Genomic Med. (2008) 2:427–433
DOI 10.1007/s11568-009-9084-7

ABSTRACTS

Chemical genomics and molecular medicine

586: Use of natural analogs as AchE inhibitor for treatment of early stage Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disease that, in its most common form, occurs as dementia in people over 65 years old although early onset form also exists. Currently it afflicts 24 million people worldwide. The disease is characterized in the brain by abnormal clumps (amyloid plaques) and tangled bundles of fibers (neurofibrillary tangles) composed of misplaced proteins. Three neurotransmitters commonly affected by AD are acetylcholine, serotonin, and nor epinephrine. The cholinergic system—the nerve cell system in the brain that uses acetylcholine as a neurotransmitter—is the most dramatic of the neurotransmitter systems affected by Alzheimer's disease. Acetylcholinesterase is one of the most crucial enzymes that hydrolyze acetylcholine in the brain to choline and acetic acid at cholinergic synapses, leading to nerve response and function. Potentiation of central cholinergic activity has been proposed as a therapeutic approach for improving the cognitive function in patients with Alzheimer's disease (AD). Increasing the acetylcholine concentration in the brain by modulating/inhibiting acetylcholinesterase (AchE) activity is among the most promising therapeutic strategies. In our studies attempts were made to produce potential natural acetylcholinesterase inhibitors. More than eight hundred compounds were taken from different natural sources. Initial in-silico screening of molecules was based on docking score between acetylcholinesterase and compounds taken from the natural sources. The hits were further analyzed and narrowed by applying Lipinski's rule-of-five analysis. Molecules with high ability to cross blood brain barrier were taken for energy minimization and quantitative structure activity studies. The biological activity of promising compounds was predicted by using PASS prediction analysis. Ferulic acid and Caffeic acid found in garlic showed very good IC50 values and satisfied the standard drug-like properties with remarkable pharmacokinetics and pharmacodynamics abilities. Thus we hypothesize that ferulic acid and caffeic acid which is obtained from garlic can be used as an effective acetylcholinesterase inhibitors in the early stage of Alzheimer's disease. Wet lab studies were conducted on the Swiss Albino mice as the animal model to check the inhibition of acetycholinestrerase by ferulic acid and caffeic acid.

587: Challenges and opportunities in the post-genomic chemical biology age: The hunt for small molecule, chemical dissectors of signaling networks

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The research in our group aims at developing methods leading to high-throughput generation of natural product-inspired compounds with the goals of identifying chemical modulators of protein–protein interactions-based dynamic signaling pathways. In addition to utilizing these compounds in a wide variety of solution cellular assays, they are also utilized in printing small molecule microarrays (SMM) in collaboration with the chemical biology team at the Broad Institute. The use of the SMM technology is highly attractive in performing high-throughput small molecule–protein binding studies. Over the years, although the high-throughput synthetic approaches have been very successful in generating a large number of small molecules but in most cases, these compounds are simple in nature and thus lack the features that are commonly found in bioactive natural products (i.e., 3D architectures, rich in chiral display of dense functional groups etc). Our team is working on the hypothesis that small molecules being generated from our program are highly likely to exhibit better biological responses (e.g., as modulators of protein-protein interactions etc.) because these compounds are anticipated to occupy the chemical space being championed by bioactive natural products known to modulate p–p interactions. Through working in collaboration with the biomedical (including the NMR structural biology) community involved in programmed cell death pathways (e.g., Bcl-2 family, Caspases, XIAPs etc) and in kinases and phosphatases signaling, several novel functional chemical probes have been identified to date. Our work in this direction will be presented in this poster/short talk.

