SYSTEMS BIOLOGY CENTER NEW YORK

POSTER SESSION

1425 MADISON AVE, NEW YORK, NY 10029 MOUNT SINAI SCHOOL OF MEDICINE

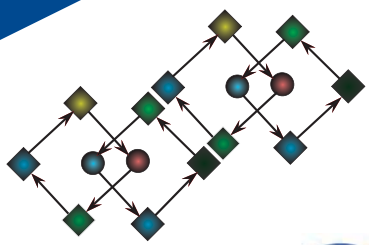
DATE: WEDNESDAY AUGUST 8 2012

LOCATION: ICAHN BUILDING 12th Floor (Outside of room 12-59A)

TIME: 1:00PM - 2:00PM

2012 SUMMER RESEARCH PROGRAM IN SYSTEMS BIOLOGY

MOUNT SINAI SCHOOL OF MEDICINE



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Regulation of cyclic guanosine monophosphate levels by calcium, nitric oxide, and calmodulin via cGMP-specific phosphodiesterases

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Cyclic nucleotides such as cAMP and cGMP are second messengers that regulate many cellular and physiological processes such as: apoptosis, ion channel conductance, and vasodilation. The production of cGMP starts with activation of neuronal nitric oxide synthase (nNOS) by Ca++-Calmodulin (Ca-CAM), allowing the production of nitric oxide (NO). NO can then bind and activate soluble guanylate cyclase (sGC). Upon activation, sGC is able to catalyze the synthesis of cGMP from GTP. Cyclic nucleotide phosphodiesterases (PDEs) are major regulators of cAMP and cGMP. There are 11 families of PDEs, each consisting of several genes and expressing multiple isoforms. In malignant gliomas, upregulation of cAMP leads to an anti-proliferative state, suggesting that inhibitors of cAMP/cGMP phosphodiesterases could be used as anti-cancer therapies. In this study we concentrated on cGMP-specific PDEs, namely PDE1, PDE5, PDE9. Although PDE2 is not cGMP specific in terms of hydrolysis, it binds cGMP with a high affinity, thereby regulating its activity and therefore it was included in our model. PDE1 has 3 isoforms, PDE1A1, PDE1A2, and PDE1B1, all of which bind Ca^{2+}-CaM and become activated as a result. cAMP-dependant protein kinase (PKA) phosphorylates PDE1A, which results in a reduced affinity for the binding of Ca^{2+}-CaM by ~50%. PDE1B is phosphorylated by Ca^{2+}-Calmodulin dependant protein kinase II (CaMKII), which itself must bind Ca^{2+}-CaM and autophosphorylate in order to be activated. Calcineurin (CaN), a calcium dependant protein phosphatase, dephosphorylates all PDE1s and is activated by the binding of Ca^{2+}-CaM. PDE2 and PDE5, when bound to cGMP, show greater catalytic activity, and PDE5 can be phosphorylated either by PKA or cGMP-dependant protein kinase (PKG) but require cGMP to be bound prior to phosphorylation. We created a mathematical model in order to better understand the complexity underlying cGMP signaling. Model predictions were compared to experimental data. Formation of cGMP in U87 gioblastoma cells was examined by using a cGMP sensor, after treatment with PDE inhibitors.

A Computational Model and Sensitivity Analysis of the VEGFR-2 Signaling Pathway

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Vascular endothelial growth factor receptors (VEGFRs) are expressed in endothelial and tumor cells and via autocrine and paracrine activation by VEGF-A ligand binding, the receptors play an essential role in tumor angiogenesis and invasiveness. Chemotherapeutic approaches to interfere with VEGFR activity include small molecule tyrosine kinase inhibitors that have been successful, at least initially, to block angiogenesis in different tumors types including malignant gliomas. To better understand the cell signaling effects of anti-angiogenic drugs, such as the multi-kinase inhibitor sunitinib, and potentially what changes occur during the evolution of drug resistance, an enhanced pharmacodynamic [ePD] model was constructed. ePD models consist of a set of ordinary differential equations (ODEs) that characterize the biochemical network involved in drug action and can account for underlying genetic alterations that influence drug activity, and thus offer a means to improve therapy in individual patients. In this study, the ePD model derived from the law of mass action and Michaelis-Menten kinetics for enzyme phosphorylation and dephosphorylation reactions included the VEGF-A:VEGFR2 reaction complex and various downstream components. The rate constants and initial cellular molecule concentrations were obtained from the literature. Simulations were run to obtain concentration-time profiles of the VEGF-A -VEGFR2P complex, PI3KP and PIP3-AktP which are often probed as targets in cancer therapy and could be instrumental in determining drug activity. Dose response curves were generated to quantify the relationship between ligand stimulus and steady state VEGF-A -VEGFR2P, PI3KP and PIP3-AktP concentration. Monte Carlo simulations and partial least squares (PLS) regression analyses were used to perform a sensitivity analysis. The results showed that a VEGF-A ligand concentration of 16 nM induced a 50% response of PI3KP and PIP3-AktP molecule, while only a 5% response of VEGF-A -VEGFR2P was elicited. The sensitivity analysis also indicated which parameters most affected each different molecule's output after reaching steady-state. This model provides an initial foundation that will be expanded to account for sunitinib's other targets [PDGFβR,cKit] and associated pathways, and will be supplemented with direct measurements of key cellular proteins in glioma models.

