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The symposium is organized and hosted by the Centre of Excellence in Molecular Biology, University of the Punjab, Lahore Pakistan. In order to build national capability in the new bioscience, University of the Punjab established a nucleus Centre for Advanced studies in Molecular Biology. Molecular Biology Laboratory Complex is spread over 60 acres of land, with covered area of 7000 square meters, including a Laboratory Block, a Teaching Block, a Hostel for PhD Research Scholars. The Laboratory Block is divided into four separate research units comprising a total of 20 Research Labs. and four Conference Rooms, one Production Unit and one Support Facilities Unit comprising a Lab-aid Section (for washing, autoclaving and media preparation), an Animal House, an Insectary, six large Plant Growth Rooms, and storage space for research materials. The Teaching Block consists of a well-equipped Library, Seminar Hall, Photography, Computer Rooms, a Conference Hall, Director's Office, Administration and Accounts Section.

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Program Schedule

DAY 1 (29th December 2015)

Time	Theme	Speaker
0830-0955 Hrs	**Registration of participants**	
Inauguration Session		
0955-1000 Hrs	**Guests to be Seated**	
1000-1005 Hrs	**Recitation of Holy Quran**	
1005-1020 Hrs	**Welcome Address**	**Prof. Dr. Tayyab Husnain** **Director CEMB**
1020-1035 Hrs	**Address by Dean Life Sciences**	**Prof. Dr. Javed Iqbal Qazi**
1035-1050 Hrs	**Address by the Vice Chancellor University of the Punjab**	**Prof. Dr. Mujahid Kamran**
1050-1105 Hrs	**Address by Chief guest**	**Dr. Farrukh Javed** Provincial Minister of Agriculture
1105-1130 Hrs	**Tea Break**	
Advances in Molecular Biology of PLANT Sciences		
	Technical Session I (Invited / International Speakers) **Chair: Prof. Dr. Kauser Abdullah Malik** **Co-Chair: Prof. Dr. Javed Iqbal Qazi**	
1130-1200 Hrs	An Introduction to the Epigenomics Shared Facility	**Dr. Shaheena B. Maqbool** Director Epigenomics Shared facility

		Albert Einstein College of Medicine, USA
1200-1220 Hrs	Strategies to develop plants tolerant to abiotic stresses	**Dr. Khurram Bashir** RIKEN, Yokohama, Japan
1220-1245 Hrs	Plant Immunity, Microbial Pathogenesis and Cytokinins: An Integrated Systems Biology Perspective	**Dr. Muhammad Naseem** Department of Bioinformatics, University of Wuerzburg, Germany
1245-1300 Hrs	**Q/A Session**	
1300-1350 Hrs	**Prayer / Lunch Break**	
	Technical Session II (PLANT Genomics) **Chair: Dr. Muhammad Ashraf** **Co-Chair: Prof. Dr. Ahmed Mukhtar Khalid**	
1345-14:00 Hrs	Use Of RAPD Markers In Comparison With Agro-Morphological Traits For Estimation Of Diversity Among Chickpea Genotypes	**Prof. Dr. Shiekh Muhammad Iqbal,** University of Haripur
1400-1415 Hrs	Insights of the phosphoenolpyruvate carboxylase (CaPPC) gene family in chickpea (*Cicerarietinum* L.)	**Prof. Dr. Syed Rehmat Shah,** HOD, Lasbela University of Agriculture, Water and Marine Sciences, Uthal, Balochistan
1415-1430 Hrs	Profiling and expressional studies of microRNAs in root, stem and leaf of the bioenergy plant	**Dr. M. Younas Khan Barozai** Assistant Professor University of Baluchistan

	switchgrass (*Panicum virgatum* L.) under drought stress	
1430-1445 Hrs	Identification of DNA markers associated with salinity tolerance in rice (*Oryza sativa* L.)	**Dr. Muhammad Saeed** Assistant Professor (GCUF)
1445-1500 Hrs	Confirmation of root-knot nematode resistant gene rmil using diagnostic marker approaches	**Dr. Musarrat Ramzan** Assistant Professor University of Sargodha
1500-1510 Hrs	**Q/A session**	
1510-1535 Hrs	**Tea Break**	
	Technical Session III (PLANT Genomics & Bisaofety) **Chair: Dr. Iqrar Ahmed Khan** **Co-Chair: Dr. Prof. Shahid Mansoor**	
1535-1545 Hrs	Advance -To-Be-Expressed, Modified Genomic Dna Isolation From *Nicotiana Benthamiana* Var *Domin.*'S Specified Germlasms In Pakistan	**Zaryab Khalid Sial** Lecturer (LCWU)
1545-1555 Hrs	Determining the expression pattern of GLP gene during somatic embryogenesis in *Oryza sativa,* L	**Samina Khan /** **Dr. Fazeelat Kramat** Assistant Professor COMSATS
1555-1605 Hrs	The Role of Gossypiumarboreum Universal Stress Protein (GUSP1) in *Gossypium hirsutum*	**Ms. Sameera Sattar** S. Research Officer CEMB

1605-1615 Hrs	CEMB Floriculture group	**Dr. Qurban Ali** Assistant Professor CEMB
1615-1625 Hrs	Biosafety assessment of transgenic crops at CEMB	**Mr. Tahir Rahman Samiullah** Research Officer cum Lecturer CEMB
1625-1635 Hrs	**Q/A session**	
DAY II (30th December 2015)		
	Technical Session IV (Invited Speakers) / PLANT Transformation) **Chair: Dr. Noor ul Islam** **Co-Chair: Dr. Altaf Hussain**	
0900-0930 Hrs	Invited Speaker (Skype Talk)	**Dr. Zafar Nawaz** Professor; Senior Associate Dean University of Miami, USA
0930-0945 Hrs	Development of transgenic wheat with higher phytase expression for improved phosphorus use efficiency	**Dr. Muhammad Irfan** Assistant Professor (FCU)
0945-1000 Hrs	Development of transgenic wheat with low phytate for increasing bioavailability of iron and zinc	**Dr. AsmaMaqbool** Assistant Professor (FCU)
1000-1015 Hrs	Biosafety studies of genetically modified maize (*zea mays* L.), its effect on soil fertility by evaluating ph, ec and micronutrients	**Ms. Rabia Saba** Lecturer, Uni. Sargodha
1015-1030 Hrs	Development of Taqman probe based qPCR for	**Dr. Azmatullah Khan**

	CLCuV particles (DNA-A, alphasatellite and betasatellite)	Research Associate CEMB
1030-1045 Hrs	**Q / A Session**	
1045-1110 Hrs	**Tea Break**	

Advances in Molecular Biology of Health Sciences

	Technical Session V (Molecular Biology of Cancer) **Chair: Dr. Zafar Aziz Khan** **Co-Chair: Dr. Shahid Mahmood Baig**	
1110-1130 Hrs	Identification of abberantly expressed microRNAs in colorectal cancer	**Prof. Dr. NihatDİLSİZ** Dean of Faculty of Arts and Sciences, Harran University, Turkey
1130-1150 Hrs	Latest developments in Cancer Genomics and its integration into Clinical Practice	**Dr. Zafar Iqbal** Assistant Professor King Saud Bin Abdulaziz University for Health Sciences, Saudia Arabia
1150-12:00 Hrs	Role of Human Interferon alpha2b Gene and Presumptive Drug Model against Breast Cancer	**Dr. Bushra Ijaz**
1200-1210 Hrs	Medicinal Plants for the Management of Breast Cancer and its Prevalence in Pakistan	**Hafiz Muhammad Asif** Department of Eastern Medicine & Surgery, University of Poonch, Rawalakot, AJ& K
1210-1220 Hrs	HCV regulated apoptosis, steatosis, and oxidative stress leads to HCC	**Dr. Shah Jahan** Assistant Professor UHS

1220-1230 Hrs	Medicinal Plants for the Management of Breast Cancer and its Prevalence in Pakistan	**Hafiz Muhammad Asif** Department of Eastern Medicine & Surgery, University of Poonch, Rawlakot, AJ& K
1230-1315 Hrs	**Poster Session and Evaluation** **Dr. Nihat Dilsiz, Dr. Shaheena B Maqbool, Dr. Khurram Bashir**	
1315-1400 Hrs	**Prayer / Lunch Break**	
	Technical Session VI (Molecular Biology of Cancer & Forensic Sciences) **Chair: Prof. Dr. Khalid Mehmood Khan** **Co-Chair: Prof. Dr. Fazal e Majid**	
1400-1420 Hrs	Riproximin: A plant protein with therapeutic properties against multiple cancers (Skype Talk)	**Prof. Dr. Martin R. Berger** Head of Toxicology and Chemotherapy Unit, German Cancer Research Center (DKFZ), Germany
1420-1440 Hrs	Therapeutic potential of chemokine network in metastatic colorectal cancer (Skype Talk)	**Dr. Asim Pervaiz** Toxicology and Chemotherapy Unit, German Cancer Research Center Heidelberg, Germany
1440-1450 Hrs	Forensic Analysis of Human Hair for Heavy Metals detection and Comparison among workers of Different Industries Using PIXE	**Dr. Saqib Shahzad** Associate Professor UCP

1450-1500 Hrs	Proteome of human oral tongue squamous cell carcinoma identified by quantitative tandem mass spectrometry	**Saira Saleem** (SKMCH&RC), Pakistan
1500-1510 Hrs	A study of SE33 locus as a reliable STR marker for forensic investigation in Pakistani population	**Dr. Munir Ahmad Bhinder** Assistant Professor UHS
1510-1520 Hrs	Pakistan and the Personalized Medicine Era	**Dr. Muhammad Ilyas** Assistant Professor AWKU
1520-1530 Hrs	Development of a miniSTR system for the analysis of highly degraded DNA	**Dr. Muhammad Shafiq** Research Officer cum Lecturer CEMB
1530-1540 Hrs	**Q/A session**	
1540-1605 Hrs	**Tea Break**	
Technical Session VII (Genetic Diseases) **Chair: Dr. Saleh Memon** **Co-Chair:**		
1605-1615 Hrs	Genomic regions 17q21 and 20p13 are associated with asthma in Pakistani population	**Dr. Farooq Sabar** Assistant Professor, CAMB
1615-1625 Hrs	Attenuation of apomorphine-induced sensitization in an animal model of stress	**Dr. Huma Ikram** Assistant Professor, University of Karachi
1625-1635 Hrs	Molecular & Genetic Analysis of Hereditary Vision Impairment	**Dr. Asif Naeem** Assistant Professor, CEMB
1635-1645 Hrs	Construction of Linkage Disequilibrium map for	**Dr. Mariam Shahid**

	17q21 region to find Haplotype association with Asthma in population of Lahore, Pakistan	Research Officer cum Lecturer CEMB
1645-1655 Hrs	Genetic basis of Intellectual disability in Pakistan	**Faiza Rasheed** Ph. D Scholar, CEMB
1655-1705 Hrs	Genetic and Molecular Characterization of Hearing Impairment in Pakistan	**Hamna Tariq** Ph. D Scholar, CEMB
1705-1715 Hrs	**Q / A Session**	

DAY III (31st December 2015)

	Technical Session VII (Stem Cells) **Chair: Dr. Nihat Dilsiz** **Co-Chair: Dr. Allah Bux Ghanghro**	
0900-0930 Hrs	**Invited Speaker**	**Dr. Faisal F. Khan** Director, Institute of Integrative Biosciences, KPK
0930-0945 Hrs	Neural differentiation of embryonic stem cells (ES)	**Dr. Sajida Batool** Assistant Professor COMSATS
0945-1000 Hrs	Cell-Material Interaction: a case of tail wagging the dog	**Dr. Arshad Jamal** Assistant Professor, COMSATS
1000-1015 Hrs	Transcription factor activating protein 2 beta (TFAP2B) mediates noradrenergic neuronal differentiation in neuroblastoma	**Dr. Fakhera Ikram** University of Cologne, Cologne, Germany

1015-1030 Hrs	Isolation, identification and antimicrobial sensitivity pattern of microorganisms found in different culture of burn patients at a Burn Center in Islamabad, Pakistan	**Dr. Aamir Ali Khattak** Assistant Professor, University of Haripur, KPK
1030-1045 Hrs	Preconditioning of stem cells for the repair of burnt skin	**Dr. Azra Mehmood** Assistant Professor, CEMB
1045-1100 Hrs	**Q / A session**	
1100-1120 Hrs	**Tea Break**	
	Technical Session VII (Medical) **Chair: Dr. Prof. Iqbal Chaudhry** **Co-Chair: Prof. Dr. M. H. Qazi**	
1120-1135 Hrs	Effects of Cyp4x1 mutation on weight gain and energy metabolism in mice: evidence for increased adiposity and metabolic syndrome	**Dr. Farhat Batool** Assistant Professor, University of Karachi
1135-1150 Hrs	Atrial fibrillation association with indicators of metabolic syndrome in subjects of our local population	**Dr. Saima Shareef** Assistant Professors, LCWU
1150-1205 Hrs	Effect of protein synthesis inhibitors and mitochondrial proteases on the abundance of mitochondrial DNA encoded transcripts	**Dr. Muhammad Waqar Hameed** Assistant Professor, University of Karachi
1205-1220 Hrs	Structural Characterization of interactions between small basic protein and accumulation associated protein- Two essential	**Madiha Fayyaz** Institute for Biochemistry and Molecular Biology, , Germany

	proteins in Staphylococcus epidermidis biofilm	
1220-1235 Hrs	Global consensus sequence development of dengue virus for peptide based vaccine design against local Dengue Virus	**Dr. Samia Afzal** Assistant Professor, CEMB
1235-1300 Hrs	**Q / A Session**	
1300-1340 Hrs	**Prayer / Lunch Break**	
	Technical Session VII (Metabolic Disorders) **Chair: Prof. Dr. Ikram-ul-Haq** **Co-Chair: Prof. Dr. Sharif Mughal**	
1340-1355 Hrs	Down-regulation of ABCA1 gene by hyperglycemia leads to dyslipidemia in type 2 diabetes	**Dr. Asifa Majeed** Assistant Professor, Army Medical college
1355-1410 Hrs	Culture media stress on the activity of Glutamate Dehydrogenase of Antidiabetic Medicinal herb	**Dr. Darima Habib** Assistant Professor, University of Haripur, KPK
1410-1425 Hrs	The role of aldose reductase in progression of diabetic retinopathy of local population	**Dr. Shagufta Naz** Assistant Professors, LCWU
1425-1440 Hrs	Drug loaded hydroxyapatite/polymer composites for sustained drug release	**Rabia Zeeshan** Lecturer, COMSATS
1440-1500 Hrs	Low BrdU Absorption Persuade SCEs and Association with GSTT1 Null Genotype in Healthy	**Dr. Shauka tIqbal** Associate Professor Capital University, Islamabad

	Individuals and Myeloid Leukemia Patients
1500-1510 Hrs	**Q / A session**
1520-1600 Hrs	**Closing ceremony**

ABSTRACT INFO

Session: Invited Speakers
S01

Authors' Affiliation:
Albert Einstein College of Medicine Of Yeshiva University, Bronx, NY, USA

***Corresponding Author:**
Maqbool SB

Presenter:
Maqbool SB

An introduction to the epigenomics shared facility

Maqbool SB*, Olea R, Ossipov M, Momin ZA, Shijun Mi, Lau K, Benard K, Greally JM

The Epigenomics Shared Facility (ESF), part of Einstein's Center for Epigenomics and an Illumina CSPro (certified service provider) laboratory, offers massively-parallel sequencing (MPS) including fully-automated library preparation, quality control and assurance, and a number of assays to study the epigenome such as ATACSeq, ChIPSeq, HELPTagging, MethylC-Seq and SeqCap Epi. Sample information is uploaded through a web-based interface (WASP) prior to sample submission, allowing LIMS and automated primary analysis of the data generated using the Wasp System software, and returning data through visualization and web links. Data analytical services are provided by the Computational and Statistical Epigenomics Group. We encourage novel research in a number of human diseases, with early emphases on cancer, neuroepigenomics, the epigenomics of infectious disease, aging research, diabetes and renal disorders.

ABSTRACT INFO

Session: Invited Speakers S01

Authors' Affiliation:
Toxicology and Chemotherapy Unit (G401), German Cancer Research Center (DKFZ), Im Neuenheimer Feld 581, 69120 Heidelberg, Germany

***Corresponding Author:**
Asim Pervaiz

Presenter:
Asim Pervaiz

Therapeutic potential of chemokine network in metastatic colorectal cancer

Asim Pervaiz*, Martin R. Berger

Chemokines, comprising a big network of proteins, perform a variety of homeostatic and inflammatory functions including embryonic development, lymphoid trafficking, angiogenesis and maturation of immune cells. Components of the chemokine network are gaining significant attention as potential therapeutic targets for metastatic cancers. We have focused on therapeutic relevance of chemokines in colorectal cancer liver metastasis, which accounts for ~50% colorectal cancer associated mortalities. Following the knockdown or blockade of chemokines by siRNAs or antagonists, respectively, we performed in vitro assays including RT-PCR, western blot, confocal microscopy, nuclear staining, cell cycle and FACS analysis to investigate functional effects of the chemokines in selected colorectal cancer cells (SW480, SW620 and CC531). An animal model mimicking colorectal cancer liver metastasis was used for the evaluation of targeting chemokines in vivo. Significant anti-neoplastic effects were identified in the investigated cells following the chemokines inhibition/blockage. These anti-neoplastic effects included inhibition of proliferation, migration and colonization of the cells. On molecular levels, mechanisms like induction of apoptosis leading to cytotoxic effects and changes in cell cycle corresponding to cytostatic effects were observed. Most strikingly, complete remission of growing tumor was observed for colorectal cancer liver metastasis in vivo. These laboratory investigations, while supported with relevant clinical data, will provide proof of concept about therapeutic potential of chemokine network in colorectal liver metastatic cancers.

ABSTRACT INFO

Session: Invited Speakers
S01

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Martin R. Berger

Presenter:
Martin R. Berger

Riproximin: A plant protein with therapeutic properties against multiple cancers

Asim Pervaiz, Michael Zepp, Martin R. Berger*

Riproximin (Rpx), a type II ribosome inactivating protein, was purified from kernels of the plant Ximenia americana. Previous studies highlighted its cytotoxicity in a variety of cancer cell lines originating from solid and non-solid tumors. In our recent studies, we investigated the mechanistic aspects of Rpx in selected breast and colorectal cancer cells. Cytotoxicity of Rpx was determined by MTT assay, while the migratory and clonogenic effects were determined by migration, scratch and colony formation assays. Apoptotic and cytostatic properties were studied by nuclear staining procedures and flow cytometry. Alterations at molecular levels, in response to Rpx exposure, were scrutinized by means of microarray and qRT-PCR methodologies. Rpx induced significant anti-proliferative effects in the selected breast (MDA-MB-231 and MCF-7) and colorectal cancer cells (SW480, SW620, and CC531). Profound inhibition of migration and colony formation was observed in all cell lines. Concomitantly, a distinct arrest in S phase and nuclear condensation/fragmentation were observed, indicating cytostatic and apoptotic effects. Genetic profiling revealed induction of the anticancer cytokine MDA-7/IL24 and ER-stress related GADD genes. Furthermore, inhibition of migration (RHO GTPases), anti-apoptotic activities (BCL family) and cell cycle relevant genes (cyclins) were observed in breast cancer cells. These studies indicate the anticancer potential of Rpx in breast and colorectal cancers and favor further evaluation of this naturally occurring plant compound as therapeutic agent.