588: S29 ribosomal protein regulates balance between cell death and survival in Hep2 cells through MAPK/ mitochondrial pathway

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Multifunctionality of proteins is one of the mechanisms accounting for the complexity of interactome networks in higher eukaryotes. There are various reports in the literature which suggest that during oncogenesis and other pathologic conditions many proteins perform additional functions without changes in three dimensional structures. One such multifunctional protein is S29 ribosomal protein. The S29 ribosomal protein is not only involved in ribosome assembly but its enhanced expression has been shown to possess antitumor properties in non small cell lung cancer H520 cells. Literature also suggest that S29 ribosomal protein increases tumor suppressor activity of RAP1A (Krev-1) gene on v-K ras-transformed NIH3T3 cells. Recently conservation and multifunctional nature of ribosomal protein S29 has considerably drawn the attention of researchers so that basic mechanism behind its function can be elucidated. Adopting biochemical and proteomic approaches we made an attempt to investigate the antiproliferative potential of S29 ribosomal protein on cultured Hep2 cells. Apoptosis was examined by Hoechst 33342 staining, FACS, increased expression of pro-apoptotic protein Bax, decreased expression of anti-apoptotic proteins Bcl-2 and Bcl-XL, release of apoptogenic cytochrome c and activation of initiator caspase-8, -9 and effector caspase-3 followed by cleavage of nuclear substrate poly (ADP-ribose) polymerase. There was also non-involvement of p53 in S29 ribosomal protein induced apoptosis and the pro-apoptotic effect was mediated by inactivation of ERK, JNK and p38 MAPK. We further assessed whether S29 over-expression could increase chemosensitivity of cancer cells to chemo-therapeutic agents. Our results showed that S29 overexpression increases Hep2 cells sensitivity to Cisplatin, Etoposide and TNF alpha; but to different extents. Our proteomic results also confirmed the proapoptotic role of S29. For the first time, we report here the potential of anti-human laryngeal activity of S29 ribosomal protein in Hep2 cells. This study also raises the possibility of S29 ribosomal protein as a promising approach to cancer therapy.

589: The effects of humanin peptides on mitochondrial functions and global gene expression

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Backgroud: Humanin (HN) is a novel antiapoptotic and neuroprotective peptide. Using bioinformatic tools, we have recently identified a host of putative nuclearly-encoded HN peptides.
Aim: To assess the biological properties of the synthetic nuclear HN peptides.
Methods: Human umbilical vein endothelial cells (HUVECs) were exposed to proapoptotic conditions triggering the BAX-related pathways (staurosporine or UV light) and incubated with different HN peptides. Global gene expression changes were assessed by custom-made Affymetrix NuGO chips, while mitochondrial function and apoptosis were monitored in real-time by high-throughput fluorescence confocal microscopy (BD Pathway Bioimager 855) using markers of viable mitochondria (TMRM, JC-1) and active caspases (specific labeled antibodies). Results: Among the studied peptides, significant differences in antiapoptotic potential were found between the Thr13 and Ile13 izoforms (c.38C>T) of the HN10b. The wild-type threonine izoform provided much stronger antiapoptotic activity then the isoleucine peptide. HUVECs treated with Ile13 HN10b succumbed significantly quicker to staurosporine than those pre-incubated with Thr13 HN10b as demonstrated by the rate of TMRM signal loss paralleled by the increase in caspase activation. Variable functional properties of the Thr13 and Ile13 HN10b variants were also reflected by differential global gene expression patterns.
Conclusion: The nuclearly-encoded HN peptides may differ in their antipoptotic potential, which is especially apparent when comparing the Thr13 and Ile13 variants of HN10b.

590: Novel paradigm in lipid metabolic network of *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (Mtb) is the etiologic agent of tuberculosis and infects approximately 32% of the human population. The cell envelope of mycobacteria is endowed with a number of unique lipids that play an important role in its virulence. Large multifunctional proteins called polyketide synthases (PKSs) catalyze the biosynthesis of these lipids. PKSs are generally involved in the synthesis of secondary metabolites in various organisms and their role in biosynthesis of virulence factors is unprecedented. PKSs require proteins called fatty acyl AMP ligases for the activation and utilization of fatty acid substrates. PKS12 is the largest open reading frame in the Mtb genome and contains at least twelve different catalytic sites on a single protein. Our systematic investigation of PKS12 protein revealed formation of a novel supramolecular assembly that catalyzes biosynthesis of a mycobacterial phospholipid antigen. Clearly, the lipid repertoire of mycobacteria necessitates a coordinated network of various biosynthetic enzymes. Insights into these molecular events provide an opportunity to develop inhibitors that would simultaneously disrupt several pathways in lipid metabolism. Due to overlapping substrate specificities and cofactor requirements of various enzymes involved in lipid biosynthesis, directed synthesis of chemical compounds targeting multiple pathways could provide new avenues for drug discovery.

