Sky Progress Report

Period: January-December 2019

Project Title: "Pancreatic cancer and pro-oncogenic collagen: Role of discoidin domain receptors, new potential therapeutics targets"

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Background: One of the striking hallmarks of pancreatic cancer (PC) is the increased deposition of collagen within the tumor tissue. This collagen-enriched (fibrotic) tumor microenvironment is a pathological characteristic of PC, which has been associated with disease aggressiveness. Indeed, evidence indicates that this fibrotic collagen-enriched microenvironment facilitates cell survival and invasiveness of PC cells. Therefore, disrupting PC-collagen interactions may elicit anti-tumor activity.

Our objective is to investigate the therapeutic potential of a unique class of collagen receptor proteins, known as Discoidin Domain Receptors (DDRs), in PC. DDRs belong to a specific type of signaling proteins known as receptor tyrosine kinases (RTKs). RTKs are like electrical switches that turn on the cell's intracellular machinery in response to external signals, which help cells to adapt to their immediate microenvironment. In cancer, RTKs are usually deregulated, and thus shutting down kinase activity can elicit potent anti-cancer effects. Among all the kinases in the human genome, the DDRs are the only ones capable of signaling in response to collagen. Accumulating evidence suggest that DDRs are key players in cancer progression. However, their roles in PC remains to be elucidated. Based on current knowledge, we hypothesize that jamming the stream of signals initiated by DDRs in PC cells residing within the collagen-rich fibrotic microenvironment of the cancerous pancreas may disrupt the pro-oncogenic effects of collagen, altering cancer cell proliferation, survival, metastatic dissemination and drug resistance. Thus, DDRs represent a new therapeutic opportunity that deserves exploration.

Current Studies and Progress:

1. Examining the Role of DDR1 in Overcoming MEK Inhibitor Resistance in PC. It is well established that PC develops drug resistance, which make these tumors difficult to treat. One key target in PC is MEK, a kinase involved in cell proliferation. While this is an important target, PC patients are known to develop resistance through activation of other kinase-dependent pathways. In our SKY project, we are examining whether sensitivity to MEK inhibition in PC cell lines can be restored in the presence of a specific DDR1 inhibitor. We are currently conducting these studies.

2. Elucidating the Pro-Malignant Effect of Collagen and DDRs in Tumor Xenografts. In this funding period, we concluded the animal experiments aimed at examining the role of DDRs and collagen in PC growth. As we reported in June 2018, we wished to examine the effect of collagen on the growth of PC cells expressing or not DDRs. To this end, we co-implanted human PC cells (MiaPaCa cells), expressing or not DDRs, within a scaffold of collagen I, with the goal of mimicking the collagen-rich environment in which PC tumors thrive. These experiments showed that both DDR1 and DDR2 significantly accelerated the growth of PC tumors only in the presence of collagen. Tumors were harvested and processed for various analyses to discover pathways involved in the promotion of tumor

growth by the DDR1/COL1 axis. Using the funds provided by the Sky Foundation, we conducted an analyses of phosphorylated proteins in the tumor extracts (phosphoproteomics analyses) in the Mass Spectrometry facility at WSU. Our goal was to identify downstream effectors of DDRs that may be responsible for the DDR/COL1 oncogenic effect. We compared DDR1/COL1 tumors vs. Control/COL1 tumors. These analyses revealed novel phosphorylated proteins specifically enriched in the DDR1/COL1 tumors. In the top of the list we find Oxysterol-binding Protein-related Protein 4 (ORP4), which is involved in lipid transport, cell signaling, and membrane trafficking, and has been shown to play a role in the survival of rapidly proliferating cells. Interestingly, in another model (fibrosarcoma), we found that the major kinase (by activity) in DDR1/COL1 tumors is Casein Kinase 2, which, interestingly, is the kinase predicted to phosphorylate ORP4 at the sites detected in the MiaPaCa tumors. Together, these new findings suggest the exciting hypothesis that DDR1 induction of PC tumor growth within a pro-fibrotic environment is mediated in part by activation of a metabolic pathway that regulates lipid profile. We will be further exploring this hypothesis in the upcoming year. We hope to elucidate some of the pathways activated by DDRs in PC tumors that contribute to disease progression in response to the fibrotic tumor microenvironment.

3. **Manuscript Submission**. In collaboration with Dr. Crawford's lab, we submitted a joint publication entitled: "Discoidin Domain Receptor 1 (DDR1) is Necessary for Tissue Homeostasis in Pancreatic Injury and Pathogenesis of Pancreatic Ductal Adenocarcinoma" to the American Journal of Pathology. The journal decision is "may be acceptable for publication" upon addressing the reviewers' comments. This is a very positive outcome. We are confident we can address the comments to achieve acceptance.

We are thankful to the Sky Foundation for their support.

Dr. Rafael Fridman