Multiscale modeling of the tumor microenvironment in vascularized tissue

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The cellular traits that demarcate cancer cells from healthy cells were succinctly outlined in the seminal "Hallmarks of Cancer" reviews by Hannahan and Weinberg. The specific manner in which these traits influence tumor behavior is strongly influenced by variations occurring *in vivo*. Spatial and temporal variations in metabolites, growth/survival factors, and cell genotypes result in a tumor microenvironment with conditions vastly different from *in vitro* conditions used in many experiments. Multiscale *in silico* models have been developed and utilized to help bridge the gap between these differing conditions. Here, we utilize a hybrid cellular automaton (HCA) model to study tumor progression in vascularized tissue. Our HCA model mathematically describes the diffusion of oxygen from vascular tissue in a field of cells, which are treated using an agent-based model. Each cell exhibits phenotypic responses to varying oxygen concentrations that correlate to its status as a healthy or malignant cell. Responses are cell-specific and include the potential for proliferation, quiescence, cell death, or movement. The cellular responses of malignant cells serve to mimic specific cancer hallmarks including sustained proliferative growth signaling, evasion of growth suppressors, resisting cell death, invasion of surrounding tissue, and reprogramming energy metabolism. By varying the strength of different cellular traits, we observe the emergence of tumor fingering morphologies and necrotic cores. These morphological traits are observed in more aggressive cancers and have not been observed in other vascularized HCA models. By extending our model, we hope to test how metabolic and cellular factors influence tumor evolution towards more aggressive phenotypes.

Rapid screening of potential carcinogenic aryl hydrocarbon receptor (AhR) agonists using an autobioluminescent yeast assay

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The aryl hydrocarbon receptor (AhR) and AhR nuclear translocator (ARNT) complex is a crucial signaling component that regulates the expression of a diverse set of genes involved in the response to exposure to xenobiotics. Perturbations of the AhR/ARNT signaling pathway have been linked to a variety of adverse health effects, including many types of cancer, deficiencies in reproduction and development, disruption of the endocrine system, neurotoxicity, immunotoxicity, and metabolic diseases. Here we report the development of a high-throughput autobioluminescent yeast assay for rapid, cost-effective, and quantitative detection of potential carcinogenic AhR agonists. We have engineered an autobioluminescent *Saccharomyces cerevisiae* reporter strain expressing AhR, ARNT, and an autobioluminescent *lux* reporter cassette under the control of xenobiotic response elements (XREs). This bioreporter is capable of self-initiating its autobioluminescent signal output upon exposure to AhR agonists without cellular lysis or exogenous addition of a light-activating agent, thus allowing for continuous, real-time monitoring of test compounds throughout the full exposure period. We have validated this bioreporter against the potent AhR ligand 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in a high-throughput 96-well plate assay format, showing a sigmoidal dose-response relationship with a half maximal effective concentration (EC_{50}) of approximately 1.1×10^{-9} M within 6 hours of exposure. These results demonstrate that the autobioluminescent yeast assay provides a rapid and cost-effective means for toxicological profiling of potential carcinogenic AhR agonists in a high-throughput fashion.

A method for identifying patients with multiple myeloma or monoclonal gammopathy of unknown significance who are at risk of developing light chain amyloidosis

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Monoclonal gammopathies are characterized by the presence of both a clonal plasma cell population in the bone marrow and intact monoclonal immunoglobulin and/or free light chain (LC) proteins in the serum. In the US, the prevalence of monoclonal gammopathy of undetermined significance (MGUS), a pre-malignant state, is ~4.2% in Caucasians over the age of 50, with 20% of those secreting only monoclonal LC (LCMGUS; ~0.6 million Americans are affected). A small percentage of people with LCMGUS will develop multiple myeloma (MM), and both conditions can lead to LC amyloid deposition—a devastating protein misfolding pathology characterized by the presence of toxic extracellular amyloid fibrils. Amyloid, in these patients, is systemic and can accumulate in any organ or tissue which ultimately leads to dysfunction and death.