591: Novel RNAi-based chemical genetic screen to identify small molecule modulators of the Wnt/wg signaling pathway

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Recent advances in functional and chemical genomics have led to vast advances in both, the understanding of disease and the potential to discover effective therapeutics to combat otherwise intractable disease states such as cancer. The Wnt/wingless (wg) pathway is one of a core set of evolutionarily conserved signaling pathways that regulates many aspects of development and homeostasis. Misregulation of the Wnt pathway can be detrimental since mutations in several components are associated with tumorigenesis of the breast, liver, colon, and the skin. It is therefore crucial to develop and implement new technologies in order to generate molecular tools that may be used to modulate the activity of the Wnt/wg signaling pathway. One of the key effectors of

the Wnt pathway is encoded by beta-catenin (B-cat). Induction by Wnt ligands leads to stabilization of cytosolic B-cat, which subsequently translocates to the nucleus to activate target genes that regulate many aspects of cell proliferation, growth, differentiation and death. Since Catenin Responsive Transcription (CRT) has been implicated in the genesis of many cancers, it makes a good target for developing therapeutics that could modulate the nuclear activity of B-cat. Recently, we employed a novel methodology of integrating a 'sensitized' chemical genetic high-throughput screen (HTS) with RNA-interference (RNAi) screening technology in order to identify specific small molecule inhibitors of the Wnt pathway in Drosophila cells. The primary screen was performed in Drosophila derived Clone 8 cells, which afforded the advantage of a non-redundant genome. Subsequent secondary screens were performed in mammalian cell lines including the mouse mammary epithelial cell line, C57 mg; human epithelial cell line, HEK 293; the colon-cancer cell line, HCT-116; and the breast cancer cell line, MCF-7. Each of the aforementioned cell types is Wnt responsive and thus allowed for heterologous systems to validate the efficacy of the compounds on Wnt-signaling. We envisage that compounds identified in ours and similar future screens will serve as prototypes for the development of anti-tumor drugs targeted to B-cat responsive transcriptional programs involved in different cancers. Furthermore, this study paves the way for the development and implementation of a new technological advance for rapid screening of large compound libraries to identify modulators of specific signaling pathways.

592: TRIM69/HSD34 protein, a RING finger protein, functions as an E3 ligase in regulating cell cycle and apoptosis by interacting with HUS1 protein

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Trim69/Hsd34 is a novel gene discovered in our laboratory by studying human primary spermatocytes by the combined use of the techniques of laser capture microdissection (LCM) with suppressive subtractive hybridization (SSH). The gene coded protein (TRIM69 protein) consists of an N-terminal RING finger, followed by a B-box zinc finger, a coiled-coil domain, and a B30.2 domain. A class of E3 ligase family consists of proteins containing RING finger domain that are involved in ubiquitin-dependent proteasome system. Thus based on its sequence, TRIM69/HSD34 protein is proposed to an ubiquitin ligase. In vivo ubiquitination assay showed that TRIM69 protein could transfer ubiquitin to itself, whereas a point mutant of RING finger domain failed to mediate this action. Moreover, subcellular localization of those mutants of TRIM69 protein without the RING domain changed from speckled around the nucleus to scattered specks in the cytoplasm. Thus the RING finger domain may play an important role both in TRIM69 protein auto-ubiquitination and its intracellular localization. This auto-ubiquitination may be an auto-regulatory mechanism and target itself for ubiquitin-dependent degradation or a modification process of its biological activity. HUS1 protein has been demonstrated to be capable of interacting with TRIM69 protein based on the yeast two-hybrid assay. The interaction was validated by a Co-immunoprecipitation assay. Immunofluorescence assay showed colocalization of TRIM69 protein with HUS1 protein. The above-mentioned data suggest that HUS1 protein might be a substrate of TRIM69 protein. HUS1 protein is known to function as a DNA damage sensor together with RAD9 and RAD1 proteins by forming RAD9-HUS1-RAD1 (9-1-1) complexes. Therefore, TRIM69 protein may be involved in cell cycle regulation and apoptosis by interacting with HUS1 protein. Since HUS1 protein exists in association with RAD1 and RAD9 proteins, it is pertinent and of interest to determine the functional relationship of TRIM69 protein with RAD1 and RAD9 proteins.