The effects of genomic differences in cancer on signaling within the EGFR pathway

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Cancer is a complex disease with multiple genomic changes which leads to aggressive cell growth and proliferation. To treat cancer effectively in every patient, the different genomic changes which drive tumor growth must be identified and targeted by personalized drug therapies. The goal of this project is to demonstrate on a small scale how genomic changes can affect signaling pathways and how genomically distinct cancers may respond to differently targeted therapies. Two cancer cell lines were chosen based on their mutational status: MDA-MB-231 breast cancer has mutant KRAS and glioblastoma U87 has a PTEN deletion. The first set of experiments was designed to observe differences in epidermal growth factor receptor (EGFR) signaling across these cell lines using Western Blot. The phosphorylation status of EGFR, Erk and Akt induced by EGF stimulation over varying amounts of time was analyzed. The results showed that, in response to EGF, Erk activity was sustained up to 60 minutes in MDA-MB-231 breast cancer cells, as expected due to the presence of the KRAS mutation. On the other hand, Akt activity was sustained up to 60 minutes in glioblastoma U87 cells, as expected due to the absence of PTEN, a negative regulator of AKT signaling. EGFR activity, for both the U87 and MDA-MB-231 cell lines, peaked at 15 minutes and returned to baseline by 60 minutes. It was observed that the Erk signal was more sustained in the MDA-MB-231 cells than in the Akt signal in U87 cells. These results indicate that constitutive KRAS activity may have a more pronounced effect on signaling compared to deletion of the PTEN-mediated negative feedback loop regulating the Akt pathway. The second set of experiments tested the effects of a Mek inhibitor (AZD-6244) and an Akt inhibitor (Akt inhibitor X) on the viability the two cancer cell lines. Cells were treated with each inhibitor at increasing concentrations (from 0.39 to 100 μM) and the cell viability was assessed at the end of 24 hours. The Akt inhibitor had an approximate IC_{50} value of 25 μM for both cell lines. The Mek inhibitor decreased cell viability in MDA-MB-231 cells by approximately 30%, at concentrations above 25 μM and had no observable effect on U87 cell viability. In summary, the Akt inhibitor had a similar in both cell lines, despite the genomic differences, and the Mek inhibitor had a partial effect in the KRAS mutant line, but not in the KRAS WT/PTEN null line. In conclusion, the results suggest that genomic changes, mutations in this instance, cause distinct differences in cellular signaling and that targeted inhibition likely depends on multiple factors which are not well ascertained by analyzing only a small part of the entire signaling pathway.

SHP2: More than a double-edged sword?

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Neurite outgrowth in Neuro2A cells is regulated by a signaling network downstream of Cannabinoid 1 receptor (CB1R) and Interleukin 6 receptor (IL-6R). STAT3 and MAPK are activated by these receptors, and play a major role in regulating neurite outgrowth. It has been previously shown that SHP2 inhibits the activity of STAT3 and promotes MAPK activity. Thus, knocking down SHP2 with siRNA enhances CB1R and IL-6R-mediated neurite outgrowth. MAPK becomes phosphorylated within the first hour of stimulating the receptors, whereas STAT3 shows a prolonged wave of phosphorylation which lasts up to 6 hours. In this experiment we tested if the effect of SHP2 siRNA could be mimicked by using a pharmacological SHP2 inhibitor (NSC-87877). We decided to add NSC-87877 at two hours after initial receptor stimulation to allow for the early wave of MAPK phosphorylation to occur. We hypothesized that delayed addition of NSC-87877 could further enhance the late wave of STAT3 phosphorylation. Surprisingly, delayed application of NSC-87877 resulted in the opposite outcome. We observed increased phosphorylation of MAPK and decreased phosphorylation of STAT3 after the addition of NSC-87877. These results could possibly be explained by additional feedback loops in the signaling network. Here we are proposing two potential mechanisms that require further investigation.

