

# **Translational Cancer Research Program**

## **Our Goals/Vision**

Our mission is to gain an insight into the mechanisms driving tumorigenesis, drug resistance, metastasis, and to develop personalized targeted therapies and precision medicine to treat both drug resistance and metastasis.

Tumor microenvironment regulates cancer cell survival, metastasis, inflammation, and immune surveillance. There is growing evidence that the lymph node (LN) microenvironment, especially lymph node stromal cells (LNSCs), play a significant role in solid tumor growth, drug resistance, and subsequent extra-nodal metastasis of many types of cancer. A comprehensive understanding of the associated molecular mechanisms and pathways that are involved in tumor progression and metastasis would have substantial benefits for high-risk cancer patients.

## **Cancer stem cell and tumor microenvironment**

Recently, a new class of cancer cells has been identified that may be responsible for tumor recurrence. Referred to as tumor initiating cells (TICs), these cells represent a small population within the original tumor that are resistant to conventional chemotherapeutic and radiation treatments. They also have the ability to produce new (recurrent) tumors that possess all the phenotypic features of the original tumors. We have identified such drug resistant TICs in colon cancer, bladder cancer, and follicular lymphoma cells. These cells are dependent on the LN microenvironment provided by LNSCs. Some of the communication between the LNSCs and TICs may occur by the transfer of microvesicles, small membrane-bound particles containing proteins, siRNAs and mRNAs which have been shown to form functional signaling pathways between cells in vivo. We have recently shown a positive effect of stromal cell-derived microvesicles and tumor progression.

## **Patient-derived orthotopic xenograft models**

To examine the role of LNSCs in tumor progression and metastasis, we established both in vitro cell culture models and in vivo human-in-mouse orthotopic xenograft models using patient tumor specimens, tagged with optical reporter genes. These models recapitulate human cancer features and provide a useful platform for investigating major TIC/LN microenvironment-specific mechanisms of cancer resistance to chemotherapeutic agents in various cancers, such as solid tumor Colorectal Cancer, Renal Cell Carcinoma, Bladder Cancer, Esophageal Adenocarcinoma, Pancreatic Cancer, and B Cell Lymphoma. With noninvasive imaging approaches, the labeled tumor cells can be tracked in vivo, which permits both macroscopic and microscopic analysis of tumor progression and metastasis. Thus our models provide a powerful mammalian experimental system to elucidate the mechanisms of cancer initiation, maintenance, progression.

## **Prognostic and predictive biomarkers and translate preclinical discoveries into effective cure for cancer patients**

The established in vitro and in vivo xenograft models will be used as platforms for dissecting the molecular mechanisms underlying the dissemination of cancer and for preclinical drug development to constitute an integrated bedside to bench approach. As supported by our recent data on renal cell cancer studies, these models can lead to support identification of biomarkers, optimization of clinical candidate selection, and explore novel therapeutic strategies targeting tumor microenvironment in general or to evaluate individual patient tumor cells and predict their response to therapies in an Avatar style approach.

## Pediatric Pulmonology Research Opportunities

Our department is involved in several research and quality improvement projects that may be of interest to you.

- We are assessing the best interventions to improve pediatricians' adherence to clinic guidelines in screening, counseling, treating, and referring smoking caregivers (of our patients) to cessation services. Our goal with this project is to decrease the prevalence of second and third hand smoke exposure. To date, we have assessed electronic medical records changes, lectures, and a story book (developed by one of our medical students). None of these interventions have significantly changed physicians' behaviour. Our next project is to provide the caregiver a questionnaire (delivered with electronic tablet) to fill-out in the waiting room. If they are interested in smoking cessation an automatic referral can be generated via the electronic tablet. This process would facilitate the referral without having to depend on the pediatrician. If successful, we will measure quit rates of referred caregivers.
- We are starting a project to improve pediatricians' screening and treatment of overweight and obese patients. Our recent study assessed pediatrician's documentation and it was unfortunately very low. Considering how significant the obesity epidemic is, it is imperative that the physicians appropriately screen and treat. Our group is submitting a proposal to assess the efficacy of a continuing medical education (CME) module provided by a local culinary medicine center. If successful, we will then measure if the intervention was successful in providing appropriate patient education and, ultimately, if markers of health improve. A similar study as above has been approved for obese asthmatics. This study will assess if a nutritional intervention improves asthma control as well as indices of metabolic disease.
- Our group is currently developing a study to look at decreasing length of stay (LOS) in patients admitted for asthma exacerbation. Many asthma care paths are available but their use is not consistent. Our goal is to standardize care to decrease LOS.
- The care of premature infants after their discharge from our neonatal intensive care unit (NICU) is not standardized. Lack of a structured follow-up has been shown to lead to increased re-hospitalizations. We are planning a chart review of NICU graduates that are followed within the Ochsner system to assess where we can improve their care. We also will be reviewing whether there are risk factors that can predict rehospitalization.

# ITR TRANSPLANT LAB PROJECTS

## Project 1

### Role of Myeloid-Derived Suppressor Cells (MDSCs) in Hepatocellular Carcinoma Recurrence After Liver Transplantation

*Basic Science Aim:* In the first phase of this study, we are using tumor cell implantation models of HCC (derived from the BNL HCC lines) to characterize the role of granulocytic MDSCs (PMN-MDSCs). PMN-MDSCs are recruited to the primary tumor site, peripheral lymphoid organs, and liver in orthotopic models and have been implicated in resistance to therapy and metastasis. Using tumor-bearing mice, we are isolating circulating BNL cells to analyze their cancer stem cell expression profile and using co-culture experiments with tumor and peripheral MDSCs to model interactions and identify molecular targets used by these two critical tumor-derived cell populations in promoting metastasis and tumor recurrence.