Ongoing clinical trials of anti-amyloid immunotherapeutics have yielded promising results; thus, accurate identification of those at risk for amyloidosis and early intervention may enhance patient survival. The ability to identify patients with MM or LCMGUS that have a propensity to develop amyloidosis before becoming symptomatic is limited.

We have, therefore, developed an assay to identify this patient population based on the inherent amyloidogenic potential of the patient's LC protein. Using an amyloid fibril recruitment assay, we evaluated patient urinary LC and found that amyloidogenic LC proteins were recruited by fibrils significantly more than LC from MM patients. Notably, one LC from a patient originally diagnosed with MM who later developed amyloidosis, was recruited by fibrils similarly to an amyloid patient-derived LC.

These data have led to the development of a pilot assay to determine the amyloidogenic potential of LC in serum as a method for discerning those patients with MM or MGUS/LCMGUS who are at risk of developing amyloidosis.

Phosphatidylserine, a lipid present at the outer membrane leaflet of cancer cells, hinders the insertion of pHLIP, a potential cancer cell marker

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The pH-Low Insertion Peptide (pHLIP) has shown the ability to insert into membranes when acidic conditions are present. pHLIP can be found in three different states: a random coil in pH 8 solution (State I), a surface bound form when lipids are present (State II), and a transmembrane helix in acidic conditions (State III). From this, pHLIP has been shown to effectively target diseases in which the extracellular pH of the tissue has become acidic. Cancer is an example of such a disease, and it has been shown that pHLIP can target cancerous cells. One concern with this targeting is that some cancerous cells are known to display phosphatidylserine (PS) in the outer leaflet of their plasma membranes, as opposed to healthy cells. However, pHLIP's tumor targeting ability is unknown in the presence of PS. Here, we study how pHLIP

properties are influenced by the presence of PS by using phosphatidylcholine (PC) large unilamellar vesicles (LUV's) that contain PS. We found that in the presence of LUV's containing PS, the insertion pK_a decreases compared to LUV's only containing PC with a midpoint at 1.22 $\pm$ 0.6 percent PS. This decrease in insertion p*K*a might limit pHLIP's targeting of mildly acidic tumors. To further understand the mechanism for the decrease in pK_a, we added sodium chloride to screen possible electrostatic interactions between pHLIP and PS. We observed that as the sodium chloride concentration is increased, the insertion pK_a in the presence of PS returns to values comparable to PC.

Detection of Cox-2-expressing early stages of bladder cancer by fluorocoxib A

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Conventional optical imaging technologies can detect advanced stages of bladder cancer, however they have limitations to detect bladder cancer at the early stages. Fluorocoxib A is a rhodamine-conjugated analog of indomethacin that selectively targets cyclooxygenase-2 (COX-2)-expressing cancers. In this study, we have used a well-established carcinogen (*N*-butyl-*N*-4-hydroxybutyl nitrosamine, BBN) -induced bladder cancer model that strongly resembles human low-grade papillary and high-grade invasive urothelial neoplasia.

The aim of this study was to evaluate a novel optical imaging agent, fluorocoxib A, for detection of the early stage of carcinogen-induced bladder cancer in mice. Specific fluorocoxib A uptake by Cox-2-expressing bladder tumor was detected using IVIS optical imaging system. After imaging, the histopathology of the bladder tissue was assessed using H&E and immunohistochemistry staining for detection of Ki67, uroplakin-1A, and Cox-2 expressions.

The specific uptake of fluorocoxib A in BBN-induced bladder cancers correlated with the progression of bladder carcinogenesis and with increased Cox-2 expression. Progression of bladder carcinogenesis from normal urothelium to hyperplasia, adenoma, and carcinoma over time was confirmed by H&E staining and by increased Ki67 and decreased uroplakin-1A expression in BBN-induced bladder cancer over time.

