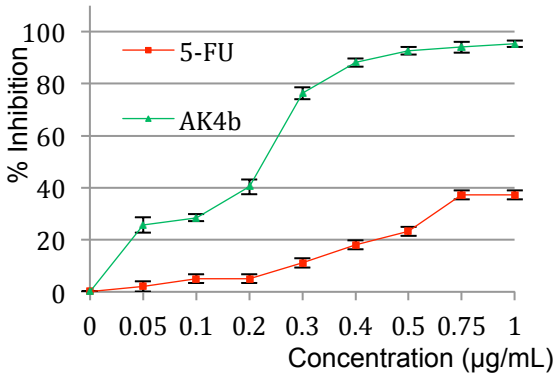

Figure 1. Antiproliferative activity of AK4b and 5FU on C6 cell line

Keywords: Coordination Complex, Anti-proliferative Activity, AK4b

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P101.Anticancer Activities and Mechanism of Action of Novel Metal Complex, $CdC_8H_{16}N_4O_2Au$

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Coordination compounds have been used in medicine for treatment of various diseases including cancer. The present study was designed to determine antiproliferative and apoptotic effect for newly synthesized cyano-bridged $\{Au^I(CN)_2\}$ coordination compound, coded as AK12a ($CdC_8H_{16}N_4O_2Au$), against on HeLa, C6 and HT29 cancer cell lines. The new coordination compound containing $Au^I(CN)_2$ was synthesized using "*brick-mortar*" method [1]. The antiproliferative and cytotoxic activities of AK12a on tumor cell lines were determined using BrdU Cell Proliferation Assay (BCPA) and lactate dehydrogenase assay (LDH assay) respectively. The mechanism of action of the AK12a was clarified using DNA laddering assay and TUNEL assay. According to BCPA and LDH test results, AK12a was significantly antiproliferative and cytotoxic on tumor cell lines compared to control anticancer drug, 5-fluorouracil (5-FU). The LDH test results revealed that AK12a was significantly cytotoxic than 5-FU, suggesting that AK12a may be detrimental to the cell membrane. The compound AK12a caused laddering of genomic DNA, indicating that it may act through inducing apoptosis on the cells. The results of the study revealed that the AK12a is a promising potent antiproliferative agent for cancer cell lines by inducing apoptosis.

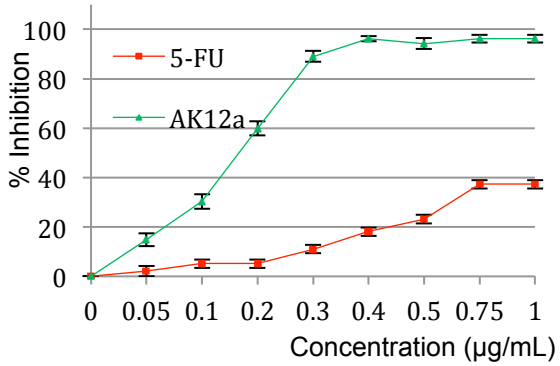

Figure 1. Antiproliferative activity of AK12a and 5FU on C6 cell line

Keywords: Coordination Complex, Anticancer Activity, AK12a

References

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P102.A New Coordination Compound Containing $Au^{I}(CN)_2$ Displays Apoptotic Effect On C6, HT29 And HeLa Cancer Cell Lines

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Coordination compounds have provides exciting the development of metal-based therapeutics. We have been exploring the anti-proliferative and apoptotic effect of newly synthesized cyano-bridged $\{Au^{I}(CN)_2\}$ coordination compound, coded as AK9a ($NiC_8H_{16}N_4O_2Au$), against on HeLa, C6 and HT29 cancer cell line. The new coordination compound containing $Au^{I}(CN)_2$ was synthesized using "*brick-mortar*" method [1]. *In vivo* cytotoxicity of AK9a was evaluated by lactate dehydrogenase assay (LDH assay) against on cancer cell lines. The anti-proliferative activity of AK9a was assessed against cancer cell lines using BrdU Cell Proliferation Assay (BCPA), 5-fluorouracil (5-FU) was used as a reference standard. DNA laddering assay and TUNEL assay were used to determine whether this compound induces cell apoptosis. According to BCPA and LDH test results, this coordination compound was inhibited the cell viability of cancer cells compared to positive control anti-cancer drug, 5-FU. Remarkably, the LDH test results disclosed that AK9a was as low cytotoxic as 5-FU, suggesting that this compound may affect by lose membrane integrity of cell as a result of apoptosis. Furthermore, the compound AK9a caused laddering of genomic DNA, indicating that it may act through inducing apoptosis on the cells. Our preliminary data strongly indicate that this compound is a potential therapeutic agent.

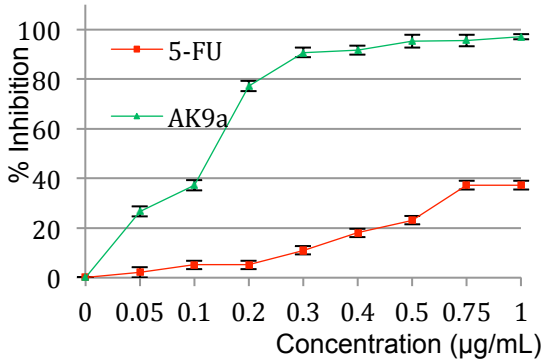

Figure 1. Antiproliferative activity of AK9a and 5FU on C6 cell line

Keywords: Coordination Complex, Anti-proliferative Activity, AK9a

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This study has supported by TUBITAK with the project No. 112T696

P103. IRF6 is a determinant in Notch induced cell fate and p63 downregulation in normal breast epithelial cells

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Abstract

Notch, an evolutionary conserved signaling pathway, is involved in breast epihelial cell differentiation and tumorigenesis, through downregulation of p63, a basal cell marker. Notch1, member of four notch receptors(1-4) in mammals, is found high level in human breast cancer cells. IRF6, a member of nine interferon regulatory factors, acts as a Notch target gene in keratinocytes and is involved in differentiation and development[2]. p63, p53 homolog, have roles in epidermal development, and in mammary bud formation. It has been establish that ΔNp63, a p63 isoform, is downregulated by IRF6 in human keratinocytes [3]. We investigate whether IRF6 is involved in Notch induced cell fate determination, p63 downregulation and cell proliferation. Activation of notch in MCF10A, normal breast epithelial cell line, increases IRF6. MTT assay results show that IRF6 is required for notch induced proliferation because amount of notch induced cell is decreased by IRF6 silencing. Luminal and basal markers are used to support effects of IRF6 on epithelial cell fate. Increasing CD24, ITGA6 and K14 expressions and decreasing K18 and ITGB4 expressions in MCF10A by IRF6 silencing indicate that IRF6 silencing alters expression of basal and luminal cell markers. These results indicate that in breast epithelial cells, Notch signaling regulates IRF6 expression, which is required for notch induced proliferation. Notch activation and IRF6 silencing have an effect on downregulation of p63. IRF6 could be involved in luminal and basal cell fate determination in breast tissue.

***Keywords*:** IRF6, Notch Signaling, p63 downregulation

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P104. Negative feedback regulation between ΔNp63 and IRF6 in normal breast epithelial cells

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Abstract

Interferon regulatory factor 6 (IRF6) is a member of IRF family of transcription factors. IRF6 is defined as a potential tumor suppressor in mammary epithelial cells due to its interaction with maspin, which is tumor suppressor in normal breast tissue. p63 is a member of p53 family of transcription factors. p63 has 6 different isoforms and these isoforms play role in embryonic development and in cancer as both tumor suppressor and tumor inducing factors. It has been implicated that p63 is essential for differentiation of basal epithelial cells during normal breast development. Recent studies in keratinocytes showed that there is a negative regulatory loop between ΔNp63 and IRF6, in which IRF6 expression is activated by ΔNp63 and IRF6 protein expression induces degradation of ΔNp63. In this study, we aim to reveal whether IRF6 and ΔNp63 regulate each other's expression in normal breast epithelial cells. For this purpose, ΔNp63 and IRF6 were knocked down by shRNA technique in MCF-10A human breast epithelial cell line which is positive for both IRF6 and ΔNp63 genes. mRNA and protein analysis were done by Real time RT-PCR and western blot respectively. qPCR analysis showed that ΔNp63 silencing resulted in up-regulation of IRF6 expression in MCF-10A cell line. MCF-10A cells were knocked down for IRF6 showed reduced ΔNp63 expression. In conclusion, our data suggest that there is a negative feedback regulation between IRF6 and ΔNp63 genes in normal human breast epithelial cell line. IRF6 activates ΔNp63 transcription and ΔNp63 regulates IRF6 transcriptionally.

P105. Biological Evaluation of New Pyrazole Derivatives for Novel Therapeutic Opportunities of Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the second most deadly and the sixth most common cancer worldwide [1]. Although chemotherapy is the major treatment method for HCC patients, chemotherapeutic agents are known to have side effects or become ineffective through drug resistance. Thus, it is important to discover non-traditional, efficient and safe chemical agents to be used in treatment of HCC. Pyrazole derivatives of CA-4 (Combretastatin A-4) are proved to exert an antimitotic activity in human cancer cells by inhibition of tubulin polymerization [2, 3]. It is aimed to define possible anticancer properties of newly synthesized pyrazole derivatives through evaluation of their cytotoxic effect on HCC cell lines and to determine the molecular mechanism underlying this effect. Cytotoxic activities of pyrazole derivatives were analyzed by SRB assay and by real-time cell analyzer on HCC cell lines. Flow cytometry and cell staining methods were used to determine the mechanism by which these derivatives were showing their anticancer effect. Among 42 tested pyrazole derivatives, 14 of them which were found to have IC_{50} values below 3 μM, were studied. Hoechst and SA-βgal staining results showed that some of the molecules were causing apoptosis and some causing senescence in HCC cell lines.

***Keywords*:** Hepatocellular carcinoma, CA-4, cytotoxicity

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P106. Androgen Receptor Mutagenesis Screen

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Abstract

Prostate cancer is an extremely common disease for Turkish men.The standard care for late-stage disease is to inhibit the activation of Androgen Receptor(AR).Antiandrogens are one class of therapeutics that directly inhibits AR activation.While treatment is initially efficient, the cancer almost always develops resistance.The recently approved antiandrogen MDV3100 has been shown to dramatically increase the survival of late stage patients who have failed hormone therapy.Yet despite this success, recent clinical studies have demonstrated that like other antiandrogens the cancer develops resistance to MDV3100 [1].In an effort to predict the clinical causes of resistance we are currently developing a novel chemical screen that can identify point mutations of the AR that cause resistance to antiandrogens.By combining random mutagenesis of AR with a strong selection model, we can test several million AR mutants in a single experiment [2].Selection is conducted with a cell line that is only resistant to puromycin when the AR is activated.If AR is inhibited by an antiandrogen or has a non-functional mutation, the cells will be sensitive to puromycin.However those cells with a mutation that causes drug resistance can proliferate in the presence of both antiandrogen and puromycin.This technique can be used to characterize resistance mechanisms in a large range of different therapeutics.

***Keywords*:** castration-resistant prostate cancer, antiandrogen, drug resistance

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P107. Does BAG-1 have a role in the autophagy decision of the cell?

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BAG-1 (Bcl-2 associated athanogene-1) is an anti-apoptotic protein found in humans that belongs to the BAG-protein family. BAG-1 has three major isoforms that are translated through the alternative initiation sites, and these isoforms form various complexes that enable BAG-1 to be involved in various cellular processes mainly related with apoptosis, cell proliferation, metastasis, cell migration, hormone action and autophagy. To date, some of the BAG-1 interacting partners were determined, and one of the well known interaction partner of BAG-1 is Bcl-2. Beclin-1 is a protein that has a central role in autophagy during periods of cell stress and extinguishes during the cell cycle. Beclin interacts with the anti-apoptotic Bcl-2. In this study, we aimed to understand the role of BAG-1 through the interactions of Bcl-2 and Beclin-1 in the regulation mechanisms of autophagy in MCF-7 and MCF-10A cells. We observed that silencing of BAG-1 in a time-dependent manner leads to the upregulation of autophagic proteins like Atg7, Atg16 and Atg5. Also Beclin-1 showed an enhanced expression with BAG-1 silencing. In conclusion, we think that BAG-1 as an anti-apoptotic protein may act as also an anti-autophagic player in the regulation of autophagy through its interactions with Bcl-2 and Beclin-1.

P108. 3'UTR Alterations in ER(+) Breast Cancers

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Alternative polyadenylation (APA) generates transcript isoforms with different 3'UTR lengths due to the position of poly(A) tail [1]. This difference may alter the stability and location of mRNA isoforms in cell [2]. APA has been observed in various cellular states: Preferential use of distal poly(A) sites occurs during development [3]. However, in proliferative normal and/or cancer cells, a global 3'UTR shortening pattern is observed [4]. In this study, we aim to investigate the 3'UTR alterations in estrogen receptor (ER)-positive breast cancer patients and cell models. Publicly available microarray datasets were analyzed by a probe-based microarray analysis tool (APADetect). Based on the means of proximal to distal poly(A) site ratios of individual transcripts, the SLR (short-long ratio) was calculated as indicator of short vs. long 3'UTR abundance. Significance Analysis of Microarrays determined significant 3'UTR shortened or lengthened genes. These genes were further analyzed *in silico* to reveal 3'UTR alterations in ER(+) breast cancer patients compared to normal breast samples. Our results suggest involvement of APA in ER(+) breast cancer. In our analyses, 32 and 35 genes were commonly 3'UTR shortened and lengthened, respectively. We plan to further investigate these APA events experimentally to further understand APA and its consequences in breast cancer.

Keywords: Alternative polyadenyletion, 3'UTR, estrogen receptor, breast cancer

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P109. 2-Chloro-1,4-naphthoquinone derivative of quercetin, exerts antitumor activity against colorectal cancer through the induction of apoptosis and oxidative stress related autophagy.

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Abstract

Phytochemical therapeutics such as Quercetin (Qc) have strong antitumor effects by inducing cell cycle arrest and apoptotic cell death, inhibiting enzymes activating carcinogens and modifying key signal transduction pathways. However, its clinical application is limited due to poor water solubility and low water availability. In a screening of novel semi-synthetic derivatives of Qc, 3,7-dihydroxy-2-[4-(2-chloro-1,4-naphthoquinone-3-yloxy)-3-hydroxyphenyl]-5-hydroxychromen-4-one (CHNQ) was the most promising in terms of biological efficacy [1]. Using a mouse model of colitis, we have previously shown that CHNQ could ameliorate the effects of acetic acid induced acute colitis more efficiently that Qç.[1]. Since chronic inflammation very significantly contributes towards neoplastic transformation, we have hypothesized that CHNQ may also have potential as an anti-cancer agent. Using colon cancer cell lines HCT-116 and HT-29, we have carried out detailed functional analyses comparing the anti-carcinogenic activities of CHNQ and Qc on cellular proliferation, cytotoxicity, cell cycle, apoptosis as well as autophagy and the associated signal transduction pathways. BrdU incorporation and cytotoxicity assays showed that CHNQ strongly inhibited cell proliferation with an IC_{50} value of $\leq$ 20 µM, which was nearly 3 folds lower than the IC_{50} value of Qc ($\geq$ 100 µM). Apoptosis was examined by Annexin V staining and flow cytometry, multi caspase activity assay and the expression of pro- and anti-apoptotic proteins using immunoblotting showing that treatment of cells with CHNQ resulted in a more efficient and robust induction of apoptosis compared to Qc. The pro- or antioxidant effect of CHNQ was examined by dihydroethidium staining analyzed by flow cytometry and NBT colourimetric assay. The results indicated that, treatment of cells with CHNQ resulted in the induction of oxidative stress as determined by the increased production of superoxide anions, leading to cell cycle arrest at G2/M. This was accompanied by the increased phosphorylation of MAP Kinases including; ERK1/2, p38 and JNK and decreased phosphorylation of Akt/PKB. Interestingly, the cells treated with CHNQ resulted in a dramatic increase in oxidative stress related autophagy as shown by increased expression and conversion of LC-3-I to LC-3II, acidic vesicle accumulation and GFP-LC-3 puncta formation. Some of these effects were also seen when cells were treated with Qc, however, the effect was weak and observed only at high doses.Overall, we propose that CHNQ, a semi-synthetic derivative of Qc, induces cancer cell death through the induction of oxidative stress and autophagy. Further studies are needed to examine the in vivo effects of CHNQ in experimental cancer models.

Keywords: Cancer, oxidative stress, autophagy

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P110. Association of DNA Methylation of $p16^{INK4a}$ (CDKN2A) and MGMT Genes and Histopathological Features of Breast Cancer

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Breast cancer (BC) is the most common tumor in women, and the second leading cause of death. Histopathology plays an important part in determining the treatment strategy for women with BC. DNA methylation is an important regulator of gene transcription [1,2]. $p16^{INK4a}$ can block G_1-S-phase progression and that mutant $p16^{INK4a}$ proteins are nonfunctional in cell cycle arrest or Cdk inhibition suggests that $p16^{INK4a}$ plays an important role in negative growth control. 0^6-methylguanine-DNA methyltransferase (MGMT) is involved in direct cleavage of mutagenic alkyl adducts within DNA (direct DNA repair). In this study the promoter methylation levels of $p16^{INK4a}$ and MGMT genes which are associated with BC were investigated by Methylation-Sensitive PCR. We analysed primary tumor core biopsies from 96 high-risk primary BC patients and their histopathologic types were associated with the methylation levels. In our study the promoter hypermethylation status were observed at different rates; $p16^{INK4a}$ and MGMT methylation frequencies were 43.8% and 34.4% respectively. The promoter hypermethylation levels of the genes found to be significant with ER positivity (+), PR (+), HER2/neu (+), E-Cadherin (+) and Ki-67 (<30%). In conclusion, our study shows that DNA methylation is a frequent event in BC and that different genes are methylated in BCs with different histopathological features.

***Keywords*:** Breast cancer, Methylation, P16, MGMT, Histopathology

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P111. The Correlation between Lymph Node Metastasis and Methylation in Breast Cancer

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Breast cancer (BC) is one of the most common malignancies with a high mortality rate among women. Invasion and metastasis are two important hallmarks of malignant tumors associated with complex genetic and epigenetic alterations that allow tumors to disseminate throughout lymphatics or blood vessels, giving rise to the colonization and growth of metastatic cells in distant organs. Considering that tumor dissemination is an early event in BC, genetic and epigenetic analysis of tumors and metastatic lesions could provide results for biomarker discovery and may improve diagnosis, prognosis and proper management of the treatment for BC patients. The contribution of aberrant DNA hypermethylation of cancer related genes to the transcriptional silencing and carcinogenesis has been demonstrated in different diseases including different cancer types [1]. We investigated the DNA methylation of MGMT, *BMP6,* $p16^{INK4a}$, *RINT1, THBS1 and TIMP3 genes* by Methylation Sensitive PCR. DNA methylation analysis of the candidate genes showed higher methylation proportion in the primary tumor tissue than that of the matched adjacent normal tissue from the same BC patients and the differences were significant for the $p16^{INK4a}$ *and RINT1 promoter* regions ($P<0.05$). MGMT and *BMP6,* ($P<0.01$). *THBS1* ($P<0.001$). Our results showed methylation heterogeneity between primary tumors and metastatic lesion. The contribution of aberrant methylation alterations of *BMP6, MGMT* and *THBS1* in lymph node metastasis might provide a further clue to establish useful biomarkers for screening metastasis.

***Keywords*:** Breast cancer, Methylation, Lymph Node

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P112. Determination of Cytotoxic, Proliferative and Anti-Genotoxic Effects of Apigenin

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Abstract

Flavonoids have protective effects against many diseases. The compound apigenin is one of the most researched flavonoid members, it possesses majority of the biological activities of flavonoids. Cancer is today's most common diseases and unfortunately it's still difficult to treat despite recent advances in cancer therapy. The aim of this study is to investigate whether cytotoxic and proliferative effects of apigenin by using the LDH and the WST-1 assays on Human Umbilical Vein Endothelial Cell (HUVEC) cultures. Optimum treatment time was also observed by Real-Time Cell Electronic Sensing (RT-CES) system. Consequently, results demonstrated that diverse concentrations of apigenin have cytotoxic and apoptotic effects on HUVEC cell line. Apigenin can be used for cancer patients as a therapeutic agent if additional antigenotoxic studies can be performed.