593: Potentiation of macrophage function by cigarette smoke against *Leishmania donovani* infection

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Background: Macrophages play important role in host defense by producing cytotoxic products, including reactive oxygen species (ROS), to kill invading organisms. They also generate cytokines, chemokines and immunomodulators that are involved in inflammatory response and cell mediated immunity. Despite these activities intracellular parasites, such as *Leishmania*, reside within the macrophage. They lower the level of ROS inside macrophage, and manipulate cellular signaling processes involved in host cell immune response, such as NF-κB, in their favour. Since cigarette smoke (CS) contains numerous oxidants and is able to induce pro-inflammatory cytokines, we examined its macrophage activation property by examining cellular parasite burden, ROS level, cytokine expression pattern and NF-κB signaling in *Leishmania donovani* (*L. donovani*) infected peritoneal macrophages.
Methods: Cultured peritoneal macrophages were either left untreated or treated with aqueous cigarette smoke extract (CSE) prior to *L. donovani* infection. Parasite burden was assessed by giemsa staining of infected cells. In vivo parasite burden was assessed by looking at the giemsa stained spleen sections obtained from either CS-unexposed or CS-exposed *L. donovani* infected BALB/c mice. Intracellular ROS level was determined by FACS analysis using DCFH-DA. Semi-quantitative RT-PCR and quantitative real time PCR were performed to analyze mRNA levels of cytokines. NF-κB activity was assessed by EMSA and reporter assay.
Results: Our results show that a restricted dose of CS and CSE limit leishmanial parasitic burden in mouse and in vitro cultured peritoneal macrophage cells respectively, without exhibiting cellular cytotoxicity. A pre-treatment with CSE causes an increase in intracellular ROS level, up-regulates the generation of pro-inflammatory cytokines, lowers the expression of immuno-suppressive cytokine and boosts NF-κB activity in *L. donovani*-infected macrophage.
Conclusion: Low concentration of CSE counteracts *L. donovani* infection-mediated suppression of macrophage function without affecting host cell viability. This study reveals a new role of CSE as an activator of macrophage function.

594: Novel mutations in sarcomeric and desmosomal genes in cardiomyopathies—An Indian study

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Cardiomyopathies, a group of disorders characterized by malfunctioning of the myocardium and clinically identified by heart failure, arrhythmias and sudden death. The Indian population is considered to be at greater risk for cardiovascular diseases with high mortality. Based on the pathophysiology, cardiomyopathies are classified as HCM, RC, ARVD/c and DCM, with the etiologies related to Sarcomere, desmosomal proteins and Ryanodine receptor, components of the cardiomyocytes. The present study included clinically confirmed 97 HCM, 86 DCM and 31 ARVD cases (213 patients) referred to various hospitals in India. Mutational Screening of MYBPC3, MYH7B, TNNI3, PKP2, RYR2 genes was carried out using SSCP method followed by sequencing.

Following novel mutations were identified—(1) HCM—(a) In MYBPC3 gene—frameshift mutation InsA11577^11578 in Exon19 in one patient (b) In MYBPC3 gene—1 novel SNP C>T at 1093 codon in exon 31 in one patient (2) ARVD—a. PKP2 gene—a frameshift mutation 2 bp del-CT in exon 3.1 was identified in two patients. b. RYR2 gene—Novel SNP A>C—5 bp upstream of acceptor splice site in exon 8 identified in six patients and one control. c. RYR2 gene—novel SNP A>G at 1079 codon in exon 28—one patient d. RYR2-Ins^G-30 bp downstream of exon 28 creating a cryptic splice site (3) DCM—a. MYH7B gene—a G>C missense mutation at 377 codon in exon 12 in one patient b. MYH7B gene—a G>A missense mutation at 787 codon in exon 21 in one patient c. TNNI3 gene—a G>A missense mutation at 91 codon in exon 5 in one patient d. TNNI3 gene—a G>C missense mutation at 74 codon in exon 5 in one patient. In silico analysis revealed structural and functional alterations of the sarcomeric and desmosomal proteins. Thus the present study highlights the unique genetic variations in Indian population indicating hotspot variations and implicating genes different from those of western populations.