In conclusion, fluorocoxib A detected the early stages of bladder cancer in carcinogen-induced mouse model. Fluorocoxib A specifically detected Cox-2-expressing bladder cancer in contrast to normal bladder urothelium where no uptake was detected. This cancer mouse model offers a translational application for evaluation of novel imaging agents to detect the early stages of bladder cancer *in vivo*.

Mutational analysis of the novel acidity-triggered rational membrane (ATRAM) peptide

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Targeted therapies have been developed to efficiently deliver drugs to tumor sites, however most delivery platforms are limited by low solubility, efficacy, or safety. The ideal tumor targeting molecule would be highly soluble, stable in serum, and specific for a property of tumors that cannot be easily selected against. We recently developed the acidity triggered rational membrane peptide (ATRAM) to target the acidic extracellular environment of tumors with high specificity[1]. ATRAM exists as a highly soluble species that interacts with the membrane interface at high and neutral pH, but inserts at acidic pH as a transmembrane α-helix into the membranes of cancer cells (and liposomes). In the present study, by altering the sequence of ATRAM we have modified acidity-targeting properties such as the Hill coefficient and the pH midpoint of insertion (p*K*). By adding positively charged residues at the N-terminus, we decreased the p*K* in phosphatidylcholine vesicles. Conversely, interaction with phosphatidylserine (PS), a negatively charged lipid that is selectively present at the outer membrane of cancerous cells, increases the p*K*. This could provide specificity towards diseased cells decorated with PS. Here we have studied the oligomerization of the soluble, membrane-bound, and membrane-inserted states of one of the ATRAM variants. ATRAM peptides do not cause membrane leakage; however, our data suggests that unlike similar peptides, oligomerization occurs in the membrane. Implications of these sequence-specific effects are discussed. By rationally adjusting the sequence of ATRAM we aim at limiting off-target insertion events and increasing efficacy. Furthermore, our work provides insight into sequence-specific properties of transmembrane domains.

1. Nguyen, V. P.; Alves, D. S.; Scott, H. L.; Davis, F. L.; Barrera, F. N., A Novel Soluble Peptide with pH-Responsive Membrane Insertion. *Biochemistry* **2015,** *54* (43), 6567-75.

Liposomal bupivacaine and pre-operative acetaminophen; Useful in minimally invasive surgery too?

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Objective: To determine if pre-operative intravenous acetaminophen (IA) and local infiltration of liposomal bupivacaine (LB) at the time of port site closure significantly improves postoperative pain control and reduces narcotic requirements following robotic hysterectomy and surgical staging for uterine malignancy.

Methods: A retrospective analysis was performed including all patients undergoing robotic hysterectomy and staging for uterine malignancy at a single institution from 2012 to 2016 (N = 243). This cohort was divided into patients receiving 1g pre-operative IA and local infiltration of LB (intervention; N = 111) and patients receiving no adjunctive intervention (control; N = 104). Demographic data including age, body mass index (BMI), diagnosis of chronic pain syndromes, and preoperative narcotic utilization were compared between cohorts. Primary outcomes included patient-reported pain scores and inpatient narcotic

utilization. The secondary outcome was the amount of narcotic prescriptions filled within 30 days of surgery.

Results: In the intervention cohort, no significant reductions in patient-reported pain scores were observed in the inpatient setting compared to controls. Despite this, narcotic utilization was significantly reduced in both PACU ($P = 0.012$) and inpatient ($P = 0.014$) settings. Patients in the intervention cohort also filled significantly less narcotic prescriptions post-operatively ($P = 0.009$). No significant differences in age, BMI, diagnosis of chronic pain syndromes, smoking status, or amount of narcotics filled in the year prior to surgery were found between cohorts.

Conclusions: While addition of local LB and pre-operative IA did not reduce patient- reported pain scores in the inpatient setting, it did significantly reduce narcotic consumption in both PACU and inpatient settings as well as post-discharge narcotic requirements in patients undergoing robotic hysterectomy and surgical staging for uterine malignancy.