***Keywords*:** Apigenin, LDH and WST – 1 assays, SCE

P113. Revealing the trastuzumab responsive miRNAs in breast cancer cell lines by qPCR-based array

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Abstract

Breast cancer is the most frequently diagnosed life-threatening cancer in women and leading cause of cancer death [1]. Small noncoding microRNAs (miRNA) regulate the expression of target mRNAs by repressing their translation or orchestrating their sequence-specific degradation. According to various studies over past decade, miRNAs are an important and prevalant regulatory layer of gene expression that acts at the post-transcriptonal level and have also been implicated to be in common diseases, including cancer [2,3]. Trastuzumab is monoclonal antibody directed against the HER2 tyrosine kinase receptor, which is overexpressed in approximently 25% of invasive breast cancers [4,5]. In this study, we investigated miRNA and miRNA target gene expression patterns in HER2(+) breast cancer to identify trastzumab responsive candidate biomarkers. Our hypothesis is that there may be a common and stable miRNA response to trastuzumab treatment in HER2(+) breast cancer cells, from which we can extract the information of trastuzumab effectiveness via miRNAs. We used Qiagen miScript miRNA PCR Arrays to identify differentially expressed miRNAs that were regulated by trastuzumab in BT474 and SKBR3 cell lines and then used miRWalk to find validated target genes. Consequently, pathway analyses predicted that these miRNAs would regulate well-described cancer-associated signaling pathways, such as PI3K/Akt, p53 pathways.

***Keywords*:** Breast cancer, miRNA, Trastuzumab, qPCR-based array

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P114. qPcr Validation Of Diagnostic Importance And Regulation By Estrogen Of Chrna5 Isoform Expression In Breast Cancer

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**equal contribution*

Abstract

Breast cancer has some subtypes depending on receptor status of tumor cells. Finding new subtypes diagnosis markers are important to decide on the best treatment approach. Transcriptional regulation of CHRNA5 has been largely studied in other cancer types. In the present study, meta-analysis of *in vivo* and *in vitro* GEO datasets was conducted in R language which helped to identify CHRNA5 as an estrogen receptor (ER) pathway modulated receptor. Finding of CHRNA5 as a biomarker was validated in wet lab by scanning human cDNA panel with specially designed primers. We developed a GUI for quality control, statistical analysis and graphical representation of cDNA panel results using MATLAB. To examine *in vitro* CHRNA5 expression, ER- and ER+ cell lines were exposed to different doses of E2, ER+ cell line to long-term E2 depletion and tamoxifen. Our results revealed that in E2 treatment, ER- cell lines did not show response to E2, while CHRNA5 expression increased in ER+ cell lines. After six months of E2 depletion, CHRNA5_v3 and ESR1 (ER gene) expression increased to compensate the lacking of E2. Our findings showed that CHRNA5 and its isoforms were regulated by E2 metabolism in breast cancer depending on its receptor status.

***Keywords*:** Breast cancer, CHRNA5, estrogen, molecular subtype, meta-analysis.

This study was funded by a research grant from TUBITAK (111T316) to Ozlen Konu.

P115. A novel tumor suppressor miRNA co-regulating EMT and cell survival in breast cancer

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Abstract

We performed a small scale miRNA mimic screen to identify miRNAs leading to inhibition of cell survival and epithelial-mesenchymal transition (EMT), both of which are key parameters for tumor growth and metastasis. We identified a miRNA (miR-X) which leads to apoptosis only in p53 mutated breast cancer cell lines. Furthermore, *in vitro* migration and invasion assays revealed that miR-X inhibits both migration and invasion by interfering with the EMT process. Then, we predicted the potential targets of this miRNA using target prediction algorithms and identified transcriptional co-repressor CTBP1 as a novel direct target which is already known for limiting apoptosis by repressing Bax transcription [1]. CTBP1 has also been reported to bind ZEB transcription factors and assists EMT by enhancing ZEB-mediated downregulation of E-Cadherin [2]. We showed that silencing of CTBP1 phenocopies the effects of miR-X in induction of p53-mediated apoptosis and blocking EMT and cell invasion. These results suggest that miR-X is a novel tumor suppressor miRNA co-regulating EMT and cell survival and it could be a potential target for breast cancer therapy.

***Keywords*:** miRNAs, p53-mediated apoptosis, EMT, breast cancer

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P116. Expression of PSMD4 Gene under Chemically-Induced Hypoxic Conditions in Different Cell Lines

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Abstract

PSMD4 (Proteosome 26S non-ATPase subunit 4) gene encodes three proteins, namely S5A, antisecretory factor (ASF) and Angiocidin. S5A functions as a major protein polyubiquitin recognition domain in 26S proteosome, that can catalyse degradation of dysfunctional proteins in eukaryotes. [1I In contrast, ASF is a secreted protein that functions in the regulation of intestinal fluid transport induced by cholera toxin. Most importantly, Angiocidin is a protein over-expressed in many different solid tumors and tumor capillary endothelial cells and inhibits angiogenesis and tumor growth [2]. However, regulation studies of this multifunctional gene are rather limited. Particularly, the expression studies in the low oxygen level are not known. Therefore, the aim of the study is to analyze the mRNA level of PSMD4 gene in normoxic and hypoxic conditions in different cell lines, namely prostate cancer cells (PC3), Endothelial Cells (HUVEC) and colon cancer cells (HT-29). A chemically-induced hypoxic condition was performed by 150 μM $CoCl_2$. Total RNA was extracted from cells treated with different Hypoxic time points, 24h, 48h and 72h along with normoxic cells as a control. qRT-PCR was performed in order to evaluate the mRNA level of PSMD4 gene. Finally, it was determined hypoxic condition induces mRNA level of PSMD4 gene.

***Keywords*:** PSMD4, S5a, ASF, Angiocidin, Hypoxia, $CoCl_2$

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P117. SIK2: A New Player in the control of Breast Tumorigenesis

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FGFs have regulatory roles in key cellular events which lead to the tumorigenic transformations in the case of aberrant signaling [1,2,3]. Our previous data indicated that SIK2 functions downstream of FGF pathway and negatively regulates it. Down-regulation of SIK2 leads to a significant increase in proliferation and migration/invasion capacity and a decrease in apoptosis. Our study aims to elucidate the potential contribution of SIK2 as the tumor suppressor in the emergence of neoplastic transformations in tumorigenesis. SIK2 levels were studied in different breast cell lines and significantly low expression was observed in tumorigenic lines compared to the non-transformed cells. In MDAMB231 tumorigenic line where SIK2 expression was enhanced, the significant decrease was seen in proliferative response and in the ERK phosphorlylation. Opposedly, the fraction of apoptotic cells increased in conjuction with decreased Akt phosphorlyation. To see tumorigenic capacity, SIK2 modulated cells were injected into the SCID mice. The significantly smaller tumors were formed by the cells with increased SIK2 in comparison to the tumors originating from control cell injections. In the non-transformed cells opposite results confirmed the in vitro data obtained from the over-expression study. These findings support the involvement of SIK2 in breast tumorigenesis as a potential tumor suppressor.

Keywords: SIK2, breast cancer, tumorigenesis, tumor suppressor

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P118. 2-Chloro-1,4-naphthoquinone derivative of quercetin, exerts antitumor activity against colorectal cancer through the induction of apoptosis and oxidative stress related autophagy.

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Phytochemical therapeutics such as Quercetin (Qc) have strong antitumor effects by inducing cell cycle arrest and apoptotic cell death, inhibiting enzymes activating carcinogens and modifying key signal transduction pathways. However, its clinical application is limited due to poor water solubility and low water availability. In a screening of novel semi-synthetic derivatives of Qc, 3,7-dihydroxy-2-[4-(2-chloro-1,4-naphthoquinone-3-yloxy)-3-hydroxyphenyl]-5-hydroxychromen-4-one (CHNQ) was the most promising in terms of biological efficacy.[1] Using a mouse model of colitis, we have previously shown that CHNQ could ameliorate the effects of acetic acid induced acute colitis more efficiently that Qç.[1] Since chronic inflammation very significantly contributes towards neoplastic transformation, we have hypothesized that CHNQ may also have potential as an anti-cancer agent. Using colon cancer cell lines HCT-116 and HT-29, we have carried out detailed functional analyses comparing the anti-carcinogenic activities of CHNQ and Qc on cellular proliferation, cytotoxicity, cell cycle, apoptosis as well as autophagy and the associated signal transduction pathways. BrdU incorporation and cytotoxicity assays showed that CHNQ strongly inhibited cell proliferation with an IC_{50} value of $\leq$ 20 µM, which was nearly 3 folds lower than the IC_{50} value of Qc ($\geq$ 100 µM). Apoptosis was examined by Annexin V staining and flow cytometry, multi caspase activity assay and the expression of pro- and anti-apoptotic proteins using immunoblotting showing that treatment of cells with CHNQ resulted in a more efficient and robust induction of apoptosis compared to Qc. The pro- or antioxidant effect of CHNQ was examined by dihydroethidium staining analyzed by flow cytometry and NBT colourimetric assay. The results indicated that, treatment of cells with CHNQ resulted in the induction of oxidative stress as determined by the increased production of superoxide anions, leading to cell cycle arrest at G2/M. This was accompanied by the increased phosphorylation of MAP Kinases including; ERK1/2, p38 and JNK and decreased phosphorylation of Akt/PKB. Interestingly, the cells treated with CHNQ resulted in a dramatic increase in oxidative stress related autophagy as shown by increased expression and conversion of LC-3-I to LC-3II, acidic vesicle accumulation and GFP-LC-3 puncta formation. Some of these effects were also seen when cells were treated with Qc, however, the effect was weak and observed only at high doses. Overall, we propose that CHNQ, a semi-synthetic derivative of Qc, induces cancer cell death through the induction of oxidative stress and autophagy. Further studies are needed to examine the in vivo effects of CHNQ in experimental cancer models.

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P119. Hypoxic Regulation of p54nrb/NonO in PC3 and HT29 Cells

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Abstract

The nuclear p54nrb/NonO protein is a RNA-binding molecule of 54 kDa containing two RNA recognition motifs [1]. NonO can bind via its HTH domain to double-stranded DNA (dsDNA) with restricted A/T specificity reminiscent of octamer motifs [2]. p54nrb/NonO was recently shown to be a component of a novel nuclear domain termed paraspeckles [3]. Paraspeckles are a relatively new class of subnuclear bodies found in the interchromatin space of mammalian cells. Paraspeckles and their components may ultimately have a role in controlling gene expression during many cellular processes including differentiation, viral infection, and stress responses [4]. In this work, we planned to analyze the expression profile of p54nrb/NonO in PC3 and HT29 cells under chemically induced hypoxic conditions. For this purpose, cells were cultured in DMEM with 10 % Fetal Calf Serum solution and hypoxic mimetic reagent, $CoCl_2$. Total cellular RNA was isolated; cDNAs were prepared and subjected to real time PCR using p54nrb/NonO and h-β-2 microglobulin specific primers. Ct values were analyzed using Livak method. Cells with normoxic conditions were used as control.

***Keywords*:** p54nrb/NonO, RNA-binding molecule, Hypoxia, $CoCl_2$

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P120. Myeloid-derived suppressor characteristics in HL-60 myeloid leukemia cells treated with ATRA or Vitamin D3

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In severe chronic inflammation, semi-mature myeloid-derived suppressor cells (MDSC) enter circulation and exert immunosuppressive activities [1]. All-trans retinoic acid (ATRA) and 1α,25-dihidroxyvitamin D3 (D3) influence myeloid cells' maturation and differentiation, including acute myeloid leukemia (AML) blasts [2]. Here, the similarities of MDSCs and immature or putatively-mature AML cells were assessed. HL-60 AML cell line was treated with ATRA or D3 for various incubation periods and stimulated with IFN-γ or LPS. Cell morphology (Giemsa staining); myeloid markers (CD11b, CD11c, CD14, CD16, CD15 CD66b, TLR4, CD62L, CD40, TRAIL, CD70, HLA-DR) and reactive oxygen species (ROS) production (flow cytometry); nitric oxide (NO) production (Griess reaction); MDSC-related gene (NOX2, MPO, COX2, IDO1, NOS2, ARG1, ARG2) expression (RT-PCR); total- and phospho-STAT3 protein levels (Western-Blot) were evaluated. Upon ATRA or D3 treatment, monocytic or granulocytic maturation of HL-60 became consistent with CD11b, CD11c, and CD14 expression, and cellular morphology. In addition, these cells gave a better response to IFN-γ or LPS. MDSC-related STAT3 pathway showed higher activity with IFN-γ. There was no difference in NO and NOS2 levels whereas ROS production, NOX2 and MPO expression were high in ATRA-induced cells. There may be a similarity between AML and MDSC cells which can improve our understanding of leukemia biology.

***Key words*:** Leukemia, ATRA, D3, MDSC, tumor immunology

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P121. THE ROLE OF PATZ1 TRANSCRIPTION FACTOR IN THE DNA DAMAGE RESPONSE

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Abstract

PATZ1 (MAZR) is a transcription factor composed of an N-terminal BTB protein-protein interaction domain, and a C-terminal zinc finger and AT-hook DNA binding domain. Recent findings indicate that PATZ1 has a crucial role in the p53 pathway and during tumorigenesis. PATZ1 interacts with p53, is upregulated in various cancers and its absence favors lymphomagenesis. We defined a role for PATZ1 as a regulator of the p53 tumor suppressor protein. We found that upon doxorubicin induced DNA damage, the protein level of PATZ1 decreases, as the p53 protein accumulates. This inverse correlation between PATZ1 and p53 protein levels led us to investigate biological relevance of these two proteins. We found that PATZ1 loss resulted in decreased proliferation rates in various cell types; while its overexpression accelerated proliferation. We demonstrate that PATZ1 inhibits the transcription activation function of p53 by luciferase reporter assays. While p53 is responsible for inducing the expression of the target genes p21 and Puma, we show that PATZ1 overexpressing cells cannot induce the expression of these genes as effectively as wild type cells. Finally, we performed genome scale RNA-Seq and microarray analysis on doxorubicin treated mouse embryo fibroblasts sufficient or deficient for PATZ1 and found that the absence of PATZ1 results in an alteration of the expression of p53 target genes. These results demonstrate that PATZ1 modulates p53-dependent cellular stress and DNA damage pathways.

***Keywords*:** PATZ1, p53, DNA Hasarı, Tümör Gelişimi, RNA-Seq

P122. Fibronectin production in breast cancer cells may influence myeloid cell maturation

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Abstract

Myeloid cells found in the tumor microenvironment favor tumor growth [1]. Certain components of extracellular matrix, e.g. fibronectin, can support myeloid cells' differentiation and maturation [2]. Here, fibronectin expression by breast cancer cells under inflammatory conditions and its correlation with myeloid maturation were determined. Breast cancer cells were stimulated with IL-1β, TNF-α, IL-6, IFN-γ; and, fibronectin expression was evaluated by RT-PCR, Western-Blot, and flow cytometry. Myeloid leukemia cell lines (U937, THP-1) served as immature myeloid cells and cultured in breast cancer cells' (MCF-7, BT474, MDA-MB-231, HCC38) supernatants. The change in myeloid markers (CD11b, CD11c, CD14, CD40, CD86), reactive oxygen species (ROS) production and phagocytosis (flow cytometry); chemotaxis capacity (Boyden chamber assay); fibronectin receptors (integrins α4,α5,αV,αX,αM,β1,β3,β7) (RT-PCR) was evaluated in myeloid cells. At mRNA level, fibronectin was found in all breast cancer cell lines and was increased with IL-1β or IL-6 stimulation. However fibronectin protein expression was not prominently affected by inflammatory mediators and was abundant in basal-like breast cancer (MDA-MB-231, HCC38) cells. Supernatants from MDA-MB-231 or HCC38 increased myeloid maturation and migration capacity, especially in monocytic THP-1. Supernatant treatment increased αX, β3, and β7 integrins. In conclusion, fibronectin may be a factor produced by basal-like breast cancer cells that influence maturation of myeloid cells.

***Keywords*:** Fibronectin, breast cancer, myeloid cell, tumor immunology,

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P123. Effect of Interleukin-1α on Carbonic Anhydrase 9 gene expression in Hepatoma cell line

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Abstract

IL-1α is a "dual-function cytokine" that is involved in the nucleus by affecting transcription, other than its extracellular receptor-mediated effects as a classical cytokine [1-2]. CA9 is a trans membrane zinc metalloenzyme that has been reported in solid tumors and several cancer type such as breast, cervical and non-small cells [3-5]. In addition, the overexpression of CA9 is a potential marker of hypoxia. The purpose of the work is to determine the effect of IL-1α on the CA9 gene expression in Hep3B cells. IL-1α was treated on Hep3B cells with serum free condition including 0,1% BSA in different time periods. IL-1α upregulates the CA9 mRNA levels at 6 hours upon stimulation. Furthermore, for the effect of IL-1α on Hep3B cell proliferation, MTT assay was performed using 50 and 500 U/ml IL-1α at different time periods. Untreated cells were used as control group. IL-1α treatment did not show statistically significant cell proliferation ($p>0.05$). The effect of IL-1α was also evaluated at transcriptional level. IL-1α was treated on the Hep3B cells transiently transected by five different CA9 promoter constructs. IL-1α increased the activity of the *CA9* promoter constructs at several levels showing the highest activity with 973bp (-935/+38) construct.

***Keywords*:** CA9, IL-1α, Hep3B (Human hepatoma cell line)

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P124. IDENTIFICATION OF PATZ1 TRANSCRIPTION FACTOR AS A NOVEL INTERACTING PARTNER AND REGULATOR OF THE p53 TUMOR SUPPRESSOR PROTEIN

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Abstract

The tumor suppressor p53 is a stress responsive, sequence specific transcription factor that regulates genes controlling the cell cycle, senescence and apoptosis. Mutation of p53 is the most common genetic event in human cancer. Moreover, p53 deficiency results in the accumulation of different types of tumors such as testicular carcinoma, soft tissue sarcoma and lymphoma in mice. The focus of this study, the PATZ1 transcription factor, has diverse roles in cancer, development and stem cell biology. Besides being a key transcriptional repressor in lymphocyte development, PATZ1 expression is misregulated in different tumor types such as testicular, colorectal and breast cancers.

Because both proteins are significant modifiers of human cancer, we aimed to link the PATZ1 protein to p53 function using a biochemical approach. In this study, we discovered that both overexpressed and endogenous p53 and PATZ1 proteins interact. We identified a p53 binding region in the C-terminal domain of the PATZ1 protein. The interaction between PATZ1 and p53 is specific, as an alternative splice variant, PATZ1Alt, which lacks this region, did not bind p53. We further delineated the interaction region by generating site directed point mutant PATZ1 variants which do not bind p53. The p53 – PATZ1 interaction is functionally significant as neither p53 nor PATZ1 can bind DNA in the presence of the other factor. Therefore, p53 and PATZ1 form a complex that is incapable of DNA binding. We examined the cellular responses controlled by p53 in cells overexpressing PATZ1. Treatment with the DNA damage inducing cytotoxic drug doxorubicin activates p53 related pathways. Overexpression of PATZ1 made cells more resistant to death by doxorubicin treatment. This study documents a novel player in the p53 pathway, a suppressor transcription factor, PATZ1.

***Keywords*:** cancer, p53, PATZ1, transcription factor, DNA damage

P125. *DEK* copy number variations and DEK expression in Multiple Myeloma

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Abstract

Multiple Myeloma (MM) is a cancer of $CD138^{positive}$ plasma cells in which about 20% of patients carry increased copy number in chromosome 6p22.3 where the *DEK* gene is located. In this study, we analyzed the *DEK* expression and copy number in $CD138^{positive}$ and $CD138^{negative}$ cells of MM (n=41) and healthy (n=3) bone marrow samples. Here we showed for the first time that copy number of *DEK* gene was increased in 4 out of 41 MM patients, and regardless of this increased copy number, *DEK* mRNA expression was significantly lower in $CD138^{positive}$ myeloma cells compared to the $CD138^{negative}$ cells of the same patients ($P<0.0001$) or healthy bone marrow samples. Immunohistochemical analysis of bone marrow samples of MM patients (n=62), and healthy controls indicated that DEK is widely expressed in myeloid and erithroid cells. However, neither in normal nor in myeloma cells ther was not detectable DEK protein. In summary, our results suggest that, DEK expression level is downregulated in normal and malignant plasma cells which is independent of the copy number of *DEK*. Lack of DEK protein might be a useful negative immunohistochemical marker for the differential diagnosis of MM.

