Online Proceedings

1M

THERAPEUTIC VULNERABILITIES OF CANCER

Session Moderator: Rodney Ho, Pharmaceutics

MGH 389

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Investigating the Role of the Extrinsic Neural Environment on the Tumorigenic Properties of Glioblastoma Stem Cells

Karan Gunwant (Karan) Rai, Senior, Neurobiology
Mary Gates Scholar, UW Honors Program
Mentor: Jan-Marino (Nino) Ramirez, Neurological Surgery
Mentor: Julia Pollak, Center for Integrative Brain Research, Seattle Children's Research Institute
Mentor: Robert Rostomily

Glioblastoma multiforme (GBM) is the most common and deadliest cancer of the central nervous system. The existence of stem-like cells within GBM tumors - Glioblastoma stem cells (GSCs) - may explain some of the malignant features of Glioblastoma multiforme (GBM) owing to their rapid self renewal and diffuse migratory behavior. Increasingly apparent is the role ion channels play in many cancer cell properties, including regulation of cell cycle progression and proliferation, tumor cell motility and invasion, and regulation of metabolic rate. We hypothesize that ion channel dysregulation contributes to GSC malignancy, including increased viability, proliferation, and migration. To test this, multiple GSC isolates derived from human GBM tumors were maintained in culture over many passages while retaining stem cell properties. RNA-seq expression profiles revealed ion channel families that were highly enriched in GSC samples. Real-time qPCR was next used to confirm increased expression of voltage gated calcium, sodium, and potassium channels, ionotropic glutamate channels and inwardly-rectifying potassium channels. Identifying ion channels uniquely enriched in GSCs led to functional analyses to determine how ion channel blockers affected their proliferative and migratory properties. We performed MTT cell viability assays to assess how ion channel blockers altered the viability of GSCs compared to a control neural stem cell line. We were able to show a significant decrease in cell viability across GSC lines compared to control at a variety of drug concentrations. Utilizing live-image cell tracking, we determined decreased migratory behavior and rates of these GSC lines with the same blockers. Finally, to elucidate the role of the extrinsic tumour microenvironment on the proliferation and migration of GSCs, unique coculture models involving GSCs cultured on mouse organotypic brain slices were utilized, and showed the affects of stimulating and inhibitory DREADD receptors to modulate local neuronal activity.

Targeting Mutation Load in Colorectal Cancer by Therapeutic Mutagenesis

Christine Katherine (Christine) Ma, Senior, Biochemistry
Mary Gates Scholar, NASA Space Grant Scholar, UW Honors Program
Mentor: Lawrence Loeb, Pathology
Mentor: Edward Fox, Pathology

Cancers are fueled by mutations which impart new capabilities to incipient cancer cells. Spontaneous mutations are, however, more likely to be deleterious than beneficial based on random chance alone. Therefore, inducing more mutations in an heavily mutated population could lead to an "error catastrophe" once the mutation level rises past a certain threshold. Accumulating too many of these debilitating mutations consequently leads to a reduction in the overall population fitness. Our hypothesis is that mutagenic versions of nucleoside analogs (modified versions of the building blocks of DNA) can induce more mutations to reach this 'error' threshold and beyond, and that this can selectively kill cancer cells. To evaluate the effectiveness of this strategy, termed therapeutic mutagenesis, we conducted cell culture experiments on various human colon cancer cell lines: HCT116 (mismatch repair deficient colorectal cancer) cells, SWS620 (non-MMR-deficient colorectal cancer), and a control normal human fibroblast line. We screened a library of candidate nucleoside analogues (e.g. O4-Ethyl-Thymidine) for mutagenicity and viability at physiologically relevant concentrations. I then quantified the short-term viability of the treated cell lines with a clonogenic survival assay. I used the highly accurate Duplex genetic sequencing methodology to monitor the changes in a

cell line's overall mutation load after treatment. After validating the concept of therapeutic mutagenesis in cancer, our next steps will be to determine whether possible animal and clinical trials would be possible in the future. Our approach may open up new therapeutic possibilities for patients in the most advanced stages of cancer, especially in the context of complementing increasingly common immunotherapies.

Targeting Protein Synthesis Control in Cancer

Jessie Lynne (Jessie) Horn, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Andrew Hsieh, Human Biology, Fred Hutchinson Cancer Research Center
Mentor: Li-Jie Wang, Human Biology, Fred Hutchinson Cancer Research Center