ABSTRACT INFO

Session: Invited Speakers S01

Authors' Affiliation:
Plant Genomics Network Research Team, RIKEN Yokohama Campus, Japan

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Khurram Bashir

Presenter
Khurram Bashir:

Strategies to develop plants tolerant to Abiotic stresses

Khurram Bashir*

Mineral nutrients are not only essential for plants growth and development but also integral for human and animal health. In recent years the concept of hidden hunger (deficiency in certain mineral nutrients despite eating enough calories) has been well established and the importance of micronutrient nutrition is increasing at a great pace. Among these micronutrients iron (Fe), zinc (Zn), and manganese (Mn) are of particular interest as these are essential micronutrients for all higher organisms. Plants have developed sophisticated mechanisms to absorb Fe from soil and to transport it from root to shoot and grain. To acquire Fe and Zn from soil, graminaceous plants secrete small molecules called mugineic acid family phytosiderophores (MAs) that solubilize Fe, and Fe(III)-MA complexes are readily taken up by specific transporter at the root surface. Besides this, plants also use other genes to uptake and transport metals in plants (Bashir et al., 2013a, 2013b). I will discuss the role of various transporters in metal absorption from soil and a particular focus on mitochondrial iron transporter in rice. I have identified the protein responsible for transporting Fe into the mitochondria of rice. It is the first report of any protein transporting Fe to mitochondria in any plant species. Not only is the identification of MIT a significant advance in understanding cellular Fe homeostasis in rice, but it may also lead to improved Fe content in this essential grain. Moreover, it will facilitate the identification of mitochondrial Fe transporters in other plant species significantly improving our knowledge.

ABSTRACT INFO

Session: Invited Speakers
S01

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Presenter:
Dilsiz N.

Identification of abberantly expressed microRNAs in colorectal cancer

Dilsiz N*

The aim of this study was to determine the differences of gene expression in normal colon (CRN) and colorectal cancer (CRC) by using microarray system.

Introduction: Cancer is the most important diseases characterized by unregulated cell growth and spread of cells from the site of origin site, to other sites in the body. Total RNA was extracted from tissue samples of 12 patients with colorectal cancer undergoing surgical resection of the colon for studying tumor specific changes in miRNA expression. MiRNA was polyadenilated by using PolyA Tailing master mix. After Flash Tag Biotin Ligation samples were hybridized, stained and washed. The arrays were finally scanned using AGCC Scan control programmer according to the manufacturer's protocol (Affymetrix).

It was found that some of miRNAs were found up or down regulated in studied CRC tissues compared to nontumor tissues. MiRNA-183, miRNA-431, miRNA-487a, miRNA-34a, miRNA-542 and miRNA-1290 were overexpressed and miRNA-3172, miRNA-422a, miRNA-378c, miRNA-497, miRNA-1244 and miRNA-378 were downregulated in patients with CRC. We have also found that some dysregulated miRNAs, which to our knowledge have not previously been associated with colorectal carcinogenesis.

Consequently, the results of this study will increase our understanding of development, progression and earlier detection and personnel treatment of colon cancer.

ABSTRACT INFO

Session: Invited Speakers S01

Low BrdU Absorption Persuade SCEs and Association with GSTT1 Null Genotype in Healthy Individuals and Myeloid Leukemia Patients

Shaukat Iqbal Malik[1*], Vasiliki Hatz[2], Khalid Akhtar[3]

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2- Institute of Radioisotopes & Radiodiagnostic Products, Health Physics Laboratory, NCSR"Demokritos" Athens - Greece

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Presenter:
Shaukat Iqbal Malik

Sister chromatid exchanges (SCEs) frequently observed in leukemia are potential indicators of genotoxins exposure and anti-leukemogenic drugs activity. However, it is not clear whether the observed increase in SCEs' incidence in leukemia patients is a sole consequence of chemotherapeutic exposure or reflects an endogenous aberration in the genetic background. Keeping in account the aforementioned consideration and the pathogenic role of detoxification gene GSTT1 polymorphism in cancer susceptibility triggered us to verify whether genetic polymorphism in GSTT1 influences the endogenous genomic sensitivity in healthy individuals and myeloid leukemia patients. The cytogenetic-based study was conducted to scrutinize the endogenous SCEs using a Fluorescein isothiocyanate (FITC)-conjugated anti-BrdU antibody in a highly-sensitive immunochemical staining method which was chosen in order to eliminate the possible BrdU-induced SCEs that result from the BrdU used in the standard FPG method. For this purpose, SCE analysis was performed on peripheral blood lymphocytes from 42 healthy non-smoking individuals and 15 myeloid leukemia patients at diagnosis. The presence or absence of the homozygous deletion in GSTT1 gene was determined in peripheral blood lymphocytes using a multiplex-PCR method. The results obtained do not demonstrate any association between the GSTT1-null genotype and the increased endogenous SCE frequencies in leukemia patients with respect to the healthy individuals. Moreover, the SCE frequency obtained in the GSTT1 genotypes in leukemia patients is comparable to its frequency in the healthy population. Hence, our results indicate that the endogenous SCEs that arise in metaphase lymphocytes of myeloid leukemia patients are independent of the GSTT1 genotype and the donors' gender.

ABSTRACT INFO

Session: Plant Genomics & Biosafety S02-S03

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Sameera Hassan

Presenter:
Sameera Hassan

The Role of *Gossypium arboreum* Universal Stress Protein (GUSP1) in *Gossypium hirsutum*

Sameera Hassan*, Aqsa Aslam, Muhammad Bilal Sarwar, Fatima Batool, Zarnab Ahmad, Muhammad Nadeem Hafeez, Sajjad Sadique, Shehla Parveen, Muhammad Umair Majid, Bushra Rashid, Tayyab Husnain

The cells of eukaryotic organisms are localized elaborately into functionally distinct membrane bound compartments, thus most cellular processes are spatially restricted to defined regions of the cell. In a previous study Universal stress protein gene (GUSP1), identified from water-stressed leaves of *Gossypium arboreum* was cloned in pCAMBIA 1301 plant expression vector by replacing reporter GUS with GFP. Transgenic plants of *G. hirsutum* were produced through *Agrobacterium* mediated transformation. The transgene was amplified through PCR and a fragment of 500bp confirmed the integration of *GUSP1* in transgenic plants by Southern blot. The gene expression was studied through ELISA and Western blot by using *GUSP1* specific polyclonal antibodies and Real-time PCR quantified 2-3 fold expression in different tissues of transgenic plants following drought stress. The location of *GUSP1* was determined through immunostaining and microscopy using *GFP* as a cytological marker. These results will be helpful in order to unravel the role of *GUSP1* as a regulatory protein under abiotic stress condition in plants.

ABSTRACT INFO

Session: Plant Genomics & Biosafety S02-S03

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Presenter:
Sheikh Muhammad Iqbal

Use of RAPD markers in comparison with agro-morphological traits for estimation of diversity among chickpea genotypes

Sheikh Muhammad Iqbal[1*], Ahmad Bakhsh[2] and Mehboob-ur-Rahman[3]

Genetic diversity was assessed among 38 chickpea genotypes on the basis of random amplified polymorphic DNA (RAPD) in comparison with agro-morphological traits. Evaluation of agro-morphological traits revealed highly significant differences between genotypes. Days to 50% flowering ranged from 92 to 118, plant height (cm) ranged from 54.16 ±2.11 to 87±2.65, Number of fruit bearing branches and pod per plant respectively varied from 4 ±1.09 – 17.25±1.47 and 7.60±1.38 – 27.4 ± 2.05, whereas grain yield per plant (gm) differed from 3.50±0.6 to 9.8±0.64. The ascochyta blight score of these genotypes, recorded on 1-9 rating scale, varied from 3-9. The cluster analysis showing relationship based on morphological traits (scale: Euclidean distance) placed 35 genotypes into five distinct groups, and three genotypes, NOOR-91, Local Mankera and BR4 were not included in any cluster. The RAPD analysis showed that 35 RAPD primers amplified a total of 212 fragments out of which 45 were polymorphic. The polymorphic bands were generated by 21 primers whereas 14 primers were monomorphic. Genetic similarity matrix based on Nei & Li's index revealed similarity coefficients ranging from 92% to 97% indicating lower level of genetic polymorphism revealed by RAPD primers. The dandrogram constructed on the basis of these coefficients grouped all the genotypes in to 2 major and 3 small clusters at 92% similarity level. Two decamers, OPC5 and OPC14 distinguished between three Desi and two Kabuli genotypes. This study showed that the level of genetic variability observed in chickpea for agro-morphological traits was not reflected in DNA polymorphism obtained by RAPD analysis.

ABSTRACT INFO

Session: Plant Genomics & Biosafety S02-S03

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Presenter:
Muhammad Saeed

Identification of DNA markers associated with salinity tolerance in rice *(Oryza sativa L.)*

Muhammad Saeed*

Rice is an important staple crop of the world. Pakistan is famous for best quality rice production. Rice production is affected by salinity stress. A research project was designed to identify DNA markers associated with salinity tolerance in rice (*Oryza sativa L.*). An F2 population, originating from the cross Pokkali × IR-36, consisting of 113 individuals were evaluated under saline and normal field conditions. F2 population was phenotyped for yield and biochemical traits. Parents of F2 population were screened for polymorphism with 553 simple sequence repeats (SSRs) markers. One hundred and eleven markers were found to be polymorphic. Linkage map was constructed by using Joinmap v. 3.0. Quantitative trait loci (QTL) mapping analysis was carried out by QTL Cartographer 2.5 software. This analysis yielded significant marker-trait associations for salinity tolerance in rice. SSR marker RM248 was found to be associated with sodium uptake under saline conditions. This marker had higher phenotypic variance explained (*R2*) value. This marker can be successfully used in rice breeding programs for salinity tolerance.

ABSTRACT INFO

Session: Plant Genomics & Biosafety S02-S03

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Presenter:
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Confirmation of root-knot nematode resistant gene rmil using diagnostic marker approaches

Musarrat Ramzan*, Riffat Ahmad, Naheed Kauser, Anis Ali Shah, Rabia Saba, Shahina Fayyaz and Saifullah Khan

Molecular studies using Simple Sequence Repeat (SSR) marker system identified six Pakistani soybean [*Glycin max.* (L) Mirr] varieties as resistant against Root Knot Nematode (RKN) out of fifteen (15) indegenous cultivars. Diagnostic SSR markers Satt-358 and Satt-492 have shown the presence of *RMi* gene in all resistance carrying genotypes. Satt-358 amplified the fragment of 200 bp and Satt-492 generated 232 bp bands in all resistant genotypes. This study confirmed the RMi gene locus (G248A-1) in all internationally confirmed resistant varieties including native ones. These investigations have revealed the effective and informative sources that can be utilized in breeding programs for the selection of RKN resistance soybean genotypes in Pakistan.

ABSTRACT INFO

Session: Plant Genomics & Biosafety S02-S03

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Profiling and expressional studies of microRNAs in root, stem and leaf of the bioenergy plant switchgrass (*Panicum virgatum L.*) under drought stress

Muhammad Younas Khan Barozai*[1,2], Zhujia Ye[2] and Suping Zhou[2]

Switchgrass (*Panicum virgatum L.*) is a perennial fodder grass that is well known as a model renewable bioenergy crop. Its high production of biomass for biofuel supply is due to fast growing and wide adaptation properties of the plant. Very little studies and data are available about the miRNAs in this important biofuel crop. This situation demands to focus and identify new miRNAs and also elaborate their expressional analysis in the management of the plant under drought stress. Both computational and expressional studies were done for the miRNAs in the switchgrass under drought stress. A total of 158 new miRNAs belonging to 83 families were identified and characterized from the switchgrass expressed sequence tags (ESTs) and Genomic survey sequences (GSS). Also five pre-miRNA clusters and four sense and antisense pre-miRNAs were resulted in this research. Furthermore, 49 miRNAs were randomly selected and subjected for qPCR expressional studies in root, leaf and stem of switchgrass under drought stress. A total of 406 putative targets were also predicted. These targets are found to play role in structural protein, metabolism, transcription factor, growth & development, stress related, signaling pathways, storage proteins and other vital processes. This baseline data can be utilized for the fine tuning of this important bioenergy plant under biotic and abiotic stresses.

ABSTRACT INFO

Session: Plant Genomics& Biosafety S02-S03

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Insightes of the phosphoenolpyruvate carboxylase (CaPPC) gene family in chickpea (*Cicer arietinum L.*)

Syed Rehmat Ullah Shah*[1], Muhammad Aslam Buzdar[2], Dost Muhammad Baloch[1] Ghulam Jilani[1]

Phosphoenolpyruvate carboxylase (EC 4.1.1.31; PEPC) enzyme is involved in β-carboxylation of phosphoenolpyruvate (PEP) to oxaloacetate and is encoded by a gene family CaPPCs. It has a vital role in diverse metabolic pathways of organic acids biosynthesis and carbon fixation. In this study we characterized the PEPC gene family in chickpea (*Cicer arietinum L.*)

Transcripts and their abundance (fragment per kilo bases per million reads, FPKM) were retrieved from online transcriptome or genome databases. Different plant type PPCs (PTPC) and bacterial type PPCs (BTPC) sequences were separated, aligned and their class and specie specific consensus were constructed in CLC Main Workbench 6.5 (CLC bio, Denmark). Blastx analysis was carried out through NCBI database. Sequence alignment and phylogeny of PPC gene family of chickpea (*Cicer arietinum L.*) was analysed through in house available softwares.

The current study revealed that PEPC enzyme in chickpea is encoded by a gene family of at least 6 members with all active site residues of C3 PEPCs. Phylogenetic analysis of the amino acid sequences showed divergence into two major groups PTPC and BTPC with further convergence of PTPC into sub groups. FPKM values of the transcripts showed tissue specific abundance in shoots, roots, flowers and pods.

Phosphoenolpyruvate carboxylase enzyme in chickpea is encoded by a small gene family. In contrast to Arabidopsis PTPC in chickpea is not restricted to 3 members and has tissue specific transcript abundance. Further studies are suggested to probe out the role of PPC genes in nodule development and its regulation under stress conditions in chickpea.

ABSTRACT INFO

Session: Plant Genomics & Biosafety S02-S03

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Determining the expression pattern of GLP gene during somatic embryogenesis in *oryza sativa*

Fazeelat Karamat*[1], Samina Khan[1], Tayyaba Yasmin[1]

Germins and Germin like proteins, display a wide spectrum of variety in their occurrence and activities in plants. They are believed to have pivotal roles in the plant germination, development and defense system i.e., biotic and abiotic stress owing mainly to the two associated enzymatic activities; oxalate oxidase (OXO) and superoxide dismutase (SOD). Among cash crops of Pakistan, rice hold top position and these proteins have been found to confer the resistance against broad spectrum diseases in this crop. Furthermore, the presence of these proteins in all organs and developmental stages of plants has been reported. On behalf of their involvement in plant growth and health it would be really insightful to examine their expression during in vitrocallus formation and somatic embryogenesis in Pakistani rice varieties. Two varieties, viz; KSK -282 and SuperBasmati were selected for callus induction in the presence of two hormonal treatments (2, 4-D alone, 2, 4-D + BAP) either in light or dark. Cali was used as explants for regeneration experiments using the BAP as growth regulator. RNA isolation from calli and various developmental stages (green spots, emerging shoots and roots) and subsequent cDNA synthesis was accomplished. Expression analysis of GLP gene would be analyzed through QPCR. The gene/s expression analysis outcome from this study would be helpful in getting perceptivity about the possible relation between the expression of GLPs at transcript level and various developmental stages of somatic embryogenesis and early development.

ABSTRACT INFO

Session: Plant Genomics & Biosafety S02-S03

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Advance-to-be-expressed, modified genomic DNA isolation from *NICOTIANA BENTHAMIANA* var domin.'s specified germlasms in Pakistan

Zaryab Khalid Sial* and Farah Khan

Most of the work done on *Nicotiana benthamiana.* Var Domin, belongs to its native climate areas where usually fresh plant parts are used to isolate the genomic DNA for further experimentation. Climate of most of the areas of Pakistan are not suitable for optimum growth of *N. benthamiana* without the help of temperature controlled facilities. For speedy workout the dried and preserved germplasm exported from abroad has become a need of researchers working on this model plant. It is also noticed that elevated temperature during the germination and growth of *N. benthamiana* not only effects the quality of extracted DNA but also leaves impact on it host pest relationship. So it was necessary to get some handy protocol/ protocols for isolating pure DNA not only from dry and preserved germplasm of *N. benthamiana* but also from the locally under controlled environment grown plants using their young, mature, fresh or dry tissues. The presence of certain metabolites like polyphenols and polysaccharides have been found to interfere more (specially during plant growth and further processing in warmer climates) with already adopted DNA isolation protocols and also with further reactions like DNA restriction, cloning and amplification. We have worked with four modified protocols for getting best quality genomic DNA extraction from dry and fresh explants of *N. benthamiana.* The best quality DNA was extracted by modifying different steps along with altering quantities of different reagents and organic solvent used during above mentioned four reported methods. The protocol without liquid nitrogen gave best results in this concern. Dry plant material was of special consideration. The isolated DNA provided distinct bands with 0.8% gel. A very efficient and simple protocol was optimized for removing highly interfering metabolites which gave the best isolated DNA of *N. benthamiana* in terms of quality and purity. We describe here the simple efficient and cost effective methods for Isolation of DNA from this model plant.

ABSTRACT INFO

Session: Plant Transformation S04

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Genetic integration and expression of insect and weedicide resistant genes in Cotton (*Gossypium hirsutum* L.)

Kamran Shehzad Bajwa*, Abdul Qayyum Rao, Ayesha Latif, Agung Nugroho Puspito, Malik Adil Abbas, Muhammad Azam Ali, Idrees Ahmad Nasir and Tayyab Husnain

Genetic engineering has many attractive interests for the development of transgenic cotton plants with economically important new traits. The main goal of current study was gene transformation in cotton for the development of bollworms and weedicide resistant varieties especially with crystal toxin and cp4 EPSPS genes protein.

In order to produce transgenic cotton resistant to insects and weeds, newly emerge embryos of MNH-786 were transformed with Agrobacterium tumefaciens strain LBA4404 harboring the plasmid pCAMBIA 1301 vector containing Cry1Ac+Cry2A and Cp4 EPSPS genes under the control of CaMv 35S promoter. Neomycin phosphotransferase (nptII) gene was used as a selectable marker.

The integration and expression of these genes were evaluated by molecular techniques such as PCR, Florescent In-Situ Hybridization and ELISA respectively. Out of 700 putative transgenic cotton plants, 10 plants (1.4% transformation efficiency) showed the presence of genes Cry1Ac+Cry2A and cp4 EPSPS through PCR analysis. Insect feed bioassay and glyphosate spray assay (1600 ml/acre) were used for measurement of mortality percentage of bollworms and weeds under field condition. The data collected of transgenic cotton plants from field condition indicated that 100% mortality of American bollworms, army worms but 39.0% to 73.0% effects on pink bollworms and also 100% mortality of weeds in glyphosate spray assay as compared to control.

The results showed that the transgenic lines were resistant to bollworms and Glyphosate, as compared to control plants. The results of this study support improvement of cotton defense systems against bollworms and natural competitors (weeds) through genome modification.

ABSTRACT INFO

Session: Plant Transformation S04

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Presenter:
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Development of transgenic wheat with higher phytase expression for improved phosphorus use efficiency

Asma Maqbool*, Samreen Mohsin and Kauser Abdulla Malik

Phosphorus deficiency is a critical issue for the agriculture world. Though use of fertilizers was considered a solution at first, it is now considered to be only adding more fuel to the problem since it adds to organic phosphorus pool of soil. Development of plant cultivars with enhanced phosphorus utilization ability via traditional breeding is very slow. With advancements in biotechnology, development of transgenic plants with better phosphorus efficiency is a better way of overcoming this problem. Thus for improved organic phosphorus utilization, transgenic wheat with root-specific phytase expression was developed. Positive transformed plants were screened via basta selection and PCR. From T1 generation onwards, phytase activity and phosphorus content of transgenic plants grown in the presence of phytate was determined. Morphological analysis of transgenic plants grown under natural conditions was also done. Plants with higher phytase activity and phosphorus content along with no adverse morphological characteristics were selected for further analysis. Results uptill now are encouraging and soon a stable homozygous transgenic line with higher phytase activity and improved phosphorus efficiency will be determined.

