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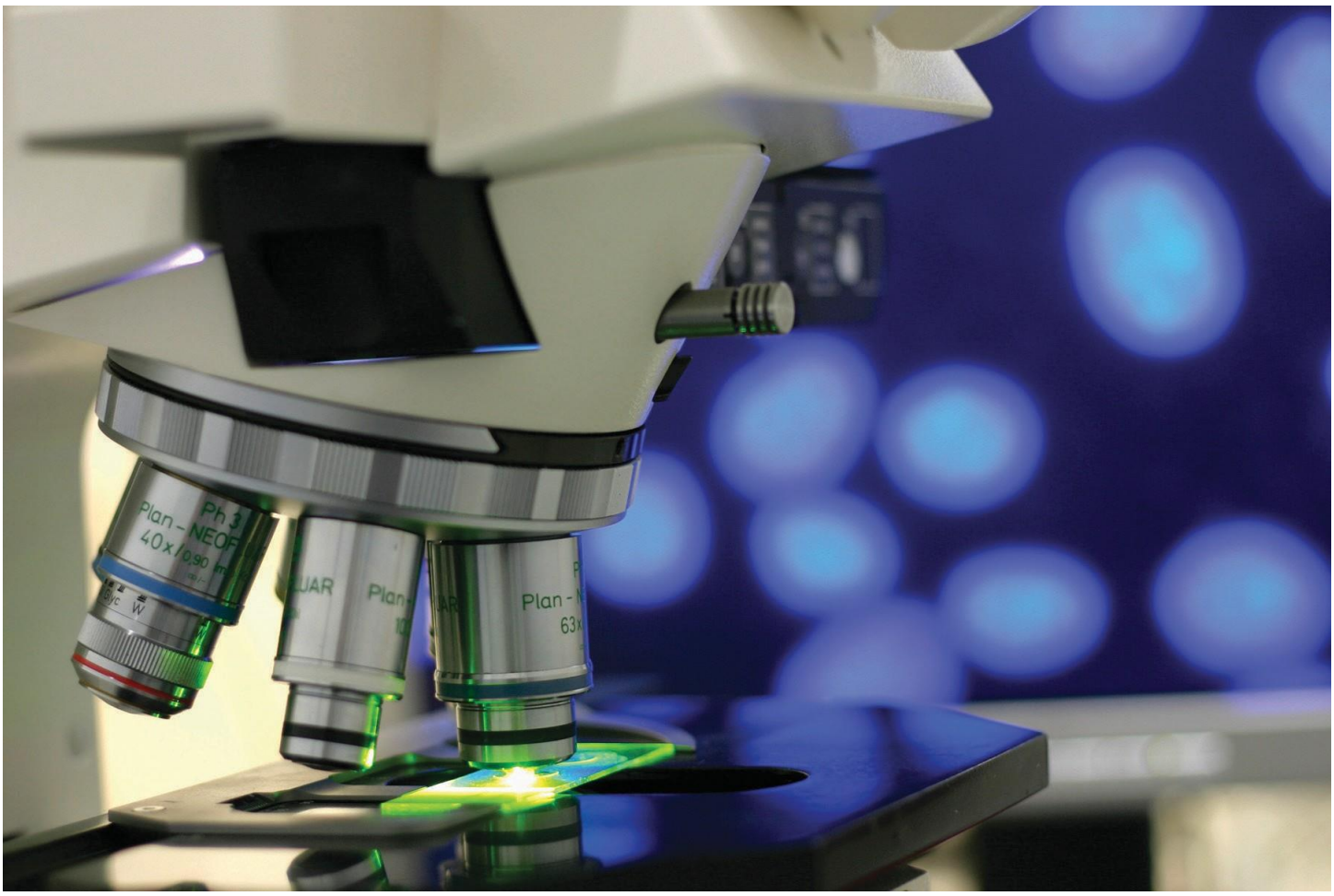
IDP Foundation Research Innovation Challenge Grants



May 2016

IDP Foundation Innovation Research Awards

Identification of Cellular Processes that Mediate the Pancreatic Cancer Metastatic Inhibition by Metarrestin

Co-Principal Investigators:
Sui Huang, MD, PhD, Associate Professor of Cell and Molecular Biology
Hidayatullah G. Munshi, MD, Associate Professor of Hematology/Oncology

Dr. Huang

With the support of the IDP Foundation Innovation Research Award, Dr. Sui Huang and Dr. Hidayatullah Munshi initiated studies to characterize the genome expression profile changes upon treatment of metarrestin in pancreatic cells (PANC1 and PC3M cells) in culture. Metarrestin is a novel, anti-metastasis drug for treatment of advanced stage pancreatic cancer. They studied this using HT12 array (Affymetrix) that probes for approximately 20,000 gene expressions and using next generation sequencing for PANC1 cells.