Anticancer properties of resin isolated from *Sciadopitys verticillata*

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The white, viscous latex-like resin of the evergreen conifer *Sciadopitys verticillata* (Japanese Umbrella Pine) is a complex mixture of solids, liquids, and volatile gases. Terpenes (α-pinene, β-pinene, β-cubebene, and tricyclene) account for more than 95% of volatiles in the resin. Communol and communic acid derivatives are primary components, but most of the components in the resin are unknown. In bioassays, the resin had an antibiotic effect on some bacteria and a probiotic effect on others. The goal of this preliminary research was to determine if the resin was active against cancer cell lines. In the first trials, U937 lymphoma cells were treated with autoclaved *S. verticillata* resin at 1:20 and 1:200 [resin: cell suspension (v/v)] concentrations for 24 and 48 h. Cell densities were reduced by approximately 50% (compared to untreated cells) after treatment with the high concentration and approximately 22-33% after treatment with the low concentration. Cell size of resin-treated U937 cells was approximately 60% of untreated cells (144 cells measured). In separate experiments, MDA-MB-231 and MCF7 breast cancer cells were treated with autoclaved resin diluted at various rates in DMSO. Cell populations were measured after 72 h. The MDA-MB-231 line was more sensitive to resin treatments than the MCF7 line. The former reached maximum inhibition at 0.5% resin, whereas the 1% dose was required for maximum inhibition of MCF7. Further research is needed to determine if this resin has true potential as a source for novel compounds that can be used to inhibit cancer cells.

MonsterPlex: A novel low cost targeted-sequencing technology to screen genetic variation and gene expression from any type of cancer

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MonsterPlex is a PCR-based targeted-sequencing technology developed at the University of Tennessee, Knoxville. The technology is easily customized to target any region of a genome and provides significant cost reduction when genotyping or genetically profiling hundreds or thousands of individual samples for 100's of genetic targets. Target regions are usually 60 to 100bp and may contain complex (e.g. clustered) SNPs or INDELS. The technology is organism independent and requires a small amount of DNA (or cDNA) per assay (e.g. 20ng total DNA, 5ng total cDNA per sample). A recent test on archival (FFPE) tumor tissue to measure gene expression for 74 cancer-related genes proved highly robust. MonsterPlex is currently being used to genotype a wide range of organisms and performs well with highly complex mixtures of template DNA. Development of novel assays for humans can be accomplished rapidly (1 week). The cost to design a novel multiplex assay is low ($15/target) and provides sufficient primers for at least 100K assays. The cost to amplify individual samples is also low ($3 to $10), with the option to sequence on any available NGS device. All work with non-profit entities (e.g. academics) is conducted at cost. We are seeking opportunities to rigorously test the technology on different disease systems, especially towards development of very low cost cancer screening and genetic profiling.

Supplemental selenium may decrease ovarian cancer risk in African-American Women

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No previous study has evaluated the associations of antioxidant intake with the risk of ovarian cancer in African-American (AA) women, who are known to have high mortality from the disease. Therefore, we sought to evaluate these associations among 406 ovarian cancer cases and 632 age- and site-matched controls of AA descent recruited from the African American Cancer Epidemiology Study, a population-based case-control study in 11 geographical areas in the US. Multivariable logistic regression models were

used to estimate odds ratios (OR) and 95% confidence intervals (CI) adjusted for a wide range of potentially confounding factors, including age, region, education, parity, OC use, menopause, tubal ligation, family history, BMI, smoking status, total energy, and physical activity. Women with the highest intakes of supplemental selenium (> 20 μg/d) had approximately 30% lower risk of ovarian cancer than those with no supplemental intake (OR=0.67, 95% CI=0.46-0.97; p-trend=0.035). This inverse association was stronger in current smokers (OR=0.13, 95% CI=0.04-0.46; p-trend=0.001). There was no association with dietary selenium. Weak indications of inverse associations with intake of total carotenoids were not statistically significant (p=0.07). We observed no association with dietary or supplemental intake of vitamin C or vitamin E. There were no appreciable differences in results between serous and non-serous tumors. These findings provide the first insights into the potential association between antioxidants and ovarian cancer in AA women, indicating potential inverse associations with supplemental selenium.