Protein synthesis is a tightly regulated cellular process important for all of life. Specific diseases such as cancer can hijack the process of protein synthesis to drive specific disease states. My research focuses on determining if the protein making factories of a cancer cell can be secifically therapeutically targeted. I have focused my studies on prostate and bladder cancer. Prostate cancer is the second leading cause of cancer death in men. A well-studied signaling pathway that is altered in prostate cancer is the PI3K-AKT-mTOR pathway. This pathway converges on and activates the apparatus necessary to start protein synthesis through a protein called the eukaryotic initiation factor 4E (eIF4E). eIF4E is an mRNA cap-binding protein which recruits another translation factor to start protein synthesis in cells. Ribavirin is an mRNA cap-mimetic that can compete with eIF4E to inhibit mRNA translation. I will present my results from a preclinical trial using Ribavirin and a mouse model of prostate cancer. In addition to my work in prostate cancer I have also initiated preclinical studies in bladder cancer. Bladder cancer is the 5^th leading cause of cancer death in men and there have been no new theurapeutics for two decades. ARID1A is an ATP-dependent chromatin modifier that is one of the most mutated genes in bladder cancer. The Hsieh lab has discovered an intimate link between ARID1A levels and mRNA translation. I hypothesize that protein synthesis machinery represents a unique vulnerability, which can be therapeutically targeted in bladder cancer. I will present data on my findings using protein synthesis inhibitors such as puromycin and an ATP-site inhibitor of mTOR in ARID1A wild-type and mutant bladder cancer cell lines. Together, my work in the Hsieh lab points to a new treatment paradigm for targeting the protein synthesis machinery in human cancers.

Anti-HER2/neu Peptide-Conjugated Iron Oxide Nanoparticles for Targeted Delivery of Paclitaxel to Breast Cancer Cells

Guanyou Lin, Senior, Bioengineering
Mary Gates Scholar
Mentor: Qingxin Mu, Pharmaceutics

Breast cancer is the most common cancer in women. Even with advancing treatment techniques, breast cancer is still the cause of tens of thousands of death in the U.S. alone. Combined treatments including surgery, radio and chemotherapy are commonly implemented for breast cancer. However, the survival rate of metastatic breast cancer remains low, so new targeted therapy strategies are needed. In recent years, nanotechnology has opened a new route for targeted therapy of breast cancer. Nonetheless, some challenges still remain for nanoparticles (NPs) used in breast cancer treatments. After loaded with anticancer drugs, fluorophores and targeting ligands, NP drug delivery systems suffer from issues of size limit, stability, target cell internalization and biodegradability. Anti-human epidermal growth factor receptor 2 (HER2/neu) monoclonal antibody is a good targeting ligand for some breast cancer cells for two main reasons. Firstly, about 1/4 of breast cancer patients overexpress HER2/neu. Secondly, monoclonal antibodies have a better targeting effect than small molecule, protein and aptamer-based targeting ligands. However, the bulky size of the antibodies can change the desired physiochemical properties of NPs. Therefore, an anti-HER2/neu peptide, which is a small exocyclic peptide derived from the antibody, is utilized for targeting purposes. Moreover, paclitaxel (PTX) is a strong anticancer drug that can restrain mitotic spindle assembly and chromosome segregation, and in turn prevent breast cancer growth. Here we report anti-HER2/neu peptide-conjugated and PTX loaded iron oxide NPs (IONP-PTX-AHNP) that are small size, highly stable and biocompatible in biological medium for breast cancer treatment. Significantly, the IONP-PTX-AHNP showed targeted cell uptake into HER2/neu overexpressed breast cancer cells and PTX-mediated cancer cell death.

Engineering M2 Macrophage-Targeting Peptide (M2pep) for Improved Serum Stability

Julio M. (Julio) Pineda, Senior, Mathematics, Bioengineering
Mary Gates Scholar, UW Honors Program
Mentor: Suzie Pun, Bioengineering

Macrophages are broadly classified as pro-inflammatory M1 and anti-inflammatory M2 macrophages. The majority of tumor-associated macrophages (TAMs) in tumors are known to express M2 phenotypes (M2-TAMs) which secrete cytokines and growth factors to promote cancer cell proliferation, tumor angiogenesis, and metastasis. Hence, depletion of the M2-TAMs is a promising adjuvant therapy to the current cancer treatments. Our lab has previously developed

M2 macrophage-binding peptide (M2pep) which can bind to both M2 macrophages and M2-TAMs. However, in vivo targeted delivery of cytotoxic drugs to M2-TAMs using M2pep as a targeting ligand was limited by poor serum stability of M2pep. Previously, we engineered and tested different linear variants of M2pep; however, none of these was able to improve serum stability while retaining M2 macrophage-binding activity. In this study, we investigated cyclization of M2pep as a strategy to improve its serum stability since cyclic peptides are usually more serum resistant than their linear counterparts due to their higher structural rigidity. To synthesize a cyclic M2pep variant, the original M2pep was synthesized with two flanking cysteines on each terminus. These cysteines were then oxidized to form a disulfide bond yielding cyclic M2pep. The serum stability of cyclic M2pep was evaluated by incubating the peptide in serum and withdrawing aliquots of the serum at various time points for analysis by mass spectrometry. Compared to linear M2pep which was rapidly degraded in serum within 4 hours, the cyclic variant was detected even after incubation in serum for more than 24 hours. In conclusion, we have developed an optimized cyclic M2pep which exhibits enhanced serum stability while retaining M2-TAM binding activity. Our peptide can be utilized as a more effective ligand for targeted delivery of cytotoxic drugs to M2-TAMs. Future development of M2pep-based TAM-depleting therapeutics would serve as an effective adjuvant platform to potentiate current cancer therapies.