ABSTRACT INFO

Session: Plant Transformation S04

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Transformation and expression studies of Cry1Ac+Cry2A and GTGene in *Zea mays*

Abdul Munim Farooq, Idrees Ahmad Nasir, Qurban Ali* and Tayyab Husnain

Zea mays is the 3rd important cereal crop grown all over the world and fulfills the needs of millions of people. Efforts to make good quality and high yielding crop are continuous. There are various biotic and abiotic stress that caused reduction in grain yield and quality of maize crop among them weeds showed the most adverse effects. Average maize production of Pakistan is 2,850 kg/ha which is very is low as compared to the developed countries like USA, which is 3420 kg/ha. In Pakistan it is planted on an estimated area of 1.083 million hectare with an annual production of 4.271 million tons (Anonymous 2006). Insect pest, like Heliothus, root bore, stem borer and weeds are the main causes of low production in Pakistan e.g. only stem borer can caused losses up to 75% (Groote, 2002). Transgenic maize having locally developed CEMB-insect resistant and weedicide resistant genes were found very effective to combat these challenges to the local maize crop. Objective of this study was to develop lepidopteron insects and Glyphosate resistance maize lines. The transformed lines will have more ability to cope with lepidopteron insects and herbicides as compared with non-transformed lines in field trials. Transformation of maize with GTGene will open new perspective for the improvement of herbicide resistant maize.

ABSTRACT INFO

Session: Plant Transformation S04

Biosafety studies of genetically modified maize (*Zea mays* L.), its effect on soil fertility by evaluating pH, EC and micronutrients

Rabia Saba, Zabta Khan Shinwari, Naseer Ahmad And Saira Karimi

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A biosafety study was conducted of genetically modified maize (*Zea mays* L.) in order to comparatively evaluate the impact of transgenic maize (insect resistance) with non-transgenic maize lines (Islamabad White and Islamabad Gold). The main objective was to measure effect of transgenic maize on soil fertility by evaluating the status of pH, EC, micronutrients in rhizospheric soil. Soil samples were collected from field trial and controlled conditions at vegetative and harvesting stage of both transgenic maize and non-transgenic maize lines the soil in which no plant was grown were referred as control. The samples were analysed for pH, EC and AB-DTPA extractable micronutrients such as Mn, Ni, Co, Cu, Cr and Fe. There was also significant difference in content of Mn (control mean 0.20, Mn (GM) mean 0.91and (non GM) mean 0.92 with significance value 0.00), Co (control mean 0.015, Co (GM) mean 0.040 and (non GM) mean 0.046 with significance value 0.043), Cu (control mean 0.064, Cu (GM) and (non GM) mean 0.099 with significance value 0.028), Cr (control mean 0.035, Cr (GM) mean 0.043 and (non GM) mean 0.15 with significance value 0.035) and Fe (control mean 0.66, Fe (GM) mean 0.56 and (non GM) mean 0.58 with significance value 0.029) in rhizospheric soil of all the plants of both transgenic maize and non-transgenic maize at all stages of both field trial and controlled conditions. So it showed that transgenic maize behave positively toward rhizospheric soil.

ABSTRACT INFO

Session: Plant Transformation S04

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Presenter:
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Improvement of cotton fibre quality by overexpression of SuS gene

Mukhtar Ahmed*, Ahmed Ali Shahid, Abdul Qayyum Rao, Salah ud Din, Sidra Akhtar, Muhammad Azmat Ullah Khan, Kamran Shehzad Bajwa, Ammara Ahad, Ambreen Gul, Malik Adil Abbas, Tayyab Husnain

The demand for long staple fibre having better strength and length is increasing with introduction of modern spinning and weaving industry in Pakistan. Work on gene discovery from developing cotton fibres has helped to identify dozens of genes that take part in cotton fibre development and several genes have been characterized for their role in fibre development. The study was focused at successful Agrobacterium mediated stable transformation of SuS gene construct in pCAMBIA 1301 into cotton variety CEMB-00. Removal of 2815bp fragment by restriction digestion with specific restriction enzymes ensures successful ligation of fibre gene into pCAMBIA 1301. Total 1000 embryos were subjected to selection free medium after inoculation with Agrobacterium harboring gene of interest. Out of 1000 total 128 embryos successfully developed shoots and roots. 45 putative transgenic cotton plants have been shifted to selection free medium in test tubes and are passing acclimatization process. Molecular analysis of these plants is under process which will further confirm their successful integration and expression will lead towards improvement of fibre characteristics when shifted to field at mature stage. Incorporation of genes related to cotton fibre length and quality can provide new avenues for fibre improvement. The utilization of this technology would provide an efficient import substitution and sustained production of long staple fibre in Pakistan to fulfill the industrial requirements.

ABSTRACT INFO

Session: Plant Transformation S04

Development of transgenic wheat with low phytate for increasing bioavailability of iron and zinc

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Iron and zinc are two most important micronutrients and according to an estimate more than two billion people are suffering from deficiency of these nutrients worldwide and especially in developing countries. The deficiencies of these micronutrients is responsible for reduced learning and working capability, increased risk of infections, anemia, retarded growth, skeletal abnormalities and depressed immune response. The bioavailability of iron and zinc in wheat can be improved by transformation of wheat with phytase enzyme, which catalyses hydrolysis of phytic acid and releases cations bound to it. Phytase transgenic wheat plants with endosperm specific expression to enhance bioavailability of Fe and Zn, were developed. Agrobacterium mediated transformation method was used to generate transgenic plants. In vitro digestion experiments revealed enhanced phytase activity, reduced phytic acid content, 1.2-1.4 folds increased bioavailable iron content and 1.3-2 folds enhanced bioavailable Zn content in dough and 2.1-3.0 folds increased bioavailable iron content and 1.3-2.2 folds enhanced bioavailable Zn content in chapatti. This development opens new ways to overcome iron and zinc malnutrition, especially women and children.

ABSTRACT INFO

Session: Plant Transformation S04

Knock down studies of *Pichia pastoris* derived OCH1 through shRNA

Zia Ur Rahman*[1], Mohsin Ahmad Khan[1], Nadeem Ahmed[1], Ahmad U Zafar[1], Tariq Nadeem[1], Rabbia Pervaiz[1], Hina Kanwal[1], Sabeela Saleem[1], Faidad Khan[1], Hamid Bashir[1], Abdul Shakoor[2]

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Outer CHain elongation 1 (OCH1) encoded α-1,6-monnosyltransferase is golgi based enzyme, which is involved in hyperglycosylation of glycoproteins, expressed in Pichia pastoris (Yeast). OCH1 initiates outer chain, on the oligosaccharides attached to glycoproteins, coming directly from Endoplasmic Reticulum. Further branching occurs on this outer chain, where bunch of mannose residues get attached, leading to hyperglycosylation. Hyperglycosylation has adverse effects on therapeutic efficacy of glycoproteins, like solubility, pharmacokinetics, pharmacodistribution, proper structural folding, receptors binding and serum half-life. There are various glycosylation engineering technologies, which has successfully engineered the N-glycosylation pathway of yeast to control hyperglycosylation. In most of these technologies OCH1 is the target of interest to knock out by homologous recombination, but this has adversely affected the cell morphology as well as volumetric productivity has decreased. In this study we have explored the possibility of alternative approach to knock down OCH1 gene via siRNA at the time of gene expression, rather than complete knock out, to counter the adverse effects. Three shRNA designed were co-transfected with OCH1 in Huh-7 transient expression system in dose dependent manner to check silencing effect of siRNA. Real Time PCR analysis shows that shRNA3 has knocked down OCH1 by 63%, while 62% by shRNA2 after 48 hours, when shRNA vector concentration was 1000ng. Almost similar results were obtained when shRNA vector concentration was decreased to 500ng. Dose reduction from 1000 ng to 500 ng has favor the silencing effect of shRNA1, almost 18% more silencing effect has been observed. Now both shRNA2 and shRNA3 can be used synergistically in a single a vector to get maximum depression of mRNA. Unfortunately it is believed that RNA interference phenomena does not exist in budding yeast. Hence, in future, first RNA interference phenomena will be reconstituted in Pichia pastoris by introduction of Dicer (DCR) and Argonaute (Ago) genes of yeast origin. Then, OCH1 gene will be silenced under inducible promotor at the time of recombinant glycoproteins expression to control its hyperglycosylation.

ABSTRACT INFO

Session: Molecular Biology of Cancer & Forensics Sciences S05

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Development of a miniSTR system for the analysis of highly degraded DNA

Muhammad Shafique*, Muhammad Saqib Shahzad, Ziaur Rehman, Ahmad Ali Shahid, Tayyab Husnain

Application of conventional STRs gives the results in full profiles where the high-quality DNA samples are available. But when the DNA is highly degraded or the fragments are lower than 250 bp due to environmental stress which fails to show conclusive profiling. For such circumstances, ten STR loci (CSF1PO, D7S820, TPOX, D18S51, D2S1338, D13S317, FGA, D5S818, D21S11, and D16S539) and amelogenin marker were selected for the development of mini-plex system. The product amplification range was defined fluorescently from 65bp to 270bp using 5-dye system on ABI3130xl Genetic Analyzer. For the stability and sensitivity of these miniSTRs DNA was artificially degraded up to 80 minutes and the all these loci were amplified successfully. Moreover ten miniSTRs were screened through 350 unrelated individuals of Pakistani population. A total of 101 polymorphic alleles were observed ranging the allelic frequency from 0.0014 to 0.4214.The power of discrimination is from 0.852 (TPOX) to 0.970 (D2S1338). The probability of matching was low as 0.0301 at D2S1338 to 0.1473 at TPOX. The combined power of discrimination (PD), combined power of exclusion (PE) and cumulative probability of matching (PM) were equaled to 0.999999999998611, 0.999753 and 1.38943x10-12 respectively. These MiniSTRs on the basis of high degree of polymorphism and fragment size reduction will be highly informative in most of the forensic cases where DNA of the samples is highly degraded, parentage analysis, mass disasters and dead body identification. Applying the p values exact test, at probability level of 0.05, significant deviations from Hardy–Weinberg equilibrium were observed at three loci (D2S1338, TPOX, and D21S11) but after Bonferroni correction, no deviations were observed. When compared the same STR loci with previously published different world populations, significant differences were also observed at these loci.

ABSTRACT INFO

Session: Molecular Biology of Cancer & Forensics Sciences S05

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HCV regulated apoptosis, steatosis, and oxidative stress leads to HCC

Shah Jahan*

Hepatitis C virus (HCV) Core protein is thought to trigger the activation of multiple signaling pathways and alteration of cellular gene expression responsible for HCV induced pathogenesis leading to hepatocellular carcinoma (HCC). Prevalence of HCV genotype 3a associated HCC is higher in Pakistan as compare to the rest of world. Approximately 40–60% of HCV infected individuals leads to chronic liver diseases, however the molecular mechanism of HCV involved in HCC pathogenesis is still unclear. This study was conducted to analyze effect of HCV genotype 1a and 3a on cellular genes involved in oxidative stress, steatosis and apoptotic pathway and cell proliferation which leads to HCC.

We examined the in vitro effect of HCV structural proteins of genotype 3a and 1a on cellular genes involved in oxidative stress, steatosis and apoptosis by real time PCR and western blotting and cell proliferation by transfecting HCV structural protiens in Human hepatocellular cell line (Huh-7). Expression analysis of cellular genes were also studied in Blood and biopsy samples of HCV chronic patients

HCV 3a Core protein over expression down regulates the RNA and protein levels of caspase and other gens which are involved in apoptosis . We found down regulated expression of these genes in HCV chronic patient with HCV genotype 3a as compare to 1a in apoptosis. Moreover, HCV 3a gene showed stronger effect in regulating protein level of p-Akt as compared to HCV 1a Core accompanied by enhanced cell proliferation in HCV 3a Core transfected Huh-7 cell line. Expression analysis of cellular genes were altered in in Blood and biopsy samples of HCV chronic patients as compare to normal which confirms our cell culture results

Reduced expression of cellular genes involved in apoptosis and increased expression of cellular genes involved in oxidative stress and steatosis in HCV patients and HCV Transfected Huh 7 cells and p-Akt involved in cell proliferation in response to HCV confirms higher involvement of HCV 3a in HCC. We suggest further studies to evaluate an interaction of HCV and cellular genes in regulating the gene expression which may provide a better understanding for genome-specific mechanisms involved in disease progression by regulating other pathways directly or indirectly increases cell proliferation leading to HCC.

ABSTRACT INFO

Session: Molecular Biology of Cancer & Forensics Sciences S05

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A study of SE33 locus as a reliable STR marker for forensic investigation in Pakistani population

Munir Ahmad Bhinder*, Muhammad Yasir Zahoor, Rukhsana Perveen, Ghulam Murtaza Anjum, Muhammad Imran

The genetic variation being an authentic discriminating marker for diverse ethnic groups in South East Asia is known throughout the inclusive context. Different genetic and forensic parameters for SE33 locus were studied in 204 individuals from different ethnic groups of Pakistani population. Genotyping of SE33 locus revealed a total of 43 alleles including 3 novel alleles. Genotype and allele frequencies for SE33 locus were not in Hardy-Weinberg equilibrium. Value for power of discrimination of SE33 locus was 0.991 making this locus a powerful marker for forensic genetics and anthrogenealogical studies of Pakistani population and neighboring ancestral populations from Iran to central Asia extending to Europe. SE33 profile is may be very useful in inconclusive cases where incomplete DNA profile is generated due to degraded evidence samples.

ABSTRACT INFO

Session: Molecular Biology of Cancer & Forensics Sciences S05

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Medicinal Plants for the Management of Breast Cancer and its Prevalence in Pakistan

Hafiz Muhammad Asif*, Sabira Sultana

Breast cancer is the most common cancer in females all over the world with approximately one million new cases each year as well as one of second leading causes of death among females. In Pakistan, the most frequently diagnosed cancer among females is also breast cancer, accounting for nearly one in nine female patients. Its incidence in Pakistan is 2.5 times higher than that in neighboring countries like Iran and India. The risk factors associated with breast cancer are age, family history, early menarche, intake of combined estrogen and progestin menopausal hormones, alcohol consumption, physical inactivity, low socioeconomic status and lack of awareness regarding the disease. Although great advancement have been made in the treatment of cancer progression, still significant deficiencies and room for improvement remain. Chemotherapy produced a number of undesired and toxic side effects. Natural therapies, such as the use of plant-derived products in the treatment of cancer, may reduce adverse and toxic side effects. However, many plants exist that have shown very promising anticancer activities in vitro and in vivo but their active anticancer principle have yet to be evaluated. Combined efforts of botanist, pharmacologist and chemists are required to find new lead anticancer constituent to fight disease. This article aims to provide awareness about breast cancer as well as an updated knowledge about the prevalence, risk factors and new lead anticancer constituent to fight against breast cancer in Pakistan.

ABSTRACT INFO

Session: Molecular Biology of Cancer & Forensics Sciences S05

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Proteome of human oral tongue squamous cell carcinoma identified by quantitative tandem mass spectrometry

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Worldwide, some oral cancers are amongst the most rapidly increasing cancers due to changes in lifestyle. The aim of this prospective study was to identify proteins associated with advancement of oral tongue squamous cell carcinoma (OTSCC). A quantitative approach was used to determine the global changes in the proteome of primary OTSCC with neck nodes positive (lymph node metastatic), neck nodes negative (non-metastatic) and non-malignant hyperplastic abrasion with chronic inflammation of tongue relative to non-cancer tissue of oral cavity origin.

A total of 10 tongue biopsies were collected from patients enrolled for surgery at SKMCH&RC and Services Hospital, Pakistan. A multi-dimensional protein identification technology (MudPIT) was used to generate proteomic profiles. For each sample, total proteins were extracted, trypsin digested and iTRAQ labelled for quantitative proteomics analysis to identify changes in protein expression Labelled peptides were combined and then separated using strong cation exchange (SCX) chromatography prior to nHPLC-MALDI-MS analyses. Peptidefragment mass lists collated in ProteinScape (Bruker Daltonik), were searched using Mascot software (Matrix Science, U.K.) against the SwissProt database.

Database searching resulted in the identification of 565 proteins (95% confidence interval threshold, $p<0.05$, Mascot score ≥ 29) with at least 2 signature peptides. A number of stagespecific up- and down-regulated proteins were identified that have not previously been associated with oral tongue squamous cell carcinoma. Of particular importance were those associated with epigenetic, apoptotic and stromal functions of squamous cells and are currently the subjects of further validation as therapeutic targets and diagnostic markers. Oral cancer represents a complex heterogeneous group of diseases emerging with both anatomical and stage-specific differences. Our study provides the first insights into tongue specific cancer progression in order to identify unique stratified disease markers and targets.

ABSTRACT INFO

Session: Molecular Biology of Cancer & Forensics Sciences S05

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Presenter:
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Forensic Analysis of Human Hair for Heavy Metals detection and Comparison among workers of Different Industries Using PIXE

M. Saqib Shahzad*[a], Muhammad Rehman[b], Javed Iqbal Bajwa[c], Azam Ali[b], Rabail Alam[b], MH Qazi[b]

The objective of this study is to determine the Aluminum (Al), Calcium (Ca), Potassium (K) and Sulfur (S) metals concentrations in the human hair of different industries workers like Pharmaceutical industry, Textile Industry and Paint Industry, the data has been presented into the three groups. Methods were developed to detect heavy metals from Human hair. In the group 1 from the pharmaceutical industry the mean for Aluminum (Al) metals was 575.1 and show significant differences the P – vale for Aluminum was $p<0.05$, the mean for Calcium (Ca) was 9.1 were non-significant different in this group the P – value was $p>0.05$, the mean for Potassium (Ca) metal was 11.1 and show non-significant differences the p – value was $p>0.05$, the mean for Sulfur (S) metal was 190.7 show significant differences and p – value was $p<0.01$. The increasing order of metals levels in was exist in pharmaceutical industry was Al>S>K>Ca. In group data 2 from textile industry the mean for Aluminum (Al) metal was 116.9 show non-significant differences the p – value was $p >0.05$, the mean for Calcium (Ca) was 1.7 and show the significant differences the p – value was $p <0.01$, the mean for Potassium (K) was 3.2 and found non-significant differences and the p – value was $p>0.05$, the mean for Sulfur (S) 17.7 show non-significant differences the p – value was $p>0.05$, the increasing order for the above four metals in the textile industry were Al>S>K>Ca. In group data 3 form brighto paint industry the mean for Aluminum (Al) was 103.7 which was non-significant the p – value was $p >0.05$, the mean for Calcium (Ca) was 1.7 which having significant differences the p – value was $p <0.01$, the mean for the Potassium (K) metal was 2.3 and was non-significant different the p – value was >0.05, the arithmetic mean for Sulfur (S) metal was 8.87 and was non-significant different the p – value was $p>0.05$. The increasing order for above metal in paint industry was the Al>S>K>Ca, it is concluded that the concentration of Aluminum (Al) metals was high in all three industries which determined that the Aluminum (Al) metal having much more exposure to industrial workers.