After ex-vivo characterization, we will employ a model of HCC recurrence following liver transplant injury by injecting circulating tumors intravenous into tumor naïve mice prior to ischemia reperfusion injury with partial hepatectomy. In this model, we will demonstrate the role of transplant injury in mediating tumor recurrence and demonstrate the pivotal role of MDSCs in recurrence using MDSC-BNL co-injection experimental groups. We will attempt strategies to antagonize this process using molecular targets identified in our ex vivo study.

In the second phase of this study, we have begun breeding animals using the Cre-LoxP breeding system to develop two liver lineage specific reporter lines (Albumin-EYFP and Albumin-Luciferase). In these mouse lines, we will use chemically-induced models of HCC to generate primary liver tumors to shed circulating tumor cells and cancer stem cells. CTCs and CSCs will be isolated and injected into tumor naïve mice  $\pm$  MDSCs as per the experiments discussed above. These animals will allow us to kinetically monitor tumor recurrence and evaluate the effectiveness of molecular targets as well as the confounding variable of post-transplant immunosuppressive drug regimens on this process.

*Clinical Aim:* We are conducting a clinical trial, in which we are collecting peripheral blood from HCC patients which are undergoing embolization therapy to (a) bridge to transplantation or (b) downstage HCC to within transplant criteria. Peripheral blood is being collected before and after the embolization procedure as well as during a <30 day followup visit. In addition to collecting plasma for cytokine, circulating tumor DNA, tumor oncogene, and tumor-derived exosome analysis we are characterizing the patients MDSC and CTC expression profiles by flow cytometry and RNA analysis. The goal of this data panel is to identify a molecular signature which identifies patients at high risk of recurrence following liver transplantation.

**Prospective student project:** The student will be trained in the isolation, characterization, and ex vivo culture:co-culture of MDSCs and CTCs from tumor-bearing mice. The student will perform molecular analysis to confirm an “activated” phenotype in the isolated cells and perform co-culture experiments to demonstrate MDSCs are capable of supporting CTC survival and activation. This will be accomplished using direct contact co-cultures and indirect co-cultures using transwell membrane inserts.

## Project 2

### **Frequency and function of NF1 mutations in hepatocellular carcinoma - implications for the use mTOR inhibitors.**

Neurofibromin 1, one the largest genes in the human genome, is mutated in ~10% of pulmonary and hepatic cancers. NF1 functions as a tumor suppressor, making loss-of-function mutations of considerable concern particularly in HCC aggressiveness especially when present with other oncogenic mutations. We have developed HCC cell lines in which NF1 expression has been disrupted using CRISPR gene editing. In this project, we are studying the role of NF1 in formation and propagation as tumor spheroids, as well as the role of NF1 in the hyperactivation of epithelial to mesenchymal transitions in metastasis. We are also characterizing the frequency of NF1 mutations in our HCC patient population here at Ochsner.

**Prospective student project:** The student will be trained in maintaining and propagating the NF1 CRISPR gene-edited and mock-edited cell lines. The student will perform spheroid formation assays, EMT induction assays, as well as molecular analysis to confirm phenotype in both assays specific to how the NF1 deletion alters these processes.

## Project 3

### Lipid-overload induced alterations in hepatocyte Cyclin D1 and IL-33 expression increases susceptibility to hypoxic injury.

Our lab has recently demonstrated that non-alcoholic fatty liver disease leads to decreased expression of the hepatoprotective cytokine IL-33 in conjunction with aberrant Cyclin D1 activation. Using primary hepatocytes and the AML mouse hepatocyte cell line, we are inducing lipid loading and hypoxia in vitro to study the mechanisms which govern IL-33 release and expression and the cross-talk of this signaling pathway with Cyclin D1 expression. Using stably transfected lines expressing Cyclin D1a, Cyclin D1b, or siRNA directed against Cyclin D1 (obtain from the Albrecht lab), we are probing the role of aberrant expression with regard to hepatocyte necrosis under lipid, hypoxia, or combinatorial stress from both variables. We will transition these observations to in vivo studies, using transient liver directed transfections as outline by Jefferey Albrechts lab. Cyclin D1 silencing and overexpression models will be used in conjunction with dietary models of liver steatosis to uncover the role of this expression profile in predicting liver dysfunction following transplant injury. We will employ these studies with the goal of developing a biomarker profile or high/low risk steatotic donor livers. Parallel studies are being designed which target autocrine verses paracrine IL-33 signaling, and the role of nuclear IL-33 as a hepatoprotective mechanism in the same models outline above.

**Prospective student project:** The student will be trained to perform lipid-loading in primary and transformed hepatocyte lines as well as hypoxia / reoxygenation studies using a sterile culture hypoxia chamber. In this model, the student will perform cell fractioning procedures to determine the expression of IL-33 in the hepatocytes as well as the concentration in the surrounding culture media. The effect of Cyclin D1a/b overexpression prior to hypoxia will be determined using transient transfection of the AML and primary hepatocytes.