***Keywords*:** Multiple Myeloma, DEK, Plasma Cell, CD138.

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P126. MIR376 family is a novel regulator of autophagy in cancer cells

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Abstract

Autophagy is one of the well known self-degradative processes that can be triggered upon different stress circumstances such as starvation and malfunctioned protein accumulation for maintaining cellular homeostasis. Understanding the process of autophagy has major significance as deregulation of this mechanism can lead to several abnormalities including cancer and metabolic diseases. microRNAs, are well known endogenous regulators of gene expression which are capable to control cellular processes. Therefore, understanding the role of microRNAs in autophagy is become crucial. According to unbiased microRNA screen, we discovered that *MIR376B* and *MIR376A* were key autophagy-regulating microRNAs. *MIR376A* is a microRNA regulating autophagic pathway through targeting *ATG4C* and *BECN1* as well as its family member *MIR376B*. Furthermore, upon exogenous expression of *MIR376A* both protein and mRNA level of ATG5 were found to be decreased. In order to illuminate the direct interaction with microRNA and targets 3'UTRs, luciferase reporter assay was performed. However, individual miRNAs were analyzed and found difference between them. This result indicated that although they share same seed sequences and target specificity, in fact, MIR376 family members can be response autophagic triggers with different kinetics. As a conclusion, we introduced for the first time MIR376 family as a key microRNA family in autophagy regulation. Impact of these findings will be discussed in cancer biology manner.

***Keywords*:** Autophagy, mammalian autophagy regulation, microRNA.

Cell biology

P127. Genotoxic effect of 4-Methylimidazole

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Abstract

4-Methylimidazole (4-MEI) is one of food coloring which is used in some alcoholic beverages as scoped by IARC (International Agency for Research on Cancer) in 2011. 4-MEI is widely used in pharmaceutical, chemical, dyes and pigment, agricultural technology. This study was aimed to investigate genotoxic and cytotoxic effects of 4-MEI in Swiss albino Mice. In this study, the mice were treated to 4-Methylimidazole as a concentrations of 100, 130, 160 mg/kg for 12 hours and 24 hours treatment periods, then bone marrow cells of the animals were used to study of substance genotoxic and cytotoxic effects by chromosomal aberrations (CA) and Mitotic Index (MI) tests. The result of this research showed 4-MEI significantly increased the percentage of CAs at 12 and 24h treatment periods with the exception of 160 mg/kg for 12 h and 100 mg/kg and 130 mg/kg for 24h treatment periods. 4-MEI at only 160mg/kg for 12h and at all concentrations for 24h decreased the Mitotic Index (MI) when to compared control. These results showed that 4-MEI can be genotoxic effect in bone marrow cells of Swiss albino mice while it showed cytotoxic effects on the bone marrow cells.

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P128. Discovery of Novel Potassium Ion Channel Blocking Peptides from Scorpion Venom

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Abstract

Scorpion venoms contain a diversity of peptides acting on several types of potassium ion channels. Since these channels are involved in many critical biological functions and play important roles in many diseases including cancer, they are considered as potential therapeutic targets [1]. The aim of this study was to optimize Invitrogen FluxOR Potassium Ion Channel Assay kit for discovery of novel potassium ion channel blocker peptides from scorpion venom. Reported work is the first ion channel screening study performed on *Protoiurus kraepelini* scorpion venom. Target ion channel subtype was determined based on categorization of scorpion peptide sequences of Uniprot database. Fluorescence based FluxOR Potassium Ion Channel assay was used for ion channel activity screening. Experiments were performed on Jurkat and NAMALWA cell lines. Clofazimine, a potent Kv1.3 inhibitor, was used as a positive control. Peptide fraction of *Protoiurus kraepelini* crude venom (0.8 mg/mL) showed no inhibitory effect on potassium ion current of Jurkat cells. Optimized assay kit provides a highly practical alternative to the established but complicated patch clamp methodology and will be used to screen other arachnid venoms studied in our research group.

Keywords: Drug discovery, potassium ion channel, scorpion, peptide, fluorescence assay

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P129. Effects of *Momordica charantia* Extract Against Induced DNA Damage

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Abstract

In this study, It was aimed that determination of protective and reparative effects of Momordica charantia aqueous extract (MCE), against DNA damage occuring cause of H_2O_2. For this purpose, haploid Saccharomyces cerevisiae BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) was used. Protective and reparative induction effects of extracts detarmined by using alkaline single cell gel electrophoresis method. The antioxidants properties were evaluated by determining total antioxidative capacity (TAC), total oxidative capacity (TOC) levels. Pre-treatments with MCE reduced DNA damage at the dilutions 1/16, 1/32 and 1/64. Thus, MCE showed cellular protection against DNA damage produced by H_2O_2. However, repairing mechanism was not good enough. Antioxidative capacity is also higest in these extracts. These results suggested that MCE have significant antioxidant activity and protective effect against DNA damage.

Keywords: *Momordica charantia*, *Saccharomyces cerevisiae,* Comet assay, DNA damage

P130. Yeast response mechanisms against boron still remain a mystery

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Abstract

Many organisms need boron for their metabolism. Although boron is an essential micronutrient, excess of it causes toxicity to the cells. The mechanisms underlying this toxicity have not been known yet. Boron stress activates the general amino acid control mechanism in yeast and activates Gcn2 kinase in an uncharged tRNA-dependent manner which then phosphorylates translation initiation factor eIF2α and inhibits protein synthesis. *GCN1* is necessary for the kinase activity of Gcn2 in boron stress. Boron causes the phosphorylation of eIF2α in mammalian cells similar to that of yeast cells. Atr1 was identified as a boron efflux pump. Gcn4 regulates *ATR1* expression which is also associated with the expression of genes involved in amino acid biosynthesis in response to boron. Transcription factors that can bind to the promoter region of *GCN4* are investigated in terms of Gcn4 and *ATR1* expressions. TOR, PKA, and SNF1 pathways are found to be likely to play roles in boron stress response. In addition, genome-wide screenings of yeast deletion library and proteomic approaches have revealed additional genes which provide boron-resistance or -sensitivity to yeast cells. These genes indicate certain intracellular pathways which will facilitate the understanding of boron toxicity and tolerance mechanisms.
This work was supported by TUBITAK Grant No. 104T213, 110T917.

Keywords: Boric acid, boron toxicity, *Saccharomyces cerevisiae*, *GCN4*, transcriptional regulation, translational regulation

P131. Zinc-Finger domain containing CXXC5 is an E2 responsive gene

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Abstract

17β-estradiol (E2), an estrogen hormone, mediates physiological and pathophysiological functions of breast tissue. E2 effects are mediated by estrogen receptor a (ERα), which is a ligand-dependent transcription factor [1]. Upon binding to E2, ERα regulates target gene expressions through genomic signaling pathways that result in alterations of cellular responses. ERE-dependent signaling constitutes one genomic pathway that denotes E2-ERα binding to specific DNA sequences, estrogen response elements (EREs) [2]. Our microarray studies suggested that *CXXC5* expression is modulated by E2-ERα through ERE-dependent pathway. Due to a zinc-finger-CXXC domain (ZF-CXXC), Cxxc5 is considered to be a member of ZF-CXXC family which binds to non-methylated CpG dinuclotides of CpG islands and mediates transcriptions [3]. Studies on the structure/function of Cxxc5 are limited. Our homology modelling suggests Cxxc5 could also be involved in transcriptions by interacting with CpGs. We therefore predict that *CXXC5*, synthesized as a primary E2-response gene, participates in secondary gene expressions, thereby in E2-mediated cellular processes. To begin examining this prediction, we cloned CXXC5 cDNA and expressed it in mammalian cells. Our findings suggest that nuclearly localized Cxxc5 may indeed be involved in transcriptions. Our ongoing studies using molecular and cellular biology approaches will highlight the importance of Cxxc5 in E2-ER mediated cellular events.

***Keywords*:** CXXC5, Zinc-Finger domain, ERα, E2

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P132. Synthesis and Investigation of Mutagenic Effects some 2-Hydroxy-1,4- Naphthoquinone Derivatives by Ames test

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Abstract

In these study, mutagenic effects of novel synthesis 2-hydroxy-1,4- naphthoquinone derivatives [2-Phenyl-2-(2- thienyl)-2,3-dihydronaptho[2,3-*b*]furan-4,9-dione(**1)** ve 2-Hidroxy-3-[(*E,Z*)-2- phenyl -2-(2- thienyl)vinyl] naphthoquinone (**2**)] were investigated by using both bacterial reverse mutation assay in *Salmonella typhimurium* TA98 and TA100 strains with or without metabolic activation system (S9-mix). 3 and 4 were dissolved in dimethyl sulfoxide (DMSO) for all test systems. Five non-cytotoxic doses of the derivatives were tested in two parallel independent experiments in Ames test. The results were also analyzed statistically by using SPSS for Windows; Mann-Whitney test was used. It was determined that 1 and 42 have no mutagenic effect on *S. typhimurium* TA98 and TA100 strains with or without S9-mix. It is, therefore, necessary to use further examination assay systemsin order to affirm their mutagenic effects.

***Keywords*:** Ames, Mutagenicity, S9, Genotoxicity, naphthoquinone

Acknowledgements: The authors wish to thank Uşak University Coordinatorship of Scientific Research Project Unit for supporting this study financially (Project No: 2014/MF003)

P133. Cellular Function of Chloride Ion Channel Proteins in Mammalian Cells

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Abstract

Cell cycle is composed of cell growth and division. Cell division is the main process for propagation of all living things. Different cellular complexes reorganize in a coordinated manner throughout cell division in order to support their dynamic structural changes such as cell rounding. At the onset of mitosis, adherent animal cells lose their flat morphology and become round. Mitotic cell rounding is necessary for the reorganization within the mitotic cells and the geometric requirements of cell division. Cells use different mechanisms to drive cell rounding including regulation of intracellular pressure. Cells use ion transporters to regulate intracellular osmolarity in the short term. Several ion transporters locate at the plasma membrane to regulate intracellular osmolarity and pressure[1]. Chloride intracellular channel proteins are examples of this sort of proteins. They localize to different compartments of the cell and insert into membranes. Chloride intracellular channel proteins form weakly selective ion channels to transport chloride ions. The purpose of this study is to investigate the cell cycle dependent regulation and the role of chloride intracellular channel proteins in mitosis. For this purpose, we performed transfection experiments in HeLa cells by using green fluorescent protein tagged wild type and mutant forms of chloride intracellular channel proteins. We used fluorescence microscopy techniques to study chloride intracellular channel proteins during cell division. We also performed RNAi mediated depletion experiments to observe the role of chloride intracellular channel proteins in the mammalian mitotic cells.

***Keywords**:* Chloride ion channel, Mammalian cell, RNAi

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P134. Cloning and Initial Protein Characterization of an Estrogen Responsive Gene: *YPEL2*

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Abstract

17β-estradiol (E2) affects physiology and pathophysiology of breast tissue. Estrogen receptor a (ERα) as a transcription factor mediates the effects of E2 that result in alterations in cellular proliferation, differentiation and death [1]. We have identified *YPEL2* as E2-ERα responsive gene in MCF7 cell line derived from a breast adenocarcinoma. *YPEL2* is a member of Yippee-like (*YPEL*) family of human with five members: *YPEL1-5* [2]. Although structure-function of Ypel proteins is unclear, our bioinformatics analyses suggest that each *YPEL* is spatiotemporally expressed while the common structural and functional properties of Ypel1-5 are important for basic cellular processes. In studies, we found that Ypel1, 2 and/or 3 are synthesized in COS7, transformed African green monkey kidney fibroblast-like cells, and localized to a region just outside of the nucleus, but not in MCF7 cells. Over-expressions of *YPEL1-5* led to DNA leakage from nucleus into cytoplasm in patterns that overlap with each Ypel in COS7 and MCF7 cells, in the latter over-expression of Ypel1-5 is associated with nuclear lamina deterioration. Thus, we suggest that Ypel2 is involved in processes regulating DNA and/or nucleus architecture during cell division and/or death. In future studies, we will address the regulation of *YPEL2* expression and Ypel2 functions in cells.

Keywords: Estrogen, Estrogen receptor, *YPEL2*, Yippee

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P135. The Effect of Connexin 26 Mutations Associated with Keratitis-Ichthyosis Deafness (KID) Syndrome on the Protein Localization and Cellular Ca^{2+} Content

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Abstract

Connexins play role in cellular communication by forming gap junctions, which facilitate the passage of small molecules between adjacent cells[1]. They are crucial for maintenance of cellular homeostasis as connexin mutations lead to human disorders such as keratitis-ichthyosis deafness (KID) syndrome. KID syndrome, that leads to hearing loss and thickening of the skin, is caused by Cx26 mutations [1]. Mutant proteins can impair various cellular processes including maintenance of cellular calcium homeostasis. Deregulation of calcium homeostasis can affect cell survival, causing cell death and necrosis[2]. Here, we characterized two KID syndrome linked Cx26 mutations, I30N and D50Y. Immunohistochemical analysis showed that Cx26-D50Y and I30N didn't form gap junction plaques at the cell-to-cell junction and while D50Y protein was mainly found in the cytoplasm, Cx26-I30N protein was observed in the Golgi apparatus. Then, internal calcium amount of cells were determined by measuring internal Ca^{2+} with flow cytometry using Fluo-3AM calcium indicator. Cells with mutant D50Y and I30N proteins had increased internal Ca^{2+} compared to cells with wild-type Cx26. In conclusion, I30N and D50Y mutations altered the cellular localization of Cx26 proteins and internal Ca^{2+} content of cells which may play role in the development of KID syndrome.

***Keywords*:** Connexin 26, Gap Junction, Ca^{2+} content, flow cytometry, immunostaining

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P136. Regulation of NFI and NFI-dependent Gene Expression in Human Neural Stem Cells

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Abstract

The Nuclear Factor One (NFI) family of transcription factors is one of the key regulators of gene expression during brain development. NFI family has four members (*NFIA*, *NFIB*, *NFIC* and *NFIX*) that are highly homologous in their N-terminal DNA binding domain. Deletion of *NFIA*, *NFIB* and *NFIX* has distinct effects on development of the different brain regions such as neocortex, hippocampus, and hindbrain, altering different developmental processes like axonal outgrowth and guidance, differentiation, neurogenesis or gliogenesis. *NFIB* specifically appears to control neurogenesis in precerebellar system of the hindbrain [1]. Previously, potential *NFIB* target genes were identified in precerebellar neuroprogenitors by mRNA expression profiling. In order to study regulation of potential *NFIB* targets, we first characterized expression of NFI family members and target genes in neural stem cells derived from H9 human embryonic stem cell line (Gibco). Expression of *NFIB*, *NFIC*, *NFIX*, *CDO* and *COL2A1* was significantly reduced while *NFIA* and *FGF19* were up-regulated during neuronal differentiation. Moreover, preliminary data show that NFIB protein expression was also decreased. In order to find out if NFIB is subject to translational regulation by miRNAs in this system, we started to study miR-153, miR-30a-5p, and miR-124a as potential regulators of NFIB during neural differentiation.

Keywords: Neurogenesis, *NFIB*, miRNAs, transcription factors

Acknowledgements: This study was supported by grants from TUBITAK (KBAG 212T017) and Istanbul Technical University Research Fund.

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P137. Reprogramming of various cell types towards a beta cell character

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Abstract

Of the various examples of direct cell type reprogramming with transcription factors, the ability to make pancreatic beta cells is of particular interest from the point of view of potential cell transplantation therapy for diabetes. This is because the clinical procedure of islet cell transplantation therapy is well established and the limiting factor is entirely the lack of suitable human beta cells. The study of Zhou et al [1], in which pancreatic exocrine cells were converted to insulin-positive cells was conducted in vivo, using immunodeficient mice. Because it is difficult to investigate molecular mechanisms in vivo, we felt it important to establish an in vitro model for the process which is more amenable to study. We selected the AR42j-B13 cell line (referred to here as B13 cells) and some other cell lines from different species (rat & mouse) with different developmental potential (stem cells & differentiated cell) for this purpose. B13 cell line is a rat cell line with a pancreatic exocrine phenotype, originally derived from a chemically induced pancreatic tumor [2]. In this study we describe the effects of *Pdx1* + *Ngn3* + *MafA*, the gene combination used by Zhou et al. [1] on different cell types to see the reprogramming potential of them.

***Keywords*:** Reprogramming, Beta cells, *Pdx1*, *Ngn3*, *MafA*

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P138. SIKs from Neural Development to Diabetes

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Abstract

Salt inducible kinases (SIKs) are Ser/Thr kinases from AMPK-RK family. SIKs are regulated by PKA signal, which regulates IRS-1 and CRTC activity. Via Akt signal, SIKs are known to inhibit CREB and CRTC, too. Beside the long known role in insulin-related pathways, these kinases are shown in mitotic control. SIKs are target of a master tumor suppressor kinase Lkb-1; which is supported by several instances, indicating SIK involvement in cancer progression and drug resistance. In this study, we model highly conserved SIK2 and SIK3 homologs in fruit fly, Drosophila melanogaster.

Like the mammalian ortholog, Drosophila SIK2 interacts with the FGF pathway in the course of insulin metabolism; while SIK3 was shown to affect glucose/lipid homeostasis, skeletal and adipocyte development. Yet the intracellular interactions and fuctions of SIKs remain largely unknown.

We started to characterize SIK2 and SIK3 in the nervous system using the Drosophila compound eyes as model. To study the SIK role spatiotemporally, we have generated the controllable expression of SIK3, SIK3 null mutants, and the fluorescent-tagged transgene of SIK2 to elucidate the expression pattern, since no specific antibody is available. Using these tools we have shown that, SIKs are expressed in the developing nervous system, in non-overlapping pattern (Figure 4). Using scanning electron microscopy, we have seen that both SIK2 and SIK3 are involved in eye development, regulating eye size and cell specification events, and required for proper development of the tissue. Using a cancer tool, Delta overexpressing flies, we proved SIKs can induce tumorigenesis in suitable niche, which can also metastasize. This suggests SIKs might be one of the cross-talks, linking glucogenic control to cancer progression.

***Keywords*:** Salt Inducible Kinase, Drosophila melanogaster, Eye development, Glucose metabolism

P139. Investigation of Alterations in Expression Levels of Heat Shock Protein Genes in Rat's (*Rattus Norvegicus*) Liver Tissues Exposed to Long-Term Iron (Fe^{+3})

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Abstract

In this study, we examined the effect of iron (Fe^{3+}) in expression profile on four small stress protein genes hspa1a, hspa4, hspa5 and hsp90aa1. For this purpose, five different concentrations of iron (0.87ppm, 3ppm, 30ppm, 300 ppm) were given to rats for 100 days. After the treatment, rats were sacrificed and liver tissues removed quickly for further experiments. The results showed that of iron, especially 300 ppm, has caused significant alterations in expressions of mentioned genes. In liver, the level of expression of hspa4 was not significantly affected by iron treatment ($p>0.05$). hsp90aa1 and hspa1a gene expression levels increased by iron exposure in tissues, highly significantly ($p<0.0001$). Finally, hspa5 gene expression levels decreased in tissues significantly ($p<0.05$). Findings of this study showed that iron may cause significant changes in regulation of stress associated genes in liver.

Keywords: HSPs, Iron, *Rattus norvegicus*, Gene Expression, Real Time.

P140. Evaluation of Zoledronic Acid effect on the *sFRP1* gene expression during osteoblast differentiation from human Mesenchymal Stem Cells(*hMSCs*)

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Abstract

Osteoblast arises from multipotentmesenchymal stem cell (MSCs) present in the bone marrow steroma. Several factors like likeWnt signaling pathway affect osteoblast differentiation from MSCs. In Wnt signaling pathway sFRP molecules such as sFRP1 act as a strong negative regulator of the canonical Wnt pathway. In this study, the effect of zoledronic acid as effective drug of osteoblastic differentiation on gene expression of sFRP1 gene as Wnt antagonist during osteoblastic differentiation was examined.After isolation of mesenchymal stem cells, osteoblastic differentiation was performed using osteogenic medium. Extraction of RNA in the first, the second and third of osteoblast cells and MSC was done. After cDNA synthesis, evaluation of the sFRP1 gene expression was carried out using Real time PCR technique. Results of flow cytometry analysis and alizarin red staining show potent effect of zoledronic acid in osteoblastic differentiation of MSCs. Also, the expression of sFRP1gene during osteoblastic differentiation was significantly decreased ($p<0.05$).Our results show that zoledronic acid treatment leads to decreasing expression of sFRP1 as Wnt antagonist and indirect activating of Wnt signaling pathway.[1,2,3,4]

Keywords: Mesenchymal stem cell, osteoblastic differentiation, Wnt signaling pathway, sFRP1 gene.