595: A High-throughput generation of flavonoid natural product-inspired chemical probes: The discovery of a novel class of cell motility inhibitor

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With the growing interest in the use of small molecules as chemical modulators of biomacromolecular interactions, a ready access to natural product-inspired structurally complex compounds with diverse architectures that could exhibit highly specific biological responses comparable to bioactive natural products has also risen. To address this need, we have developed a highly practical enantioselective synthesis of a new class of benzofuran-derived cyclic β-amino acids, which could further be utilized in generating a diverse set of flavonoid-inspired, different bicyclic, tricyclic and tetracyclic architectures. Through combining two important structural features (i.e., benzofuran scaffold and cyclic β-amino acid functionality), we were interested in generating a variety of flavonoid-inspired small molecules and check their potency as modulators of physiological processes; in particular, the protein signaling network involved in cancer invasion. In one study, these compounds were tested in a search for small molecule modulators of cell migration using phenotypic assays. Our preliminary screening efforts have identified a novel benzofuran-based derivative as a modestly potent cell motility inhibitor (IC50 $\sim$40 μM by chamber cell migration assay).

597: Heterozygous I171 V mutation of the nibrin (NBN) gene as a risk factor for solid malignant tumors

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Homozygous mutation 657del5 of the NBN gene is responsible for the majority of Nijmegen breakage syndrome (NBS). Several studies have focused on searching for an association between mutations in the NBN gene and cancer incidence. Heterozygous carriers of the NBN 657del5 mutation have been shown to have an increased risk for breast cancer, melanoma, colon and rectum cancer. Other studies have found no association between NBN gene mutations and Hodgkin's lymphoma or non-Hodgkin's lymphoma. The aim of the study was to analyse the frequency of another heterozygous missense I171 V mutation in NBN gene in 270 women with breast cancer, 176 patients with larynx cancer, 81 with second primary tumors of head and neck, 131 with colorectal carcinoma and 1274 healthy individuals. I171 V mutation was present in 17 cancer patients compared with only 8 in healthy individuals. Since DNA was isolated from non-malignant cells, all mutations found in cancer patients appeared to be germinal origin. It can be concluded I171 V mutation in NBSI gene is associated with predisposition to malignancies and NBN allele I171 V may be a general cancer susceptibiity gene.

598: Exploring the Indoline alkaloid natural product-inspired space in a search of chemical probes of FAK signaling networks

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With the goals of identifying chemical probes of protein–protein (p–p) interactions involved in signaling pathways, the research in our group aims at developing methods leading to high-throughput generation of natural product-inspired compounds. Inspired by bioactive indoline alkaloids that have a proven record of modulating p–p interactions in many cases, we embarked a program that is aimed at developing modular high-throughput approaches in producing different forms of polycyclic architectures varying in 3D shape and in different display of chiral functional groups. These indoline-alkaloid

insured compounds were engineered in a way that they could be easily utilized in printing small molecule microarrays (in collaboration with the chemical biology team at the Broad Institute). In addition to this, the highly diverse indoline-alkaloid like collection was also tested in a search of chemical probes of Focal Adhesion Kinase (FAK) signaling networks in collaboration with the Lady Davis Institute Team at McGill. FAK is an adapter protein and plays a crucial role in regulating cell survival, proliferation, migration and invasion in the development and progression of cancer. The over expression of FAK is commonly found in a wide variety of human cancers. Given its important role in a large number of biological processes related to metastasis and survival signaling, FAK is regarded as a potential target in the development of anti-cancer drugs. This poster will highlight (1) the modular design and the synthesis program in this area, (2) various solution and solid phase methods developed to date, (3) the fabrication of small molecule microchips utilizing these small molecules, and (4) the progress made to date in the discovery of small molecule chemical modulators of FAK activity.