Dr. Munshi

The initial analyses comparing data from both approaches and across different cell lines shows consistent gene expression changes either up- or down-regulated in cells treated with metarrestin. Western blot analyses of some of the factors confirmed the changes of their protein levels upon treatment with metarrestin including TXNIP. TXNIP is a multi-functional tumor suppressor involved in metabolism, ROS level regulation, and tumor growth inhibition. Preliminary modulation of the levels of TXNIP through siRNA knockdown or over-expression did not show significant impact on perinucleolar compartment (PNC) structures, indicating that TXNIP alone is not sufficient in regulating PNC structural integrity. E2F2 is a well-known positive regulator in tumor growth. The significant reduction of these proteins could help inhibit tumor growth.

In addition, proteins that bind metarrestin were identified through affinity purifications using biotin-metarrestin, which maintains its efficacy against PNC in culture cells. Competition analyses using unlabeled metarrestin and proteomic analysis identified eukaryotic translation elongation factor (EEF1A) as the most abundant metarrestin binding protein. EEF1A has two isoforms, EEF1A1 and EEF1A2. While EEF1A1 expresses in all tissues, EEF1A2 only expresses in brain, heart, and skeletal muscle in normal adults and its over- or re-expression has been described in cancers, including pancreatic cancer, suggesting a role in cancer metastasis. Binding of metarrestin to EEF1A was further confirmed in cells by using a cellular thermal shift assay (CETSA), which shows an increase in the aggregation temperature of EEF1A upon metarrestin treatment. Western blot analyses show that total EEF1A protein level did not change significantly in three cell lines upon treatment of metarrestin.

EEF1As are multi-functional proteins implicated in actin bundling, nuclear transport, tRNA export, etc. To evaluate whether metarrestin impacts cancer cell through interfering with EEF1A function, the levels of EEF1A were manipulated through either over-expression or knockdown approaches. Although EEF1A is not sufficient to induce the formation of PNCs in PNC negative cells, it does enhance PNC structures. Unfortunately, selective reduction of EEF1A has not been successful.

These preliminary observations further support that metarrestin targets multi-cellular processes and reinforce the importance to investigate changes of various cellular pathways upon exposure to metarrestin at cellular and tumor levels. Drs. Huang and Munshi plan to use these preliminary findings to write an R01 grant. Additionally, they are submitting a manuscript to *Cancer Cell* that summarizes the anti-metastatic efficacy of metarrestin and its characterized mechanisms of action. The IDP Foundation is acknowledged for its support of this work in the paper.

Effect of Radiation and DNA Alkylator Chemotherapy on Host Immune Response to Intracranial Tumor

Co-Investigators:
C. David James, PhD, Professor of Neurological Surgery and Biochemistry and Molecular Genetics
Derek A. Wainwright, PhD, Assistant Professor of Neurological Surgery and Microbiology-Immunology
Orin Bloch, MD, Khatib Professor of Neurological Surgery, Assistant Professor of Neurological Surgery and Neurology
Jeffrey J. Raizer, MD, Professor of Neurology and Hematology/Oncology


Dr. James

This project is directed toward understanding how standard of care therapy for glioblastoma, which involves treatment with radiation and temozolomide subsequent to surgical debulking of tumor, influences the efficacy of an investigational immunotherapy in which patients receive heat shock protein (hsp) vaccine obtained from their own tumor.

To address this question, Drs. James, Wainwright, Bloch, and Raizer are using two syngeneic, immunocompetent engraftment models for this research, where brain tumors are established in mice by intracranial injection of tumor cells. One of the models is the GL261-C57BL6 tumor cell-mouse strain combination, and the second involves glioblastoma cells from a genetically engineered mouse tumor having Rb, p53, and PTEN inactivation. The first is the most commonly used syngeneic engraftment model, which is often used for studying brain tumor immunology and immunotherapies. The second model is important for addressing consistency, or lack thereof, of results from distinct tumor cell-mouse host pairings. Following is a progress report based on the team's work accomplished since July 2015, when the project was initiated in association with institutional approval of the associated animal research protocol.