ABSTRACT INFO

Session: Molecular Biology of Cancer & Forensics Sciences S05

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Forensic Analysis of GSR of Locally manufactured ammunition in Pakistan on different fabrics

Muneeba Butt*, Makhdoom Saad

Gunshot residue (GSR) examination is one of the most imperative field of forensic Investigation in Pakistan. In the present study, simple and rapid techniques of presumptive color tests (Modified Griess Test and Sodium Rhodizonate test and Diphenylamine test) and contemporary sophisticated analytical method computer-controlled Scanning Electron Microscope (SEM) coupled with Energy Dispersive X-rays detector (EDX) were involved. This work concentrated to evaluate the collection efficiency of gunshot residues (GSR) as a function when different types of fabrics were exposed to shooting from various distances. The samples were stubbed to collect GSR following a standard operating procedure. The main factors explaining that difference was probably the rate of saturation of inter fiber spaces of the fabrics and ammunition used. The study was designed in following fashion; (1) firearms discharged from variable distance (0, 1, 3, 5, and 7 ft), (2) using different five types of target fabrics (Cotton, K.T. cotton, polyester, silk and raw silk), (squares sizes 18x18 inch)., (3) type of firearms used (9mm caliber pistol and 7.62x39 AK-47 riffle), (4) Modified Griess test and Sodium Rhodizonate tests were performed for the detection of the Nitrites and Lead in the form of the intensity of orange and red color respectively, (5) SEM/EDX was used for the particle size determination of GSR. The intensity of the color was found to be proportional to the GSR on fabrics. Modified Griess test and Sodium Rhodizonate test gave corresponding color for nitrite and lead respectively, while DPA test produced characteristic color (greenish blue).The results revealed not only the presence of GSR components but also strong correlation between the retain-ability and the collection efficiency of GSR on different types of fabrics. The SEM/EDX analysis of GSR revealed the dependency of GSR particles size on distance and type of weapon used. It is concluded that the persistency of GSR residues on different fabrics depends not only on the variable firing distances but also on the texture of the fabrics. The intensity of GSR pattern found better on cotton in comparison with polyester and silk in colored tests at fixed distance. This work will help to reconstruct the shooting scene to estimate muzzle-to-target distance up to 7 feet.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders
S06

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Presenter:
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Pakistan and the Personalized Medicine Era

Muhammad Ilyas*

The new era of pharmacogenomics has been raised where we use genetic information of an individual for the development of personalized medicine. Here, we analyzed the whole genome sequence of a Pakistani individual to get his pharmacogenomics profile. We found a number of SNPs associated with methotrexate dose response, docetaxel and thalidomide, and several other variants of clinical relevance. The current study provides a workflow example to use personal genomes for developing personalized medicine protocols. The pharmacogenomics association to neurological disorders, depression and hypertension discovered here can provide the most fundamental information for a Pakistani individual to change his way of life and carry out precautionary actions for a better life ahead.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders
S06

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Presenter:
Samia Afzal

Global consensus sequence development of dengue virus for peptide based vaccine design against local Dengue Virus

Samia Afzal*, Muhammad Idrees, Iram Amin, Muhammad Shahid

Dengue virus (DENV) is the member of Flaviviridae and causative agent of 300 million clinical infections each year. Dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are serious forms of Dengue fever (DF) and cause 500,000 infections annually. Every year, around 70% of the world population is at risk, due to epidemic episodes orchestrated by one or more of its serotypes. So, a tetravalent DENV vaccine is needed which may induce the immune response against all four DENV serotypes. In this study, B-cell and T-cell epitopes have been predicted from the DENV envelope glycoprotein (Eg) using a consensus based approach in complement with the physico-chemical property (PCP) conservancy analysis. Through DENV-Eg analysis, a total of 7 PCP conserved, water soluble, in vitro and in vivo stable epitopes were predicted which may induce the B-cell and T-cell mediated anti-viral immune response. The DENV-Eg is highly conserved protein and appropriate candidate for the multivalent vaccine design. Furthermore, the features of solubility and stability of the DENV-Eg based epitopes are the additive advantages which may lead to effective manufacturing and development of the immune response.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders
S06

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The role of aldose reductase in progression of diabetic retinopathy of local population

Shagufta Naz*, Saima Sharif, Afia Iqbal , Tasnim Farasat, Ayesha Jamil

Diabetic retinopathy is one of the leading cause of acquired blindness all over the world. It affects the 80 % of diabetic patients in Pakistani population. It is an ocular manifestation of diabetes with poor long term glycemic control. Our study was planned to analyze the serum amount of aldose reductase from blood samples taken from the patients of diabetes and diabetic retinopathy from Diabetic management center Services hospital, Lahore. Control healthy objects were also included for comparison. This research was conducted on diabetic retinopathy patients during the period of Jan 2014 to June 2014. The study population was comprised of 90 subjects categorized into control (n=14) diabetic (n=29) and diabetic retinopathy (n=47).

Demographic data was compiled on excel sheet. Data was collected through self designed questionnaire, filled by patients. The questionnaire contained various parameters such as the height, weight time duration of disease, family history of disease, associated diseases, vision impairment, sugar level of patient and other complications.

The blood samples were collected and serum was separated by centrifugation. The blood glucose level was measured by Glucometer. For quantitative analysis of aldose reductase enzyme from the serum of blood ELISA was performed by using commercially available kit. This analysis was performed in the laboratory of Lahore College for Women University. Statistical analysis was done by SPSS. The comparison among groups was performed with ANOVA and gender difference was determined with the help of T. test.

The results showed that as age of subjects and duration of diabetes increases the risk factor of developing the DR also increases and as BMI of DR group was 25.6 kh/m^2 higher than diabetic group 24.2 kg/m^2. Correlation analysis between sugar level and concentration of aldose reducatse revealed that concentration of aldose reducatse is directly proportional to the sugar level that leads to severity of diabetic retinopathy.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders S06

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Presenter:
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Atrial fibrillation association with indicators of metabolic syndrome in subjects of our local population

Saima Sharif*, Tasnim Farasat, Saira Rafaqat , Shagufta Naz, Farkhanda Manzoor

Atrial fibrillation (AF) is the common cardiac arrhythmia in which heart beats irregularly usually too fast. The heart rate is greater than 100 beats per minutes. AF is well-documented public health problem causing substantial mortality and morbidity. AF progresses from short, rare episodes, to longer and more frequent attacks. The risk factors for the development of AF are aging, hypertension, diabetes, heart failure, coronary artery disease, Hyperthyroidism, obstructive sleeps apnea and obesity. Metabolic syndrome (MS) is a collection of metabolic risk factors like diabetes, hypertension, dyslipidemia, obesity and impaired glucose level that exist in one person. Cardiovascular diseases are the primary outcome of metabolic syndrome. The aim of study was to find the relationship of AF with metabolic syndrome and to determine the diagnostic value of early detection of insulin resistance in AF patients. The study was conducted in Punjab Institute of Cardiology, Lahore. 100 AF subjects were sampled with fasting, 50 with metabolic syndrome and 50 without metabolic syndrome. 25 healthy control subjects were also included for comparison. Blood sample was collected and serum was separated and used for the measurement of fasting blood glucose, total cholesterol, triglycerides levels and HDL-C by using the kits on chemical analyzer. The insulin level was also measured by Enzyme-Linked Immunosorbent Assay (ELISA) in the laboratory of LCWU. Data was analyzed statistically by using the SPSS software. The fasting blood glucose levels, blood pressure, HDL-C, insulin and IR were showed the highly significant difference ($p \leq 0.05$) among the AF groups without MS and with MS and control groups. The BMI, WHR, TC, TG and LDL-C were also significantly higher in AF group with MS as compared with AF group without MS and control group. It is concluded that insulin resistance is an important risk factor for the progression of AF and it is directly related with several other risk factor of AF. Our study proved the relationship between the Metabolic Syndrome and Atrial Fibrillation. In summary, in our large community based study we observed a significant association between IR and incident AF.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders
S06

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Presenter:
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Effects of Cyp4x1 mutation on weight gain and energy metabolism in mice: Evidence for increased adiposity and metabolic syndrome

Farhat Batool*[1,2] , Francis Ebling[2] and David Kendall[2]

The novel cytochrome P450 4x1 (Cyp4x1) is expressed at unusually high levels in the brain. Although the precise function of this protein is unknown, it has been hypothesised to regulate metabolism of fatty acids and to affect the activity of endocannabinoid signalling systems which are known to influence appetite and energy metabolism. The objective of the present investigation was to determine the impact of the Cyp4x1 mutation on body weight and energy metabolism. A line of transgenic Cyp4x1-null mice (Al-Anizy, 2006, FEBS Journal 273:936–947) and control mice of the same genetic background were studied in their home cages. Both male and female Cyp4x1-null mice gained significantly more body weight on normal lab chow compared to controls. This was associated with significantly higher fasting blood glucose at 10 and 20 weeks of age, and significantly impaired glucose clearance ($P<0.001$), as revealed by a glucose tolerance test (2g/kg BW, ip) at 20 weeks of age. At necropsy, Cyp4x1-null mice had significantly greater intra-abdominal fat depots ($P<0.01$), and evidence of fatty liver pathology suggestive of hepatic steatosis. Mice (n=6 per group) were monitored in a CLAMS at 14 weeks of age when Cyp4x1-null mice were significantly heavier than controls ($P<0.005$), to determine metabolic rate and fuel utilization. Metabolic rate as inferred from VO2 was not significantly affected by the mutation in either gender at this age. However, light phase locomotor activity levels were significantly reduced in both sexes ($P=0.01$). A gender x genotype interaction revealed that respiratory exchange ratio was significantly decreased in male mice ($P<0.05$), suggesting a greater degree of fax oxidation, consistent with their higher adiposity.

We conclude that the Cyp4x1-null mouse demonstrates a number of characteristics of metabolic system. Given the growing prevalence of metabolic syndrome this transgenic mouse line may provide a valuable animal model for identifying strategies to reduce adiposity and increase insulin responsiveness.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders
S06

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Culture media stress on the activity of glutamate dehydrogenase of antidiabetic medicinal herb

Darima Habib*, Muhammad Zia and Muhammad Fayyaz Chaudhary

Activity of Glutamate Dehydrogenase (GDH) enzyme were studied in complete life cycle of antidiabetic medicinal herb *Argyrolobium roseum* from in vitro developed conditions until the regenerated phase produced through indirect organogenesis. Culture medium was supplemented with different hormonal combinations along with additives to enhance the cell culturing process. Glutamate dehydrogenase showed inverse pattern during callogenesis as compared to in vitro grown seedlings. High activity of GDH was observed in callus as compared to in vitro studied explants. NADPH-GDH showed maximum activity at end of callus development and continued this pattern in proliferation phase. Leaf derived calli exhibited 25% increase in enzyme level as compared to stem 53% and roots 42%. NADPH-GDH showed descending pattern during regeneration period. Regenerated leaf exhibited 13.3% decline in enzyme level as compared to stem (17.05%) and roots (19.9%). Leaf, stem and roots showed 9.76%, 17.9%, 32.9% activity respectively, at the end of acclimatized stage studied. High activity of NADPH-GDH was recorded in regenerated plants as compared to in vitro developed plants.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders S06

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Down-regulation of *ABCA*1 gene by hyperglycemia leads to dyslipidemia in type 2 diabetes

Asifa Majeed*, Raheela Yasmin, Amir Rashid, Suhail Razak

Diabetes mellitus is recognized as a major public health problem worldwide with an estimation of 6.3 million people diabetic in Pakistan. Dyslipidemia is the most common complication seen in type 2 diabetes and an increase risk of cardiovascular and cerebrovascular diseases. ATP binding cassette protein (*ABCA1*) maintains the homeostasis of HDL-C and involved in reverse cholesterol transport in human body. Imbalance of this homoeostasis leads to dyslipidemia in type 2 diabetes. The objectives of the study were determination of expression level and identification of genetic variations in *ABCA1* gene and further association with HDL-C levels. The enrolled subjects were (I) diabetic patients with dyslipidemia (II) diabetic patients and (III) healthy individuals. Biochemical analyses were done to measure the glucose and lipid profile. RNA and DNA extracted from leukocytes. Quantitative PCR was done for expression of *ABCA1* gene and automated DNA sequencing performed for the identification of mutations in *ABCA1* gene. The relative quantification showed remarked decrease in *ABCA1* gene expression in group-1 compared to group-II & III. The HDL-C and TG levels were significantly deranged in group-I. The spearman coefficient statistics showed strong association between low expression of ABCA1 gene and decreased levels of HDL-C in type 2 diabetes patients with dyslipidemia. The expression of *ABCA1* gene was also lower in group II patients compared to controls. This study concluded that hyperglycemia has an important role in the down-regulation of *ABCA1* gene that leads to dyslipidemia in type 2 diabetes patients.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders
S06

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Assistant Professor

Effect of protein synthesis inhibitors and mitochondrial proteases on the abundance of mitochondrial DNA encoded transcripts

Muhammad Waqar Hameed*

Mitochondria are semi-autonomous organelles that are evolved through endosymbiosis. During evolution most of the endosymbiont's genes were either lost or transferred to the nuclear genome of the host cell. Despite to this huge translocation modern mitochondria still retain a subset of genes chiefly encoding components of the electron transport chain complexes and transcription translation machinery. A number of studies were conducted to investigate the regulation of mitochondrial genome but very little is known about the regulation of transcription in mitochondria and almost nothing is known about regulation of translation in mitochondria. Therefore, to investigate mitochondrial transcription and translation in more detail we have investigated the effects of protein synthesis inhibitors and mitochondrial ATP dependent proteases in the regulation of mitochondrial gene expression. We found significant up-regulation of mitochondrial transcripts after treatment of cells with protein synthesis inhibitors (homoharringtonine and puromycin) whereas the level of tested mitochondrial (ND1, COX2, ATP6) and nuclear (RPL5) proteins remained unaffected. Since, mitochondrial proteases especially the ATP dependent proteases are responsible for the serine protease activity-dependent cell death we investigated if mitochondrial proteases (HtrA1 and HrtA2) could play part in the regulation of mitochondrial genome expression. We triggered the expression of HtrA proteases by heat shock (55°C) and found that Hsp60 and mitochondrial (except for cytB and atp6) transcripts were significantly up-regulated. Our analysis shows that mitochondrial transcripts respond generally to protein synthesis inhibitors but specifically to mitochondrial proteases.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders
S06

Interleukin 6 receptor gene polymorphisms in rheumatoid arthritis patients from Pakistan

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Presenter:
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Increased production of interleukin 6 (IL-6) is associated with rheumatoid arthritis that acts through its receptor, IL-6R (Interleukin 6 receptor). Various single nucleotide polymorphisms in IL6R gene confer susceptibility to rheumatoid arthritis have been identified in various population yet these associations have not been not fully established. The present study was pursued with the aim to evaluate a possible association between three single nucleotide polymorphisms (*rs2228145, rs4537545, rs4845617*) of *IL-6R* gene and rheumatoid arthritis in Pakistani patients. For this purpose, we recruited 60 patients diagnosed with rheumatoid arthritis and 60 healthy age and gender matched controls. Blood samples were collected and DNA was extracted. Sanger DNA sequencing was performed to evaluate the SNPs in *IL6R* and the data were statistically evaluated using chi-square test. Results of chi-square test of independence showed that allele C of *rs2228145* was significantly associated with rheumatoid arthritis in patients (Odds ratio or OR= 6.78, chi-square or χ^2=43.26, p=0.0001). Moreover, the G allele of *rs4845617* was also significantly associated with rheumatoid arthritis in patients (OR=2.28, χ^2=8.98, p=0.0027). However, we could not find any association between *IL6R* (*rs4537545)* with rheumatoid arthritis in Pakistani population. Our results signify that these polymorphisms may be associated with rheumatoid arthritis susceptibility in Pakistani patients.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders
S06

Drug loaded hydroxyapatite / polymer composites for sustained drug release

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Lecturer

Cefixime, a third generation antibiotic has been known to be potent against a number of gram positive and gram negative bacteria. This drug has a short half- life and therefore is removed rapidly from the body. A delivery mechanism that would facilitate efficient and site specific incorporation of cefixime in countering infection, is highly anticipated.
This study focuses on employing properties of Hydroxyapatite and polymers to fabricate a biomaterial with the essential properties of biodegradation, biocompatibility and non-toxicity. Cefixime loaded composites showed a sustain release pattern upto 7 days. These composite biomaterials harnessed the ability to carry the drug and promote sustain release. A distinct zone of inhibition on agar plates and bacterial growth suppression were demonstrated by these drug loaded composites. Cytotoxicity evaluation was mediated by MTT assay with human cell lines MCF-7 and MDAMB231.Relative growth of these two cell lines in the presence of the three composite biomaterials remained unaffected. Further physical characterization including Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy FTIR shed light on the structural aspects of these composites. The results indicate drug loaded composites show great potential in bone regeneration.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders S06

Structural characterization of interactions between small basic protein and accumulation associated protein- two essential proteins in Staphylococcus epidermidis biofilm Formation

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Presenter:
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Staphylococcus epidermidis, a coagulase negative opportunistic bacterium is a leading pathogen in nosocomial infections. Small basic protein (17KDa) and accumulation ssociated protein (140KDa) play important role in biofilm formation on implanted medical devices in *Staphylococcus epidermidis*. These proteins have been found co-localized on biofilms so it was hypothesized that Sbp and Aap may interact with each other. To understand the interaction between Sbp and Aap, we recombinantly expressed Sbp and a construct (G5EG5EG5) of domain B of Aap after cloning them. The production and purification of miligram quantities of both proteins were done by Ni^{+2} affinity chromatography and gel filteration. After stabilizing proteins in specific buffer, monodisperse properties and the prediction of secondary structures along with the folding states of proteins were analyzed by dynamic light scattering (DLS) and circular dichroism spectroscopy respectively. DLS of the mixture of Sbp and Aap construct demonstrated that they associate with each other in the presence of 5mM ZnCl2. Size exclusion chromatography and native mass spectrometry also confirmed the interaction between them according to their molecular weights. Furthermore, hydrodynamic radius by DLS and radius of gyration of Sbp by Small angle X-ray Scattering (SAXS) confirmed that Sbp oligomerizes with increasing concentrations and thus helps in forming biofilm surface. The ab initio model of Sbp has also been generated by SAXS. We conclude that recombinant, correctly folded Sbp and Aap construct associate with each other. These findings pave the way for the structural characterization of Sbp alone and in complex with Aap which can ultimately help in designing a drug against such a hospital acquired infection.

ABSTRACT INFO

Session: Genetic and Metabolic Disorders
S06

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A comprehensive analysis of human proteins' binding sites and its implications for drug design

Ammarah Anwar*, M. Qaiser Fatmi

Analysis of protein binding pockets offers considerable insights into unique microenvironments for ligand binding and catalysis. Volume identification significantly assists in ligand optimization as per size and shape requirements of pocket. The basic aim of this study is to determine the volume and area of binding pocket of human proteins, and to identify the pocket residues involved in mutations, which affect the structural and physico-chemical parameters of binding pocket. The work includes extensive Computed Atlas of Surface Topography of Proteins (CASTp) analysis of 13,855 human proteins, providing a range of smallest and largest binding pocket size, and determining the effects on volume and area of binding pocket due to the occurrence of mutations and ligand induced changes. The data set has been classified into 6 enzymatic categories having a considerable number of small, medium and large, liganded, nonliganded, mutated and wild-type proteins bound to drugs of variable sizes. It has been observed that small and medium sized proteins possess binding pocket volume within the range from 200-500 Å3 while the large sized proteins have pocket volumes >1000 Å3 with 1-2 mouth openings. CASTp analysis of human proteins provides an evidence for decrease in volume and area of protein with ligand and mutation induction. However, an increase in volume and area was observed due to binding of exceptionally large sized ligands in the active site. Modified amino acid residues i.e. MSE and CSE also tend to increase the pocket volume and area. The observed results will be helpful in designing the nature of the potential ligand that may bind to the target site with a greater affinity.

ABSTRACT INFO

Session: Genetic Diseases
S07

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Attenuation of apomorphine-induced sensitization in an animal model of stress

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An important role of 5-hydroxy tryptamine (5-HT; serotonin)1A receptors is there in the pathophysiology of depression as well as addiction. A supersensitivity of these receptors may impair adaptation to stress and lead to depression. Whereas, a desensitization of these receptors is suggested to be helpful for adaptation to stress and attenuation of addiction. Present study was designed to monitor apomorphine-induced behavioral sensitization in rats exposed to restraint stress. Experiment was carried out in two phases and apomorphine was experienced during restraint stress in phase I, but was not experienced during restraint stress in phase II. Activities in novel and familiar environments, as well as daily food intakes were recorded. Present study revealed that apomorphine-induced sensitization was greater in animals which experienced apomorphine during restraint stress. However, animals which did not experience apomorphine during restraint stress, apomorphine-induced sensitization was attenuated. Apomorphine potentiated adaptation to repeated restraint stress if experienced after restraint stress, but not before it. The study therefore suggests that apomorphine and other psychostimulants could be used for the treatment but not for the prevention of stress-induced depression.

ABSTRACT INFO

Session: Genetic Diseases
S07

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Genomic regions 17q21 and 20p13 are associated with asthma in Pakistani population

Muhammad Farooq Sabar*

Asthma is chronic inflammatory disorder characterized by repeated cough, wheezing and chest tightness. Over 300 million people worldwide suffering from this disease and 200 genes have been identified to be associated with it in different populations. Some genomic regions are known to be significantly associated with this disease in multiple populations. In Pakistan very rare research work has been done on asthma studies especially on molecular level. CAMB asthma research group has identified some genomic variants associated with asthma in local Pakistani population. Initially we have investigated two regions, 17q21 and ADAM33 gene in 20p13 region. Twenty five SNP markers in 17q21 and eight markers in 20p13 region were analyzed in 200 patients and 100 controls using Applied Biosystems mini-sequencing (SNaPSHOT) technology and capillary based genetic analyzers. SHEsis v 1.0 was used to construct Linkage Disequilibrium map and finding associated haplotypes.