3D genome organization of metastatic cancer cell migration

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Most cancer deaths are caused by metastasis, the ability of cancer cells to spread from the primary tumor site into surrounding tissue. In order to metastasize, cancer cells must squeeze through tight junctions which requires the deformation of the nucleus. Consequently, the chromosomes inside of the nucleus have to accommodate deformations that happen during migration. The three-dimensional organization of the chromosomes in the nucleus into loops, domains, and territories is essential in maintaining successful cellular processes like gene expression, DNA replication and DNA repair. Little is known about the influence and role of the 3D organization of cancer cells in metastasis. To study the organization of cancer genome, we are using Chromatin Conformation Capture (HiC) and live-cell imaging techniques to identify drastic changes that happen during migration. Using a photoconvertible fluorophore, Dendra2-H4, we can draw patterns on migrating cell nuclei and monitor the changes during migration. Our preliminary data demonstrate changes in these patterns, indicating spatial reorganization of the genome during cell metastasis.

Additionally, previous work has suggested that altering the epigenetic state of the chromatin inhibits cancer cell migration. We are using histone deacetylase (HDAC) inhibitors, which are epigenetically active chemotherapeutics, to study their effect on genome structure and cell migration. By utilizing wound healing assays and transwell migration assays, our preliminary data suggests that genome decondensation prevents the passage of the nucleus through narrow 3D spaces. However, a condensed genome organization is not necessary for 2D cell motility, where nuclei are unconfined.

Ensemble docking towards the design of novel inhibitors for Histone Deacetylase 4

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Histone deacetylases (HDACs) catalyze the deacetylation of lysines present in histones tails. This deacetylation results in the compaction of DNA leading to the suppression of transcription. HDAC4 has been associated with multiple cancers (Colon, Gastric, Breast) over the years and has emerged as a major drug target. HDAC4 is enzymatically dead, but it is believed to act as a scaffold for the recruitment of multi-protein complexes containing enzymatically active HDAC3. Previous studies have shown that the enzymatic activity associated with HDAC4 is dependent on its interaction with these complexes. HDAC4 binds to these complexes via Nuclear receptor co-repressor (NCoR). All the currently known HDAC inhibitors (HDACi) target the catalytic pocket by chelating the zinc atom to inhibit the enzymatic action. However, due to similarity of the catalytic site, these HDACi bind promiscuously with all HDACs which results in issues with toxicity and adverse side effects. In order to reduce these side effects, selective HDACi are needed. In our current study, we aim to computationally predict compounds that disrupt formation of the HDAC4:NCoR complex, thus preventing its association with HDAC3. We perform high throughput *in-silico* screening using VinaMPI of NCI dataset 5 to an ensemble of HDAC4 structures. The ensemble was constructed from molecular dynamics (MD) simulations, accelerated molecular dynamics (aMD) simulations and HDAC4 crystal structures. The binding affinity of compounds were calculated using AutodockVina scoring function. Our final list consists of 156 compounds which could potentially disrupt the formation of the HDAC4:NCoR complex.

Efficacy of two non-cardiotoxic doxorubicin derivatives, AD312 and AD198 treatments in bladder cancer