Dr. Wainwright

To achieve the indicated objectives of this research, the group has been working on a protocol for isolating heat shock protein peptide complex-96, known as (HSPPC96), from mouse glioblastoma. The investigators initially focused on isolating HSPPC96 complex from a normal mouse brain in order to refine and optimize their protocol prior to isolating complex from tumor-bearing brain. They were successful in isolating HSPPC96 from a normal brain. The protein is not present in the other three samples (pellet wash, pellet RT, and supernatant RT), suggesting that the protein bound to the conA-sepharose beads and did not dissolove prematurely.


Dr. Bloch

They next isolated HSPPC from GL261 intracranial tumors in C57BL/6 mice. In total, 22 micrograms of protein were isolated using this protocol from two GL261 bearing mouse brains. To increase the amount of vaccine needed for experiments in which vaccine will be administered with radiation and/or temozolomide, the group has engrafted GL261 tumor cells in the brains of 20 mice. The brains from these mice will be used for preparing a substantial batch of HSPPC vaccine.


Dr. Raizer

Initial experiments will involve testing vaccine efficacy and specificity by treating mice bearing GL261 intracranial tumors with vaccine isolated from either naïve brain-lacking tumor versus vaccine from brain with tumor. Once the investigators have established GL261 vaccine anti-tumor efficacy, they will proceed to experiments involving the co-administration of vaccine with radiation and temozolomid, as detailed in the original project proposal.

The progress that has been made in this project opens the door for exciting research involving a variety of HSPPC combinatorial drug treatments, in addition to those with radiation and temozolomid. Of particular interest are combination treatments with immune checkpoint inhibitors, and with inhibitors of IDO1, whose enzymatic activity is known to be immunosuppressive.

Embryonic Renal Tumors: A Model for Epigenetic Transcription Regulation in Early Stem Cells

Principal Investigator:
Elizabeth Perlman, MD, Professor of Pathology


Dr. Perlman

There are three tumors of the childhood kidney that cause the majority of deaths. Wilms tumors are the most common tumor of the pediatric kidney. Rhabdoid tumors and clear cell sarcomas of the kidney are less frequent, however, they also are more deadly. Little progress has been made in the treatment of these tumors in the last decade. This has been due to the lack of knowledge regarding the genetic underpinnings of these pediatric renal tumors. In addition, there are no *in-vitro* models of these tumors that allow for in-depth studies or for the development and testing of new, more effective drugs. In the last five years, Dr. Perlman and her colleagues have led the High Risk Renal Tumor studies of the National Cancer Institute's TARGET initiative, which comprehensively analyzed the genomic changes of these three tumors. This has led to several major discoveries regarding the genetic causes of these tumors. During the last year, the IDP Foundation's funding has not only enabled these discoveries to be published in high-impact journals, but also has helped the group begin the process for identifying the appropriate *in-vitro* models to further investigate these discoveries. By doing so, they hope to increase the survival, and decrease the toxicity of therapy, in children that are afflicted.

Project 1: MLLT1 YEATS Domain Mutations in Distinctive Favorable Histology Wilms Tumors

Dr. Perlman and her group have identified mutations in the *MLLT1* gene in Wilms tumors that have never been previously reported in any other tumor. *MLLT1* is involved in the basic machinery of all our cells; therefore, this was a very important discovery. The IDP Foundation's support helped the group to complete the studies that enabled the group to publish this effort in *Nature Communications*. This publication shows that *MLLT1* causes a subset of Wilms tumor that have a particularly high death rate. The primary goal of the proposal was to find a suitable cell line in which to study the effects of *MLLT1* mutation during early kidney development. To this end, they identified two mouse cell lines, mk3 and mk4, that were developed from the embryonic kidney. The goal was to place the mutant *MLLT1* into mk3 and mk4 cells and evaluate the expression of selected genes involved in renal development. These efforts were successful, and resulted in abnormal expression of genes involved in early renal development. Data from these studies confirm the appropriateness of the mk3 and mk4 cell lines as a model upon which to build a grant studying Wilms tumor development. This will provide sufficient preliminary data for a grant planned for October.

In addition, Dr. Perlman and her team are analyzing the effect of *MLLT1* mutation in a zebrafish model during early renal development. This system will enable the group to study the potential tumor-causing effects of the mutant gene in an intact organism.

