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ABSTRACTS

# Chemical genomics and molecular medicine



## 088: Novel paradigm in lipid metabolic network of *Mycobacterium tuberculosis*

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*Mycobacterium tuberculosis* (Mtb) is the etiologic agent of tuberculosis and infects ~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.

## 089: 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 anticancer 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 μM 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.

## 090: 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.

## 091: 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.

## 092: 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 anti-apoptotic 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.

## 093: 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 overexpression could increase chemosensitivity of cancer cells to chemotherapeutic 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.

## 094: 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.