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P141. IDENTIFICATION OF UPSTREAM REGULATORS OF INTERFERON REGULATORY FACTOR 4 (IRF4) EXPRESSION IN MELANOMA CELL LINES

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Melanoma is derived from melanin pigment producing cells that are melanocytes. Late stage, metastatic melanoma is considered as one of the most aggressive malignancies with one of the worst prognoses [1]. IRF4 (also known as LSIRF, ICSAT, MUM1) belongs to interferon regulatory family member transcriptional regulators. IRF4 expression, which is regulated by mitogenic stimuli in lymphocytes, is associated with certain cancers such as T-cell leukemia, multiple myeloma and B cell lymphoma [2]. In addition to immune cell derived malignancies, IRF4 upregulation is found in melanoma cell lines and melanoma patient samples [3]. Additionally, dependency of melanoma cell lines to IRF4 expression was recently found [4]. Moreover, genome wide association studies (GWAS) identified a SNP in IRF4 locus as high risk factor for melanoma [5]. Herein, we asked how IRF4 expression is regulated, and which pathways are playing role in its upregulation in melanoma. If we gain insights to its regulation, this might lead to a therapeutic intervention strategy for melanoma. Most of our knowledge about upstream regulators of IRF4 comes from hematologic cells. Based on a candidate approach, a number of signal transduction pathways and immunomodulatory drugs that are known to regulate IRF4 expression in other contexts (such as hematologic cells) were tested by using small molecular inhibitors and dominant negative regulatory proteins. Our preliminary results implicate that a major lymphocyte-related signaling pathway plays role in IRF4 expression in melanoma cell line models.

Keywords: Melanoma, IRF4, signal transduction.

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P142. Induction of gene expression and cellular proliferation with directional polarity by designer monotransregulators

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Abstract

The main circulating estrogen hormone 17β-estradiol (E2) contributes to the initiation and progression of breast cancer. Estrogen receptor α (ERα), as a transcription factor, mediates the effects of E2. ERα is a modular protein consisting of a DNA binding (DBD) and transcription regulatory domains (TRDs). The interaction of E2-ERα with specific DNA sequences, estrogen response elements (EREs), of genes constitutes one genomic pathway necessary for cellular alterations. Based on DBD of ERs and TRDs of other transcription factors, we engineered designer transcription factors with activator (monotransactivator) or repressor (monotransrepresor) functions to specifically regulate ERE-driven genes. Using adenovirus infected ER-negative MDA-MB-231 cells derived from a breast adenocarcinoma, we found that monotransactivators but not the ERE-binding defective counterparts, mimicked the ability of E2-ERα to regulate ERE-driven gene expression and cellular proliferation. On the other hand, monotransrepressors modulated ERE-driven gene expressions with directional polarity that resulted in alterations in DNA synthesis, cell cycle phases and proliferation opposite to those observed with E2-ERα and monotransactivators. Thus, monotransregulators could offer a protein engineering platform to modulate gene expressions and cell growth with directional polarity to combat breast cancer.

***Keywords*:** Estrogen, Estrogen receptor, Estrogen response elements, transcription

P143. Nitrogen and Glucose Signaling Pathway Regulates the Transcription of *NTH1* Gene in S. *cerevisiae*

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Abstract

Trehalose is an essential component of the yeast metabolism. It is synthesized by trehalose synthase enzyme complex and degraded by neutral trehalase enzyme, encoded by *NTH1* gene of *S. cereveisiae* [1]. Trehalose biosynthesis is activated by unfavorable growth conditions. The cytoplasmic level of trehalose is strictly controlled by continuous recycling, which involves trehalose synthase and neutral trehalase enzymes. Previous studies have clearly shown that the neutral trehalase activity is regulated at post translational level by phosphorylation. In this study, we have shown that the expression of *NTH1* gene is also regulated at transcription level by glucose and nitrogen signaling. Our results indicated that the transcription of *NTH1* gene is activated nearly 6-fold by nitrogen starvation. In addition, our results also showed that the transcription of *NTH1* gene is activated by RAS-cAMP pathway. *NTH1* transcription is activated 2 to 3-fold in alternative carbon sources which does not cause glucose repression. Moreover, transcription of *NTH1* gene is activated 8 to 10-fold in Σ strain of *S. cerevisiae* that has a very active RAS-cAMP pathway. Apart from the nutrient signals, our results indicated that heat stress also activates the transcription of *NTH1* gene in 2-fold. Furthermore, we have analyzed the trehalose and glycogen levels in the Σ strain and compared it to the BY4741 strain of *S. cerevisiae*. Trehalose level in the Σ strain is at least 2-fold higher than BY4741 strain. Our results indicated that the expression of *NTH1* is regulated at transcription level by nutrient signaling and stress pathways.

***Keywords*:** Trehalose, *NTH1*gene, Glucose signaling, Nitrogen signaling, cAMP.

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P144. Growth Stage and the Amino Acid Starvation Regulates the Expression of the *EST3* Gene in *S. cerevisiae*

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Abstract

The *EST3* gene of the yeast *S. cerevisiae* encodes the regulatory subunit of the telomerase enzyme complex [1]. It encodes a 181 amino acid long protein. Translation of the full length Est3p requires +1 ribosomal frameshift. In the absence of the programmed ribosomal frameshift, the translation of *EST3* mRNA yields a 93 amino acid long truncated product with no known functions [2]. In this study, it was found that the translation of the full length Est3p is regulated by the growth conditions in *S. cerevisiae*. Under normal growth conditions, the frameshift efficiency of *EST3* mRNA measured as 12.7% in the sigma strain of *S. cerevisiae*. However, induction of amino acid starvation by 3-AT or by boric acid resulted with at least 2-fold activation of programmed ribosomal frameshift in *EST3* mRNA. Moreover, induction of pseudohypeal growth by low ammonium decreased the frameshift rate down to 6%. In addition to unfavorable growth conditions, it was shown that the growth stageof the yeast cultures also affects the frameshift efficiency in *EST3* mRNA. While the frameshift efficiency is 12.7% in the logarithmically growing yeast cultures, it was found that it decreases to 4% in the stationary stage yeast cultures. These results indicated that the growth conditions and the growth stage regulates the expression of EST3 gene in *S. cerevisiae*.

***Keywords*:**Ribosomal frameshift, Telomerase, EST3 gene, Translational regulation.

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P145. Genome-wide Localisation Analysis for IRF4 Target Gene Identification in Melanoma Cell lines

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Abstract

With increasing rate of diagnosis worldwide, melanoma is one of the most lethal and aggressive form of skin cancer. Melanoma originates from accumulation of genetic alterations and transformation of melanocytes which gives them abnormal proliferation, invasion and metastasis abilities. [1] Interferon Regulatory Factor 4 (IRF4), is a transcriptional regulator, with functional roles in differentiation and function of immune cells. Previous studies have demonstrated that IRF4 expression levels are elevated in some lymphoid cancers and IRF4 is one of the key transcription regulators for the survival of multiple myeloma and ABC-type diffuse large B-cell lymphomas (ABC-DLBCL) cells. [2, 3] Recent studies from our lab and elsewhere demonstrated high expression levels of IRF4 in melanocytes and melanoma cells, [4, 5, 6] which encouraged us to investigate the functional roles of IRF4 in melanoma. In this study, we investigate the genome-wide localisation of IRF4 via high-throughput sequencing of immunoprecipitated chromatin (ChIP-seq) in melanoma cell lines, to help identify IRF4-regulated genes and pathways in melanoma. Initial results from ChIP-qPCR experiments in our lab demonstrated IRF4 binding on Tyrosinase gene which is one of key developmental genes in melanocytes.

***Keywords*:** IRF4, Melanoma, ChIP-seq

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P146. Functional Analysis of the Human ADAMTS-3 Gene Promoter

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ADAMTS-3 is the member of ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) gene family and plays an important role in the processing and biosynthesize of type II fibrillar collagen molecules [1-2]. Although procollagen processing activity of the ADAMTS-3 gene was studied, there isn't any study on its own transcriptional regulation. To elucidate the transcriptional regulation of the ADAMTS-3 gene a putative promoter region (1380 bp), and three truncated promoter constructs were cloned into luciferase based vector system to determine transcriptionally most active region. Transient transfection studies were performed to identify basal promoter activity in Saos-2 cell line, a human bone osteosarcoma model by Fugene or calcium phosphate precipitation. Effects of overexpression of SP1, USF and C/EBP transcription factors on ADAMTS-3 promoter activity in Saos-2 cells were also evaluated by co-transfection studies by simultaneous transfections of promoter and expression plasmids. Transcriptional activity obtained from co-transfection assay was compared to basal activity of ADAMTS-3 promoter constructs. ADAMTS-3 mRNA level was also determined from the SP1, USF and C/EBP overexpressed cells by qRT-PCR. As a conclusion, overexpression of these transcription factors differentially upregulate the truncated promoter constructs. They might be involved in the transcriptional regulation of ADAMTS-3 gene in osteosarcoma cells.

***Keywords*:** ADAMTS-3, osteosarcoma, promoter, transcriptional regulation, type II collagen.

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Immunology

P147. CRAM-A decoy chemokine receptor expression and function in breast cancer cells

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CCRL2 is the newest member of decoy (atypical) chemokine receptor family. Human CCRL2 gene has two variants; namely, CRAM-A and CRAM-B. CRAM-B binds to CCL5, CCL19 and chemerin; decreases their local concentration [1]. Here, expression and the functions of CRAM-A isoform in breast cancer cells were examined. CRAM-A and CRAM-B expression was determined with RT-PCR in breast cancer cell lines under IFN-γ, LPS, IL-1β, TNF-α, IL-6 stimulation. pCRAM-A-IRES2-EGFP recombinant DNA was constructed and confirmed by PCR, restriction digestion and sequencing. This recombinant plasmid was transfected into HEK293T and MDA-MB-468 and BT-474 breast cancer cell lines. GFP and CRAM expression were examined by flow cytometry. For functional analyses; Ca^{2+} flux (FuraRedII staining), ligand binding, receptor internalization and ligand removal assays were performed with CCRL2-blocking antibodies. Recombinant CRAM-A was de novo expressed in the cells (GFP^{+} $CCRL2^{+}$ 66.3-84.22%). As expected, CCL5, CCL19 and chemerin did not stimulate intracellular Ca^{2+} flux whereas ionomycin ionophore did. On breast cancer cells, CRAM-A expression was specifically increased upon IFN-γ stimulation. In the presence of chemokine ligands, CRAM-A internalization was determined in ~30 minute-intervals. CCL19 was efficiently removed from the environment. Therefore, CRAM-A expression may serve as an immune evasion mechanism that mitigates T cell influx towards the breast tumors.

Key words: Decoy receptors, breast cancer, chemokines

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P148. IDENTIFICATION AND CHARACTERIZATION OF IL1R SMALL MOLECULE INHIBITORS

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Abstract

Numerous autoimmune disorders such as Behcet's Disease and Familial Mediterranean Fever are believed to occur through the aberrant activation of the IL1R pathway [1]. While there are several protein-based treatments that inhibit this critical pathway, there are no IL1R small molecule inhibitors. Therefore, in an effort to identify novel IL1R inhibitors we conducted an *in silico* screen of the ZINC database. From the computational predications several chemicals were selected and tested in a cell-based IL1R assay. A hit molecule was identified (low-μM IC_{50}) and several hundred chemical derivatives were synthesized and tested. Through the modification of different chemical groups we were able to improve the potency of the inhibitor ~100x (mid-nM IC_{50}). Further, with the large number of tested derivatives we developed a docking-based Structure Activity Relationship (SAR) model that proposed a potential binding site of our small molecule inhibitor on the IL1R. We are currently conduct alanine scanning mutagenesis to validate the predicted mechanism of action.

***Keywords*:** IL1β, auto-inflammatory diseases, IL1R signaling, small molecule inhibitors

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P149. Identification of the Relationship Between NLRP7 Protein and Immune Privilege

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NOD Like Receptor (NLR) proteins are cytoplasmic pattern recognition receptors (PRRs), which process pro IL-1b by forming inflammasome complexes upon detection of pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). In mammals, more than 20 NLR proteins take role in inflammation. NLRP7 is a primate specific protein and consist of PYRIN, NACHT and LRR domains. High expression of NLRP7 has been linked to testicular seminoma and several cancer types. Also, mutations of NLRP7 give rise to recurrent hydatidiform moles, which lead to stillbirths and abortions.

In this project, we aim to elucidate the possible role of NLRP7 in immune privilege and inflammation. According to our findings, NLRP7 forms an inflammasome by interacting with Caspase 1 and ASC and activates IL-1b. Co-IP studies also showed that NLRP7 has an interaction with Caspase 1, ASC and Caspase 5. Polyclonal antibodies against PYRIN and full length NLRP7 were produced. By using these antibodies we identified several different cell lines increased in NLRP7 expression, especially in immune privilege sites. NLRP7 inflammasome activators have been identified by treatments of pathogen associated molecular patterns (PAMP), danger associated molecular patterns (DAMP), heat killed bacteria in different cell lines and endogenous CO-IP studies are still in progress. As a result, NLRP7 forms an inflammasome to activate the IL-1b by binding to ASC and Caspase-1. NLRP7 directly interacts with ASC, Caspase-1, Caspase-5 and certain PAMPs and DAMPs increase NLRP7 expression in THP-1 cells.

Keywords: Innate immunity, inflammasome, NOD like receptors, NLRP7.

P150. Development of Recombinant DNA Vaccines

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Abstract

The vaccine types can be classified as live (attenuated), killed (whole cell), and subunit vaccines. A DNA vaccine is a class of recombinant subunit vaccines composed of bacterial plasmid DNA molecule carrying the gene(s) encoding for immunogenic protein(s) of a pathogen under a eukaryotic promoter for the expression in mammalian cells [1]. The efficiency of gene expression is checked first via transfection of mammalian cells using purified endotoxin-free plasmids under a reporter such as GFP [2]. DNA vaccines are delivered as coated gold microparticles using a biolistic system (gene gun) [3] or via injection directly into the muscles for eliciting both humoral and cellular immune responses against the immunogenic protein [2]. DNA vaccines are relatively easy in production and cost less with a good preclinical efficacy. Due to increased cellular immunity, DNA vaccines are promising for prevention of diseases caused by intracellular pathogenic bacteria and viruses. The lower protection capacity might be overcome by utilization of proper adjuvants or prime-boost strategies.

***Keywords*:** DNA vaccine, immune responses, pathogenic bacteria, recombinant vaccine

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P151. Expression of Key Transcription Factors in Th17 Differentiation

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Abstract

Naive CD4+ helper T cells can differentiate into distinct T helper (Th) lineages including Th17 cells. At present, the transcription factors of Th lineages that have been identified include T-bet/Stat4 (Th1), GATA-3/Stat5 (Th2) Foxp3/Stat5 (Treg) and RORC2/Stat3 (Th17) respectively [1]. However, emerging data suggest that there are many more critical transcription factors in the regulatory network for T-cell fate determination. Therefore, the aim of the study is to measure kinetics of transcription factors of CD4 T cells in Th17 differentiation. In this study, human naive CD4 T cells were purified from healthy donors' PBMC. Cells were cultured with Th17 polarizing stimulants at various times (0-9 days). Th-specific transcription factors were monitored by flow cytometry. Data showed that RORC2 was increased until 6th day (15%), following days it stayed stable and decreased. Increase of T-bet was also observed at 6th day (8%). Foxp3 was high at day 3 and continue to increase up to 9% at 6th day and then it slowly decreased. Additionally, IL-17 expressing Th17 cells (20%) were not concomitantly express IL17/IFNγ (1.63%) and IL17/Foxp3 (0.29%). Overall data suggested that there is a fine-tuning of transcription factors presence and interactions during Th17 differentiation from naïve CD4 T cells. This work was supported by TUBITAK (Grant Number 110T412).

***Keywords*:** Th17 differentiation, RORC2, transcription factors

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P152. Monitoring Apoptotic Markers of Activated T cells in Th17 Polarizing *in vitro* Culture Condition

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Abstract

Th17 cells are subset of CD4 T cells which express an inflammatory cytokine IL-17. The players of human Th17 cells' apoptosis are not fully known yet. Thus, the aim of the study is to measure the apoptotic markers of activated T cells during differentiation of naïve CD4 T cells into Th17 effector cells. In this study, human naive CD4 T cells were isolated from healthy donors' peripheral blood mononuclear cells (PBMCs) and cultured under Th17 differentiating conditions. At 7th day, expression of CD4, CD25 (IL-2 receptor), Bcl-2 and active caspase-3 molecules were measured in cells by flow cytometry. Data showed that naive CD4+T cells were activated and expressed CD25 (29%, $p<0.01$) and differentiated into IL-17 cytokine producing cells (43%, $p<0.01$). Interestingly, there was a significant level of down regulation of Bcl-2 (52%, $p<0.05$) within CD25+CD4+T cells compare to negative controls (98.8%, $p<0.05$). Additionally, activated CD4+ T cells were also active-caspase-3 positive (8.7%, $p<0.01$) compare to negative controls (0.3 %, $p<0.01$). Altogether the data indicate that apoptosis of activated CD4+ T cells in Th17 differentiating conditions is mediated by IL-2 receptor (CD25) upregulation and changes in Bcl-2 and active caspase-3 levels. This work was supported by TUBITAK (Grant Number 110T412).

***Keywords*:** Th17 differentiation, apoptosis, CD4 T cells

P153. The effects of Sulforaphane isoforms on inflammatory response in murine microglia

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Abstract

D,L-sulforaphane (1-Isothiocyanato-4-methylsulfinyl-butane) is the synthetic form of the naturally occurring compound from cruciferous vegetables. It is chemically racemic mixture of both D and L isomers. Microglia is the main inflammatory glial cells in the central nervous system (CNS). Activation of microglia trigger inflammatory responses initiated by transcription factor NF-κB and the NLRP3 (Nod-like receptor family, pyrin domain containing 3) inflammasome. The purpose of this study is to evaluate the effects of sulforaphane isoforms on inflammatory responses in murine microglia. N9 microglia cell line was used and cells were treated with LPS or pure LPS following ATP with or without sulforaphane. At the end of incubation period, cytokine and NO levels were analyzed. We showed that sulforaphane isoforms differentially attenuate inflammatory response in microglia.

***Keywords*:** Inflammasome, ATP, microglia, DL-sulforaphane

P154. Testing the hypothesis that chronic stress accelerates brain aging in a mouse model

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Abstract

Epidemiology studies state that chronic stress can increase the risk of age-related neurodegenerative disease [1,2]. In addition, studies on animal models demonstrate that chronic stress create cognitive and histological effects that show parallel effect during normal aging [3]. Here our aim is to directly test the hypothesis that chronic stress accelerates brain aging in a mouse model. We planned to measure the effects of chronic stress on short term memory and make transcriptome measurements of prefrontal cortex during aging. In our chronic stress model, restraint stress and predator stress (rat exposure) was applied periodically 4 hours everyday during 2 months. A radial maze test was applied to determine short and long term memory changes before and after chronic stress exposure. At the molecular level, once before stress and twice during the stress period, the blood of the animals was collected to detect corticosterol levels. After the animals reach late age, we plan to collect transcriptome data which will aid us to see the effects of chronic stress and aging on brain and blood transcriptoms. We expect to observe increase in short term and long term memory errors, also rise in the interindividual molecular and phenotypic variation after the chronic stress.

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***Keywords*:** Chronic Stress, Short Term Memory, Aging, Transcriptome.

P155. The Cloning of a Reference Standard for Detection of Reduced Penetrance Alleles in Huntington Disease

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Abstract

Huntington disease (HD) is an autosomal dominant progressive neurodegenerative disease which has a global prevalence rate ranging between 0.005% and 0.01% [1]. The disease occurs with expanded and unstable CAG trinucleotide repeats in the first exon of *HTT* gene [2]. CAG-repeat expansion mutations account for >99% of cases of HD. Therefore, molecular tests that effectively detect and measure the CAG-repeat region of the *HTT* gene are >99% sensitive [3]. The alleles in the 36-39 repeat range are reported to have reduced penetrance. In molecular diagnostic tests of HD, it is important to determine the exact repeat numbers especially in this range for the clinical assessment. In this study, we aimed to perform a TA cloning of a reduced penetrance allele (36-39 repeats) for use as a control in molecular diagnosis of HD. Therefore, we selected 2 HD probands with 38 and 43 repeats containing alleles; performed PCR and TA cloning by using pGEM®-T Easy Vector System II (Promega Inc). The alleles including 38 and 43 repeats were successfully cloned. The inserts were checked by HD-PCR. Sequencing results of inserts in pGEM®-T vectors are being expected. Finally, the control sample for the detection of trinucleotide repeats in critical range was provided.