Results of this study indicate that five SNPs in 17q21 genomic region are significantly associated with asthma in the studied population while one SNP out of eight studied SNP markers in 20p13 region is also associated with the disease. This study shows that there is great potential to find possible causes of asthma manifestation on molecular genetics level in Pakistan. This study may be a blink in the ocean of information regarding genetic variants associated with asthma in Pakistani population. Further studies on larger sample size will follow this in future which may result in the better understanding of the disease, its management and treatment.

ABSTRACT INFO

Session: Genetic Diseases
S07

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Molecular and genetic analysis of hereditary vision impairment

Muhammad Asif Naeem*, Bushra Rauf, Bushra Irum, Inayatullah, Raheela Nadeem, Hira Iqbal, Firoz Kabir, Tayyab Husnain, Shaheen N. Khan, Sheikh Riazuddin

Hereditary vision Impairment (HVI) affects human of all ages and societies worldwide. There are 45 million blind individuals and 135 million have severely impaired bilateral vision worldwide. Most notable of all diseases that cause blindness are retinitis pigmentosa (RP), congenital cataracts (CCs) and congenital glaucoma (CGL). The major rationale of our study is to find out reported/novel mutations in the genes involved in HVI through the applications of linkage analysis, genome-wide scan (GWS), next generation sequencing (NGS) & exome sequencing. This is done by ascertainment of families having affected individuals of HVI like RP, CCs and CGL. The major work done during current year includes identification of eleven novel & reported mutations of CYP1B1 in primary congenital glaucoma through Sanger sequencing of genomic DNA from 23 familial cases. For the first time, two novel recessive mutations of CRYAB were reported in two autosomal recessive CC families through GWS. Nine casual mutations including six novel variants associated with retinal degeneration were reported through NGS. Last but not least, the development of exomeSuite (1st user-friendly freely available software) that facilitates identification of casual mutations in the NGS data and reduces data sets to less than 1% of original size. In Pakistan, high rate of consanguinity along with ethnicity and geography has significant implication for increasing the prevalence of recessive genetic disorders. These multiplex pedigrees provide a rich genetic source for linkage studies and identification of novel mutations in monogenic autosomal recessive and X-linked disorders. As part of an ongoing program, investigation of genetic basis of these diseases was done in 700 Pakistani families, discovery of 27 novel loci, 14 novel genes and 69 pathogenic variants, diagnostic screening and provision of genetic counseling to families affected with HVI. Overall 35 articles have been published in peer reviewed international journals. A specifically trained manpower has been produced to work on genetic diseases beyond Vision Impairment.

ABSTRACT INFO

Session: Genetic Diseases
S07

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Genetic and molecular characterization of autosomal recessive hearing impairment in Pakistan

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Hearing impairment (HI) is the commonest sensory disorder that affects 1 in 1000 child births worldwide, of which approximately 60% cases are attributable to genetic factors. Non syndromic hearing loss is predominant and is inherited as recessive trait. In Pakistan, however, the prevalence of bilateral hearing loss is estimated at 1.6 per 1000. Mapping loci for the non-syndromic hereditary hearing impairment may lead to identification of genes that are essential for the development and preservation of hearing. To date 103 recessive deafness (DFNB) loci and 60 of the corresponding genes have been mapped, out of which 36 deafness loci and 22 genes were localized in large inbred and consanguineous Pakistani families. Pakistani population is invaluable resource of study due to its geographic location and diverse ethnicity. Here consanguineous marriages or marriages within the same ethnic groups are very frequent therefore number of affected individual is high as compared to other populations. Research on hearing impairment at CEMB has largely contributed in identifying several reported/new genes and loci.

The present study was aimed to elucidate the molecular basis of autosomal recessive hearing impairment by using highly inbred families from all over Pakistan. So far 60 families segregating autosomal recessive hearing impairment with 3 or more than 3 affected individuals were enrolled from urban as well as rural areas of Pakistan. 10 families were found to be linked to reported genes/loci (DFNB1, DFNB2 and DFNB3). To achieve our goals new hearing impaired families are being enrolled and the unlinked families have been subjected to search for novel locus/gene or mutation.

It is further anticipated that results deduced from these findings will pave the way to better understand the harmonial symphony of sound transduction in the ear and will also play a pivotal role to alleviate the sufferings of ailing HI individuals.

ABSTRACT INFO

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Genetic and molecular basis of intellectual disability in Pakistani population

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Intellectual disability (ID) is a clinically and genetically heterogeneous disorder, defined by the presence of significant limitations in intellectual capability and functional skills, affecting 2-3% of the general population. The research for the genetic causes of ID has recently gained momentum, with the use of Next Generation Sequencing (NGS) in intellectual disability. identification of pathogenic mutations that cause autosomal recessive ID (ARID) has lagged behind, predominantly due to non-availability of sizeable families. The inbred population provides an excellent resource for the identification of underlying molecular causes of ARID. Up till now 215 X-linked ID conditions have been described with >90 genes and autosomal recessive form of intellectual disability is associated with 50 MRT loci. Pakistani population is a repository for search of novel genes due to consanguinity and large family structures and only few have been discovered till now and most of the genes have to be discovered.

This study has enrolled 45 families with 2 or more affected individuals segregating either ARID or XLID from different areas of Pakistan. Nineteen families were studied through linkage analysis and two were found to be linked with reported autosomal recessive non-syndromic ID loci. Four Novel Linkage Intervals have been mapped through Genome wide scan. The confirmation of these regions and search for candidate genes located in these regions are in process of confirmation.

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Clinical Utility of the Mendeliome across Disciplines of Medicine

Khushnooda Ramzan*

Single gene mutations that are sufficient to cause clinical diseases (Mendelian mutations) represent the most medically actionable class of human genetic variants, and are encountered by every discipline of medicine. For most diseases, the genetic risk is both modest and strongly influenced by environmental risk factors. In order to understand the contribution of Mendelian mutations to the burden of diseases that are suspected to be genetic in origin, we developed a next-generation sequencing-based multiplexing assay that encompasses the ~3,000 known Mendelian genes (The Mendeliome) and tested 2,202 patients referred with suspected genetic diagnoses from virtually every medical specialty. A likely causal mutation was identified in 922 patients for an overall clinical sensitivity of 42%. This compares favorably with whole-exome sequencing but offers the advantage of reduced running and interpretation costs. Although our study population is enriched for consanguinity, 198 (21%) of solved cases were autosomal dominant and 34 (4%) were X-linked in nature so our assay is likely applicable to outbred populations as well. The current version of the Mendeliome accounts for a large proportion of suspected genetic disorders, and offers a translational diagnostic opportunity as well as significant practical advantages over more comprehensive genomic tools.

ABSTRACT INFO

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S07

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Mutation in *LPAR6* Gene Underlies Autosomal Recessive Hypotrichosis in Two Consanguineous Pakhtoon (Pakistani) Families

Anwar Kamal*, Muhammad Humayun, Sher Alam, Saadullah Khan, Noor Muhammad

Autosomal recessive hypotrichosis/woolly hair is a rare genetic hair loss disorder characterized by sparse scalp hair/woolly hair, sparse to absent eyebrows and eyelashes, sparse axillary and body hair in affected individuals. This form of hair loss results from mutations in the genes involved in LPA pathway. We have recently identified mutations in *LPAR6* gene that is associated with autosomal recessive hypotrichosis/woolly hair. To study the underlying genetic causes of autosomal recessive hypotrichosis/woolly hair in Pakistani population.

Genotyping in 2 families was carried out using polymorphic microsatellite markers linked to genes causing autosomal recessive hypotrichosis/woolly hair phenotype. To screen for mutations in *LPAR6* gene, Sanger sequencing was performed using an automated DNA sequencer.

Genotyping with polymorphic microsatellite markers showed linkage in both families to *LPAR6* gene located on chromosome 13q14.11 -21.32. Sequence analysis revealed substitution of G with A at nucleotide 436 (c.436G>A, p.Gly146Arg) in all affected individuals of both families. Comparison of the haplotype generated by typing *LPAR6* linked microsatellite markers in both families suggested common founder nature of this mutation.

Mutations identified in the present study extend the body of evidence implicating *LPAR6* gene in pathogenesis of human hereditary hair loss.

ABSTRACT INFO

Session: STEM CELLS S08

Transcription factor activating protein 2 beta (TFAP2B) mediates noradrenergic neuronal differentiation in neuroblastoma

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Neuroblastoma is an embryonal pediatric tumor that originates from the developing sympathetic nervous system and shows a broad range of clinical behavior, ranging from fatal progression to differentiation into benign ganglioneuroma. In experimental neuroblastoma systems, retinoic acid (RA) effectively induces neuronal differentiation, and RA treatment has been therefore integrated in current therapies. However, the molecular mechanisms underlying differentiation are still poorly understood. We here investigated the role of transcription factor activating protein 2 beta (*TFAP2B*), a key factor in sympathetic nervous system development, in neuroblastoma pathogenesis and differentiation. Microarray analyses of primary neuroblastomas (n=649) demonstrated that low *TFAP2B* expression was significantly associated with unfavorable prognostic markers as well as adverse patient outcome. We also found that low *TFAP2B* expression was strongly associated with CpG methylation of the TFAP2B locus in primary neuroblastomas (n=105) and demethylation with 5-aza-2´-deoxycytidine resulted in induction of *TFAP2B* expression in vitro, suggesting that *TFAP2B* is silenced by genomic methylation. Tetracycline inducible re-expression of *TFAP2B* in IMR-32 and SH-EP neuroblastoma cells significantly impaired proliferation and cell cycle progression. In IMR-32 cells, TFAP2B induced neuronal differentiation, which was accompanied by up-regulation of the catecholamine biosynthesizing enzyme genes DBH and TH, and down-regulation of MYCN and REST, a master repressor of neuronal genes. By contrast, knockdown of *TFAP2B* by lentiviral transduction of shRNAs abrogated RA-induced neuronal differentiation of *SH-SY5Y* and SK-N-BE(2)c neuroblastoma cells almost completely. Taken together, our results suggest that *TFAP2B* is playing a vital role in retaining RA responsiveness and mediating noradrenergic neuronal differentiation in neuroblastoma.

ABSTRACT INFO

Session: STEM CELLS S08

Preconditioning of stem cells for the repair of burnt skin

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Presenter:
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Burn injuries are globally responsible for about 5% of total mortality and the overall global annual cost was estimated around 500 billion US dollars. Stem cells have shown promising potential for the repair of many debilitating diseases including damaged skin. However, stem cells after transplantation in injured/ diseased organ face harsh microenvironment which is non-conducive for stem cell engraftment and survival. This causes reduction in potential outcome of stem cell therapies. We aimed to improve the ability of placental, adipose and skin derived stem cells to counteract such stresses prevalent after severe skin burns by modulating stem cells using differentiation and preconditioning strategies. For this purpose, all the cell types were characterized by their specific markers. Placenta derived stem cells were in-vitro differentiated into epidermal and dermal lineage cells. Whereas, adipose tissue derived stem cells and skin derived cells were preconditioned with an antioxidant followed by exposure to in-vitro thermal and chemical burn stresses. The cells were then evaluated for cell viability, cell damage (by lactate dehydrogenase release), cellular senescence, and alteration in survival (Bclxl, VEGF, PCNA) and skin specific markers (FGF23, FGF7, Col7) by semi-quantitative Real Time PCR. The differentiation and anti-oxidant preconditioning of cells resulted in decrease in cellular damage, improvement of cell survival, and up-regulation of expression of survival (Bclxl, VEGF, PCNA) and skin specific markers (FGF23, FGF7, Col7). Further, in-vivo evaluation of antioxidant preconditioned adipose derived stem cells after transplantation in burn wounds in rats, demonstrated greater ability of these cells for rapid re-epithelialization and wound closure in comparison to non-preconditioned cells. In conclusion, in-vitro differentiation and preconditioning strategies could be used to gain better outcome of transplantation of stem cells for the repair of damaged skin.

ABSTRACT INFO

Session: STEM CELLS S08

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Presenter
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Neural differentiation of embryonic stem cells (ES)

Sajida Batool*

Embryonic stem cells are pluripotent capable of developing into any cell type originating from all three germinal layers and hence hold potential to be used in transplantation medicine besides being used in research to study embryonic development. This study was undertaken to direct differentiation of mouse embryonic stem cells into neural lineage. Mouse embryonic stem cells were differentiated toward neural lineage by forming embryoid bodies (EBs) first. These EBs were then dissociated and cultured in differentiation medium to promot neural differentiation. Morphology changes together with expression of lineage-specific markers studied by semi quantitative PCR and immunocytochemistry in cells undergoing differentiation showed the existence of neural phenotype in culture. Given the increasing prevalence of neurodegenerative disorders, optimization of conditions to grow neural cells in vitro may pave the way for their use in clinical medicine.

ABSTRACT INFO

Session: STEM CELLS S08

Isolation, Identification and Antimicrobial Sensitivity Pattern of Microorganisms found in different culture of Burn Patients at a Burn Center in Islamabad, Pakistan

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Infections in Burn patients are the major complication among them nosocomial infections are most frequent one after initial period of shock. Greater than 70 % of mortality in burn patients is mostly contributed by infection specially nosocomial infections. This study was designed to check microbes associated with burn infections and their pattern of antibiotic sensitivity.

This study was conducted from 2005 to 2009 at Burn Center of NESCOM hospital, Islamabad Pakistan. Various culturing samples were isolated to culture with intention to observe most prevalent pathogens in burn wound infection, urinary tract infection and in blood. About 243 individuals were enrolled with age ranges from 1.4 to 71 years. Isolation, identification and antimicrobial sensitivity pattern of microorganisms was done by following standard procedures.

Out of 243 clinical isolates, highest frequency of pathogens isolated were *Pseudomonas aeruginosa* 163 (67%), *Staphylococcus aureus* 40 (16%), *Escherichia coli* 14 (6%), *Candida albicans* (4%), *Klebsiella species* (2%), *Salmonella species* (1%), *Proteus species* (1%), (0.4 %) of cultures were positive for *Streptococcus* pyogenes while (1 %) yields no growth. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* showed 80±10 % resistance against Ampicillin, Augmentin, Amoxicillin, Clarithromycin, Methicillin, and Vancomycin.

High prevalence of nosocomial infections and common pathogens isolated from burn wounds were *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Drug resistant microbial infections become common and difficult to treat especially in burn patients. So this study recommends continuous monitoring and strategies development to avoid complications of burn infections in order to prevent antimicrobial resistance.

ABSTRACT INFO

Session: STEM CELLS S08

Cell-Material Interaction: a case of tail wagging the dog

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Tissue engineering (TE) and regenerative medicine aims to regenerate damaged tissues by combining cells obtained from the body with biodegradable, bioresorbable, biomimetic and biomaterials. Biomaterials play pivotal role in influencing the biological processes towards achieving the goal of TE. TE is a truly multidisciplinary field, which exploits fundamental principles of biology, medicine, material sciences and engineering in order to improve the quality of life. Modern cell isolation and culturing techniques is the core of TE. Naturally derived and synthetic biomaterials when molded into different forms such as scaffolds, pellets, membranes and particles etc. when implanted with cultured cells, provide a template that facilitates the formation of new soft and hard tissues. During this process the biomaterials gradually degrade and are eventually metabolized.

We have thus far established different material-mediated in vitro cytotoxicity assays, cell attachment/adherence on scaffolds, drug delivery, decellularization of tissues and organs etc. Moreover we carry out mesenchymal stem cell (MSC) extraction from rat bone marrow and human umbilical cord tissue. We are aiming to study the response of in-house developed biomaterials in animal models and subsequently in humans.

ABSTRACT INFO

Session: CEMB

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Genetic heterogeneity of hepatitis C virus determined by next-generation sequencing

Sana Saleem*

The hepatitis C virus (HCV) shows great heterogeneity in infected patients, referred to as a quasispecies. Through next-generation sequencing (NGS) technique, currently it is feasible to study quasispecies at much more detail.

Using a novel ultra-deep sequencing technique, we studied the viral diversity in 13 chronic HCV patients. Ultra-deep sequencing determined thousands of reads of the HCV genome and exposed high genetic heterogeneity in the genotype 3a HCV population. We sequenced two regions at very high coverage. After NGS error correction, average number of reads for HVR1 quasispecies sample contains 1508 unique sequences, 6910 reads, the major has a population frequency of 22% and a nucleotide diversity of 0.0290 9. In average for NS3 the quasispecies sample contains 416 unique sequences, 1172 reads, the major has a population frequency of 19%, and a nucleotide diversity of 0.011. The average nucleotide diversity of NS3 is less than HVR1.

Ultra-deep sequencing technology showed great genetic variability of HCV, which has significant implications regarding the treatment response and outcome of antiviral therapy.

ABSTRACT INFO

Session: CEMB

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Transformation of Amplicon based RNAi construct in cotton to target Begomoviruses

Aneela Yasmeen*[1], Sarfraz Kiani[1], Afshan Butt[1], Abdul Qayyum Rao[1], Faheem Akram[1], Aftab Ahmad[1], Idrees Ahmad Nasir[1], Tayyab Husnain[1], Shahid Mansoor[2], Imran Amin[2], Muhammad Zubair[2], Muhammad Numan Tahir[2], Jody Scheffler[3] and Brian Scheffler[3]

Transformation of RNAi based gene construct namely C2 targeting V2 region of CLCuD responsible for virus movement, cloned under 35S promoter was done in two elite cotton verities i.e. MNH-786 and VH-289. Eighty six plants were screened on MS medium containing 50mg/µl kanamycine. Shoot apex cut method of gene transformation in cotton was applied by using Agrobacterium strain LBA4404. The transformation efficiency was found to be 3.75 and 2.88 % respectively in MNH-786 and VH-289 cotton varieties. PCR analysis by using gene specific primers confirmed the positive integration of RNAi gene construct in T0, T1 and T2 generations. The Single copy number was achieved in both varieties by using Florescent in Situ Hybridization and karyotyping in T2 generation. A comparison of Virus Disease Index and Virus titer calculation from absolute quantification through real time PCR confirmed the CLCuD tolerant transgenic cotton events with minimum symptoms and low virus titer. From the results it can be concluded that knocking down of V2 gene to control CLCuD is a useful strategy.

ABSTRACT INFO

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Genetic association analysis of the MAOA-uVNTR and 5HTTLPR, candidate genes for aggression in maltreated individuals

Hasib Aamir Riaz*, Ahmad Ali Shahid, Muhammad Shafique, S M Nauman Jillani and Tayyab Husnain

Human aggression is known to have strong gene-environmental underpinning. Molecular geneticists have proved significant role of Gene-Gene and Gene-Environment interactions in shaping aggressive/violent behavior. It includes different physiological and neural processes that play role in neural plasticity. The varying level of neurotransmitters in corticolimbic system because of genetic-environmental factors has strong linkage with antisocial behavior. Neurotransmitter serotonin remains primary determinant for its well-known role in psychiatric disorders. Monoamine oxidase A (MAOA) catabolizes neurotransmitter signaling by catabolizing serotonin and other monoamines. Serotonin transporter gene (5-HTTLPR) also regulates serotonin level by its reuptake from the synaptic cleft. Maltreatment is an environmental pathogen which effects brain structures and functions by changing brain chemicals' level specially neurotransmitter level. The varying serotonin level due to genetic or environmental factors play significant role to regulate human mood, cognition, behavior and attitude. The aim of the study was to investigate interaction of MAOA-uVNTR, 5-HTTLPR and maltreatment in the development of aggression. Blood samples of unrelated males along with self-survey report were collected and genotyped. The low activity MAOA-uVNTR was closely associated with aggression maltreatment. Moreover, the short 5-HTTLPR allele was found to be associated with aggression but not with maltreatment. Adverse childhood conditions play crucial role in development of aggression. Child maltreatment may trigger two folds more aggression risks as compared to non- maltreated conditions. In future, this study will assist understanding the gene-environment interactions of aggression development in humans.