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Doxorubicin (Dox) is a widely used chemotherapeutic drug for treatment of bladder cancer. The next generation anthracyclines, including AD312 and AD198, demonstrate distinct mechanisms of actions compared to Dox. AD312 inhibits topoisomerase II activity in the nucleus of the cells, whereas AD198 localizes in the cytoplasm and induces apoptosis through the PKC pathway. Studies in mice confirm that AD312 and AD198 are non-cardiotoxic compared to Dox. Mutation and inactivation of multiple genes were reported and identified as potential targets in over 70% of the bladder cancer, including p53 and c- myc. In this study, we assessed the efficacy of these drugs in human (UMUC-3, T24, RT4, J82, 5637) and canine (K9TCC#2Dakota) transitional cell carcinoma (TCC) cells by measuring cellular viability and changes in c-myc and p53 protein expressions. We observed that AD198 was the most effective treatment in inhibiting cell proliferation compared to AD312 and Dox in all tested TCC cells as assessed by MTS assay. Our results also demonstrate that AD198 decreased cell viability in TCC cells through the upregulation of p53 and suppression of c-myc proteins. A p53-wild type cell line RT4 showed significant increase in p53 expression after all drug treatments as compared to TCC cells with p53 mutations, further suggesting an important role of p53 in inhibiting cell growth. Dox and AD312 were not efficient in inhibiting c-myc expression. Overall, our results demonstrate that AD312 and AD198 are more effective in inhibiting bladder cancer than Dox and are promising candidates for the treatment of bladder cancer.

The Pre-clinical Bioimaging Core Research Facility at the University of Tennessee-Knoxville

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The Bioimaging Core (http://ceb.utk.edu/bioimaging-core-facility/) at the University of Tennessee – Knoxville provides cutting-edge bioluminescent and fluorescent biological imaging to the UT-Knoxville and surrounding research community. Our primary bioimaging instrumentation consists of two in vitro/in vivo/ex vivo/in planta PerkinElmer IVIS Lumina imaging systems that enable visualization of light emission from fluorescent and bioluminescent proteins, dyes, and nanomaterials directly within living animals, tissues, cells, whole plants, and biomaterials. For cancer research in particular, these imaging systems are supporting a number of research projects that involve the in vivo visualization of tumor growth, monitoring drug delivery, tracking cell migration dynamics, assessing the biocompatibility of three-dimensional tissue scaffolds, and brain imaging. Our newest IVIS Lumina K instrument provides fast frame capture to enable animals to be imaged as they freely move about their enclosure without anesthetization. Staff is available to provide individual to classroom-level instrument training and assistance with data analysis to ensure that you and your lab's investigational and developmental needs are optimally met.

A mathematical model for reciprocity and reversibility of epithelial-mesenchymal transition

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Plasticity of epithelial cells is critical for development and carcinoma progression. A prime example of the epithelial plasticity is a process called epithelial-mesenchymal transition (EMT), in which epithelial cells convert to mesenchymal cell types during embryogenesis. Partial EMT that enhances epithelial cell motility and invasiveness is also strongly implicated in cancer progression. Information suggesting the intricate relationship between EMT and metastasis has accumulated in recent years, but mechanisms underlying this cellular reprogramming event remain elusive. In particular, it is unclear how cells coordinate the loss of epithelial (E) identity and the gain of mesenchymal (M) identity, and what determines the reversibility of this transition. We recently found that the loss of *ZEB1* gene uncouples these two reciprocal processes in MCF10A cells, i.e. cells can express both E and M genes simultaneously upon the *ZEB1* knockout. In addition, exogenous ZEB1-induced EMT is irreversible whereas this irreversibility is lost in the knockout condition. Using mathematical modeling of gene regulatory network that controls EMT, we found that a stable hybrid EMT state emerges in the absence of *ZEB1*. Also, we predict that both E and M identities can be suppressed if *ZEB1* is overexpressed and TGF-β signaling is blocked, providing an additional uncoupled EMT state. We show that the positive feedback loop between ZEB1 and TGF-β governs an irreversible transition from E to M state. Our model highlights the importance of ZEB1 in controlling the reciprocity and reversibility of the EMT, and provides potential strategies to interfere the plasticity of epithelial cells during cancer progression.