***Keywords*:** Huntington disease, TA cloning, Reduced penetrance, Intermediate allele.

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P156. microRNA-34a regulates neuroprotective effects of lithium in SH-SY5Y Cells

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Abstract

Neurodegenerative diseases are characterized by slow progressive loss of neurons in the central nervous system and their pathological mechanisms remain uncertain. Today, several neuroprotective agents are being investigated with the purpose of slowing or preventing further cell loss. Lithium is used as a treatment agent for a wide range of psychiatric and neurological conditions. Previous studies have suggested that lithium has neuroprotective effects against various damages, but the mechanisms of the neuroprotective effect of lithium have not been fully clarified. SH-SY5Y cells were pretreated with various concentrations of lithium at different time points. First we analyzed the effects of lithium treatment on brain-derived neurotrophic factor (BDNF), apoptosis related genes (Bcl-2, Bax, Bcl-xl) also, NF-E2-related factor 2 (Nrf2) transcription factor and its target genes' expression with real-time PCR. Our result showed that lithium induced the expressions of Bcl-2, BDNF and Bcl-xl mRNA, while decreasing Bax mRNA levels. More importantly; lithium induced nuclear translocation of Nrf2 and activated Nrf2 transcription factor. In addition, lithium treatment upregulated HO-1, GCS and NQO1 expression levels. We also assessed Nrf2 gene function by using siRNA to determine whether Nrf2 knockdown affects lithium-induced neuroprotection. Our results showed that Nrf2 suppression led to a significant reduction in anti-apoptotic and anti-oxidative effects of lithium. Finally, we examined the role of apoptosis-related miR-34a in the protective effect of lithium. We found that miR-34a overexpression significantly reduced lithium-mediated neuroprotection. Altogether, our results provide insight into the different pathways mediating the neuroprotective effects of lithium.

Keywords: Lithium, SH-SY5Y, neuroprotection, gene expression, microRNA, transcription factor

P157. Serum miR-16 levels show negative correlation with amyloid-beta$_{1-42}$ levels in Alzheimer's disease patients

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease, and represents the most common form of dementia. AD has no known cure, and eventually leads to death. The disease is characterized by the formation of amyloid-beta plaques, and neurofibrillary tangles (NTF). Alterations in microRNA (miRNA) expression profiles have been linked to AD pathogenesis, and several studies suggest that altered miRNA signatures could serve as diagnostic markers for AD. We have previously performed a microarray-based screening by using serum samples from AD patients (n=10) and controls (n=10), and found several dysregulated miRNAs in AD patients. Of note, miR-16 showed the highest level of overexpression in AD patients, compared to healthy controls. Here, our aim was to validate the microarray data on a larger patient cohort. Quantitative real-time PCR analysis showed that miR-16 expression was approximately 3.5-fold higher in AD patients (n=24) compared to healthy controls (n=24). Moreover, we analyzed amyloid-beta expression by using ELISA, and we compared $A\beta_{(1-42)}$ levels and miR-16 expression levels by Spearman's Rank Order correlation test. Correlation analysis revealed a significant negative correlation between $A\beta_{(1-42)}$ levels and miR-16 expression levels.. Altogether, our results suggest that elevated miR-16 levels and low $A\beta_{(1-42)}$ levels can serve as a novel biomarker signature for AD patients.

***Keywords*:** Alzheimer's Disease, miRNA, Amyloid-beta, Biomarker

P158. EVALUATION OF TRACE ELEMENTS AND OXIDANT/ANTIOXIDANT STATUS IN GENETICALLY ABSENCE EPILEPTIC RATS

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Abstract

Epilepsy is characterized by recurrent seizures, which has been shown to increase in productionof reactive oxygen species in brain.Oxidative stress can occurs as a consequence of prolonged epileptic seizure and may contribute to seizure-induced brain damage.It is known that oxidative stress and distribution of trace elements play an important role in pathophysiology of epilepsy.The aim of this study was to investigate the levels of zinc(Zn),copper(Cu),iron(Fe),superoxidedismutase(SOD) and catalase(CAT) in brain cortex suffered absence epileptic seizure in WAG/Rijrats. 2weeks old,1, 3and 6-mo old female WAG/Rij(n=28)rats were used in study.The levels of trace elements in brain were analysed by Spectroblue ICP-OES.CAT and SOD activities in brain were measured by biochemical methods inall study groups.The brain activities of SOD and CAT in6- mo old WAG/Rij rats were found to be significantly higher than1-mo old WAG/Rij rats.The levels of Fe and Cu in6-mo old WAG/Rij group were found to be significantly higher than the 2weeks old,1 and 3-mo old WAG/Rij rats.However, the brain levels of Zn in6-mo old WAG/Rij group were found to be significantly lower than the 2weeks old and 1-mo old WAG/Rijrats.In this study, we demonstrated the presence of significantly changed levels of trace element and antioxidant enzymes activities in seizure-induced oxidative stress.

***Keywords*:** Epilepsy, WAG/Rij rat, Zn, Cu, Fe.

P159. THE EXPRESSION OF FETUIN-A IN BRAIN TISSIUES OF WAG/RIJ RATS, GENETIC RAT MODEL OF ABSENCE EPILEPSY

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Abstract

Epilepsy is a widespread chronic neurological disorder affecting about 50 million people worldwide with epilepsy prevalence ranging from 0,2%-4,1% [1,2]. It is characterized recurrent seizures that causes neuronal activity in the brain.Lately, several studies have reported that epileptic seizures contribute to increase inflammatory mediators plasma cytokine levels in animal models as well as in human patients with epilepsy[3].Fetuin-A as a negative acute phase reactant, is involved in many inflammatory processes by inhibiting pro-inflammatory cytokine production [4,5].However, the relationship between epilepsy and Fetuin-A had never been studied yet.In this study, we aimed to investigate the expression of Fetuin-A in an age dependent manner in absence epilepsy. 1, 3 and 6-months-old female WAG/Rij(n=21) rats were used in our study. All animals were decapitated using transcardiac perfusion. The cortex and thalamus was dissected from brains of WAG/Rij rats in all group.Fetuin-A protein expression levels were assessed with Western blot. Western blots were performed using standard techniques at 1 month old WAG/Rij rats, 3 and 6, respectively.p values <0.05 accepted as statistically significant. In conclusion, we obtained no statistically differences of Fetuin-A protein expression levels in 1,3 and 6-months-old rats.Therefore this study shows that Fetuin-A is not effective in the pathophysiology of absence epilepsy.

Keywords: Fetuin-A, absence epilepsy, Wag/Rij rats.

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P160. Screening for mutations in *PSEN1* gene and *APOE* genotyping in patients with Alzheimer's disease.

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Abstract

Aim: The aim of this study is screening for mutations in *PSEN1* gene and determining *APOE* genotype in patients with Alzheimer's disease. **Methods:** *PSEN1* gene coding regions were screened by using DNA sequence analysis in 30 late onset of Alzheimer's disease (LOAD) cases diagnosed based on DSM-IV criteria and 40 non-dementia controls. Additionally, genotype and allele frequencies of ε2, ε3 and ε4 polymorphisms of *APOE* gene were determined by using PCR-RFLP methods in both groups. **Results:** We did not find any mutations in the coding regions of *PSEN1* gene. On the other hand, rs165932 (G / T) polymorphism was found in intron 8 of *PSEN1* in 26 patients. There was no substantial difference in genotype and allele frequencies of intronic polymorphism between control group and patients ($P > 0.05$). The frequency of ε3/ε4 genotype was substantially higher in patient group ($P < 0.05$) while frequencies of ε4 allele were substantially higher among the patients with LOAD ($P < 0.05$). **Conclusions:** We suggested that there was a potential association between LOAD and *APOE* ε4 allele, there was no association between *PSEN1* gene polymorphism and disease pathogenesis.

Keywords: Alzheimer's disease, polymorphism, mutation, *PSEN1*, *APOE*.

P161. Immunoregulatory and Inflammatory microRNA Expression Signatures are Altered In Subacute Sclerosing Panencephalitis

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Abstract

Subacute sclerosing panencephalitis (SSPE) is a chronic, progressive disease caused by a persistent infection of the measles virus. Despite the attempts to identify the underlying molecular mechanisms of neurodegeneration in SSPE, the exact mechanism of neurodegeneration remains obscure. Although many factors are emphasized in SSPE pathogenesis, the exact mechanism of neurodegeneration remains unknown. MicroRNAs are essential for normal immune system development, and alterations in microRNA expression levels have been linked to various chronic inflammatory disorders. Thus, our aim was to analyze immunoregulatory and inflammatory microRNA expression levels in SSPE. For this purpose, we used quantitative real-time PCR to analyze microRNA expression levels in SSPE patients (n=39) and healthy age-matched controls (n=41). Our results showed that miR-146a, miR-155, miR-181a, miR-196b, miR-296-5p, and miR-431 expression levels were significantly higher in SSPE patients compared to healthy controls. Of note, miR-155 expression was significantly higher in Stage 3 SSPE patients. Overall, altered miRNA expression signatures may serve useful to identify the molecular mechanisms underlying SSPE pathogenesis, and can serve as potential biomarkers for SSPE.

***Keywords*:** Subacute sclerosing panencephalitis, microRNA, biomarker, inflammation.

P162. SIK2 Involvement in Müller Cell Transdifferentiation

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Abstract

Müller cells are the radial glia of the retina. A common multipotent retinal progenitor gives rise to all neurons and Müller glia. Müller cells proliferate, dedifferentiate and produce new neurons upon injury and they are thought to be the source of injury-induced neurogenesis. The regenerative potential of Müller glia is evolutionarily conserved, however, the efficiency of regeneration is not. Compared with fish, the ability of Müller cells to transdifferentiate into neurons in mammals is limited. Limited transdifferentiation ability might stem from non-neurogenic environment of the mammalian retina[1]. Mammalian Müller glia transdifferentiate more efficiently when cultured in the presence of growth factors. MIO-M1 cells, a spontaneously immortalized Müller cell line isolated from human retina, express progenitor and neuronal markers with FGF2 treatment[2]. We aim to investigate the involvement of SIK2, which is proposed to regulate Erk1/2 activation and Müller proliferation under short term FGF2 treatment, in MIO-M1 transdifferentiation. Increased numbers of proliferating cells were observed after 1 day of FGF2 treatment. 22% increase in Pax6 expressing cells was observed after 4 days. Vimentin expressing cell population decreased 19% and 10% decrease in SIK2 expression was detected after 7 days. In the same time frame 30% increase in calretinin expression was evident.

***Keywords*:** Müller Cells, Transdifferentiation, FGF2, SIK2

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P163. Whole Exome Sequencing Analysis in Recessive Hereditary Spastic Paraplegia Patients from Turkey

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Abstract

Hereditary Spastic Paraplegia (HSP) is a neurodegenerative disorder, characterized with lower limb spasticity and progressive weakness. Inheritance can be autosomal dominant, autosomal recessive, or X-linked. There are 41 genes related to autosomal recessive HSP (ARHSP) showing the genetic heterogeneity of HSP. Whole exome sequencing (WES) was performed for one affected individual from each of six ARHSP families from Turkey to determine causative mutations in the known or novel HSP genes. GEM.app[1] was used for variation filtering and Sanger sequencing was performed for segregation analysis. In family H52, a C.901G>A (p.Arg301Gly) variation was determined in a novel HSP gene, *KIF1C.* A c.2239G>A (p.747R>W) mutation is identified in another novel HSP gene, in family H53. In two further families, mutations were identified in two known HSP genes: a c.325_326insTGTC insertion in *ALS2* (family H59) and a c.4321C>T (p. A1394X) variation in *Spastizin* (family H61). For family H65, a c.405G>T (p.W135C) variation in GJC2 gene was chosen as candidate and segregation analysis is being performed. WES data of the last family H57 is still under investigation. This small cohort greatly represent the clinical and genetic heterogeneity of the HSP that is increased with the two novel HSP genes identified in this study.

***Keywords*:** Hereditary spastic paraplegia, HSP, whole exome sequencing, WES.

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P164. EFFECTS OF YC-1 ON SELENıUM LEVELS AND OXIDANT/ANTIOXIDANT SYSTEM PARAMETERS IN BRAIN TISSUES OF PENTYLENETETRAZOLE-INDUCED EPILEPTIC RATS

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Abstract

Epilepsy,one of the neurological conditions,is known that oxidative stress and certain trace element balance play an important role in it's pathophysiology,and certain antioxidant treatment strategies for seizure have been proposed.YC-1,a synthetic bezylindazol derivate 3-(5-hydroxymethyl-2-furyl)-1-benzyl-indazole(YC-1),that has been demonstrated to have neuroprotective effect on neurons.The aim of the study was to evaluate the effects of YC-1 on the levels of lipid peroxidation(MDA), nitric oxide(NO), reducted-glutathione(GSH) and selenium(Se) in brain cortex in a single-dose pentylentetrazole(PTZ)-induced epilepy model. 28 Wistar rats were divided into four groups:Group1-received PTZ(55 mg/kg,i.p.) and YC-1(1mg/kg,i.p.),Group2-single dose PTZ-recevied rats,Group 3-YC-1(1mg/kg,i.p.) as control group and Group4-0.1%DMSO(vehicle) treated group.Se levels in brain were determined by Spectroblue ICP-OES.MDA,NO and GSH levels in brain tissues were determined by biochemical methods in all study groups.The brain levels of MDA in group2 were found to be significantly higher than the group1 and 4. Furthermore, the levels of NO in group2 brain were found to be significantly higher than the group1.In contrast the brain Se levels were significantly lower in group2 than in the group1,3 and 4.In conclusion, YC-1 administration caused protection against the single dose PTZ-induced brain oxidative toxicity by inhibiting reactive oxygen species production,and supporting selenium element homeostasis and oxidant/antioxidant redox system on epileptic seizures.

Key Words: Epilepsy, YC-1, Se, MDA, NO

P165. Effects of Iron Ions (Fe^{3+}) on Expression Levels of Synapsin III, Syntaxin IV, Synaptogyrin I and Synaptogyrin IV Genes in RAT (*Rattus norvegicus*) Brain Hippocampus and Cortex

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Abstract

More iron that can be used by metabolism has a toxic effect. Degeneracy of normal iron metabolism makes brain tissue vulnerable to oxidative stress damage. A lot of evidences show that transition metal ions like iron, zinc and copper do not have a strong effect on neurodegenerative sicknesses. At present, there are many findings available which give impression that alzheimer, parkinson and schizophrenia result from defects in that synaptic transmission. In this thesis study, iron ions, given orally to Rattus norvegicus, are examined by comparing expression levels of Synapsin III, Syntaxin IV, Synaptogyrin I and Synaptogyrin IV which undertake important roles in synaptic transmission in the hippocampus and cortex tissues of those animals with expression of GAPDH gene. The changes at Stx4 gene expression level in cortex tissue were found significantly. The reduction occuring at Stx4 gene expression levels in hippocampus tissue was found significantly. The increase at Syngr1 expression levels in cortex tissue and Syn3 gene in cortex and hippocampus tissue was found significantly. It was observed that there was significant changes Syngr4 gene expression levels in cortex and hippocampus tissue.

P166. WHOLE GENOME SEQUENCING OF 16 TURKISH GENOMES REVEALS FUNCTIONAL PRIVATE ALLELES AND IMPACT OF GENETIC INTERACTIONS WITH EUROPE, ASIA AND AFRICA

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Turkey is a crossroads of major population movements throughout history and has been a hotspot of cultural interactions. Several studies have investigated the complex population history of Turkey through a limited set of genetic markers. However, to date, there have been no studies to assess the genetic variation at the whole genome level and without ascertainment bias. Here, we present whole genome sequences of 16 Turkish individuals resequenced at high coverage (32x-48x). Our study provides the first map of common genetic variation in western Asia. We show that the genetic variation of the contemporary Turkish population clusters with South European populations, as expected, but also shows signatures of relatively recent contribution from ancestral East Asian populations. In addition, we document a significant enrichment of non-synonymous private alleles, consistent with recent observations in European populations. A number of variants associated with skin color and total cholesterol levels show frequency differentiation between the Turkish populations and European populations. Variations in the Human Leukocyte Antigen (HLA) loci between different regions of Turkey are also detected. Furthermore, we have analyzed the 17q21.31 inversion polymorphism region(*MAPT* locus) and found increased allele frequency of 31.25% for H1/H2 inversion polymorphism when compared to European populations which shows about 25% of allele frequency. Our data will help develop population-specific experimental designs for studies investigating disease associations and demographic history in Turkey.

P167. The association between the IVS4+44C>A polymorphism in the DMT1 gene and myocardial infarction risk

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Abstract

Iron is physiologically essential despite of dangerous element for the metabolism. Iron catalyzes production of free radicals inducing oxidation of lipids production. Therefore, iron overload may increase the risk of myocardial infarction through lipid oxidation [1,2]. It has been demonstrated that high iron accumulation in the body causes 2.2-fold risk factor of acute myocardial infarction [3]. The divalent metal transporter-1 (DMT1) is a protein that functions in transport of ferrous iron and some divalent metal ions across the plasma membrane and/or out of the endosomal compartment. It has been reported that functional polymorphism in the DMT1 gene may lead to excessive iron storage and iron overload [4]. We aimed to investigate the association between the IVS4+44C>A polymorphism in the DMT1 gene and myocardial infarction risk. Genomic DNA isolated from peripheral leukocytes of the 100 patients with myocardial infarction and 100 controls. Genotyping was performed using the PCR-RFLP method. Comparisons of the groups were analyzed by chi-square tests. There was no statistically difference in the frequencies of the IVS4+44C>A genotypes (χ^2=0.32, p=0.57) and alleles (χ^2=0.28, p=0.59) between healthy controls and patients with myocardial infarction. Our findings suggest that the IVS4+44C>A polymorphism is not associated with myocardial infarction in Turkish population. If the increased number of patients with familial myocardial infarction and controls are used, different results may be obtained.

***Keywords*:** Divalent metal transporter-1, IVS4+44C>A polymorphism, Myocardial infarction

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Others

P168. ASSOCIATION BETWEEN NPY GENE POLYMORPHISMS AND ALCOHOL DEPENDENCE

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Abstract

Background: *NPY* is one of the candidate genes responsible for alcohol dependence. This gene encodes a neuropeptide that is widely expressed in the central nervous system and influences many physiological processes. Previous studies in the different populations showed that rs16139 (T/C) and rs16147 (T/C) polymorphisms in the *NPY* gene were associated with alcohol dependence.**Aim:** To determine relationship between *NPY* gene polymorphisms and alcohol dependence in our population. **Methods:** rs16139 (T/C) in exon 2 and rs16147 (T/C) in the *NPY* promoter region were screened using PCR-RFLP method in 192 patient and 111 control subjects. rs16147 amplicon was digested with *CviK-I* enzyme and rs16139 amplicon was digested with *BsrI* enzyme. Results were confirmed in randomly selected 50 samples for each polymorphisms by using DNA sequencing.R**esults:** The distribution of each of the genetic variants met the conditions of the Hardy–Weinberg equilibrium. For *NPY* rs16139 polymorphism, there were no significant differences between groups ($p<0.005$). For *NPY* rs16147 polymorphism; genotype frequencies were found different between the groups ($p<0,05$).**Conclusion:** It was shown that rs16147 polymorphism was associated with alcohol dependence in our population. Our results which shows relationship between NPY gene polymorphisms and alcoholism are important in terms of being first study in Turkish population.
***Keywords*:** Alcoholism, polymorphism,*NPY*

P169. Exogenous selenium mitigates osmotic, heat stress and their combination toxicity by up-regulation of antioxidant enzymes in *Zea mays* roots

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Abstract

The aim of study was to elucidate the influence of exogenous application of selenium (Se) in alleviating detrimental effects of osmotic stress (PEG), heat (H) and their combination in *Zea mays* cultivars (Ossk-713 and Safak). Three-weeks-old maize seedlings were treated with 25% PEG-6000 and heat (44^{o}C) with/without Se (5 and 15 mM) treatments for 8 h. Both individual and combined used stress decreased growth rate (RGR) and osmotic potential (Ψ_{π}). Moreover, a significant increase in proline (Pro) and hydrogen peroxide (H_2O_2) contents was observed under the stress treatments. Although superoxide dismutase (SOD) increased with stress, superoxide anion radical increased at all the stress treatments. Also, Ossk and Safak had higher H_2O_2-scavenging enzyme activities in PEG-treated roots, including activities of catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX). However, only POX activity was induced in both cultivars with H stress. Therefore, the enhanced levels of lipid peroxidation (TBARS) during stress were more pronounced under H stress than PEG. Also, the combination of PEG and H caused a higher accumulation of H_2O_2 and TBARS. However, exogenous application of Se mitigated significantly PEG and/or H-induced stress injury. Under PEG stress, the oxidative damage which was measured as TBARS content was lesser in Se-applied maize roots that were associated with greater activities of SOD, APX and glutathione reductase (GR). On the other hand, the exogenous application of Se in the presence of H stress improved the activities of SOD and CAT at both cultivars. As well as the level of antioxidant enzymes, Se application under stress enhanced RGR and Ψ_{π}. According to these findings, it is possible to say that Se could be used to alleviate the damaging effects of PEG and/or H stress (especially in PEG) by improving antioxidative system in roots of maize cultivars.