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Preconditioning of insulin producing cells renders them resistant to hyperglycemic stress

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Stem cells derived insulin producing cells (IPCs) have shown promising potential for the treatment of diabetes. However, hyperglycemia induced oxidative stress micro-environment prevalent in the diabetic pancreas affects the survival and proliferation of the transplanted cells leading to reduced outcome of cell therapy. Preconditioning of stem cells with growth factors has been shown to improve their ability to cope various stresses such as thermal, hypoxic or hyperglycemic stresses. The current study aimed to explore the effect of preconditioning of ADMSCs (mesenchymal stem cells isolated from adipose tissue) derived IPCs with combination of basic fibroblast growth factor (bFGF) and stromal derived factor 1 α (SDF1α) to improve their tolerance against high glucose stress in-vitro. ADMSCs were isolated, cultured, and differentiated into IPCs. The IPCs were preconditioned with either SDF-1α (50 ng/ml), or bFGF (50 ng/ml) or a combination of both (25 ng/ml each) for 48 hrs, followed by exposure to media with 5.5, 17, and 33 mM glucose concentrations. Results revealed that cells preconditioned with a combination of bFGF and SDF1α exhibited maximal decrease in senescence, apoptosis and cell damage with concomitant increase in insulin release ability and proliferation in response to 33 mM glucose stress. Semi-quantitative Real Time PCR analysis also revealed that the increase in expression of pancreatic markers (Insulin 1, Ngn3, Insulin 2, Nkx 6.2 and Pdx1) and reduction in expression of apoptotic marker, caspase-3 was most significantly observed in IPCs pretreated with combination of SDF1α and bFGF. In conclusion, preconditioning of ADMSCs derived IPCs with a combination of bFGF and SDF-1α improves their ability to tolerate high glucose stress by increasing cell survival, glucose dependent insulin release and enhancing expression of pancreatic survival genes in IPCs. Hence, IPCs preconditioned with combination of SDF1α and bFGF may have improved potential to repair diabetes.

ABSTRACT INFO

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Expression of bacterial cellulose synthase (BCS) gene in cotton

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The cotton industry is challenged with problems in cost of production and requirements for high quality in the product. Among the fiber quality parameters, fiber strength, fiber length, and fiber fineness are the primary quality properties that influence textile processing. Microbial cellulose differs from plant cellulose with respect to its high crystallinity and purity, high water-absorption capacity, and mechanical strength. As the major component of cotton fiber is cellulose therefore introduction of bacterial cellulose synthase (Bcs) gene into cotton fibers can bring revolutionary changes in the quality of cotton fiber. This study is aimed at expression of bacterial cellulose synthase genes in cotton fiber under fiber specific promoter in order to enhance the fiber length and fiber strength of the targeted cotton variety via Agrobacterium mediated transformation. Cloning of genes responsible for bacterial cellulose synthesis has been done. Removal of 6586bp fragment through restriction digestion analysis confirmed the successful cloning of bacterial cellulose synthase genes in pCAMBIA 1301 vector under fiber specific promoter. Agrobacterium mediated transformation of the gene construct into non-transgenic cotton variety was done. As a result of transformation, around 30 putative transgenic plants have been obtained and shifted to selection free medium in test tubes. Acclimatization of the putative transgenic plants and molecular confirmation of genes in the transgenic plants is in process which will further confirm the successful integration and expression of the transformed genes leading towards improvement of fiber characteristics at mature stage of the transgenic plants under field conditions.

Fiber qualities of local cotton cultivars can be improved by transforming with bacterial cellulose synthase genes. This would ultimately lead to the enhancement of the cotton fiber length and strength and hence would reduce the import of high quality cotton fiber by fulfilling the desired level of the cotton fiber quality for the Pakistan textile industry.

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Role of Human Interferon alpha2b Gene and Presumptive Drug Model against Breast Cancer

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Cancer is the most challenging and still inaccessible ailment faced by human body. Although a considerable research data is available on occurrence of cancer and genes related to cancer induction and suppression. There is colossal need to explore new genes in our population. Hence attempt was carried out to analyze expected risk factor i.e. human interferon alpha-2b (hIFNα-2b) gene mutation and also applying computational tools to generate predicted protein model for proposed drug against breast cancer. In present study two different sets of primer (IFN1F & IFN2R and NIF & N2R) were used to amplify biopsy samples of breast cancer patients. Amplified products were proceeded for sequencing, by using different available online bioinformatics tools: Chromas 2.33 software for the peak correction, Clustal-W 2.0 for multiple sequence alignment and ExPASY for translating DNA sequence in order to study the changes at amino acid levels. Amplified product also showed mutation at amino acids level which were analyzed for local and general fold pattern changes and receptor (IFNAR), ligand (hIFNα-2b protein) interaction through Z-DOCK (3.0.2) server.

In present research total of 60 biopsy samples of breast cancer patients were carried out to amplify hIFNα-2b gene, 38 (63.33%) samples were shown to be PCR positive. The amplified products were subjected to gene sequencing; results showed frequent alterations after 400bp, where mostly adenine was found to be replaced with other bases. The sequenced files with nucleotide alterations were translated by ExPasy tool. Out of 38 amplified products 19 showed mutation at amino acids level which were further analyzed for local and general fold pattern changes and receptor (IFNAR), ligand (hIFNα-2b protein) interaction through Z-DOCK (3.0.2) server to find out the binding pattern of ligand and receptors. The variant model of protein is synthesized by superimposition of 19 amino acids sequence which signifies that variant model of hIFNα-2b protein may be the risk factor in addition to other known risk factors associated with breast carcinoma. Recombinant hIFNα-2b variant therapy to cancer patients may prove to be more potent anti-cancerous drug than normal interferon.

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Whitefly Resistance in Cotton (*Gossypium hirsutum*) through micro-RNA against *Sxl*, *AChE* and *ORC* genes

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Artificial miRNA based construct namely C1 targeting three housekeeping genes of whitefly i.e Sex lethal gene (Sxl), Orcokinin gene (ORC) and Acetycholine esterase (AchE) genes were transformed into two elite cotton varieties MNH-786 and VH-289 by using Agrobacterium mediated shoot apex method, ninety five plants were screened on MS medium containing 50mg/µl kanamycine. The transformation efficiency was found to be 3.78 and 2.89 % respectively in MNH-786 and VH-289 cotton varieties. PCR analysis by using gene specific primers confirmed the positive integration of artificial miRNA gene construct in T0, T1, T2 and T3 generations. Random integration and variable copy no. of artificial miRNA gene was found by Florescent in Situ Hybridization and karyotyping in T2 generation. Two copy numbers in one of the MNH-786 transgenic event while in a VH-289 transgenic event one copy number of the gene was confirmed. These results were further elaborated by Southern analysis which showed similar results. A comparison of artificial miRNA expression and Viral disease index from relative quantification through real time PCR confirmed the CLCuD tolerant transgenic cotton events with minimum symptoms and low viral disease index. From the results it can be concluded that knocking down of whitefly genes to control CLCuD is a useful strategy.

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Induction of mitochondrial proteases also stimulates mitochondrial gene expression in human cancer cell lines

Narjis Fatima* and Muhammed Waqar Hameed

Mitochondria are cell organelles involved in ATP generation and other cellular processes like signal transduction and apoptosis. These organelles are unique since they contain their own genome and independent transcription translation machinery. Mitochondria house a number of different proteases that are important for the regulation of mitochondrial biogenesis. They chiefly degrade misfolded and un-assembled polypeptides but they are believed to be directly involved in the development of various malignancies. HtrA is a family of mitochondrial ATP dependent proteases that is located in the inter-membrane space and are found to be upregulated during stress and tumor growth. But to our knowledge no one has ever attempted to investigate the level of mitochondrial transcripts and proteins in conditions of HtrA induction. Therefore, in the current study we have investigated the role of a mitochondrial protease in the regulation of mitochondrial gene expression using colorectal (HCT-116) and prostate cancer cell lines (PC-3). We found differential abundance of mitochondrial transcripts in conditions that induced HtrA expression levels. However, we found no detectable changes in the abundance of mitochondrial proteins under condition of HtrA induction. Given the rapid response of transcripts to the environmental condition applied to provoke HtrA, a transcriptional mode of induction of mitochondrial transcripts seems to be operative.

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Antifungal response of Chitinase from *Hordeum vulgare* L.

Anwar Khan*, Bushra Tabassum, A. Munim Farooq, Muhammad Tariq, Khadija Aliya, Sameera Hassan, Ahmad Ali Shahid, Shafique Ahmed, Zahida Qamar, Muhammad Bilal, Idrees Ahmad Nasir, Tayyab Husnain

Chitinases are antipathogenic proteins that are involved in plant defense responses to pathogen infection. Plant chitinases are also expressed in response to environmental stress and also take part in physiological processes of plants such as embryogenesis and ethylene synthesis.Chitinase gene belong to PR-3 family, many of them with molecular mass range from 24 to 35 kDa is reported. Different pathogen related protein family have been identified and cloned that can be used to enhance disease resistance in plants. In this study we overexpressed chitinase gene in *E. coli* and after purification of recombinant protein checked the inhibitory effect against pathogenic fungi in-vivo. For this purpose the sequence verified gene was expressed in prokaryote expression vector, expression was optimized and the heterologous protein was purified along with his taq using affinity chromatography. The protein was used for inhibition of Rhizoctonia solani, Alternaria alternate and Fusarium spp. The objective of this work was to analyze the inhibitory effect of protein against filamentous fungi and further recommend it for transformation in plant to protect them against fungi.

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Alteration of color development pathway can produce multiple colors in cotton

Ammara Ahad*, Aftab Ahmad, Nadeem, Sidra Akhtar & Ambreen Gul

Green activities are famous for their non-toxic and non hazardous effects. This approach to introduce eco-friendly products has been extended to textile stuff, especially for those which directly come into contact with the skin. Naturally colored cotton has pigmented fibers that grow in shades of green and brown. Because of less dying procedures, it is friendlier to environment than white cotton. Bioinformatics studies evaluation of pigment genes has provided a concrete avenue to alter the pigmentation pattern in cotton lint. For high expression in cotton (*Gossypium hirsutum*) the retrieved gene sequences from the gene bank were subjected to cordon optimization according to crop genetic makeup. The pigment genes were chemically synthesized with their own enhanced promoter and terminator regions in a single cassette. The particular cassette was successfully cloned in pCAMBIA 1301, plant expression vector. Removal of 4000bp by using specific restriction enzymes confirm successful ligation of pigment related genes in plant expression vector. Total 2000 embryos were subjected to selection free medium after inoculation with Agrobacterium harboring gene of interest. Out of 2000 total 288 embryos successfully developed shoots and roots. Twenty putative transgenic cotton plants have been shifted to selection free medium in test tubes and are passing acclimatization process. Molecular analysis of these plants is under process which will further confirm their successful integration and expression will lead towards color development when shifted to field at mature stage.

Thus, by modifying the natural color producing pathways there is a potential to alter cotton fiber pigment. By producing high quality pigmented fiber, a country can save roughly 12 million rupees (cost of dyes imported used in dyeing units), energy and water.

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Control of Cotton Insect through Modified Combination of Bt and Plant Lectin Genes

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The advent of the vegetative insecticidal proteins is considered as the best possible way to overcome the limitations of the first generation Bt insecticidal crystal protein resistance build up in major chewing insects. Sucking insects are the second largest group of insects invading cotton crop by sucking the phloem sap and damaging 2 million cotton bales each year. No report determining combined strategy against resistance builds up and control of sucking insects altogether has been documented in recent past. Use of Vip3a in combination with ASAL a mannose binding lectin protein has provided solid way to control such problem in combination when evaluated through bioinformatics tools. The sequences of insecticidal genes; Vip3A and ASAL were retrieved from the gene bank and the codons were optimized to get high expression in cotton (*Gossypium hirsutum*). The genes were chemically synthesized with their own promoter and terminator regions in a single cassette and with specific flanking sequences at 5' and 3' end of the cassette that is supposed to integrate at a specific chromosome in cotton genome. The 4.5 kb cassette was successfully cloned into the plant expression vector pCAMBIA 1301 in XhoI and HindIII sites. The germinating embryos of cotton (*G. hirsutum*) were transformed with Vip3A+ASAL construct without any selectable marker using Agrobacterium mediated shoot apex method of transformation. The transgenic plants have been acclimatized in the field conditions and will be analyzed for the successful integration and expression of the genes through different molecular biological techniques such as Southern blot analyses, real-time PCR, fluorescence *in situ* hybridization (FISH). The current study is aimed to transform vegetative insecticidal protein (Vip3a) gene and *Allium sativum* leaf agglutinin (ASAL) gene to successfully control the chewing and sucking insect attack in cotton.

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Prediction of host-derived miRNAs with the potential to target PVY in potato plants

Muhammad Shahzad Iqbal[1], Muhammad Nadeem Hafiz*[1], Idrees Ahmad Nasir[1] and Tayyab Husnain

Potato virus Y has emerged as a threatening problem in all potato growing areas around the globe *PVY* reduces the yield and quality of potato cultivars. During last 30 years, significant genetic changes in *PVY* strains have been observed with an increased incidence associated with crop damage. In current study, computational approaches were applied to predict Potato derived miRNA targets in *PVY* genome. *PVY* genome is about 9 thousand nucleotides approximately which transcribes 6 genes CI, NIa, NIb-Pro, HC-Pro, CP and VPg. Total 343 mature miRNAs were retrieved from miRbase database and searched for their target sequences in *PVY* genes using minimum free energy (mfe), minimum folding energy, sequence complementarity and mRNA-miRNA hybridization approaches. Identified Potato miRNAs against viral mRNA targets have antiviral activities leading to either translational inhibition by mRNA cleavage, mRNA blockage or both. We have found 86 miRNAs targeting *PVY* genome at 151 different sites on *PVY* genome. Moreover, only 36 miRNA potentially targeted the *PVY* genome at 101 loci. CI gene of *PVY* genome was targeted by 32 miRNAs followed by complementarity by 26, 19, 18, 16 and 13 miRNAs respectively. Most importantly, we found 5 miRNAs (miR160a-5p, miR7997b, miR166c-3p, miR399h and miR5303d) could target CI, NIa, NIb-Pro, HC-Pro, CP and VPg genes of *PVY*. The predicted miRNAs can be used for development of *PVY* resistant potato crops in future.

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Expression, purification and bioinformatics analysis of NDV capsid proteins

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This study consist of isolation and *E. coli* expression in vector carrying an N- terminal His-Tag sequence of viral capsid gene. The objective of this study was to generate the recombinant protein and edible vaccine by isolation and characterization of immunogenic genes of NDV by purification in order to control this disease.

Collection of confirmed NDV viral strains was done from Veterinary Research Institute, Lahore on request. Both F and HN genes were ligated into pET 30a expression vector and transformed in *E. Coli* Rosetta cells, specific for protein expression. Cloned genes were confirmed by sequencing and BLAST in accordance with Mukteswar strain. Induction by adding IPTG generated increased yield of protein and SDS Polyacryamide gel electrophoresis was performed to confirm presence and size of genes. Proteins was transfered on nitrocellulose membrane in western blotting. Protein purification using IMAC affinity chromatography was performed and 2D and 3D structural analysis of F and HN protein through IEDB analysis resource tool. Amplification of 1662 bp for F gene and 1712bp for HN gene confirmed NDV immunogenic gene in collected samples. Gene sequence and size was confirmed by sequencing and BLAST. SDS Polyacryamide gel electrophoresis confirmed the presence and size of protein (67kDa for F and 69 kDa for HN). Further western blotting confirmed specificity of protein through antigen antibody reaction at proper size. IMAC affinity chromatography and 2D and 3D structural analysis of F and HN protein revealed that more than 70% of its sequence is antigenically active and the predicted protein regions behave as epitopes. The key achievement of this study can lead towards recombinant/ edible vaccine production in future studies.

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Transformation of bleaching herbicide resistant hydrilla verticillata Phytoene desaturase gene in cotton

Muhammad Azam Ali*, Adnan Iqbal, Kamran Shahzad Bajwa, Aleena Khalid, Sallah ud din, Abdul Qayyom Rao, Ahmad Ali Shahid, Tayyab Husnain

This study was aimed to develop herbicide resistant transgenic cotton against bleaching herbicide like Norflurazon. Transformation of mutated *pds* (*Phytoene desaturase)* from *Hydrilla verticillata* at position Arg304 was done in cotton variety CEMB-33 which has been reported to cause resistance against bleaching herbicides. An *Agrobacterium tumefaciens* strain LB4404 was used for transformation. In constructs *Phytoene desaturase* gene of *Hydrilla verticillata* mutated at Arg 304 position (Threonine – Thr, Cysteine – Cys) were cloned in pCAMBIA 1303 vector under CaMV35S promoter. Successful transformation of ppdCYS1303 and ppdTHR1303 was obtained in *Gossypium hirsutum* variety CEMB-33 with transformation efficiency of 1.31%. The putative transgenic plants were subjected to initial screening by adding selection drug hygromycin in the growth medium. The screening of plants was also done by PCR using gene specific primers. Plasmid was used as positive control while non-transgenic cotton plants as negative control.

Amplification of 418bp was obtained in five plants out of total eighteen plants analyzed and plasmid as positive controll, while no amplification was obtained in negative control. Expression of the gene was also taken at mRNA level by Real Time PCR with an efficiency of 95%. GAPDH was used as (house-keeping) internal gene for normalization. Results showed 1.5 to 7 folds higher expression level of transgene *pds* in transgenic plants as compared to the control plants.

This study is first ever report of introducing bleaching herbicide resistance gene in cotton. Use of bleaching herbicides can be an alternative to many other herbicides already being used in cotton.

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Transformation and expression of Virus (CLCuV), insects and weeds resistant genes in cotton variety VH-289

Adnan Iqbal*, Naila shahid, Muhammad Azam Ali, Amina Yaqoob, Abdul Qayyum Rao, Ahmad Ali Shahid, Tayyab Husnain

There are many threats to cotton such as insect, weeds and viruses. To overcome the problem of cotton crop, development of transgenic cotton and the expression of transgenes in adequate quantity is the need of time. Transgenic cotton variety VH-289 was developed through Agrobacterium mediated transformation of GTG, pk2Ac and NIBI plasmids, containing GTG, (Cry1Ac, Cry2A) and (GroEL, Zinc finger AZP and G5) genes respectively. Evaluation of integration and expression of these transgenes was done through molecular analysis in T0 and T1 generation.

In T0 and T1 generation transgenic cotton plants with six genes were confirmed through molecular analysis like ELISA and PCR which determined that nine plants were positive for the presence of three genes i-e Cry2A, Cry1A and GTG while out of these nine plants six were found to be positive for the presence of all six genes i-e Cry1AC, Cry2A, GTG, GroEL, Zinc finger AZP and G5. Protein quantification by ELISA for Cry1AC and Cry2A gene was determined. After the glyphosate assay the survival of cotton plants confirmed the presence of GTG gene. In all categorised plants, based on CLCuV symptoms, no significant difference was observed in DNA-A quantity and the quantity of DNA-A was also found to be very low compared to the copies of Betasatellite. High copies of Betasatellite were observed in virus susceptible plants than virus moderately resistant plants and least quantity was observed in virus resistant plant and vice versa for G5 mRNA expression. Negative correlation was found between virus resistant gene G5 and Betasatellite.

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Overexpression of Aspartate in Cotton against Sucking Insects

Ambreen Gul*, Abdul Qayyum Rao, Azmat ullah Khan, Ayesha Latif, Kamran Shehzad Bajwa, Tahir Iqbal, Mukhtar Ahmed, Ammara Ahad, Tayyab Husnain

Whitefly feeding results in honeydew secretion which favors the sooty mold growth on cotton plant. The only available method to control the populous growth of whiteflies is the foliar application of insecticidal sprays which are hazardous for human consumption. Glutamic acid and the aspartic acid are the excitatory neurotransmitters in the sucking insects. The increased levels of aspartic acid can cause the destructive effects on the insect nervous system since it can cause neurotoxicity at the synaptic vesicles in neuromuscular junctions.