Early Markers in Mechanosensitive Signaling

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Cells have long been known to respond to external stressors by enacting a series of defensive mechanisms, by up- or down-regulating the necessary proteins to adapt to the particular form of strain they are subjected to. The same pattern of allocating resources to adapt to external pressures has been observed in the differentiation of adult stem cells upon growth on a variety of substrates with varying elastic moduli (Engler et al., 2006), and the mechanism by which this is done has been attributed to the "mechanosensing" of the cell of the matrix it is situated on.

There have been many studies examining just how the cell is able to respond to external forces, and a variety of signaling pathways have been decoded for how substrate stiffness correlates to myosin and actin activity and function, and thus, by extension, overall activity and differentiation of the cell. Just recently, however, has the discussion turned from observing cells when they are sitting stagnant on a plate and adapting to their environment, to observing cells while they are being physically stretched in real time. These new studies in mechanosensation have been instrumental in identifying signaling pathways that translate mechanical stimuli to cellular responses. Of particular interest is the role of the focal adhesion complex, as it is the facet of a cell presumed to be directly responsible for this signal transduction. The current model presumes that upon application of a physical force, focal adhesion kinase (FAK) is stimulated (Wang et al., 2001), which in turn activates a variety of other proteins that will eventually regulate cell proliferation and motility, i.e. MAPK and Src proteins.

Although signaling networks for this proliferation of signals have been deduced, the importance and effect that each individual component has on the overall network has not been extensively studied. In this project, we focused on the regulation of Rho GTPases; using a custom designed indentation stretch bioreactor, the activity of early markers in the network that regulate Rho GTPases were probed. Serum starved human dermal fibroblasts were subjected to 8% equibiaxial stretch at 1 Hz for 45 minutes. Cells were lysed at 0, 5, 10, 15, 30 and 45 minutes and activity of early signaling markers were measured with Western blotting. Accordingly, significant increases in Src, Csk and FAK activity were observed as well a rapid and transient activation of MAPK upon equibiaxial stretch.

In the future, we will use similar stretch experiments to focus on several other aspects of cellular mechanoresponse: effect of stretch on cell morphology, activity of phospho-Myosin (a direct indicator of cellular contractility), effect of external inhibitors on upstream signaling elements (i.e. inhibitors of kinases/phosphatases), and spatiotemproral distribution of focal adhesion proteins. These data will allow us to develop a more detailed model of early mechanotransduction and mechanoresponse.

Interactions between phosphodiesterases 3 and 4 affect cAMP production

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Phosphodiesterases (PDEs) degrade cyclic adenosine monophosphate (cAMP) and cyclic guanine monophosphate (cGMP) through hydrolysis of the 3'-5' phosphodiester bond, and thus control intracellular concentrations and shape downstream signaling. There are 11 families of PDEs and each family consists of several genes. Here we focus on the cAMP-specific PDE4 family and dual-specific PDE3 family. Within the PDE4 family, there are four genes, and the PDE3 family consists of two genes, and through the use of alternative splices multiple isoforms are expressed differing in size. The activity of long isoforms of PDE4 are upregulated by protein kinase A (PKA) and downregulated by mitogen-activated protein kinase (MAPK) whereas short forms' activity are upregulated by MAPK and have no interaction with PKA. PDE3, also known as cGMP inhibited phosphodiesterase, has a higher affinity for cGMP but a Vmax that is a tenth of cAMP. The activity of PDE3 is upregulated by both PKA and protein kinase B (PKB). In malignant gliomas, upregulation of cAMP leads to an anti-proliferative state, suggesting that inhibitors of cAMP/cGMP phosphodiesterases could be used as anti-cancer therapies. While the effects of individual PDE types have been studied, the combined contribution of multiple PDEs on cAMP production has not been explored. Given the complexity of PDE regulation, a computational approach was used. Here we modeled the PDE3 and 4 networks using Virtual Cell. Theoretical data was then confirmed with experimental data. The cAMP FRET probe ICUE3 was used to examine the effects of PDE4 inhibitor rolipram and PDE3 inhibitor trequisin in U87 glioblastoma cancer cells stimulated with forskolin.

Understanding Parameters of Ventricular Myocyte Models Utilizing Markov Pathways

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Markov models are used to describe systems whose future state depends exclusively on the state it currently occupies. Several models of the cardiac action potential (AP) have been developed using voltage-clamp data for individual ion channels, which implement Markov pathways to describe their molecular structure and function (2). However, given the intricate relatedness of these pathways, it becomes difficult to isolate the effects of individual parameters, and few studies aimed at identifying the physical analogues at the cellular level of each rate parameter have been performed. Since the differences in transition rates cause discrepancies in cellular phenotypes, understanding the meaning of these rates is crucial in analyzing further diseased-state models that utilize this type of pathway. This project is aimed at developing novel computational analyses of Markov models, and subsequently determine how changes in transition rates at the molecular level scale up to influence changes in the cellular level.