599: A High-throughput generation of natural product-inspired compounds: The discovery of chemical modulators of cell death pathways involving Bcl-XL/Mcl-1

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Bcl-2 is the founding member of a group of proteins that includes anti-apoptosis proteins such as Bcl-2, Bcl-XL and Mcl-1 and pro-apoptosis proteins such as Bax and Bak. Although Mcl-1 is a homologous protein related to other anti-apoptotic proteins such as Bcl-2 and Bcl-XL, it has a distinctly different structure and specificity for its binding to other pro-death Bcl-2 members. Cancer cells that are highly resistant to small-molecule inhibitors of Bcl-2/Bcl-XL can become highly sensitive when Mcl-1 activity is eliminated by sRNAi, indicating the critical importance of inhibiting both Mcl-1 and Bcl-XL/Bcl-2 for successful chemotherapy. Targeting Mcl-1 is therefore an important strategy in a search of a class of anti-cancer agents based on overcoming the apoptosis resistance. With the goals of identifying small molecule modulators of protein–protein interactions, our group is developing high-throughput methods leading to library generation of natural-product inspired small molecules. In addition to testing these small molecules in solution, they are also utilized in printing small molecule microchips in collaboration with the Chemical Biology Program at the Broad Institute. Through utilizing the small molecule microchip technology, we identified a novel, natural product-inspired scaffold as a small molecule binder to Mcl-1. This study then led us to initiating collaboration with the Gehring team to carry-out low-throughput screening of several analogs as small molecule binders to Bcl-XL and Mcl-1 by NMR using the fragment-based approach. This led us to identify a novel chiral tetrahydroquinoline scaffold as a weak binder 200 UM to both Bcl-XL and Mcl-1. To our delight, by simply making only nine derivatives of this scaffold, we were able to discover the lead compound as a low uM binder to Mcl-1. Interestingly, this compound also binds to Bcl-XL but with nearly threefold less affinity.

600: Novel neurotrophic factors for the treatment of neurodegenerative diseases

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Neurotrophic factors (NTF) are small secretory proteins that promote differentiation and maintenance of neurons in developing and adult vertebrate nervous system. NTFs can also protect degenerating neurons in neurodegenerative diseases. In Parkinson's disease there is a progressive loss of functional dopaminergic neurons projecting from substantia nigra to caudate putamen. The most potent NTF, the glial cell line-derived neurotrophic factor (GDNF) delivery into the putamen of Parkinsonian patients has resulted in significant clinical improvement in some patients, but in double-blind clinical trial no symptomatic improvement compared to placebo was reported. We have identified a novel survival factor for dopaminergic neurons, the conserved dopamine neurotrophic factor (CDNF), which was strongly neuroprotective and neurorestorative in an experimental model of Parkinson's disease in rats. CDNF is a secreted protein with eight conserved cysteine residues with the unique protein fold and defining a novel, evolutionarily conserved neurotrophic factor family. Other members of this family include the mesencephalic astrocyte-derived neurotrophic factor (MANF) and invertebrate MANFs. Our results demonstrate that CDNF might be beneficial for the treatment of Parkinson's disease.

601: In-silico designing of novel triazole derivatives as substitute for novel fungicides

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Sterol 14α-demethylase is an enzyme involved in metabolism of endogenous and xenobiotic substances. The antifungal effect of azoles is due to inhibition of sterol 14α demethylase in fungi, thereby blocking the biosynthesis of ergosterol. The ergosterols provide structure, modulation of membrane fluidity, and possibly controls some physiologic events. Inhibition of this critical enzyme in the ergosterol synthesis pathway leads to the depletion of ergosterol in the cell membrane and accumulation of toxic intermediate sterols, causing increased membrane permeability and inhibition of fungal growth. Due to the prolonged use of some marketed triazoles most of the fungal infections have become resistant. This is mainly due to acquired mutations by the fungi for self defence. Therefore there is need to develop new modified fungicides which can specifically bind to the sites involving mutation. We have used in silico methods to design and screen some such novel triazole fungicides whose synthesis is feasible in laboratory. Instead of synthesizing molecules for hit and trial experiments, it is a much cost effective approach. The synthesized triazoles can be field tested for their efficacy for the resistant varieties.