In vivo cancer bioimaging with substrate-free autobioluminescent cells

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In vivo bioluminescent imaging (BLI) enables the visualization of an optical signal from living cells and tissues to provide a unique perspective toward the understanding of biological processes as they occur within an authentic *in vivo* environment. Bioluminescence-producing cells have to date primarily relied upon the integration of firefly luciferase constructs whose bioluminescent signal is dependent upon the extraneous addition of substrate (luciferin), thereby requiring repetitive animal injections and consequent intermittent snapshots of imaging data. To overcome this obstacle, we have developed a synthetic luciferase cassette that enables cells to continuously produce a bioluminescent signal without the need for extracellular stimulation. Based on the bacterial luciferase gene cassette of *Photorhabdus luminescens*, this system encodes both a luciferase protein, as well as a short synthetic pathway for transforming natural intracellular products into luciferin substrates. This autobioluminescent system has been validated for its expression across a suite of cell types (kidney, breast, colon, liver, pancreas, and bladder) and demonstrated to self-modulate its output signal concurrent with the host's metabolic activity level. Results from assays employing this autobioluminescent signal therefore correlate strongly with those from existing metabolic activity assay systems (i.e., MTT assays and commercially available firefly luciferase-based bioluminescent assays), but can also be applied towards the dose-responsive correlation of autobioluminescent output with hormone-induced cell proliferation, and dose-dependent autobioluminescence induction in response to specific chemical targets through the use of target-specific genetic controls. Autobioluminescently labeled cancer cells have also been demonstrated to allow for noninvasive tracking of tumor growth and response to drug treatment over time without the need for injecting substrate at each and every time point of interest.

Protein-nanoparticle interactions and the effect of metabolic state on nanoparticle biodistribution

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Nearly 70% of the U.S. adult population is overweight and about 30% are obese. Consequently, patients that will potentially be administered drug delivery vehicles (e.g. nanoparticles) to localize to cancerous tumors will have significant amounts of white adipose tissue (WAT), low-density lipoproteins and enlarged livers. However, the majority of studies investigate the biodistribution and efficacy of drug delivery vehicles in lean mice with healthy metabolic states. These studies may not emulate the state of most patients in the clinic. Our work is therefore aimed to examine nanoparticle-protein interactions in conjunction with the biodistribution of drug delivery vehicles as a function of mouse diet, weight, and metabolic condition. The *in vitro* studies with plasma proteins and nanoparticles demonstrated that nanoparticle surface chemistry plays a role in the binding activity of these proteins. In the *in vivo* work, the uptake of nanoparticles in lean mice (C57) was mainly in the liver and spleen, and as the weight of these mice increased – through a high fat diet – the localization to these organs also increased. In obesity models, *ob/ob* mice and A^y mice with non-alcoholic fatty liver disease (NAFLD) showed decreased accumulation of nanoparticles in their livers. This was in contrast to lean, medium and heavy mice. From these observations, we postulate that the metabolic condition of a patient will significantly change the biodistribution of current anti-cancer nanoparticle technologies.

A novel soluble peptide with pH-responsive membrane insertion

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Several diseases, such as cancer, are characterized by acidification of the extracellular environment. Acidosis can be employed as a target to specifically direct therapies to the diseased tissue. We have used first principles to design an acidity-triggered rational membrane (ATRAM) peptide with high solubility in solution that is able to interact with lipid membranes in a pH dependent fashion. Biophysical studies show that the ATRAM peptide binds to the surface of lipid membranes at pH 8.0. However, acidification leads to the peptide inserting into the lipid bilayer as a transmembrane α-helix. The insertion of ATRAM into membranes occurs at a moderately acidic pH (with a p*K* of 6.5), similar to the extracellular pH found in solid tumors. Studies with human cell lines showed a highly efficient pH-dependent membrane targeting, without causing toxicity. Here we show that it is possible to rationally design a soluble peptide that selectively targets cell membranes in acidic environments.