This research work is designed to biochemically target the whitefly population by altering the aspartate amino acid content in the plant sap. Aspartate aminotransferase and asparaginase genes are responsible for production of aspartate from glutamate and asparagine respectively. Both of these genes will be cloned and transformed into cotton plant to see their effect against whitefly infestation. Cloning of genes responsible for overexpression of aspartate aminotransferase and decrease in conversion of asparagine has been done. Restriction digestion and removal of 1407bp (*Nco*I,*Bgl*II), 1233bp (XhoI) and 1564bp (*Sac*I,*Hind*III) fragments confirmed the successful cloning of aspartate aminotransferase and asparaginase genes in pCAMBIA 1301 vector under CaMV35S promoter. The transformation of the above gene constructs into non-transgenic cotton variety has been done by using *agrobacterium tumefaciens*. Around 40 embryos were obtained for each constructs after initial screening on MS media with cefotaxime selection. Acclimatization and molecular confirmation of genes in transgenic plants is in process which will be confirmed by successful control of sucking insects in transgenic plants.

Overexpression of aspartate can lead to significant reduction in the whitefly infestation and subsequently improvement in the cotton yield.

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Biosafety assessment of transgenic crops at CEMB

Amina Yaqoob*, Ahmad Ali Shahid, Tahir Rehman Samiullah, Abdul Qayyum Rao, Muhammad Azmatullah Khan, Kamran Shehzad Bajwa and Tayyab Husnain.

Genetically modified (GM) plants with insecticidal Bacillus thuringiensis (Bt) genes are widely accepted but their commercial utilization highlights the biosafety issues worldwide. The risk assessment of GM crops demonstrates their impact on ecosystem as well as non-target organisms (NTO's). Among the NTO's, plant growth promoting rhizobacteria (PGPR) demand more critical experimental studies since they are playing significant role in plant growth.

A comparative study of Bt with non-Bt cotton rhizosphere was conducted, regarding isolation of representative PGPR strains (Pseudomonas and Bacillus spp.), their biochemical characterization, auxin biosynthesis, analytical profiling (API NE) and molecular characterization to assess the risks of Cry proteins on non-target PGPR strains.

The horizontal gene flow (HGT) of *Cry1AC* gene was also observed in three of the total experimental strains (03-N9, 01-1 and 03-N4) whereas no significant difference was observed in the colony morphologies, biochemical activities, auxin biosynthesis and API NE enzymatic reactions during this comparative analysis. Sequence homology searches of bacterial strains through 16S ribotyping showed ≥90% similarity to Pseudomonas and Bacillus spp. However, phosphatase activity in bacteria from Bt rhizosphere was lower than that of non-Bt.

The findings of this study, favor the use of Bt products and show no harmful impact of Bt toxins (*Cry1AC*) on non-target soil microorganisms despite the HGT of respective genes into their genomes. The results showed that neither the biochemical nor the molecular characteristics of PGPR strains are being affected by Bt cotton when compared with that of control strains.

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Circulating micro-RNAs expression profiling in HCV infected patients: A novel insight into diagnostic and therapeutic research

Hamna Imtiaz*, Muhammad Idrees Khan

Hepatitis C is one of the major global public health problems. Hepatitis C virus is a major causative agent for chronic hepatitis which can lead to hepatocellular carcinoma and liver cirrhosis. Thus, extensive mortality and morbidity is produced by the infection of HCV. Worldwide almost 200 million people have HCV infection, which is around 3.3 percent of the total world's population.

MicroRNAs (miRNAs) are naturally abundant, evolutionarily conserved, regulatory, small, non-coding RNAs which inhibit the gene expression in a sequence-specific manner at post-transcriptional level. Today, above 1500 miRNAs are identified in human genome and their biological functions are under investigation. In spite of the important progresses accomplished to reveal their part in regulating different pathological and physiological processes, the understanding of their implications in response to viral infection (hepatitis C virus for instance) is in its early stages. However, human miRNAs are recently found of having important role in viral replication by intervening in virus–human interactions.

In the present study the expression profile of six human miRNAs (miRNA-21, miRNA-141, miRNA-124, miRNA-126, miRNA-200c and miRNA-320) in HCV infected persons has been compared with the healthy ones. The modifications in expression patterns of these miRNAs, who are found to be involved previously in HCV or HCV-related diseases, were selected to identify miRNAs with the potential value as prognostic /diagnostic markers. Among the selected miRNAs miRNA-21, miRNA-126 and miRNA-124 are upregulated while miRNA-R141, miRNA-200c and miRNA-320 are down regulated in persons with HCV infection in the population of Pakistan. The results of such studies can be used as potential biomarkers due to the stability of miRNA in the body fluids, and moreover can be useful for further pathological studies.

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CYP1B1 mutations in patients with primary congenital glaucoma

Muhammad Umer Khan*[1], Haiba Kaul[2], Saqib Mahmood[3], Ali Ammar, Raima Rehman[4].

Primary congenital glaucoma is a significant cause of blindness in children. In PCG the developmental defects of the trabecular meshwork of the eye lead to the obstruction of aqueous outflow and consequent increased intraocular pressure (IOP) resulting in optic nerve damage and ultimately permanent loss of vision. The incidence of PCG varies geographically with higher occurrence in populations where consanguinity is common. CYP1B1 is major contributing gene for PCG. The present study was aimed to investigate the mutational spectrum of the CYP1B1 in local patients affected with PCG. The study comprised of 35 participants clinically diagnosed with PCG. DNA was extracted from blood samples of PCG patients enrolled in the study and all the coding regions of CYP1B1 were amplified by polymerase chain reaction (PCR) using specifically designed primers. PCR products were purified and directly sequenced to find out the mutations in CYP1B1 gene. Sequencing analysis revealed one mutation, c.1169 G>A in exon 3 of CYP1B1 gene. This missense mutation replaces Arginine with Histidine at codon 390. This homozygous mutation was observed in 20% of the PCG patients enrolled in the study. In addition two missense sequence variants c.1294G>C (2 patients), c.1358A>G (4 patients) and a synonymous variant c.1347T>C (18 patients) were also seen in the present study. The results of the current study reflect that CYP1B1 mutation is one of the cause of primary congenital glaucoma in Pakistani patients.

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Evaluation of the antimicrobial activity of aqueous, ethanolic and methanolic extracts of Nigella sativa (kalonji) against gram positive and gram negative bacterial isolates

Ujala Nasir* and Saba Irshad.

Nigella sativa (kalonji) has been used since ancient times as a nutritional supplement and for the treatment of various infections and chronic ailments. As the pathogens are becoming resistant to most of the drugs, kalonji can be used as an alternative compound to be used in modern medicines. The antimicrobial activity of kalonji was determined through disc diffusion and agar well diffusion method. All types of extracts of kalonji showed antimicrobial activity against all bacterial isolates with different zones of inhibition. In case of disc diffusion method, methanolic extract showed maximum antimicrobial activity against B. subtilis, B. cereus and *Corynebacterium sp.* Ethanolic extract showed highest antimicrobial activity against E. coli. Both ethanolic and methanolic extracts represented maximum antimicrobial activity for *Enterococcus sp.* In case of agar well diffusion method, ethanolic extract showed maximum antimicrobial activity against all of the bacteria except *E. coli*, where methanolic extract exhibited the maximum activity. Aqueous extract gave the least activity against all bacterial strains through both methods except for S. aureus by disc diffusion method where it exhibited the maximum antimicrobial activity. The statistical analysis showed the agar well diffusion method (M=17.46 ± 2.02 mm) and methanolic extract (M=16.08 ± 3.61 mm) to be most effective against bacteria. The aqueous extract (M=12.75 ± 2.90 mm) showed least activity. The Enterococcus sp. (M=16.75 ± 3.50 mm) was found to be most sensitive and S. aureus (M=12.75 ± 6.04 mm) was found to be least sensitive to the extracts of kalonji. Phytochemical screening showed the presence of flavonoids, tannins, saponins, alkaloids and steroids depicting the antimicrobial and antioxidant properties of kalonji.

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Correlation of clinical marker, viral load with liver fibrosis stages and grades in chronic HCV patients with genotype 3a

Muhammad Shahid*, Muhammad Idrees, Iram Amin, Samia Afzal.

Hepatitis C virus (HCV) is one of the most important causes of chronic liver diseases, which include inflammation, fibrosis, cirrhosis and hepatocellular carcinoma. Several factors have been proposed to determine the clinical outcome of HCV infection. The accurate mechanism by which HCV damages the liver remains poorly understood. In chronic hepatitis C patients, the relation between serum clinical markers, HCV viral load and histological liver injury remain controversial.

The objective of this study was to examine the relation between serum clinical markers, HCV viral load and the degree of liver damage in patients with chronic HCV.

Liver biopsies were performed on 79 of a total of 100 enrolled patients. The histological activity was evaluated by the METAVER scoring system. HCV RNA quantification was performed by quantitative real-time PCR, and HCV genotyping was performed by nested PCR. Clinical markers were measured with biochemical instruments.

HCV viral load were significantly correlated with aspartate aminotransferase (AST) (P=0.004), alkaline phosphatase (ALP) (P=0.001) and total bilirubin (P=0.012) levels. HCV viral load were also significantly correlated with a progression of the fibrosis stage (P=0.000), but no correlation was observed with the change in inflammatory grades. It was observed that bilirubin levels were higher in later fibrosis stages as compared with the initial stage (P=0.000). Results revealed that in different fibrosis stages, the levels of AST (P=0.000), ALP (P=0.000) and alanine aminotransferase (ALT) (P=0.008), the age at diagnosis (P=0.000), the present age (P=0.000) and the BMI (P=0.009) were statistically significant. In the case of the inflammatory grade, levels of bilirubin (P=0.000), ALP (P=0.000), AST (P=0.016) and ALT (P=0.000) were statistically different between the inflammatory grades.

Serum HCV viral load were correlated with AST, ALP and total bilirubin. Levels of ALT, AST, ALP and bilirubin had significant relation with the liver fibrosis stage and the inflammatory grade in genotype 3a. Hence, our study suggests that HCV viral load, AST, ALP and ALT correlate with liver damage.

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Identification and Characterization of the Type Six Secretion System in Helicobacter pullorum Poultry Isolates

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Helicobacter pullorum is a relatively less characterized *Helicobacter species* that causes vibrionic hepatitis in chickens. The infection may be transmitted to humans via contaminated meat where it is associated with gastroenteritis and Crohn's disease. Although a few virulence factors in *H. pullorum* have been reported, the mechanism by which the bacterium causes disease is still unclear. Recently research has shown presence of the Type VI secretion system (T6SS) in *Helicobacter pullorum*. Studies on T6SS from different bacteria show that it plays a major role in bacterial pathogenesis and adaptation. Reports from these studies suggest that Hcp and VgrG are the important proteins which play a central role in the structural formation of the T6SS pilus, as well as act as effector proteins in certain bacteria. In this study, we have isolated Helicobacter pullorum from liver and caecum samples of broiler chickens and determined the presence of T6SS. We will also study the association of Helicobacter pullorum on chickens suffering from vibrionic hepatitis. Genus specific primers were used to identify Helicobacter isolates, next *H. pullorum* specific cdtB primers were used to identify specie and for the detection of T6SS, Hcp and VgrG primers are employed. Furthermore, using bioinformatics tools we have compared Hcp and VgrG proteins of bacteria with previously characterized bacterial T6SSs to determine homology of these proteins. Lastly, antibiotic resistance profiling will be performed to test the sensitivity and resistance of *H. pullorum* poultry isolates.

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Vertical Transmission Of Dengue By *Aedes aygepti*.

Iram Amin*, Muhammad Idrees, Muhammad Shahid, Samia Afzal

Dengue virus has now become a serious threat to humans. As yet, no vaccine and proper treatment is available for dengue virus, so control of vector is the only option left to control dengue infection. Aedes mosquitoes (*Aedes aegypti* and *Aedes albopictus*) are well known vectors for this virus but some studies show that dengue virus can also survive in Culex mosquitoes. Larvae act as reservoir of dengue virus during inter-epidemic period. Laboratory reared adults of Aedes aegypti (60), *Aedes albopictus* (45) and Culex (204) were collected from Institute of Public Health, Lahore. To determine the role of developmental stages of *Aedes* in dengue infection, 523 larvae were collected from different localities of Lahore from March to mid of June, 2014. Larvae and adult were divided into 47 pools and each containing 1-15 larvae. Molecular analysis was done to detect the dengue virus. Of the total of 47 pools of larvae, 15 pools of *Aedes* aegypti were found positive for dengue. Only one pool of laboratory reared adult mosquitoes was found positive. Detection of dengue virus from field caught larvae and laboratory reared adult mosquitoes confirm the existence of vertical transmission of dengue virus in nature by *Aedes aegypti*. DENV-2 was the most prevalent serotype and DENV-1 was not found in any pool. While all pools of Aedes albopictus and Culex were negative for dengue virus. Our study showed that the main cause of spreading of dengue virus was *Aedes aegypti* and there is need to conduct detailed studies to establish the vertical role of *Aedes albopictus* and Culex for dengue virus.

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Development of Oligonucleotide Microarray for the Identification of Drought Responsive genes in Cotton

Sajjad Sadique, Zarnab Ahmad, Muhammad Bilal Sarwar, Bushra Rashid*, Tayyab Husnain.

Crop improvement can be made efficient by studying novel genes and pathways in different species and making a comparison among them. *Gossypium hirsutum* (the upland cotton) and *Gossypium arboreum* (the desi cotton) are two very important species. The former has a high yield and great fiber quality whereas the latter is rich in stress tolerant transcriptome. The oligonucleotide microarray was developed and used for identification of drought tolerant genes in cotton and for the transcriptome profiling of *Gossypium hirsutum* and *Gossypium arboreum* under drought stress. Different cotton genotypes were obtained from CCRI Multan and screened under drought stress condition. The stress was confirmed by IRGA analysis by calculating the photosynthetic rate, transpiration and stomatal conductance. Total RNA was isolated and aminoallyl labelling of cDNA was carried out. The samples were then labelled with Cy3 for the final hybridization and then scanned. The acquired data was analyzed and we obtained 118 upregulated genes in *Gossypium hirsutum* and 66 in *Gossypium arboreum*.These sequences were then analyzed for GO annotation and pathway studies using Blast2GO Pro software. The results obtained were correlated and were found to be in accordance with our initial hypothesis, showing an upregulation in stress responsive genes and pathways. This cross-species analysis will further help in understanding the drought responsive pathways and developmental kinetics in cotton. This technique can also be extended to study and analyze other crops and their stress responsive and tolerating varieties for the ultimate crop improvement.

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Involvement of transcription factor (TF) in fiber development of Extra Long Staple (ELS)

Fozia Akhtar[1*] and Uzma Qaisar[1].

Global transcriptional analysis using microarray or next generation sequencing produce overwhelming amount of data and full utilization of that information is beyond the capacity of a single scientist. We made use of unutilized public microarray raw data produced by cotton fiber development studies and performed meta-analysis using bioinformatics tools in GeneSpring 13.0 -GX (Agilent Technologies). After normalization between experiments, the transcriptome of various varieties of *Gossypium hirsutum* (producing short and long fiber) and Gossypium barbadense (producing extra-long fiber) was compared and identified 1431 genes differentially expressed among fibers of different lengths. 574 genes showed upregulation while 844 genes were down regulated in G. barbadense as compared to G. hirsutum.

In order to validate meta-analysis results, expression of 5 genes was checked in local germplasm of *G. barbadense* and *G. hirsutum* along with desi cotton by RT-real time PCR. The expression of all tested genes validated microarray data. The expression pattern of an ethylene responsive transcription factor (TF) and a vacuolar processing enzyme gene completely correspond to fiber lengths in cotton. TF is previously reported to be specifically expressed in cotton boll and involved in the seed oil biosynthesis. However, present study highlights that TF shows significantly higher expression in ELS as compared to *G. hirsutum* and *G. arboreum* during fiber development. TF transcription is enhanced in fiber while reduced in seeds during different stages of boll development. As TF shows minimal expression in desi cotton, thus introduction of this gene in desi cotton can lead to improvement in fiber quality trait.

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Mitochondrial translational inhibitors induces the expression of mitochondrial transcripts in Hela and SH-SY5Y cell lines

Hina Shakeel* and Muhammad Waqar Hameed.

Mitochondrion is an essential organelle present in virtually all eukaryotic cells. They are vital to the eukaryotic cell as they generate ATP required for cellular processes. Human mitochondria house a circular double-stranded DNA (mtDNA) of ~16,600 base pairs. It encodes 2 rRNAs, 22 tRNAs and 13 protein coding sequences of the ~90 proteins required for the assembly of oxidative phosphorylation (OXPHOS) complexes. Mitochondria are therefore heavily reliant on the nucleus of the cell to provide the remaining proteins required for its function.

A number of eukaryotic and prokaryotic translational inhibitors are known that could potentially effect translation of mitochondrial proteins within the mitochondrium. Among these, puromycin is a translational elongation inhibitor that interacts with larger subunit of mammalian mitochondrial ribosome and leads to premature termination of translation. Homoharringtonine binding at the A-site cleft in the peptidyl transferase center in the 80S ribosomal subunit and blocks protein synthesis. The effect of these translational inhibitors on mitochondrial gene expression is not very well established. Therefore, in this study, we have investigated the effect of translational inhibitors on the expression of human mitochondrial gene expression. We have observed a significant induction in the level of mitochondrial transcripts in response to these translation inhibitors in both SH-SY5Y (neuroblastoma) and HeLa (cervical) cells however, the level of tested mitochondrial proteins remained unaffected. This indicated that these translational inhibitors might not directly affect the abundance of mitochondrial proteins

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Matrix assisted refolding and purification of placenta derived recombinant human interleukin-6 produced in *Escherichia coli*

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Biological activity of human interleukin-6 (IL-6) is associated with vast number of diseases like rheumatoid arthritis, sepsis and severe inflammatory diseases. In this study, human interleukin-6 cDNA was isolated from a cDNA library that was constructed with mRNA derived from human placental tissue. Subsequently, the complete human interleukin-6 cDNA was cloned and expressed in BL21DE3 cells. Recombinant human IL-6 (rhIL-6) protein was expressed in form of insoluble inclusion body. Inclusion bodies were solubilized under denaturing conditions and purified by immobilized metal affinity chromatography (IMAC) with gradual on-column refolding by gradient elution method (6 M to 0 M urea). Protein was purified to apparent homogeneity of about 99% with a yield of 50 mg/L. The purity was assessed by SDS-PAGE, size exclusion HPLC and Western Blot Analysis. The bioactivity was assessed by proliferation assay of TF-1 cells in dose dependent manner. The present study, confirms the expression of placenta derived IL-6 gene in prokaryotic expression system and matrix assisted on-column refolding and purification of rhIL-6 by immobilized metal affinity chromatography. This method for purification of recombinant IL-6 might be help full for downstream process development of IL-6 under current good manufacturing conditions (cGMP).

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Bioactivity Studies Of Huh-7 Cells Derived Human Epidermal Growth Factor Expressed in *Pichia pastoris*

Khan MA*, Ahmed N, Zafar AU, Khan MI, Khan F, Parvaiz R, Rehaman, Z, Husnain T

Human Epidermal Growth Factor (*hEGF*) is a key member of Epidermal Growth Factor family having role in cell proliferation and differentiation in vivo as well as in vitro. Human Epidermal Growth Factor gene has been isolated from different tissues, but the procedure is invasive and technically complicated as it deals with biopsies.

In this study we report cloning of hegf gene from *Huh-7* cell line. Three rounds of PCR amplification were performed to amplify mature *hegf* gene from Huh-7 cell line derived cDNA. Sequencing of amplified *hegf* gene showed 100% sequence homology with wild type *hegf* gene.

The cloned gene was expressed in *Pichia pastoris* (yeast) expression system successfully. Molecular characterization of the Pichia pastoris expressed *hEGF* was done with special reference to its glycosylation profiling and bioactivity studies. Densitometric scanning of SDS-PAGE separated extracellular proteins from hEGF recombinant *Pichia pastoris* strain indicated that about 84% of the extracellular proteins were glycosylated. Size exclusion chromatography using Superdex-75 prep grade column was successfully utilized to separate fractions containing glycosylated and non-glycosylated extracellular proteins. Both glycosylated and non-glycosylated fractions were found reactive to anti-hEGF antibodies in dot blot. Bioactive studies revealed that both glycosylated and non-glycosylated fractions were bioactive as determined by cell proliferation assay and semi-quantitative real time PCR analysis. The semi-quantitative real time PCR also showed that hEGF present in non-glycosylated fraction was found relatively more bioactive then *hEGF* present in glycosylated fraction.