602: Predicting the activity and druggability of compounds from medicinal plants through in silico approach

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In silico approaches can help in identifying better drug candidates by multiple screening methods. These bioactive components are extracted from the medicinal plants and show their extensive biological action and used in treating several diseases like diffrent type of cancers, neurotoxicity, skin diseases, diabetes and immune disorder etc. Biological activities and druggability of these chemical compounds are predicted against Lukemia by applying in silico tools. Leukemia is a cancer of the white blood cells. There are two main type of Leukemia: slower (called chronic) and faster growing (called acute). High levels of exposure to radiation are known to increase the risk of leukaemia. About 6,000 people in India and 6,755 cases in the UK are diagnosed with leukemia and blood-related cancers. The druggability of these compounds was checked by Lipinski's scoring function by calculating molecular weight (MW), LogP value, number of hydrogen bond donor (Hdon) or hydrogen bond accepter (Hacc). In the scoring function, if the predicted activity (Pa) of the lead compound is >0.7, the substance is very likely to exhibit pharmaceutical agent properties. Furthermore, targets for these bioactive compounds have been screened out from disease pathway. Important genes/enzymes, obtained in the crucial step of disease metabolism. The binding sites on the target were complementary with their ligand in term of volume, topology and physicochemical properties, only certain ligand have been bound to the target those had high binding affinity. The ligand or bioactive compound must have the desired physicochemical properties to be a better drug candidate. The result of this In silico studies have identified important lead molecules from 12 medicinal plants used for the treatment of Leukemia on the basis of their pharmaceutical and clinical properties.

603: RSA-14-44 interacts with PSMB5, a proteasome subunit, in spermatogenic cells

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RSA-14-44 is a novel gene expressed exclusively in rat testis and has previously been identified by suppressive subtractive hybridization (SSH) between cDNAs of laser capture micro-dissection(LCM) trapped rat primary spermatocytes and round spermatids. PSMB5, a catalytic subunit of proteasome, is a protein that putatively interacts with RSA-14-44 when screened by a yeast two hybrids assay of testis cDNA library. Here we confirmed the occurrence of an interaction between RSA-14-44 and PSMB5 by the GST-pull down and co-immunoprecipitation assays. Moreover, the result of an immunofluorescence experiment demonstrated that RSA-14-44 and PSMB5 showed exactly the same pattern of localization in a series of cells, exemplifying different developmental stages of spermatogenesis. We also found that RSA-14-44 is associated mainly with pro-type PSMB5, based on a gradient sedimentation analysis, suggesting that it might be involved in PSMB5 processing and that cysteine 190, a putative site for palmitoylation modification in the C-terminal of RSA-14-44, is required for mediating the interaction between RSA-14-44 and PSMB5. Hence, the present findings suggests that RSA-14-44 is involved in spermatogenesis through its interaction with PSMB5.

604: A novel PEG cross-linked PEI nanoparticle: An efficient transfection agent in vitro and in vivo

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Gene therapy is a rapidly growing field of medicine in which genes are introduced into the body to modify or replace the disease-causing genes of certain cells in order to correct a genetic defect. Genetic material such as DNA, RNA etc. have been used as molecular medicine and are delivered to specific cell types with the aim to either inhibit some undesirable gene expression or express therapeutic proteins. The basic requirement in gene therapy is the effective gene transfer followed by adequate gene expression. An ideal delivery system for nucleic acids should be a nontoxic and non-immunogenic that can protect nucleic acids during the delivery process, prevent rapid elimination from the body, and release nucleic acid intracellularly at the site of action. The serious drawbacks of the prevalent viral delivery systems are related to security problems, severe immune or inflammatory response, secondary oncogenesis and potential pathogenicity because of the possible viral recombination. Hence the development of biologically inactive non-viral methods have been encouraged for gene delivery. Low immunogenecity, the absence of endogenous recombination, low production costs, and reproducibility are benifits of nonviral vectors, furthermore, they have no size limitation in DNA packaging. Unfortunately, nonviral DNA delivery vehicles often affect cell viability and have poor transfection efficiency. In order to overcome these shortcomings, we have developed a nanoparticulate carrier system, by covalently crosslinking Polyethylenimine (PEI) with bisaldehyde-Polyethyleneglycol (bisald-PEG) and further covalently coated the nanoparticles with hydrocarbon chains with the aim of improving transfection efficiency and enhancing the cell viability. The nanoparticles were characterised spectroscopically, by AFM and DLS for size and morphology and zeta potential for surface charge. The nanoparticles were tested in various cell lines for the cytoxicity and were found to be nontoxic with enhanced transfection efficiency (several folds compared to commercial transfection agents). The proposed system was also found to deliver siRNA efficiently into cells, resulting in $\sim$90% suppression of the targetted gene. In summary bisald-PEG crosslinked PEI nanoparticles being virtually nontoxic in the optimal concencentrations, and having good transfection efficiency are promising non-viral vector for systemic in vivo gene delivery.