Protease dead separase inhibits chromosome segregation and RAB-11 vesicle trafficking

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Separase cleaves cohesin to allow chromosome segregation. Separase also regulates cortical granule exocytosis and vesicle trafficking during cytokinesis, both of which involve RAB-11. We investigated whether separase regulates exocytosis through a proteolytic or non-proteolytic mechanism. In *C. elegans*, protease-dead separase (SEP-1PD::GFP) is dominant negative. Consistent with its role in cohesin cleavage, SEP-1PD::GFP causes chromosome segregation defects. Partial depletion of cohesin rescues this defect, suggesting that SEP-1PD::GFP impairs cohesin cleavage by a substrate trapping mechanism. SEP-1PD::GFP causes cytokinesis failure that is synergistically exacerbated by depletion of the t-SNARE SYX-4. Furthermore, SEP-1PD::GFP delays furrow ingression, causes an accumulation of RAB-11 vesicles at the cleavage furrow site and delays the exocytosis of cortical granules during anaphase I. Depletion of syx-4 further enhanced RAB-11::mCherry and SEP-1PD::GFP plasma membrane accumulation during cytokinesis, while depletion of cohesin had no effect. In contrast, centriole disengagement appears normal in SEP-1PD::GFP embryos, indicating that chromosome segregation and vesicle trafficking are more sensitive to inhibition by the inactive protease. These findings suggest that separase cleaves an unknown substrate to promote the exocytosis of RAB-11 vesicles. Overall, our findings provide insights into the molecular control of separase in exocytosis and indicate that separase has substrates involved in multiple cellular roles including chromosome segregation and vesicle trafficking.

Changing the quantitative paradigm in PET/CT

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Region of interest measurements in PET yield standard uptake values (SUVs). These values are the activity concentration measured by the scanner normalized by the patient's weight and the radiopharmaceutical dose injected. These units, although, the clinical standard for nearly 20 years suffer from a number of issues. First, the SUV is only proportional to the true metabolic activity of a given compound. Second, they have a reasonable degree of variability due to biological factors, such as kidney function differences between patients. Third, is that they suffer from additional biological variability which has been estimated to be ~10% even for scans repeated on the same patient in a short time frame. These issues are alleviated with the use of dynamic PET imaging, which enables calculation of true metabolic rates. These methods, however, typically require long scan times (1+ hours), and invasive blood sampling. Our group has developed novel methods for whole-body dynamic imaging to enable routine use of whole-body kinetic studies in a clinical setting with no arterial blood sampling and requiring only a 15 minute PET scan. This is accomplished through the use of external detector systems to estimate the arterial input function and multi-pass continuous bed motion PET imaging to acquire the necessary whole-body patient data. This enables calculation of true glucose metabolic rates which are more robust quantitative units for assessment of patient disease and disease response to therapy.

Nicole McFarlane – waiting for abstract

Enhanced guide RNA design and analysis for precise CRISPR/Cas-based cancer therapy

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The development of effective cancer therapies relies on the ability to accurately and precisely target cancerous cells while disregarding healthy cells. To accommodate CRISPR utilization for applications in cancer identification and treatment, we have developed a novel general framework for identifying on- and off-targets with enhanced predictability to assist in the genome editing for a variety of organisms and endonucleases. Utilizing this framework, we demonstrated a 146% improvement over the conventional method for determining sites of off-target activity. Analysis of the human genome for target sites using multiple endonucleases increases the ability to precisely target oncogenic mutations while avoiding healthy cells. Further we used this framework to design guide RNAs for genome editing across a consortium of organisms. Our analysis identified shared and unshared targets that enable genome editing of single or multiple genomes simultaneously in a consortium of interest. A comparison of the genome of *Clostridium thermocellum* with a series of related microbes showed the co-habitant *Thermoanerobacterium thermosaccharolyticum* shares nearly double the number of sequences on average as other *Clostridia* species, suggesting such analysis may be important to consider when looking to apply the CRISPR system to natural consortia, such as the human gut microbiome, which has been shown to play a role in the development of a variety of cancers. Our improvement in predictive capabilities in conjunction with endonuclease flexibility will aid in the development of CRISPR derived therapeutics for targeting and repairing oncogene mutations.