Keywords: Antioxidant enzyme; Heat stress; Osmotic stress; Selenium; *Zea mays*

P170. Exogenously applied selenium in *Zea mays* subjected to osmotic, heat and their combination: Effects on the water status, osmotic adjustment and carbonhydrate accumulation

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Abstract

The aim of our study was to examine whether exogenously applied with selenium (Se) enhances the tolerance of maize cultivars to polyethylene glycol (PEG)-induced osmotic stress and/or heat (H) stress. After two maize cultivars, Ossk-713 and Safak, were hydroponically-grown for three weeks, seedlings were treated with 25% PEG-6000, heat (44^{o}C) and their combination with/without Se (5 and 15 mM) for 8 h. PEG had greater reduction in growth rate (RGR) than that of H stress in both cultivars. Also, PEG and H stress caused a reduction in osmotic potential (Ψ_{π}) and water content (RWC). The both stress treatments decreased the maximum photochemical efficiency (F_v/F_m), the photochemical quenching coefficient (qP) and the actual quantum yield (Φ_{PSII}), even more pronounced at PEG in Ossk. Also, a notable increase in lipid peroxidation (TBARS) was observed with PEG, H stress and their combination. The sensitivity of plants to stresses was higher in Ossk than in Safak. Proline (Pro), glycinebetaine (GB) and choline (Cho) were induced to a greater extent by osmotic and H stress. There was no change in total carbonhydrate accumulation PEG-treated plants, but it decreased in H stress and PEG+H treatment. However, compared with the stress-treated plants alone, Se added to stress-treated maize cultivars significantly decreased TBARS and increased RGR, RWC, Ψ_{π}, F_v/F_m, qP and Φ_{PSII}. The carbonhydrate accumulation was also induced with Se plus stress treatments. It could be concluded that the Se concentrations alleviated PEG and H toxicity by increased the water status, photosynthetic efficiency and carbonhydrate accumulation in maize.

Keywords: Carbonhydrate accumulation; Lipid peroxidation; Osmotic adjustment; Osmotic stress; Selenium; *Zea mays*

P171. Identification of Salt Tolerance Genes in Sea Beet

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Soil salinity, excessive accumulation of salt in soil, is one of the agricultural problems that affect the crop yield through damaging germination, growth and fruit production. High concentrations of salt ions have three different effects on plants basically: Water stress, salt stress and ionic imbalance stress. Salt tolerant (halophytic) plants can develop many alternative survival pathways against salinity stress. In this project, we isolated and characterize salt tolerance genes in *Beta vulgaris* sp. plant by using functional genomics technique. For this purpose, Beta vulgaris cDNAs over expressed in yeast and screened under toxic salt condition. After screening, 11 colonies detected. Sequencing of these 11 cDNAs revealed different proteins: 1- S-Adonesyl methionine(SAM), 2- uridylate kinase 3- unknown function gene. We are trying to characterize and relate function of these genes and relationship with salt tolerance on Beta vulgaris sp.

P172. Transcriptional profile of the human prostate cancer cell line in response to a small nettle (*Urtica urens*) chloroform sub-extract

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Alternative and complementary therapies are frequently used among patients not only in Turkey but also all over the world. Therefore, popularity of herbal preparations as alternative and complementary therapies has been ever growing. Although herbal extracts are suggested to have benefits, they have some risks for human health as well. Therefore, effects of this extracts on all of the biological activities should be determined in order to analyze both beneficial and side effects. Although *Urtica urens L.* (Small Stinging Nettle) is one of the most commonly used plants in alternative and complementary treatment of cancer patients in Turkey, the literature on its biological activities are limited. In this respect, this study is undertaken to evaluate the potential effects of chloroform sub-extract of small nettle in different cellular pathways by whole-genome transcriptome analysis. For this purpose, first, chloroform sub-extract of the dried stinging nettle leaves was prepared by the soxhlet method. The cytotoxicity of this sub-extract in LNCaP (human prostate cancer cell line) cells was investigated using WST-1. It was found that EC50 of the sub-extract was 70 mg/ml. This dose was applied to cells for 48 hours and then cells were harvested. cRNA samples were prepared, and whole genome transcriptome analysis was performed by using Human HT-12 V4 BeadChip in Illumina iScan platform. Results of Transcriptome analysis showed that 847 different probes were significantly changed ($p<0.05$). Pathway analysis revealed that the sub-extract altered a group of genes involved in DNA replication, repair and division, activation of NFkB by phosphorylation and activation of IKKs complex, energy metabolism, gastrin-CREB signaling pathway via PKC and MAPK, regulation of Hypoxia inducible Factor (HIF), GPCR ligand binding and downstream signaling. These results provide a comprehensive view of the changes in gene expression when prostate cancer cells was exposed to urtica extract, and shed light on its complicated effects on these cells.

This work is supported by TUBITAK 111T515.

Key words: whole genome transcriptome, *Urtica urens*, Human Prostate Cancer Cell Line (LNCaP), pathway analysis.

P173. Cytotoxicity of Cetuximab in Lung Cancer Cell Lines Expressing Different Level of EGFR

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Abstract

This study aimed to assess the cytotoxic effect of cetuximab (C225, Erbitux_, a chimeric anti-epidermal growth factor receptor (EGFR) monoclonal antibody) in vitro on cancer cell lines (P-H1299, R-H1299, A549, A431) expressing different levels of EGFR. The cytotoxicity of cetuximab were evaluated with 2 in vitro test which are CellTiter-Blue^{R} Cell Viability Assay and Lactate dehydrogenase (LDH) assay. R-H1299 cells viabilty were found higher than the other cells against cetuximab's cytotoxicity at IC_{50} cetuximab concentration for 72 hours, while A431 cells were found the most sensitive cells. The lactate dehydrogenase activity of cells were found to order of A431> P-H1299> A549>R-H1299. R-H1299 cell had the highest resistant against antitumor activity of cetuximab in all cells.

Cytotoxic effect of cetuximab changed depends on cetuximab concentration, EGFR expressing capacity and drug resistant properties of cells.

P174. The degree of oxidative damage and antioxidative changes due to exposure to boron deficiency and toxicity in *Arabidopsis thaliana*

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Abstract

Till now, the possible oxidative damage and protective mechanisms such as antioxidative system following boron (B) toxicity and deficiency conditions have not been satisfactorily elucidated in *Arabidopsis thaliana* as a model organism. For this purpose, we measured contents of malondialdehyde (MDA), H_2O_2, free proline, flavonoid, anthocyanin, total soluble and insoluble sugars as well as activities of catalase (CAT) and glutathione reductase (GR) under 0, 1 and 3 mM H_3BO_3 conditions. The levels of proline, total soluble and insoluble sugars increased significantly under only 3 mM B whereas flavonoid and anthocyanin contents were elevated during both 1 and 3 mM B. However, MDA content increased at 1 mM B but not at 3 mM B. Conversely, 3mM B caused a significant increase in H_2O_2 content whereas it was stable in 1mM B. Coordinately, CAT activity was remarkably higher at 1 mM B than respective controls. Interestingly, GR activity was steady under all B conditions. On the other hand, B deficiency did not cause any significant change in all parameters as mentioned above. In conclusion, these results suggest the occurence of oxidative damage and respective antioxidative response in excess B but not in B deficient condition in *Arabidopsis*.

Keywords: Antioxidant enzymes, Arabidopsis thaliana, boron deficiency, boron toxicity, oxidative stress

P175. THE EFFECT OF GERANIOL ON LIVER REGENERATION AFTER HEPATECTOMY IN RATS

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There are many studies about the protective effects of Geraniol on liver. in our study, after %70 partial hepatectomy on rats, geraniol's effect on liver regeneration was demonstrated in vivo.

For this purpose, by using wistar albino type male rats, 9 groups were formed by arranging as n=10. While Group I is the control group, remaining 8 groups received single dose saline, silymarin and geraniol intraperitonel injections separately and immediately after the surgical operation. While on groups II and III only laparotomy was performed, on Groups IV, V, VI, VII, VIII and IX %70 partial hepatectomy was performed on animals in addition. 24 and 48 hours after the surgical operations, for biochemical analysis, blood serum samples were taken and their ALT values were examined.

According to the data we've obtained, Geraniol has a significant role during the regeneration process which has started with the liver hepatocyte proliferation related to the TNF-α and IL-6 48 hours after the partial hepatectomy.

Keywords: Geraniol, Partial Hepatectomy, Liver Regeneration, TNF-α, IL-6

P176. PURIFICATION AND CHARACTERIZATION OF GLUTATHIONE S- TRANSFERASE FROM LAUREL FRUİT *(Prunus Laurucerasus)* RESEARCH THE EFFECTS OF SOME PESTICIDES ON ENZYME ACTIVITY

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In this study; the glutathione S-transferase (EC 2.5.1.18) was prufied and characterized from the laurel fruit. Purification was performed in one step by affinity chromatography [1]. SDS-polyacrylamide gel electrophoresis was applied for control of the glutathione S-transferase. In kinetic studies on the enzyme; optimum pH, optimum temperature, optimum ionic strength, Km and Vmax for glutathione (GSH) and 1- chloro 2,4- dinitrobenzene (CDNB) studies were carried out [2,3]. Km and Vmax values for GSH and CDNB 0,194 mM, 0,038 EU/ml and 0,353 mM, 0,099 respectively. The effect of pesticides were researched on enzyme activity. Of these pesticides; diklorvos, cypermethrin, imidacloprid, fenoxaprop-p-ethyl, glyphosate isopropilamine salt's to exhibit inhibitory properties; haloxyfop-P-methyl, lambda-cyhalothrin and 2,4-dichlorophenoxyasetic acid dimethylamine salt's were found to act as the activator.

Keywords: Glutathione S-Transferase, glutathione, inhibition, purification, laurel fruit

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P177. The influence of IFN-gamma-induced myeloid maturation on ICOS-L expression

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T-cell activation proceeds by the support of costimulatory molecules. ICOS-L, the ligand for inducible costimulatory (ICOS) receptor, is generally found on myeloid cells [1]. Here, expression kinetics of ICOS-L in myeloid cells whose activation and maturation was simultaneously induced with IFN-γ treatment was evaluated. AML cell lines (HL-60, U937, THP-1) of different maturation stages and peripheral blood monocytes (PB-mono) were used. Monocytic maturation marker CD14 and ICOS-L expression was determined by flow cytometry. The changes in ICOS-L mRNA expression were also assessed by real-time RT-PCR. ICOS-L levels were inversely correlated with myeloid cells' maturation state (HL-60>THP-1>U937>PB-mono). Myeloid cell lines, especially HL-60, showed a gradual increase in CD14 expression upon treatment with IFN-γ. Except for HL-60, both protein and mRNA levels of ICOS-L were increased (especially at 8-16h). Prolonged exposure to this inflammatory cytokine decreased the ICOS-L gene expression in all cell lines to a certain extent. Alternatively, only a slight modulation at mRNA levels was observed in HL-60 whose maturation was explicitly induced upon IFN-γ treatment. In conclusion, ICOS-L mRNA is promiscuously found in myeloid cells; whereas, its expression was inversely correlated with the maturation stage. This modulation in ICOS-L expression might have implications for the regulation of immune responses.

Keywords: leukemia, ICOS, ICOS-L, T-cell activation

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Cancer Biology

P178. ARID3B Expression in Breast Cancer Cells

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Abstract

ARID3B (AT-rich interaction domain 3) is a member of the ARID proteins that are evolutionarily conserved transcription factors implicated in development, differentiation and cell growth. ARID3B has been linked to immortalization, mesenchymal transition, developmental processes and tumorigenesis. We examined ARID3B expression in breast cancer cells.

Nuclear, cytoplasmic and low levels of membranous ARID3B staining were detected in 63 invasive breast tumors. Nuclear ARID3B staining positively correlated with estrogen receptor status and negatively correlated with tumor grade, mitotic index and ERBB2 status of patients. Increased nuclear abundance of ARID3B was confirmed breast cancer cell lines expressing ERα vs ER (-) cells. In addition, 2 out of 3 ERBB2 positive breast cancer cell lines lacked full length ARID3B. 3 other isoforms of ARID3B were shown to be present in normal breast and in breast cancer cells.

We report a positive correlation between ER positivity and nuclear ARID3B expression in breast cancers along with a negative correlation with the ERBB2 status. Similar correlations were verified in breast cancer cell lines. ARID3B levels has emerging roles in relation to cancer associated proteins and microRNAs. Therefore understanding ARID3B expression and functions may shed better light on major molecular interactions and potentially important cancer related networks.

***Keywords*:** ARID3B, breast cancer, ER, estrogen receptor, immunohistochemistry

Cancer Biology

P179. 3'UTR Alterations in ER(+) Breast Cancers

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Alternative polyadenylation (APA) generates transcript isoforms with different 3'UTR lengths due to the position of poly(A) tail [1]. This difference may alter the stability and location of mRNA isoforms in cell [2]. APA has been observed in various cellular states: Preferential use of distal poly(A) sites occurs during development [3]. However, in proliferative normal and/or cancer cells, a global 3'UTR shortening pattern is observed [4]. In this study, we aim to investigate the 3'UTR alterations in estrogen receptor (ER)-positive breast cancer patients and cell models. Publicly available microarray datasets were analyzed by a probe-based microarray analysis tool (APADetect). Based on the means of proximal to distal poly(A) site ratios of individual transcripts, the SLR (short-long ratio) was calculated as indicator of short vs. long 3'UTR abundance. Significance Analysis of Microarrays determined significant 3'UTR shortened or lengthened genes. These genes were further analyzed *in silico* to reveal 3'UTR alterations in ER(+) breast cancer patients compared to normal breast samples. Our results suggest involvement of APA in ER(+) breast cancer. In our analyses, 32 and 35 genes were commonly 3'UTR shortened and lengthened, respectively. We plan to further investigate these APA events experimentally to further understand APA and its consequences in breast cancer.

Keywords: Alternative polyadenyletion, 3'UTR, estrogen receptor, breast cancer

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Topic: Biotechnology, Microbiology

P180. Insecticidal Activity of Recombinant Cry1Ab Toxin on Mediterranean Flour Moth, *Ephestia kuehniella* larvae

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Abstract

Bacillus thuringiensis (Bt) is a gram-positive soil bacterium producing crystal inclusions during sporulation. Cry proteins of *Bt* exert insecticidal activity on agricultural and forest pest insects from different orders. Commercial products of *Bt* are used as biocontrol agents for many years. This bacterium carries more than one *cry* gene on its plasmids. Larvicidal activities of their proteins may differ from each other. In the present study, to determine individual activity of *cry1Ab* gene from *Bt* SY49-1 strain, recombinant pET-*cry1Ab* was transferred into *E. coli* and expressed to search for its activity on *E. kuehniella* larvae. *E.coli BL21(DE3)* carring recombinant pET-*cry1Ab* was induced with IPTG and resulting proteins are used in bioassays at the doses of 10, 25, 50, 100, 250, 500 and 1000µg/g. The dead larvae were recorded during ten days. Mortality rates increased depending on the doses applied. It was conluded that Cry1Ab protein of native *Bt* SY49.1 strain effectively controlled the larvae of *E. kuehniella*.

Key words: *B. thuringiensis* SY49-1, *cry1Ab,* clonning, Cry protein, larvae

Acknowledgement: This study was supported by the Erciyes University Scientific Project Unit under the codes of FBD11-3634 and ÖNAP-3638, and also funded by Bilim, Sanayi ve Teknoloji Bakanlığı (TGSD-0802).

Topic: Biotechnology, Microbiology

P181. Cloning and Expression of *cry2A* Gene from *Bacillus thuringiensis* SY49-1 Strain and Its Toxicity on *Culex pipiens* (Diptera) Larvae

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Abstract

Mosquitos suck blood from humans and many vertebrate animals, and also are the vectors of several important diseases. Although Chemical control is commonly used woldwide in controlling mosquitos, biological control agests such as bacteria, viruses, and fungi were also used in controlling these pests. House mosquito (*Culex* spp.) is the most common mosquito species and various formulations of Cry proteins of *Bacillus thuringiensis* is applied in combating this pest. In this study full sequence of *cry2A* was amplified. PCR products were cut with the *EcoRI* and *SalI* restriction enzymes. The pET28a(+) expression vector to be used in clonnig process were also cut with the same restriction enzymes and sticky ends were obtained. The recombinat pET28a(+)-*cry2A* plasmid DNA was obtained after ligation process. pET28a(+)-*cry2A* plasmids were then transferred to *E.coli BL21(DE3)*, made competent with $CaCl_2$. After transformation process, the colonies carring recombinant pET28a(+)-*cry2A* plasmid DNA were induced with IPTG to produce Cry2A protein. The isolated total protein were then lyophilized and used at 50µg/ml 100µg/ml, 250µg/ml and 500µg/ml doses against the *Culex pipiens* larvae. The activity of the toxins were screened for ten days. Although the 500µg/ml dose of *Bt* SY49-1 spore crystal mixture caused 97% mortality, the recombinat *cry2A* had 40% mortality rate at the same dose. The results indicated that the high influence of the *Bt* SY49-1 may result from combined effects of spores and several *cry* gene products, including Cry2A.

***Key words*:** *B. thuringiensis* SY49-1, *cry2A,* Cloning, Cry protein,,*Culex pipiens*

Acknowledgement: This study was supported by the Erciyes University Scientific Project Unit under the codes of FBD11-3634 and ÖNAP-3638, and also funded by Bilim, Sanayi ve Teknoloji Bakanlığı (TGSD-0802).

Topic: Biotechnology

P182. Molecular Characterization of Two Entomopathogenic Nematodes and Their Bioactivity on Pine Processionary Moth, *Thaumetopoea wilkinsoni* (Lepidoptera) Tams

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Abstract

Two new entomopathogenic nematode strains (SK-17 and SK-71) were isolated and characterized from soil samples in Adana and Kahramanmaraş regions of Turkey. 28S rDNA region were sequenced (577bp for isolate SK-17 and 575bp for isolate SK-71) and phylogenetic trees constructed. BLASTN homology searches and phylogenetic tree results indicated that SK-17 and SK-71 isolates were belong to *Steinernema afine* and *Steinernema feltiae* species, respectively. Increasing mortality effects of strains on *T. wilkinsoni* larvae were recorded upon using IJs at laboratory conditions. *S. affine* and *S. feltiae* strains caused 80 and 90% mortality at 100 IJs/larvae in foliar treatments, respectively. Complete mortality was achieved using 500 IJs for each strain. On the other hand, soil treatments indicated that *S. affine* and *S. feltiae* strains caused 30 and 33.34% mortality at 80 IJs/cm^2, respectively. It was concluded that these strains were promising in controlling forest pest *T. wilkinsoni.*

***Keywords*:** Phylogenetic analysis, *Stenernema affine*, *Steinernema feltiae,* biological control, *Thaumetopoea wilkinsoni*

Acknowledgements: This study was supported by the Erciyes University Research Foundation (FBD-10-3267)

Topic: Biotechnology, Microbiology

P183. Controlling *Plodia interpunctella* (Hübner) (Lepideptera: Pyralidae) with Recombinant Cry1A Protein

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Abstract

Plodia interpunctella (Indian meal moth) larvae fed by spreading on their food environment and cause product damage. In addition, they impair the quality of the product through leaving impurities, remains of molting and the head capsule. Their primary damage is on stored products such as fig, raisin, chestnut, walnut, hazelnut, pistachio, peanut, almond, sesame, sunflower, oilseeds, cereals, cocoa, milk powder, spices, and locust. It is well known that chemicals used to control the pest brings serious negativities on human health and environment. Many alternative methods are developed and used to eliminate or reduce those harmfull effects. In an alternative way environmentally friendly *Bacillus thuringiensis* (*Bt*) *cry* genes can be cloned and expressed in a host for obtaining desired toxin for controlling a spesific insect species. By this way non taget organisms are saved and their environmental benefits are preserved. In the current study, Lepidopteran spesific *Bt* Cry1A protein is used to control the *P. interpunctella* larvae. For this purpose *cry1A* gene from native *B. thuringiensis* strain SY49.1 was cloned into *E.coli* BL21(DE3) host and on expressing the desired gene its opportunity for controlling *P. interpunctella* larvae was investigated. Results indicated that recombinant Cry1A protein exerted 40% mortality (LC_{50}:1.320 mg/g) on *P. interpunctella* larvae at 1mg/g.