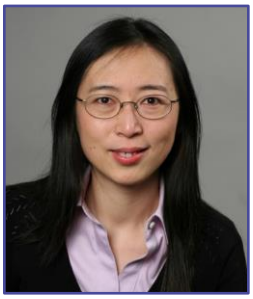
Project 2: Impact of SMARCB1 Loss in Rhabdoid Tumors

Rhabdoid tumors are rare, deadly kidney tumors of infants that are caused by mutation in the *SMARCB1* gene. The group's hypothesis is that *SMARCB1* loss had to appear at a very small window of time in the earliest stages of human development in order to result in rhabdoid tumor. Dr. Perlman and her colleagues therefore studied *SMARCB1* loss within embryonic stem cells, which are cells able to rapidly multiply and to maintain an undifferentiated state (known as pluripotency) until they are triggered to differentiate. They demonstrated that loss of *SMARCB1* may result in retained pluripotency, increased cellular proliferation, and delayed evidence of neural differentiation following the appropriate trigger (all features of rhabdoid tumors).

While these findings are exciting and support their hypothesis, the particular method they used was not sufficiently stable to allow robust analyses. Therefore, Dr. Perlman's group is currently using another technology (known as CRISPR). These studies are nearing completion and should be available for preliminary data for grant submission in October.

Project 3: TCF21 hypermethylation in Clear Cell Sarcoma of the Kidney (CCSK)

CCSKs represent the third high-risk tumor of the childhood kidney. Dr. Perlman and her team recently published the identification of decreased expression of an important tumor suppressor gene *TCF21* in CCSKs. The group initially planned to develop a cell line with loss of *TCF21*, similar to the above studies. However, more recently a unique mutation of another gene, BCOR, was identified in approximately 85 percent of CCSKs. These BCOR mutations may be the direct cause of *TCF21* decreased expression, and hence CCSK development. This publication as well as budgetary constraints led to the discontinuation of their efforts in this project. However, in the near future, the group plans to introduce the BCOR mutation into the mk3 and mk4 cell lines, followed by analysis of *TCF21*. They hope to complete these studies by October.



Dr. Cheng

New IDP Research Innovation Challenge Grant Recipients Announced

A generous supplementary donation from the Sherman Fairchild Foundation has allowed the Lurie Cancer Center to fund an additional IDP Research Innovation Challenge Grant in 2015. The IDP Challenge Grant will support the study *Cytoarchitectural Dynamics Drive Breast Cancer Invasion*. The following scientists are collaborating on the study:

Chonghui Cheng, MD, PhD, Assistant Professor of Hematology/Oncology
Kathleen Green, PhD, Joseph L. Mayberry, Sr., Professor of Pathology and Toxicology, Professor of Pathology and Dermatology
Brian Mitchell, PhD, Assistant Professor of Cell and Molecular Biology

Dr. Green

Cytoarchitectural Dynamics Drive Breast Cancer Invasion

More than 90 percent of breast cancer-related mortality is due to tumor metastasis, and efforts to develop effective therapies have been hampered by a poor understanding of the molecular mechanisms driving this process. In order to metastasize, cancer cells undergo morphological and behavioral remodeling to acquire an invasive and migrative phenotype. This remodeling is largely controlled by regulation of cell to cell adhesion and cytoskeletal connections.

Dr. Mitchell

The purpose of this project is to investigate the mechanism of this remodeling and its role in breast cancer metastasis. The group's ultimate goal is to develop novel strategies based on the knowledge they gain from this study to successfully treat breast cancer metastasis.

In this period of funding, Drs. Cheng, Green, and Mitchell have established the BioID technology in Project 1. This assay is crucial for identification of interacting proteins in the network of APLP2 splice isoforms that modulate cell adhesion and migration.

For Project 2, they have established the 3-dimensional mouse mammary organoid culture system and have successfully performed immunostaining for core desmosomal proteins, including desmosomal cadherins and desmoplakin, as well as for specific markers for the luminal and myoepithelial layers. The group is currently generating viruses that are required to perform the desmoplakin mutant and silencing experiments in both 2-dimensional and 3-dimensional cell culture systems. Subcellular microtubule stabilization is an important feature of directed cell migration.

In Project 3, they have made considerable progress in their efforts to understand the regulation of asymmetric subcellular accumulation of the microtubule binding protein CLAMP and the role for this localization in establishing asymmetric microtubule stabilization. The team's efforts have included identifying a novel CLAMP interacting protein (Prickle2), developing novel imaging paradigms to visualize microtubule asymmetry, and developing novel quantifiable metrics for computational-based imaging.