605: Application of small molecule screening to investigate binding specificity of protein families

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Availability of a generic screening method, which is not dependent on the activity of proteins and can rapidly identify small molecules that interact with proteins, greatly facilitates analysis of the binding

specificity of individual members of any family of proteins. We have employed various high throughput small molecule screening methods to investigate the binding specificity of different members of families of human proteins such as kinases and sulfotransferases. These methods include differential static light scattering (DSLS), a label free method, and differential scanning fluorimetry (DSF), a fluorescence based method. Creating chemical fingerprints for each protein provides the opportunity to compare the small molecule binding specificity of different members of each family and enables us to identify potential inhibitors that bind to only a subset of proteins and discriminate between different members. Using DSLS we have also screened three membrane proteins: ABC transporter MsbA, Mg++ channel CorA and histidine kinase CpxA, and detected the stabilizing effect of some ligands. We identified ligands, including ATP and its analogues, for MsbA by screening it against a library of 38 selected compounds and also detected the binding of Mg++ and ADP to CorA and CpxA respectively. Combination of these available techniques provide an opportunity to investigate protein-ligand interaction genome-wide.

606: SPAG8, a testis specific protein, decreased the cell proliferation and prolongated G2/M phase in cell cycle

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SPAG8 is a testis-specific protein, the expression of which is highly related to the progression of spermatogenesis. The distribution of SPAG8 shows a phase-specific pattern in cell cycle, which could be regulated by the interaction between SPAG8 and α-tubulin, a component of microtubule. CHO-K1 cells expressing GFP-SPAG8 and those expressing GFP were synchronized at early S phase by the double thymidine block. After the release from the block, the tested cells showed 16.9% in G2/M; in contrast to control cells having 4.1% in G2/M phase while the remainder of 75.4% had progressed into G1 phase. The result of the flow cytometry assay showed that a G2/M phase delay occurred in CHO-K1 cells stably expressing GFP-SPAG8, during which the phosphorylation level of Tyr15 on Cdc2 was increased significantly compared to that of CHO-K1 cells stably expressing GFP. Furthermore, the results of MTT assay suggested the cell proliferation of CHO-K1 cells stably expressing GFP-SPAG8 was also retarded. Our findings demonstrate that SPAG8 might play an important role in spermatogenesis through regulating the cell cycle of spermatogenic cells based on its interaction with microtubules.

607: GADD153, a critical nodal point protein in selenite-induced apoptosis of NB4 cells

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Selenium, an essential trace element possessing anti-carcinogenic properties, can induce apoptosis in various cancer cells. Our previous studies have discovered that 20 μM sodium selenite induced apoptosis of human acute promyelocytic leukemia NB4 cells, and mitochondrial apoptotic pathway played a significant role in this process. Based on those findings, the present study have found that endoplasmic reticulum (ER) stress apoptotic pathway was also involved in apoptosis of NB4 cells induced by sodium selenite. In the early stage, sodium selenite induced activation of unfolded protein response (UPR), which involves three pathways, PERK-eIF2α-ATF4, IRE1-XBP1S and ATF6, thus initiated protective mechanisms against ER stress to promote cell survival. However, as the stress level was aggravated until too severe to be repaired, the apoptotic signals will be triggered. GADD153 is a key pro-apoptosis transcription factor that is closely related with ER stress. Selenite induced expression of GADD153, while its specific siRNA remarkably inhibited cell apoptosis induced by selenite, suggesting a critical role of GADD153 in selenite-induced apoptosis of NB4 cells. Our further study found that selenite suppressed activation of anti-apoptotic kinase AKT, while silencing of the expression of the GADD153 gene, DDIT3, by siRNA prevented selenited-induced inactivation of AKT. Since activated AKT can induce phosphorylation of Bad, leading to inhibition of mitochondrial apoptotic pathway, these data suggest that as a nodal point protein, GADD153 can transmit signals from ER to mitochondria, linking ER stress apoptotic pathway and mitochondrial apoptotic pathway together.