Keywords: Gene cloning and expression, Recombinant Cry1A protein, *P. interpunctella*

Acknowledgement: This study was supported by the Erciyes University Scientific Project Unit under the codes of FBD11-3634 and ÖNAP-3638, and also funded by Bilim, Sanayi ve Teknoloji Bakanlığı (TGSD-0802).

Cancer Biology

P184. Understanding the Structural Interactions between Androgen Receptor and Androgen Receptor Variants

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Abstract

The androgen receptor (AR) is critical for the progression of all stages of prostate cancer. Variants of the AR (ARV) can arise through alternative splicing of cryptic exons. Lacking a ligand-binding domain these modified nuclear receptors do not require androgens and are both constitutively active and intrinsically resistant to AR antagonists. Interestingly the expression of ARV's is markedly increased in during androgen deprivation treatment suggesting a potential role of these ARV's in late-stage prostate cancer. It has been previously shown that full-length AR requires both intramolecular and intermolecular interactions during transcriptional activation. Given that the ARV's lack the necessary domains to form these interactions it is unclear how they can initiate gene transcription. Therefore the goal of our ongoing studies is to reveal the structural interactions within ARV's or potentially between ARV and full-length AR. To characterize these interactions we have prepared molecular constructs of both AR and ARV fusion proteins that contain Venus and Cerulean at the 3' and 5' end. With these FRET pairs we are currently characterizing the mechanism of ARV activation using live-cell microscopy.

***Keywords*:** Castration Resistant Prostate Cancer, CRPC, FRET, nuclear receptor

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P185. *ARABIDOPSIS THALIANA* CARBOXYL-TERMINAL DOMAIN PHOSPHATASELIKE1 (CPL1) MEDIATES RESPONSES TO IRON DEFICIENCY

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Abstract

The expression of genes that control iron (Fe) uptake and distribution (i.e., Fe utilization-related genes) is under a strict regulation. Fe deficiency strongly induces Fe utilization-related gene expression; however, little is known about the mechanisms that regulate this response in plants. In this research, a RNA metabolism factor, RNA POLYMERASE II CTD-PHOSPHATASE-LIKE1 (CPL1) is shown to localize to the root stele, and to be involved in the regulation of Fe deficiency responses in *Arabidopsis thaliana*. An analysis of multiple *cpl1* alleles establishes that *cpl1* mutations enhanced transcriptional responses of Fe utilization-related genes, e.g. *IRON-REGULATED TRANSPORTER1* (*IRT1*), to low Fe availability. In addition to the lower Fe content in the roots, but higher Fe content in the shoots of *cpl1-2* plants, the root growth of *cpl1-2* shows improved tolerance to Fe deficiency. Genetic data indicates that *cpl1-2* likely activates Fe deficiency responses upstream of both *FE–DEFICIENCY-INDUCED TRANSCRIPTION FACTOR* (*FIT*)-dependent and -independent signaling pathways. Interestingly, various osmotic stress/ABA-inducible genes are up-regulated in *cpl1-2*, and the expression of some ABA-inducible genes is controlled by Fe availability. Overall, CPL1 functions as a negative regulator of Fe deficiency signaling at the crosstalk with a branch of osmotic stress/ABA signaling pathway.

***Keywords*:** iron, Arabidopsis, RNA metabolism, transcription, osmotic stress

Cancer Biology

P186. Alternative Polyadenylation Dependent 3'-UTR Shortening in Triple Negative Breast Cancer

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Abstract

Triple Negative Breast Cancers (TNBCs) are aggressive and highly proliferative tumors characterized by lack of estrogen (ER) and progesterone (PR) and human epidermal growth factor 2 (HER2) receptors [1]. Due to high proliferative indices of TNBCs; we hypothesized alternative polyadenylation (APA) to play a role in TNBC. APA has newly emerging roles in gene expression regulation by causing shortening or lengthening of 3'-UTRs (Untranslated Regions) [2]. To address 3'-UTR shortening events in TNBCs, a probe based meta-analysis tool (APADetect) was used. When we compared 520 TNBC and 82 normal breast tissue samples with APADetect, we detected 113 3'-UTR shortening events. After a detailed *in silico* analysis, we focused on candidate genes showing significant 3'-UTR shortening events. For further validation, first we confirmed the existence of short 3'-UTR isoforms by RACE. Later, we used RT-qPCR to analyze 8 TNBC cell lines and a panel of breast cancer patient cDNAs. Increased protein levels due to mRNA shortening in TNBC cell lines were shown by Western blotting. Our results, for the first time, show a possible role of APA and a significant shift to shorter 3'-UTR usage in TNBCs. In addition to gene expression analysis, identification of post-transcriptional regulations such as APA could improve current knowledge on the molecular etiology of TNBCs.

Keywords: Alternative polyadenylation, Triple negative breast cancer, 3'-UTR

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Stem Cell Research

P187. Alternative Polyadenylation Dependent 3'-UTR Shortening in Triple Negative Breast Cancer

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Abstract

Background: The recent discovery of cardiovascular stem/progenitor cells (CPC) in adult hearts as well as embryonic stem cell (ESC) cultures opened a new avenue for basic and translational research. Although, various CPC populations have been identified, molecular mechanisms in self-renewal and differentiation of these progenitors still remain to be resolved. In this study, we investigated signaling pathways in generation of heart muscle cells from a novel population of cardiovascular progenitors characterized by expression of Islet-1 (Isl1) transcription factor.

Methods and Approach: Isl1-expressing CPCs were isolated from ESC lines derived from a transgenic mouse, that allows for Isl1-CPC identification by GFP expression. GFP+ Isl1 progenitors were isolated by FACS-sorting and were stimulated with various signaling molecules. In order to characterize CPCs and their progeny at the molecular level, qRT-PCR and immunocytochemical analysis were employed. The electrophysiological properties of CPC-derived cardiomyocytes were determined by patch clamp analysis and by measuring calcium transients.

Results: Our results showed that purified CPCs had the ability to differentiate into cardiomyocytes, smooth muscle cells and endothelial cells. Isl1-CPC derived cardiomyocytes exhibited embryonic-like electrophysiological characteristics both in action potentials and calcium transients. Moreover, Bmp signaling lead to a robust enhancement of cardiomyogenesis in Isl1-CPCs through enhancing proliferation and preventing apoptosis. Furthermore, Bmp-mediated CPC differentiation resulted in upregulation of Tbx transcription factors.

Discussion: An important challenge for regenerative strategies for cardiac repair is to identify optimal cell source and to employ efficient derivation approaches. This study is the first to show that Bmp signaling induces cardiomyogenesis in a pure populations of cardiac progenitors. Further characterization of CPCs in the human system and Bmp-mediated tissue engineering techniques has the potential to enhance cardiac muscle formation for heart repair. To this end, ESC-derived cardiac progenitors and differentiated cardiac cells can provide a renewable and ideal source for basic research and for cell-based therapies.

Reference: Cagavi E., Bartulos O., Suh C., Sun B., Yue Z., Jiang Z., Yue L., Qyang Y., "Functional cardiomyocytes derived from Isl1 cardiac progenitors via Bmp4 stimulation" Plos One (Under revision).

Neuroscience

P188. Cell Proliferation in Zebrafish Brain

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Abstract

Adult brain cells were long thought to be incapable of proliferating but we now know that cells continue to be renewed throughout life. Many studies investigate cell proliferation zones that are active in the adult brain, in hope for understanding cognitive decline and age-related diseases. We are interested in changes during normal aging in females and males. Gender has a significant effect on differential gene expression [1], and we assessed samples by taking gender into consideration. In this study, we aimed to identify proliferating cells in the brain by using young and old, male and female zebrafish as models. We injected bromodeoxyuridine (BrdU) intraperitoneally and after four hours, we dissected the brains, fixed and obtained slices, which were then immunostained [2]. We cut through the telencephalon and studied the whole area in consecutive slices. This gives us an understanding of the entire telencephalon, which has corresponding areas to human brain regions such as hippocampus and amygdala. Findings of our study will enable us to build up a regeneration map of critical regions in the brain. Further, we aim to manipulate this processes of cell proliferation with non-genetic interventions, such as caloric restriction.

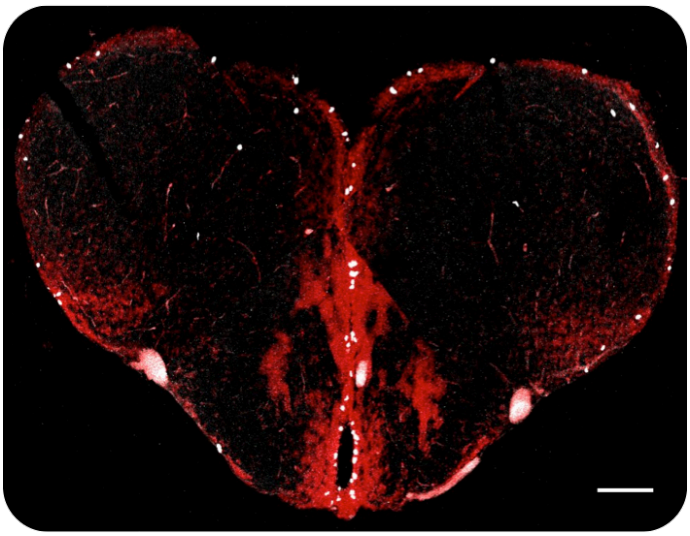

Figure 1. BrdU (white) and 7-AAD (red) labeling in zebrafish brain slice. Zeiss LSM 510 microscope, scale bar 100 uM.

***Keywords*:** Zebrafish, Brain, Neurogenesis

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P189. Functional Analysis of the Human ADAMTS-3 Gene Promoter

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ADAMTS-3 is the member of ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) gene family and plays an important role in the processing and biosynthesize of type II fibrillar collagen molecules [1-2]. Although procollagen processing activity of the ADAMTS-3 gene was studied, there isn't any study on its own transcriptional regulation. To elucidate the transcriptional regulation of the ADAMTS-3 gene a putative promoter region (1380 bp), and three truncated promoter constructs were cloned into luciferase based vector system to determine transcriptionally most active region. Transient transfection studies were performed to identify basal promoter activity in Saos-2 cell line, a human bone osteosarcoma model by Fugene or calcium phosphate precipitation. Effects of overexpression of SP1, USF and C/EBP transcription factors on ADAMTS-3 promoter activity in Saos-2 cells were also evaluated by co-transfection studies by simultaneous transfections of promoter and expression plasmids. Transcriptional activity obtained from co-transfection assay was compared to basal activity of ADAMTS-3 promoter constructs. ADAMTS-3 mRNA level was also determined from the SP1, USF and C/EBP overexpressed cells by qRT-PCR. As a conclusion, overexpression of these transcription factors differentially upregulate the truncated promoter constructs. They might be involved in the transcriptional regulation of ADAMTS-3 gene in osteosarcoma cells.

***Keywords*:** ADAMTS-3, osteosarcoma, promoter, transcriptional regulation, type II collagen.

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Microbiology

P190. Antibiofilm Properties of *o*-Coumaric Acid in the Human Pathogen *Listeria monocytogenes*

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Abstract

Biofilms are a major problem in both medical and food industries. The formation of biofilms on every type of surfaces makes it very dangerous for human health. Biofilm structures provide an environment for the bacteria to withstand against thousand folds of lethal antibiotic doses. Food borne pathogens that survive in biofilms can contaminate foods and feeding tubes which results food poisonings, outbreaks and infections. *Listeria monocytogenes* EGDe is one of the dangerous food borne pathogens with ability to form biofilms and cause listeriosis among children, elderly people, immunocompromised patients or pregnant women, which result lethal consequences. Herein, the antimicrobial and antibiofilm effect of *o*-coumaric acid was examined on *Listeria monocytogenes* EGDe via microtiter plate methods. The antimicrobial assay revealed the MIC value of *o*coumaric acid against *Listeria monocytogenes* EGDe as 10mM. The initial attachment of the 10^6cfu/ml bacterial cells to a polystyrene surface was reduced 58% by addition of 10mM *o*-Coumaric acid and showed inhibitory effect on preformed biofilm as 56%. Microscopy studies supported this data. Furthermore, protein profiles of the bacteria were investigated in the presence and absence of *o*-coumaric acid.

***Keywords*:** *o*-Coumaric acid, *Listeria monocytogenes* EGDe, Biofilm

Others (Molecular genetics, medical genetics)

P191. A novel nuclear envelopathy-related gene: mutation in *TOR1AIP1* encoding LAP1B causes muscular dystrophy

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Abstract

Muscular dystrophy is a heterogenous group of hereditary muscle disorders characterized by progressive muscle weakness and degeneration. We identified a homozygous frameshift mutation in *TOR1AIP1* (torsinA-interacting protein 1) encoding LAP1B (lamina-associated polypeptide 1B), in three individuals affected by a myopathy with contractures and cardiomyopathy [1]. It has been shown that the c.186delG mutation leads to a premature stop codon and causes the loss-of-function of a specific LAP1 isoform normally expressed in skeletal muscle, liver and kidney. A 5-fold decrease in expression of LAP1 transcript has been detected and molecular analyses showed that the 66 kDa isoform of LAP1 was absent in patient's skeletal muscle. Ultrastructural studies showed nuclear envelope abnormalities in the patient's muscle. LAP1 is a transmembrane protein of the inner nuclear membrane binding A- and B-type lamins and regulating the activity of torsinA ATPase with LULL1 (luminal domain like LAP1). Interestingly, LULL1 was overexpressed in the patient's skeletal muscle, highlighting a possible compensatory effect in the absence of LAP1. Mutations in genes encoding proteins of the nuclear envelope such as laminA/C, emerin and nesprins are known to cause muscular dystrophy. Therefore, this study expands the spectrum of the genes related to nuclear envelopathies presenting with a muscle phenotype.

***Keywords*:** muscular dystrophy, homozygosity mapping, LAP1, TOR1AIP1, nuclear envelopathy

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Biochemistry

P192. Gene Expression and Activity of Some Antioxidant Enzyme in Oxidative Stress Induced by Long-Term Iron Overload in Rat Liver and Kidney.

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Abstract

Although the trace elements such as iron are vital for various cellular events, excessive intake of iron causes the production of reactive oxygen species (ROS) [1, 2] that induces hepatoxicity [3] and nephrotoxicity [4] in living organisms. Since ROS are highly reactive and oxygen-containing molecules, it has to be neutralized by antioxidant defense system to protect cells against its damage [5, 6, 7]. The present study was designed to examine the effect of long-term iron overload on renal and hepatic antioxidant system at the enzymatic and molecular level. A reduced glutathione (GSH) level, which is a marker for oxidative stress, markedly decreased with a long-term iron overload in rat kidney and liver. While the content of iron in the blood and kidney was not affected, hepatic iron content was increased by iron toxicity. The gene expression of antioxidant related enzymes was affected by iron overload. However, the actual effect of long-term iron overload on renal and hepatic antioxidant system is observed at protein level. The gene expression of antioxidant related enzymes did not correlate to enzyme activity. Furthermore, the impact of iron on the renal antioxidant system is different from its effect on the hepatic antioxidant system [8, 9, 10].

Keywords: iron, toxicity, antioxidant enzymes, mRNA expression, enzyme activity.

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Acknowledgments

This work was financially supported by the Atatürk University Scientific Research Projects Coordination Commission (ATAUNI-BAP) with project number 2010/277, 2010/279 and 2011/84.

P193. Determination of Anti-mutagenic and anti-oxidant potencies of Cladonia chlorophae

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Abstract

Lichens have long been investigated for their biological activities such as antitumor, antimicrobial and antioxidant activities. Many positive results were obtained in this previous research. Thus, in this study, we aimed to investigate antimutagenic and antigenotoxic potential of Cladonia chlorophae. The results of our studies showed that investigated lichen species has antimutagenic and antigenotoxic properties in the Ames (Salmonella typhimurium TA1535, TA1537) and WP2 (Escherichia coli) and sister chromatid exchanges test systems. Additionally, we have evaluated the levels of antioxidants of human lymphocytes in order to clarify the possible mechanisms that may contribute to the anti-genotoxic activity of the lichen extract. Consequently, results of this experiment have clearly shown that Cladonia chlorophae has strong antioxidative and antigenotoxic effects that are associated with its antioxidant nature. Detailed studies may provide useful outcomes which will extend the use of lichen extracts in food and pharmacy industries as it has antioxidant, antigenotoxic and antimutagenic properties.

***Keywords*:** Cladonia chlorophae, antioxidative, antigenotoxic

Microbiology

P194. Antimicrobial Activities of Soil Isolate Microorganisms on Some Pathogens

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Abstract

In recent years, one of the problem arising in health sciences is that pathogen microorganism gaining resistance against antibiotic drugs. Therefore, currently used many antibiotic almost lost their effectiveness on microorganism. That is why new antibiotic drugs (mainly synthetic) is introduced to the market. On the other hand, it is very well known that most of the microorganism on earth are still unknown, hence, their antimicrobial potentials. For this purpose, soil samples were inoculated on to three different agar; nutrient broth agar, patato dextraose agar and actinomycete isolation agar. Inoculated agars were incubated at three different temperatures; 28, 35 and 50 °C. All actinomycetes and microorganism showing clear zone around it self were isolated as pure culture. After obtaining pure culture, they were tested for antimicrobial activities against some pathogens by using cross-streak method, namely, *Bacillus subtilis, Esherichia coli, Staphylococcus aureus, Salmonella* ve *Agrobacterium tumefaciens.* Among 60 bacteria, 22% of them showed antimicrobial activity agains *B. subtilis*, and only the 1% of them had activity on *S. aureus*. In total 17% of them has the ability to produce antimicrobial chemicals. For further analysis, they will be grown in fermentation medium and the metabolites will be investigated by GC-MS.

***Keywords*:** Soil microorganism, Antimicrobial acitivity, Actinomycete

Acknowledgement: This study was supported by BAP-10-M-13 project.

P195. Determination of anti-mutagenic and anti-oxidant potencies of Cetraria aculeate

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Abstract

In this study, the mutagenic and anti-mutagenic effects of methanol extract of Cetraria aculeata was investigated by using E.coli-WP2, Ames-Salmonella (TA1535 and TA1537) and sister chromatid exchange (SCE) test systems. The results obtained from bacterial test systems demonstrated that methanol extracts of lichen species has strong anti-mutagenic potencies on TA1535, TA1537 strains and to a lesser extent on E.coli-WP2 strain. The antioxidant level of human lymphocytes cells was determined in order to clarify the mechanism underlying the anti-mutagenic effects of lichen species. Co-treatments of 5, 10 and 20 µg/ml concentrations of these three lichen species with AFB decreased the frequencies of SCE and the level of MDA and increased the amount SOD, GSH and GPx which decreased by aflatoxin. The findings of this work have clearly demonstrated that Cetraria aculeata, has significant anti-mutagenic effects which are thought to be partly due to the antioxidant activities and the interaction capability of lichen extracts with mutagen agents (Sodium azide, acridin, N-methyl-N′-nitro-N-nitrosoguanidine and aflatoxin B_1).

***Keywords*:** Cetraria aculeata, antioxidative, antigenotoxic

Gene Regulation

P196. Up-regulation of Human *ADAMTS-2* via IL-6 in Osteosarcoma Cells

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Abstract

Extracellular matrices (ECM) are secreted molecules that constitute the cell microenvironment and provides the shape and strength of many tissues. Collagen is one of the major component of the ECM [1]. ADAMTS-2 (A disintegrin and metalloproteinase with thrombospondin type 1 motif 2) plays crucial roles in the processing of fibrillar collagen precursors allowing correct fibril conformation. ADAMTS-2 is essential for maturation of type I collagen fibrils in the skin. The functional analysis of the ADAMTS-2 protein have been largely studied so far because of the relation with EDS type VIIC, but the studies on the role of the cytokines on one of the main extracellular matrix component, ADAMTS-2, is rather limited [2]. IL-6 (Interleukin-6) is a major pleitropic and pro-inflammatory cytokine that plays a significant role in bone remodeling [3]. In this study we investigated effects of IL-6 to ADAMTS-2 expression levels in two different osteosarcoma models, namely Saos-2 and MG-63, displaying different osteoblastic characteristics. Here we elucidated that IL-6 stimulation up-regulates ADAMTS-2 mRNA and protein expression levels in these cells. IL-6 also increases the transcriptional activity of the ADAMTS-2 gene promoter. As a result of the pathway inhibition studies ADAMTS-2 upregulation by IL-6 was mediated by JNK pathway.