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GC–MS analysis and evaluation of antibacterial activity of *Nigella sativa* oil against diverse strains of *Salmonella*

Arslan Sarwar* and Zakia Latif

Salmonella resistance is becoming a worldwide serious health issue in these days; therefore, it is an urgent need to develop some alternative approaches to overcome this problem. Twenty bacterial strains were isolated and purified from different environmental sources and confirmed as *Salmonella* by morphological and biochemical analyses. Further confirmation was done by 16S rDNA sequencing. Antibiotic susceptibility test was performed by well diffusion assay against different concentrations of Ceftriaxone and Ciprofloxacin. The behavior of both antibiotics was different against diverse strains of *Salmonella*. *Salmonella* strains resistant to both antibiotics were analyzed for antibacterial activity of natural extracts of *Nigella sativa* (black seeds). *N. sativa* oil was found to be more effective against *Salmonella* species for which even Ceftriaxone and Ciprofloxacin were ineffective. Gas chromatography and mass spectrometry analysis of *N. sativa* oil was also accomplished, exhibiting 10 compounds including thymoquinone, p-cymene, cis-carveol, thymol, a-phellandrene, a-pinene, b-pinene, trans-anethole, a-longipinene and longifolene.

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Molecular and Behavioural Profile of Repeated Methylphenidate Administration in Rats

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Methylphenidate (MPD) is the most regularly prescribed psychostimulant for patients with Attention-deficit hyperactivity disorder (ADHD). Although dopamine is the main neurotransmitter involved in the pathophysiology of drug abuse, serotonin (5-hydroxytryptamine; 5-HT) can modulate addictive effects of drugs of abuse. The present study was designed to check MPD-induced behavioural sensitization and cognition along with the 5HT-1A receptor expression in the nucleus accumbens and prefrontal cortex of repeated MPD treated rats. Twenty four male albino Wistar rats (180-220 gm) were used to determine dose related effects of MPD (0.5, 2.5 and 5 mg/kg) on cognition in water maze test. Acquisition of memory was assessed after two hours of the three successive training sessions. After 20 hours of drug administration, retention of memory was assessed. In another experiment, twelve male albino Wistar rats were randomly assigned to two equal groups. Water and methylphenidate (2.5 mg/kg), were administered orally to the respective groups. Animals were exposed to 12 (one daily) place conditioning sessions of 30 min each. Motor behaviour during this session was also recorded. Reinforcing effects of methylphenidate were monitored in a balanced design during the test session on day 13. On day 14, drug/water was administered and learning acquisition was done after training sessions. Retention of memory was assessed after 24 hours of the drug administration. Decapitation was done on the next day and brains were micro-dissected to collect the nucleus accumbens and prefrontal cortex for 5HT-1A receptor expression. 2.5 mg/kg MPD found to enhance cognitive effects in Morris water-maze test. Conditioned place preference (CPP) test on day 13 revealed that repeated administration of MPD (2.5 mg/Kg) produced reinforcement as well as the behavioural sensitization. While repeated administration of MPD moderately enhanced acquisition of memory while significantly increased memory retention. We also report that 5HT-1A receptor expression was also down regulated in methylphenidate treated rats both in the nucleus accumbens and prefrontal cortex. These findings may help to improve pharmaco-therapeutics in treating ADHD.

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Antibiotic resistance pattern and distribution of mec and cna gene among biofilm producing methicillin resistance *Staphylococcus aureus* (MRSA) isolated from wounds

Muhammad Sohail* and Zakia Latif

MRSA is considered as a major cause of hospital-acquired and community acquired infections due to its high antibacterial resistance. Biofilm formation is a well-known pathogenic mechanism in MRSA infections, since sessile bacteria are protected in an extracellular matrix of exopolysaccharide. The expression of collagen adhesion protein (*cna* gene) can be important for biofilm formation by MRSA. The purpose of this research was to evaluate the antibiotic resistance pattern and distribution of the *cna* gene among biofilm-producing MRSA isolates obtained from pus of wounds. One hundred isolates of MRSA were obtained from wound pus. MRSA isolates were identified using standard bacteriological practices. Drug susceptibility test was performed by disk diffusion method for all the isolates against twenty nine antimicrobial agents and MIC of vancomycin was determined by E test. Biofilm formation was measured by microtiter plate assay. Polymerase chain reaction (PCR) was used to identify the presence of the mec and *cna* gene among the isolates. Biofilm formation was observed in 60% of the MRSA isolates. The potential formation of biofilm was significantly associated with resistance to antimicrobial drugs resistance. In addition, the *cna* gene only existed in biofilm-producing isolates with a frequency of 35%. The findings of the present study well demonstrated that the MRSA biofilm-producing isolates were more resistant to the tested antibiotics. Furthermore, because of wide distribution, it seems that the *cna* gene is associated with biofilm formation.

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In planta biometric screening of local rice (*Oryza sativa*) cultivars under saltstress and development of their cell lines for molecular analysis

Bushra ijaz[1]*, Tayyaba Yasmin [1], Muhammad Zeeshan Hyder[1], Fiorella Lo Schiavo[2]

Pakistan ranks among the top leading countries for rice production in Asia. Pakistani rice varieties are mostly cultivated in areas of Punjab and Sindh. Reports have shown that these areas are being polluted with salinity at high rate. To cope up the future threats,responses of five Pakistani rice varieties (KSK 133, KS 282, Super Basmati, Shaheen Basmati and DilRosh) were analyzed to determine their salt sensitivity. Physiological analysis was conducted for total plant height, length & number of leaves and senescence rate in each variety.On the basis of results, out of the five varieties, one tolerant and one sensitive to salt stress variety were selected to determine the root architecture. WinRhizo Pro Software was used to analyze the data for total root length, average root diameter, surface area and total number of tips. Results showed that the sensitive cultivar produced longer but thinner roots with less surface area and less number of tips as compared to the control and tolerant cultivars in saline environment. Additionally, the rice plant, as experimental system, doesn't allow an accurate study of the early events in salt stress response because of immediate induction of transduction pathways. To overcome this problem, cell cultures suspension for both varieties have been established and stabilized. Among components involved in the signaling pathway induced by salt, the role of nitric oxide and hydrogen peroxide will be investigated in cell cultures. At molecular level, RNA sequence analysis, aimed to compare the transcriptional profile of the two cultivars, will be performed. Key Words: Salt stress, rice, root analysis, cell cultures, hydroponics.

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Acetylcholinesterase Inhibition of two Insect Species, *Apis mellifera* and *Tribolium castaneum*, by *Azadirachta indica* derived Bio-pesticides

Basit Jabbar*, Hina Ejaz and Amtul Jamil Sami

Compounds from the plant Azadirachta indica are known to be toxic for insect pests and hence are useful as bio-pesticides, yet can also be detrimental to crop-friendly insects at higher doses. Exploring the mechanism of insect inactivation by plant-derived biopesticides is an important avenue in plant biotechnology. Bio-pesticides can target insect enzyme Acetylcholinesterase (3.1.1.7) which is involved in regulation of acetylcholine and vital for proper functioning of nerve impulses. We homogenized AChE from two insects- Apis mellifera and Tribolium castaneum- to solubilize free and membrane bound AChE and determined AChE activity by Ellman's assay. Solubilized enzymes, AmAChE and TcAChE, were subjected to inhibition assays using neem extract and neem-derived saponins as inhibitors and percentage inhibition was evaluated. Significant percentage inhibition of AChE activity from T. castaneum was observed while the same concentration of neem extract and saponins caused only minor inhibitory effect on A. mellifera AChE. For gaining insights into the kinetics of inhibition by saponins, AChE assays were performed using a range of substrate (ATC) concentrations. Results of inhibition kinetics indicated reduced Vmax for AmAChE while similar Km value for inhibited and un-inhibited enzyme suggesting non-competitive inhibition. For TcAChE, kinetic studies revealed competitive inhibition by neem-derived saponins indicative of inhibitor binding at the active site of TmAChE. Computational analyses and docking studies were performed to relate it with the experimental work. We have discussed comparative features of AChE of insect pest (*T. castaneum*) and crop pollinator (*A. mellifera*).

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Effect of Tris Citrate & Skim Milk extender on buck semen during liquid storage

Anam Mushtaq*, Muhammad Rafi Ullah, Nadir Hussain, Mushtaq Ahmad, Zahid Tahir, Nasim Ahmad*

Artificial insemination is a great tool for wide spread use of high genetic potential and use of liquid semen is less laborious and economical way of semen extension. The objective of present study was to compare the effect of Tris Citric acid Egg Yolk (TCEY: Tris 1.73gm,Citric Acid1.93gm, Egg Yolk 20ml) and Skim Milk Tris Citric Acid (SMTC: Skim Milk 0.9gm, Tris 1.73gm, Citric Acid 1.93gm) semen extenders on buck semen parameters (Motility, Live Dead Ratio & Membrane Integrity) at 4 ˚C from 0 to 96 hours. Semen was collected from two Beetal bucks once in a week for four consecutive weeks (4 replicates) through artificial vagina. The ejaculates qualifying the minimum criterion were pooled and divided into two aliquots i.e. one diluted with TCEY and second with SMTC. After extension semen samples were cooled to 4º C and then incubated at 4º C for 96 hours. Motility, live ratio (Eosin and Nigrosine staining), HOS (Hyper Osmotic Swelling) & NAR (Normal Apical Ridge) were assessed after every 12 hours interval from 0 to 96 hr of incubation. Semen samples were pre-warmed at 37˚C before evaluation. Data was analyzed by repeated measure ANOVA in SAS Enterprise Guide 4.2.Motility and HOS were higher ($p<0.05$) from 12 to 96 hours in SMTC as compare to TCEY (17.5% ±3.22 vs. 3.75% ±2.39; 33.75% ±0.75 vs. 25.5% ±1.94 respectively at 96 hours).Moreover, live ratio and NAR were higher ($p<0.05$) from 0 to 24 hours in SMTC (72% ± 2.38 vs. 60.75% ±2.95 ; 70.25 ±1.93 vs. 55% ±1.87 respectively at 24 hours) than TCEY. It is concluded that skim milk based extender can maintain higher sperm quality during liquid storage at 4ºC upto 96 hours skim milk based extenders.

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Elemental sulfur crystallization-a plant reaction to microbial infections

Shazia Kanwal Malik*[1], Zaheer-Ud-Din Khan[2], Farah Khan[1]

Sulfur is the element with the largest number of allotropes (30), but only a few are found in nature and occur in biological systems. Elemental sulfur as fungicidal is well documented in certain specialized prokaryotes but has rarely been detected in eukaryotes. In the current work isolation, purification and identification of crystalline sulfur (α-sulfur, S_8) was done from the infected fruits samples of *Putrangiva roxburghii* Wall, *Pterospermum acerifolium* Linn. and *Diospyros malabarica* Kostel. Samples were dried and solvent extraction was done by maceration.

Different allotropic forms of elemental sulfur (monoclinic S_8 and orthorhombic S_8) were crystallized from fruit extracts. Isolation of pure elemental sulfur was done by recrystallization. Physico-chemical properties and microscopy of different sulfur crystals were determined. Structure analysis of refined sulfur crystals was done by X-Ray Diffraction Analysis (XRD). Elemental analysis of infected fruits samples were done by CHNS elemental analyzer. X-Ray Diffraction Analysis and CHNS confirmed the accumulation of sulfur in infected parts of fruits that crystallizes in allotropic forms (monoclinic S_8, orthorhombic S_8).

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Skin diseases spread through makeup testers and used cosmetics

Shazia Kanwal Malik*[1], Farah Khan[2], Kiran Zahid[3]

In the present study investigation was done to look at skin ailments created by utilized beauty care products and cosmetics testers. Twenty makeup specimens (Lipsticks) were collected from a super market. Each specimen underwent clinical examinations and laboratory tests such as direct KOH (20 %) analysis and culturing on Potato Dextrose Agar plates. Incubation of plates was done at 28 °C for 3 to 7 days. The fungal strains were subjected to microscopy and identification. Microscopic examination was done with fungal hyphae and spores. Macro-conidia of *Tinea nigra* and *Malassezia furfur* of brown colour were seen in the specimen. *Tinea nigra* is a superficial fungal infection that causes dark brown to black painless patches on the palms of the hands and the soles of the feet. *Malassezia furfur* causes most skin infection like skin rash in people, including the most widely recognized reason for dandruff and dermatitis. From the present work it is presumed that used lipsticks and cosmetics testers reasons skin maladies (rashes and dandruff).

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Introducing ApoE-Modified Liposomal Nanoparticles for altering the expression of Cholesterol Metabolism-Associated Genes in HCV Infected Liver

Hibba Ansar*

Hepatitis C virus (HCV) infection, alters cholesterol metabolism due to activation of sterol regulating element-binding protein (SREBP) that ultimately increased the expression of 3-Hydroxy 3-Methyl-Glutaryl coA Reductase (HMGR) results in cholesterol accumulation. Due to high replication of HCV it is difficult to control its growth and infectivity. So it may be useful to suppress host factors that promote replication of virus. Thus ApolipoproteinE (ApoE)-modified liposomal nanoparticle containing cDNA of HMGR gene is delivered to infected hepatic cells to suppress expression of HMGR gene. By using ApoE/YSK-MEND cholesterol biosynthesis may be lowered due to suppression of HMGR gene. As concentration of HMGR enzyme can be reduced in treated cell lines as compared to HCV infected cells. Our research, may lower the cholesterol biosynthesis that has been upregulated in HCV infection, by using Nanoparticle technology. Also, it may help to evaluate the effective drug delivery to hepatocytes via LDLR by ApoE modified liposomal nanoparticle.

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Computational analysis of dengue virus protein interaction network with its host *homo sapiens*

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With almost 50% of the world's population living in the dengue epidemic regions, about 390 million people suffer from Dengue Fever annually. Protein-protein interaction network of dengue with its host Homo sapiens can give us significant systems-level insights. Although research has been done to decipher the interaction between dengue and its host proteins, the reliability of these interactions are still questioned. In this study, we gathered available dengue-human protein interactions from current literature and publically available datasets. This includes data from Yeast-2-hybrid (Y2H), Affinity purification-mass spectrometry (AP-MS), and computational prediction methods. Our analysis showed that dengue-human protein interaction data is still noisy and incomplete. Combining available data and constructing a network is a step forward toward the completing of this network. An integrated dengue-human protein interaction network was constructed, containing 1444 proteins and 10,576 interactions. The hubs protein in our network identified important host proteins in the network, which might have a significant role in dengue infection. Integration of gene expression data on our network indicates 141 protein co-expressed during the infection. Moreover, enrichment analysis of the network helps us identify strong candidates for in vitro and in vivo characterization. Enrichment analysis indicates that most of the host proteins in the network are involved in blood coagulation, homeostasis and the complement pathway. This network will provide a starting point for future lab experiments in the drug design.

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Plant Immunity, Microbial Pathogenesis and Cytokinins: An Integrated Systems Biology Perspective

Muhammad Naseem*

A dynamic relationship of evolvability exists between microbial pathogens and their cognate plant hosts, formerly called gene-for-gene interaction and now known as the **zigzag** model of plant immunity. The pathogen attack disrupts cellular organization at multiple levels and this demands integrated systems biology approaches to resolve the underlying complexity in host-pathogen interactions. Establishing the concerted, multi-level plant immune response from genome, transcriptome, and interactome data, we transformed the **zigzag** model of plant immunity into an integrated semi-quantitative dynamic model of the Arabidopsis interaction with *Pst* DC3000. These analyses integrates signaling cascades, protein-protein interaction networks and metabolic reconfiguration taking different pathogen virulence strategies into account. The applicability of these models was exemplified on the role of plant hormone cytokinins (CK). Despite great advances in unravelling the signaling circuitry of CK in the last three decades, many unanswered questions regarding its functions still remain to be addressed. Most notably, the role of CK in plant immunity beyond host susceptibility to tumor-gall causing pathogens was not congruently analysed. We unravel hub points of immune interaction mediated by CK signaling in *Arabidopsis* after infection with *Pst* DC3000. Moreover we traced the connection of CK to salicylic acid (SA) and jasmonic acid (JA) mediated central back bone of plant immune system. However, the ubiquitous distribution of CKs in biosphere due to their enlarged production by plants, plant and animal associated microbes (Mycobacteria, Staphylococcus and Salmonella etc.) as well as some insect species implicate this purely plant specific growth regulator as potent candidate molecule for cross-kingdom (mammals, plant, insects and many microbes) communication. Future efforts deciphering the implications of CK in communication biology will help to manipulate biological interactions for sustainable benefits; such as crop production, yield protection against microbial pathogens and noxious herbivores, the targeting of animal diseases and skin care applications.

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DC-SIGN and Dengue; DC-SIGN, -336A/G association with Dengue Fever and Dengue Hemorrhagic Fever

Humeira Naeemi, Sumra Batool, Syed Mohsin Raza, Shagufta Khaliq, Saba Khaliq*

Dengue is a mosquito born viral disease; Dengue fever (DF) and Dengue hemorrhagic fever (DHF) are its more severe forms. Dengue virus interacts with various immune cells including dendritic cells (DCs) which mediate immune response leading to innate and acquired immunity. DCs specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) has a key role in the interaction between pathogen and DC, their migration and pathogen uptake. The -336A/G SNP (rs4804803) polymorphism in the promoter of CD209 modulates DC-SIGN expression and is associated with the progression and severity of dengue infection in a number of different populations. We investigated the putative association of this polymorphism with DENV infection and its pathogenesis. The rs4804803 SNP association with a susceptibility to dengue fever (DF) and/or dengue hemorrhagic fever (DHF) through genotyping analysis was done using PCR-RFLP technique. A total of 400 DNA samples were genotyped, including 160 DF, 40 DHF and 200 healthy population. A control sample of 200 individuals were selected through a clinical investigation for absence of past DF infection. A strong association between GG/AG genotypes of rs4804803 and risk of DHF was found when compared among DF and controls ($p<0.05$). The AA genotype was associated with protection against dengue infection compared to controls ($p<0.001$).
The rs4804803 SNP in the CD209 promoter contributed to susceptibility to dengue infection especially DHF.

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SOCS3 mRNA expression, polymorphisms and response to treatment in HCV genotype 3a infected patients

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SOCS3 belongs to SOCS family and it is one of the negative regulators of cytokine signalling and interferon (IFN) response that function via the JAK-STAT pathway. SOCS3 genetic polymorphism influences the pathogenesis and outcome of antiviral treatment. This study was designed for analysis of SOCS3 gene expression and polymorphism in Pakistani HCV patients. This was a descriptive study, which included 250 subjects positive for HCV antibodies (ELISA positive) and HCV RNA (PCR positive). The study population was divided into three major groups on the basis of therapeutic response i.e. early virologic response (EVR), late virologic response (LVR) and non-responders/relapsers (NR). SOCS3 gene expression analysis was done by using Real time PCR technique, and data was analysed using SPSS 20.0. ARMS PCR technique was used for SOCS3 gene polymorphisms, 8464 A/C (rs12952093), -4874 A/G (rs4969170) and -1383 A/G, (rs4969168) analysis. There was a statistically significant difference in the levels of AST and ALT between all three groups. Gene expression analysis of SOCS3 showed that there was statistically significant increase of 1.92, 2.63 and 3.72 folds in relative gene expression ($p = 0.02$) of EVR, LVR and NR respectively, where GAPDH was used as an internal control for normalization of data. On comparison the allele frequency of rs4969168 was statistically significant between EVR vs. LVR ($p = 0.012$) and EVR vs. NR ($p = 0.013$). The distribution of rs4969168 genotype frequencies between all three groups was statistically not significant. On comparison rs4969170 genotype frequency showed no statistically significant difference. Only the allelic frequency of rs4969170 was highly significant ($p = <0.0001$) when patients of NR group were compared with patient EVR and LVR. Genotype and allelic frequency of rs12952093 showed no association in any group when compared with each other. Results regarding response to HCV therapy in our population is diverse i.e. EVR, LVR and NR after completion of therapy. To conclude the results, it can be said that genetic factors i.e. polymorphisms alone are not involved in response to therapy in our local population.

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