Multifunctional Iron Oxide Nanoparticles for Targeted Delivery of Paclitaxel and Gemcitabine for Glioblastoma Therapy

Victoria Kletke (Victoria) Patton, Senior, Chemical Engr: Nanosci & Molecular Engr
Mentor: Qingxin Mu, Pharmaceutics

Glioblastoma multiform (GBM) is the most common and aggressive malignant primary brain tumor and accounts for about 70% of new cases of primary brain tumors in the US annually. Current treatments are very aggressive and have a high risk of systemic toxicity due to non-specificity of current cancer therapeutic agents. Iron oxide nanoparticles (IONPs) have shown great promise as versatile targeted drug delivery vehicles for effective cancer treatment. In this research presentation, the development of two multifunctional IONPs for targeted GBM therapy will be discussed. The first is an IONP covalently conjugated with anti-cancer drug, gemcitabine (GEM), using hyaluronic acid as a bridging molecule. The second is an IONP conjugated with carboxylated ß-cyclodextrin and loaded with paclitaxel (PTX) via hydrophobic interactions. Both IONPs were conjugated with chlorotoxin (CTX), a GBM targeting agent that can cross the blood-brain barrier (BBB), and fluorescent dyes. Through characterization by TEM, DLS, and HPLC, the IONPs showed small size, high stability, and proper drug loading. More importantly, the GEM-conjugated IONPs showed prolonged blood circulation time and BBB permeability *in vivo*. The PTX-conjugated IONPs showed enhanced cell killing of the GBM cells and drug-resistant GBM cells compared to the free drug. The two multifunctional IONPs have shown great promise for effective treatment of GBM.

Optimizing Peptides for Targeting Tumor-Associated Macrophages

Jonathan Lik Wing (Jonathan) Yu, Senior, Bioengineering
Mary Gates Scholar, UW Honors Program
Mentor: Suzie Pun, Bioengineering
Mentor: Gary W. Liu

Macrophages are cells of the immune system that play key roles in surveillance, destruction of pathogens, and tissue repair. In the context of cancer, macrophages can infiltrate solid tumors and adopt an anti-inflammatory profile resembling that of the "M2" macrophage subclass. These M2-like tumor-associated macrophages (TAMs) promote tumor growth and metastasis by suppressing the immune response. Therefore, selective destruction of TAMs using a biomolecule that targets and binds to TAMs may have therapeutic benefit in cancer. We have previously identified a novel peptide, M2pep, that selectively binds to M2 macrophages over other immune system cells. In other experiments, a specific pattern of amino acids, named the M2pep motif, was observed to be critical for peptide binding to TAMs. This project seeks to improve M2pep binding and selectivity to TAMs by constructing and screening a library of peptides containing the M2pep motif for binding to M2 macrophages. The library was constructed by genetically engineering bacteriophages to display all possible peptides containing the M2pep motif. This was achieved by: *(1)* designing DNA sequences encoding for peptides with certain amino acid positions "fixed" with the M2pep motif, and the other positions randomized; *(2)* inserting these sequences into bacteriophage DNA; and *(3)* incorporating the engineered DNA into bacteria to generate bacteriophages that display the peptide library on their outer surface. Then, the peptide library was screened for binding to M2 macrophages, and the peptides that bound were sequenced. Several candidates were evaluated for M2 macrophage-binding and compared with M2pep to identify sequences with improved binding and selectivity. This method of peptide library construction and screening using a bacteriophage platform can be a powerful tool for future drug development, and enable further refinement of binding molecules with established binding motifs.

Detecting Gene-Gene Interactions that Underlie Cancer using the R Package algstat

Melissa Maria Stadt, Senior, Mathematics
Undergraduate Research Conference Travel Awardee
Mentor: Luis David Garcia-Puente, Mathematics and Statistics, Sam Houston State University

Interactions between single nucleotide polymorphisms (SNPs) and complex diseases have been an important topic throughout epidemiological studies. Previous genome-wide-association studies have mostly focused on gene variables at a single locus, but did not obtain strong evidence for SNPs with complex diseases such as cancer. In our project, we performed a focused candidate gene study to test the interaction of multiple SNPs with the risk of different types of cancer. Using the R package algstat, developed by Kahle, Garcia-Puente, and Yoshida, we developed an algorithm which can test for independence between several variables and the disease. We applied our methods to the study of gene-gene interaction on cancer data obtained from the European case-control study Gen-Air. Previously pairs of SNPs were tested for independence versus certain cancers in this study, but it was not computationally feasible to test triplets of SNPs with the methods available. The algorithm developed was significantly faster so we were able to test for independence of SNP triplets and found strong evidence to reject independence of many triplet combinations of SNPs with the disease, suggesting correlation between having a certain combination of SNPs and risk of certain cancers. These results can be used for further testing of associations of these SNP combinations and certain cancers. Outside of the study of SNP-cancer association, this algorithm can be easily adjusted to perform other studies using arbitrary log-linear statistical models.