***Keywords*:** ADAMTS-2, IL-6, Saos-2, MG-63, Collagen.

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P197. Cloning and In Silico Analyses of URG-4 cDNA

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URG-4 (Up-regulated Gene 4) was a novel gene that found to contribute Hepatocellular Carcinoma by effecting HBxAG protein. URG-4 gene covered 50.000 bp (43,915,501-43,967,000 position) at chromosome 7. It has five spiced variants (Access. No: *Variant 1* NM_017920, *Variant 2* NM_001077664, *Variant 3* NM_001077663, *Variant-4* NM_001290076 and *Variant 5* NM_001290075). However, there is no information avalaible on the expression profile of URG-4 variant Bioinformatics analysis showed that the sequence of Variant 2 and Variant 4 has similarity. To determine expression profile of variants in various cancer cells, specific primers were designed for PCR strategy. We observed that, all variants' cDNA were expressed in various cancer cells such as Hep3B, PC3, MCF-7, HT-29 and DU-145. Full length of URG-4 *Variant-1* cDNA was cloned into pCDNA3.2 expression vector by PCR based approach. URG-4 gene was transiently transfected to Hep3B and stably transfected into Saos-2 cells. URG-4 expression level from transfected cells was confirmed by using RT-PCR, qPCR and western method. MTT and colony forming assays were carried out to determine cytotoxic and proliferative effect of this gene. MTT and colony forming assays, show that the ectopic expression of URG-4 gene induces proliferative effects of cells.

Keywords: URG-4, variant, cDNA, cloning

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Microbiology

P198. Investigations on the effects of *opcA* mutation in *Synechocystis* sp. PCC 6803

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Abstract

In cyanobacteria *opcA* gene has always been considered to play a role in oxidative pentose phosphate cycle [1-4]. Such a conclusion depends on the mutation analyses of the gene in some cyanobacterial strains. In these strains, *opcA* gene is always follows the *zwf* gene encoding glucose-6-phosphate dehydrogenase and is organized into same operon. Therefore it is not clear whether the enzyme activity was affected by only *opcA* mutation or polar effect of the mutation on the *zwf* gene. Since *opcA* gene is located away from *zwf* gene, *Synechocystis* sp. PCC 6803 provides an ideal system for mutagenesis of both the genes. In this study, we mutated *opcA* gene of this strain and analyzed the affect of the mutation on G6PDH activity. The results from the mutant cells showed that no G6PDH activity decrease occured at higher substrate concentrations. Contrary to the almost complete loss in the strains analyzed before, the activity decreased gradually with the lower substrate concentrations and disappeared completely at the end. Weakly reproducible dark viability experiments did not provide conclusive data. This study shows in conclusion that *opcA* mutation affects G6PDH activity in different levels depending on substrate concentration, not like a complete loss of activity as reported previously.

***Keywords*:** *opcA* mutation, *Synechocystis* sp., glucose-6-phosphate dehydrogenase activity, dark viability

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Topic: Other

P199. CARDIAC GLYCOSIDES: FROM HEARTH TO LIVER

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Abstract

Hepatocellular carcinoma is the second most deadly and sixth most comman cancer worldwide [1,2]. Therefore it is vital to develop therapeutic agents against hepatocellular carcinoma. Cardiac glycosides are steroid-like compounds and used for treatment cardiovascular disorders and were previously shown to induce apoptosis in cancer cells [3,4,5]. The bioactivities of six Digitalis Ferrugiana originated glycosides were examined by NCI-Supforhodamine B (SRB) assay and real-time cell electronic sensing assay (RT-CES) on liver cancer cells. Highly cytotoxic, Lanatoside A, Lanatoside C and Glucogitorosid, were analyzed for their effect on cell cycle and were shown to induce G2/M arrest. Hoechst staining showed condensed apoptotic nuclei characteristics in treated cells. Apoptosis was further confirmed by western blot analysis for apoptosis specific PARP and caspases. Lanatoside C was further shown to induce reactive oxygen species (ROS) production. Mechanisms of action on signaling pathways was examined by western blot revealing Lanatoside C inducing oxidative stress and activate stress-activated protein kinases (SAPK)/Jun amino-terminal kinases (JNK), resulting in extrinsic apoptotic pathway induction. Finally, in vivo xenograft experiments on nude mice depicted both chemopreventive and chemotherapeutic actions of Lanatoside C. Consequently, Lanatoside C might be contemplated in cancer chemoprevention or cancer therapy in future in clinics.

Keywords: Digitalis, hepatocellular carcinoma, apoptosis, nude mice,

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Microbiology

P200. Glycolysis Regulatory Factor Gcr2p Involves in the Regulation of *TPS1* and *NTH1* Genes in the yeast *S. cerevisiae*

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Abstract

Gcr2p is the co-activator of glycolytic genes and associates with transcription factor Gcr1p. Mutants of *GCR2* gene is viable and shows increased levels of respiration and biomass yield but lower level of ethanol production with respect to the wild type yeasts [1]. Reserve carbohydrates trehalose and glycogen is synthesized from glycolytic metabolites. Their biosynthesis tightly regulated by growth conditions. Trehalose also acts on hexokinase and restricts uncontrolled glucose entry to the glycolytic pathway. *TPS1* gene encodes the regulatory subunit of trehalose synthase enzyme complex. *NTH1* encodes neutral trehalase enzyme which degrades cytoplasmic trehalose and prevents trehalose over accumulations. Hence there is a continuous regulatory interaction among glycolysis, biosynthesis and degradation of reserve carbohydrates in the yeast *S. cerevisiae* [2]. In this study, we have analyzed the effect of Gcr2p on the reserve carbohydrate metabolism in yeast. Our results indicated that transcription of *TPS1* gene is down regulated in the *gcr2* mutant yeast strain. Contrary to *TPS1*, transcription of *NTH1* activated more than 50% in the *gcr2* mutant yeast strain. Duplication time of *gcr2* mutant strain is also longer than the wild type strain. Moreover, it appears that the lag stage of the growth is much longer in *gcr2* mutant than the wild type strain, indicating that overall nutrient sensing is not effective in this mutant which results in the delay for the log stage entry. We have also determined the levels of trehalose and glycogen in the wild type and isogenic *gcr2* mutant yeast. Our results indicated that *gcr2* mutant strain has similar level of trehalose and glycogen both in the log stage and also at stationary stage of the growth. In addition, our results indicated that the glucose consumption rate is much lower in the *gcr2* mutant than the wild type yeast.

***Keywords*:** Trehalose, Glycolysis, GCR2 gene, Transcriptional regulation.

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MICROBIOLOGY

P201. Disruption of *Pseudomonas aeruginosa* biofilms by human serum paraoxonase 1 (hPON1)

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Abstract

Human serum paraoxonase (hPON1, EC 3.1.8.1.) is a high density lipoprotein (HDL) associated, calcium dependent enzyme that hydrolyses aromatic esters, organophosphates and lactones [1]. The recent studies showed that hPON1 enzyme, known as lactonase can play a role in degredation of signal molecules used on regulation of biofilm formation *Pseudomonas aeruginosa* [2,3] and it may play an important role in the fight against bacterial biofilm formation. In our study, hPON1 was purified by using ammonium sulfate precipitation and Sepharose 4B-L-tyrosine-1-Naphtylamine hydrophobic interaction chromatography. Purified enzyme was shown a single band with 45 kDa in SDS-PAGE [3]. The biofilm forming ability of the *P. aeruginosa* ATCC35032 at different hPON1 enzyme concentrations (0,1; 1; 2,5; 5; 10 $mgml^{-1}$) was determined by microscopic and spectrophotometric analyses using 96-well polystyrene plates [4]. The hPON1 enzyme was proved as an antibiofilm agent against *P. aeruginosa* ATCC35032. At different concentrations, the purified enzyme caused 12.5-58.9% inhibition of biofilm formation. Beyond the inhibition, the enzyme was also effective in degradation of mature biofilm by disrupting the exopolysaccharide (EPS), an essential component in biofilm architecture. This study proved that hPON1 enzyme can be used to inhibit/disrupt the biofilms of *P. aeruginosa* and beholds much promise in clinical applications.

***Keywords*:** Antibiofilm, Biofilm, hPON1, *P. aeruginosa*

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P202. Is *Helicobacter Pylorus* a Frequent Infectious Agent also for Extragastric Tissues as well as Stomach?

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Abstract

Gastric infections with *Helicobacter pylori* can lead to gastritis, duodenal or gastric ulcers and adenocarcinomas. This organism can invade gastric cells and so, can protect itself against antibiotics and then, may cause reinfections after treatment [1]. Recent studies have been focused on oral cavity and gallbladder as extragastric reservoirs of *H.pylori* [2,3]. This study aims to investigate the prevalence of *H.pylori* in the stomach, gallbladder and palatine-tonsils of different patients with gastritis, cholecyctitis, and tonsillitis, respectively by using real-time PCR. Bacterial DNA was extracted using a QIAampDNAminikit. Species specific primers were targeted CagA region. Sixty-five patients were included. Distribution of the samples was as the following: 30 from stomach with gastritis, 22 from gallbladder with cholecyctitis, and 13 from palatine-tonsils chronically infected. Of 65 samples, 27 were positive. Thirteen, 10 and 4 positive samples were stomachs, gallbladders and tonsils, respectively. *H.pylori* prevalence was 43.33% for stomach, 45.45% for gallbladder, and 30.77% for tonsil. There was no statistical difference between three organ groups in respect to *H.pylori* percentage. Our data suggest that *H.pylorus* is also an important pathogenic agent in examined extragastric organs apart from the stomach. More studies are needed to determine mechanism of pathogenesis of *H.pylori* in extragastric diseases.

Keywords: Helicobacter pylori, real-time PCR, Stomach, Gallbladder, Tonsil

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Cancer Biology

P203. Apoptotic Pathway Activation and Cytotoxicity of Melatonin in Cultured MCF-7 Cells

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Abstract

Breast cancer is one of the most common types of cancer in the world and is located at the top of death rates releated to cancer [1]. An innovation of a novel agent for the treatment of this disease is among the primary goals of many researchers. In this study, we aimed to examine the effects of melatonin on breast cancer. Melatonin is known as a powerful antioxidant with versatile effects in the modern medicine [2]. To determine the cytotoxic doses of melatonin, it was applied on MCF-7 cell line at intervals of 10 nM–100,000 nM concentrations. After the treatment with various concentration of melatonin, cell viability was analyzed by MTT assay and effective doses were determined. Then, apoptotic activity (Bax and p53 immunopositivity) and expression levels of p53 gene were investigated. The results showed that melatonin inhibited MCF-7 cell proliferation. It also increased the p53 gene expression and Bax protein synthesis at the end of the 24-hour-incubation, suggesting that melatonin treatment may induce apoptosis in MCF-7 cells. These findings reveal that melatonin may be a candidate agent for the treatment of breast cancer.

***Keywords*:** Melatonin, MCF-7 cells, Apoptosis, Bax, p53 gene, real-time PCR, cytotoxicity

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P204. Potential Effect of Traditional Preparation from Some Medical Plants on Wound Healing

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Abstract

Dermal wounds emerge as a result of cuts, burns and ulcers. If not repaired, the barrier function of skin may dwindle and the tissues may be exposed to microbial infections. The aqueous combination of three major extracts from traditional preparations of plants was investigated for its putative treatment of wounds as traditional healer. Although this combination is used in traditional medicine, any scientific research for each extract is present that support its therapeutic application and mechanism of action. In the current study, we perform "*in vitro* scratch assay" to examine the attitude of keratinocytes in the process of wound healing in the presence of the special combination of *Olea europaea, Nigella sativa* and *Rosemarinus officinalis* extracts [1]. Cell proliferation and migration were observed using an inverted microscope and pictures were taken at different time intervals (0h, 12h, 24h. 36h and 48h). The findings showed that the extract combination induced proliferation and migration of keratinocytes. The wound closure was significantly faster in the treatment group in comparison to the control group. Our study suggests that this extract combination can accelerate wound healing by increasing keratinocyte proliferation and migration. Further studies are needed to explain its effect mechanism *in vivo*.

***Keywords*:** Plant extract, Kerationocytes, Wound healing, Cell migration

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P205. Mutations are biased to GC in *Deinococcus radiodurans*

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Abstract

Mutations are the ultimate source of genetic variation and their rate and spectrum are essential for understanding all evolutionary processes. Mutation events are known to be not entirely stochastic, but with certain biases like mutation rate being influenced by the genome position and the flanking nucleotides. Mutations were also previously thought to have universal AT-bias, especially GC→AT transitions dominating AT→GC transitions, in wild-type bacteria with mutation spectra resolved. However, we find that *Deinococcus radiodurans*— the most radiation-resistant life form known— lacks this mutation bias: the GC→AT mutation rate is actually lower than the AT→GC rate, as well as the GC→AT transition rate being no higher than the AT→GC one. The possible causes for this mutation spectrum is that the exceptionally abundant UDGs in *D. radiodurans* effectively remove uracils created from deaminated cytosines, or the absence of Dcm, since methylated cytosine deamination at Dcm sites is the major cause of AT mutation bias. Also, Dam non-canonical or potential target sites contribute to AT→CG mutations. All these indicate that genes involved in DNA oxidation repair and methylation in bacteria could shape mutation spectrum. The genomic mutation rate of 0.0015 per generation agrees well with the modified Drake's rule. The only four-fold mutation rate elevation upon MMR deficiency confirms the low efficiency of the *D. radiodurans* MMR system. The fact that MMR only repairs transitions but not transversions suggests that an additional unidentified post-replication mismatch repair pathway for transversions exists in *D. radiodurans*.

Keywords: mutation accumulation, mutation bias, DNA mismatch repair

P206.

CHARACTERIZATION OF NLRP13 IN INFLAMMASOME ACTIVITY AND IMMUNE PRIVILIGE

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Nod-like receptors are members of a protein family that sense infection, cellular damage and pathogens. NLRs consist of N terminal interaction domain (PYRIN), central oligomerization domain (NACHT/NOD) and leucine rich repeat domain (LRR) at the C terminus. The inflammasomes are multiprotein complexes consisting of caspase 1, ASC, NLR and sometimes caspase 5. Depending on the activator, components of the inflammasome assemble and maturation of inflammatory cytokines such as IL-1β and IL-18 occurs. We found that NLRP13 can interact with caspase 1, ASC and IL-1β. However, the effect of NLRP13 on Caspase 1 cleavage, IL-1β expression and secretion are not well known. Our preliminary results show that NLRP13 negatively regulates IL-1β maturation and secretion. Immune privilige sites such as brain and placenta are suggested to be tissues with NLRP13 expression and this may be a clue for the immune suppressive role of NLRP13 in these tissues. We produced NLRP13 antibodies for endogenous experiments and made NLRP13 sh-knockdown cell lines to understand possible roles of NLRP13 in inflammasome activity and immune privilege.

P207. Rotavirus and Adenovirus Prevalence in Children under Five Years

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Abstract

Rotavirus and adenovirus have been recognized as the most common pathogens causing acute gastroenteritis among children [1]. Rotavirus and adenovirus found in high concentration in the stool of children with gastroenteritis [2]. To determine the prevalence of rotavirus and adenovirus, and infection under five years old children attended hospital with acute gastroenteritis in region was aimed in this study. This study is conducted with the stool specimens of 188 children under five years old, attended our hospital from September 2012 to August 2013. Antigens of virus were detected by ELISA. One hundred thirty five (72%) male and fifty three (28%) female of patients were found. Thirty-eight of the collected samples (20%) were positive for Rotavirus and fourteen of the collected samples (7%) were positive for Norovirus. Increase the number of positive cases of rotavirus antigen patients were viewed in winter and spring, but most frequently were found in December. In our study, the most common virus antigen positivity was observed in the age group 7-12 months. As a result, children between 0-5 years of age an important factor in the etiology of acute gastroenteritis in epidemiological monitoring of rotavirus and adenovirus infection were thought to be guiding the selection of diagnosis and treatment.

***Keywords*:** Rotavirus, Adenovirus, prevalence

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P208. Isolation and Identification of Thermophilic Bacteria from Different Geothermal Areas in Turkey

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Abstract

The thermophilic microorganisms can survive in high temperatures. Due to the enzyme systems of these organisms significant advances have been recorded especially in biotechnology and molecular biology. This property of thermophilic bacteria has been caused an increase in isolation, identification and characterization studies to determine unknown microbial content of geothermal sources [1]. In this study; water and sludge samples were taken from Bursa-Bademli Garden, Tokat-Sulusaray, Sivas-Hot Cermik and Balıkesir-Bostanci village springs. Isolation, phenotypic, genotypic characterization of thermophilic bacteria was carried out and protease activity of these isolates was determined by petri dish method. At the end of the conventional tests, isolates were determined as Gram positive, mobile-rod shaped, aerobic, oxidase, catalase and endospor positive. As a result of the molecular analysis; FAME analysis was particularly insufficient for diagnosis of thermophilic microorganisms ,but rep-PCR (GTG5) method was successful in seperation of organisims at species and even subspecies levels. According to the result of 16S rRNA sequencing analysis, the isolates were found to be similar to *Bacillus pumilus, B. licheniformis, B. thermoamylovorans, Aeribacillus pallidus, Anoxybacillus kaynarcenesis, A. gonensi, Thermomonas hydrothermalis* ranges between 97 to 99 %.

***Keywords*:** Thermophilic bacteria, FAMEs, rep-PCR, 16S rRNA sequencing, Protease activity

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Biotechnology

P209. Purification and Characterization of Thermostable Alkaline Protease Enzyme from *Bacillus licheniformis* A10

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Proteases (EC.3.4) are enzymes that hydrolyze proteins to produce small peptides and catalyze peptide synthesis in the presence of organic solvents [1,2]. Proteases have diverse applications in various industrial products and processes [3,4]. Thus, characterization and purification of novel proteases is the most important for the enzyme industry. In this study, purification and characterization of a new alkaline protease produced by *Bacillus licheniformis* A10 isolated from Ayder Hot Spring was investigated. The enzyme was purified from the culture supernatant to homogeneity using ammonium sulfate precipitation and ion-exchange chromatography with 1.38-fold and 9.44% recovery. The molecular weight of the purified enzyme was estimated to be 40.55 kDa by SDS-PAGE. Optimal pH and temperature were determined for the enzyme as 9.0 and 70ºC, respectively. The enzyme was extremely stable in the pH range of 7.5–9.5 and between 60°C and 70°C temperatures. The enzyme was completely inhibited by PMSF which is a general serine protease inhibitör. The activity of the enzyme increased in the presence of Mg^{2+}, Mn^{2+}, K^{+}. It is also observed that the enzyme lost its activity in the presence of surfactants, oxidants and organic solvents. The V_{max} and K_M for the enzyme was 0.033 mg/ml and 8.17 µmol. $ml^{-1}.dk^{-1}$ respectively.

***Keywords*:** Thermophilic bacteria, Alkaline protease, *Bacillus licheniformis*

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Acknowledgments.*This work was financially supported* by Atatürk University Scientific Research Projects Coordination Commission (ATAUNI-BAP) with project number 2013/297.

CANCER BIOLOGY

132B. EPIGENETIC MODIFICATIONS OF ANDROGEN RECEPTOR SIGNALING IN CASTRATION-RESISTANT PROSTATE CANCER (CRPC)

Abstract

Prostate cancer (PCa) is one of the most common forms of cancer in Turkish and European men. In all stages of the disease the including so-called castrate resistant prostate cancer (CRPC), the androgen receptor (AR) has been demonstrated to be critical for progression [1]. There are several potential reasons for the conversion from androgen- sensitive PCa to CRPC. Previous work has demonstrated that epigenetic modifiers such as EZH2 and LSD1 can mediate the sensitization of androgen receptor in CRPC [2]. However, only a small subset of epigenetic modifiers has been characterized. To better understand the role of histone modification on CRPC, we conducted a large scale shRNA screen of epigenetic modifying enzymes in a model of CRPC to identify those genes that prevent androgen-independent proliferation.From this screen several histone modifying enzymes have been found that are critical to the proliferation of androgen independent but not dependent PCa cells. The shRNA knock-down of these hit genes was confirmed by western blot and qRT-PCR. Our preliminary studies suggest that the decrease in proliferation may be due to a down-regulation of AR-mediated cell cycle regulatory proteins that are critical in CRPC.

Keywords: CRPC, AR, epigenetic modifications

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