In order to accomplish this, several models of individual ion channels applying standard voltage-clamp protocol have been implemented; these include Na+ (2), various K+ (4), and different L-type Ca2+ (2,3) ion channels. Parameters from each model were randomized and collected. Repeated trials were simulated in which specific features of whole-cell ionic current, such as peak current and time constant of depolarization decay were then collected as well. Subsequent multivariable partial least squared regression was then performed, and the results were validated. Once we determined that our initial results were valid, the data was then analyzed qualitatively with respect to each output collected for all models. Additionally, reverse regression analysis (5) was performed, which allows us to simplify a complex Markov pathway by identifying the parameters with high inverse coefficients. Through this analysis we were able to identify specific parameters that have counterintuitive effects on relevant channel physiological properties; this in particular emphasizes the necessity of our approach in determining parameter effectiveness. Furthermore, we were able to use reverse regression analysis to distinguish between high-impact parameters, and those that have little role in each pathway. This approach gives us a novel method to characterize the quantitative aspects of Markov chains. Our results provide a better method for understanding the propagation of change from the molecular level of single-ion channels to the cellular level AP.

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Network Analysis of Retinal Degenerative Disease Genes

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Retinal Degeneration (RD) is a diverse group of diseases defined by both clinical manifestations and etiology. Subcategories of RD have evolved through the study of their subtle differences in phenotypic characteristics and genetics. RD affects approximately 1 in 4,000 and is a leading cause of blindness worldwide. We focused on Retinitis Pigmentosa (RP), a heterogeneous subgroup of RD involving all modes of inheritance. We compiled lists of known RD and RP disease genes from literature and online databases to assess their overlap. Then, we utilized the seed lists of RP and RD gene lists to build functional gene-gene and protein-protein interaction subnetworks to elucidate clusters of interactions that connect these disease genes within functional modules. In addition, we extracted differentially expressed genes from two relevant studies in order to construct additional subnetworks that were compared to the subnetworks created from the seed RD and RP disease gene lists. Lastly, whole-exome sequencing (WES) data was obtained from one RP patient whose causative gene is unknown. Using the collective assembly of prior background knowledge about RP gene-lists, subnetworks and pathways, we prioritized potential mutations from the patient. Hence, network analysis was used to assess newly identified mutations by studying their relationship to known RP disease genes, whereby the most highly ranked novel mutations are predicted as the best candidates to be further validated in cells and mouse models. In conclusion, the RD and RP disease gene-lists and subnetworks can be used to help RP and RD research by providing a consolidated web-based resource to study these diseases and prioritize newly identified mutations discovered by deep sequencing.

Soluble IL-6 Receptor: The Missing Link in STAT3 Signaling

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Interleukin-6 (IL-6) cytokine-mediated Jak/STAT3 signaling pathway has been long implicated in promoting neurite outgrowth in neuronal cell lines. While the original studies in PC12 cells showed that IL-6 can promote neurite outgrowth on its own, other groups were unable to reproduce these results, and suggested that additional factors such as nerve growth factor may be required. These variable results were later explained by uneven expression patterns of IL-6 receptor subunits on the cell surface. The IL-6 receptor consists of one IL-6R subunit, which binds the IL-6 ligand; and two gp130 subunits, which transduce the signal into the cell. The IL-6R subunit can be expressed in both cell-bound and secreted form, and has been implicated to be the limiting factor of IL-6-mediated signaling. Interestingly, the soluble form of IL-6 (sIL-6R) enhances the effect of IL-6 stimulation, by binding IL-6 and bringing it to the membrane bound gp130 subunits, allowing them to transduce the signal into the cell. The Iyengar laboratory has previously shown that stimulation of Neuro2A with low concentrations of IL-6 results in low levels of STAT3 phosphorylation, and minimal neurite outgrowth. Therefore, we hypothesized that adding sIL-6R along with IL-6 may enhance STAT3 phosphorylation and neurite outgrowth in Neuro2A cells. Our results show that the addition of sIL-6R to IL-6 in Neuro2A cell line substantially increases the effect that IL-6 alone has on downstream signaling. Western blot analysis confirms that even a low concentration of sIL-6R in combination with IL-6 will induce phosphorylation of STAT3 greater than IL-6 alone. We are planning to further investigate a potential role of sIL-6R in neurite outgrowth induced by IL-6R/ cannabinoid receptor 1 (CB1R) - mediated signaling network. Further research may be done in combination with CB1R to see the effect sIL-6R will have when combined with IL-6R and HU210 (a CB1R agonist).