Sky Progress Report

Period: January-December 2020

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). Because DDRs signal in response to collagen, these receptors have been implicated in tumor-cell collagen interactions and therefore they may play a role in PC progression. Based on current knowledge, our studies are focused on elucidating the role and therapeutic potential of DDRs in PC.

Current Studies and Progress:

1. Defining the Pro-Oncogenic Signaling Networks Driven by the DDR1/Collagen Axis in PC. As we reported earlier, we demonstrated that inoculation of human PC cells embedded within a collagen scaffold, designed to mimic the collagen-rich microenvironment of PC tumors, promotes tumor growth and formation of liver metastasis when he cells express DDR1 or DDR2. During this funding period, PC tumors with or without DDR1 expression from xenografts growing within a collagen scaffold were subjected to two major analyses: RNAseq and high-throughput kinase activity analyses to determine the profile of gene expression and the kinases involved in bailer of the DDR1/collagen axis to promote tumor growth. These analyses have concluded, and we are currently testing several candidates identified in these screens.

PDPK1 a new downstream effector of DDR1 and therapeutic target: We are particularly excited by the results of the kinase activity assays. For these analyses we collaborated with Dr. Jean-Phillipe Coppe at the University of California at San Francisco, who developed a high- throughput kinase activity mapping (H-KAM) assay capable of simultaneously measuring the activity of multiple kinase enzymes in biological samples. This assay utilizes 450 biological peptides that correspond to multiple cancer signaling nodes (including >100 kinases, >900 kinase-substrate nodes, and 100's of pathways). We used the H-KAM assay to identify the activity of kinases specifically regulated by DDR1 and collagen in the PC tumor xenografts. These analyses revealed that the activity of PDPK1, a kinase known to be at the root of the PI3K/AKT pathway, is specifically elevated in DDR1-expressing tumors. PDPK1 is a major activator of AKT and thus it has a fundamental role in the initiation of the signaling pathways that contribute to PC progression. Interestingly, recent studies reported that Collagen 11, a major collagen

of fibrotic tumors including PC, promotes PDPK1 stability, thus supporting its kinase activity. This suggests that Collagen 11 in fibrotic PC tumors supports PDPK1 activity to promote malignancy. Importantly, Collagen 11 can activate DDR1 (our data). Collectively, our findings in the DDR1-expressing tumors place DDR1 as a likely mediator of the effects of Collagen 11 on PDPK1 activity. Because a *DDR1* deficiency dampens PC progression (shown in our recently published study), this finding also suggests that targeting PDPK1 in PC may represent a new approach that is worth exploring. Indeed, in spite that PDPK1 is upstream of key oncogenic networks operative in PC, this kinase has been barely explored in PC. Therefore, this is a unique opportunity to examine this novel pathway in DDR1-mediated PC progression. To this end, we plan to examine the Collagen 11/DDR1/PDPK1 network in PC cell lines, xenografts, mouse model of PC, and human samples. We will also test the therapeutic impact of PDPK1 inhibitors in conjunction with DDR1 inhibitors on PC progression in our models. In addition, the RNAseq data, the phosphoproteomics profiles

These Sky Foundation-supported studies are a collaborative effort of three research labs (Fridman, Crawford, Coppe) that share knowledge, reagents and technologies to foster PC research. We conduct weakly meetings to discuss progress and experiments and our planned submission of a joint R01 for early next year.

2. Other Studies in Progress: DDR1 Regulation of Hyaluronic Acid Remodeling. As we reported previously, we continued our studies examining the regulation of KIAA199 (also called CEMIP1) and TMEM2 (CEMIP2) by DDR1 in PC cells, KIAA1199 and TMEM2 are two recently discovered hyaluronidases, enzymes capable of degrading hyaluronic acid (HA), a major component of the extracellular matrix (ECM) and of the fibrotic stroma of PC tumors. HA degradation in the fibrotic stroma facilitates tumor cell invasion by creating a permissive ECM that doesn't constrain tumor expansion. In addition, evidence indicate that the resultant HA degradation fragments regulate cell behavior. Consistent with these concepts, our data suggest that DDR1 expression upregulates the expression of two hyaluronidases in PC cells, which consequently create a permissive ECM that facilitates tumor cell proliferation and invasion. These enzymes were identified in our gene expression analyses of tumor cells expressing DDR1 and treated with collagen, indicating that DDR1 upregulates these proteins. We are currently examining the expression, localization and activity of these two hyaluronidases in PC cells and tumors and their role in regulation of malignant activities, including remodeling of hyaluronic acid in the ECM. In addition, Dr. Marco Prunotto (University of Basel), a collaborator in this project, is producing specific inhibitor to KIAA1199 activity, which we hope to test in our PC models.

3. Other Accomplishments: Publications and Grants. Our paper entitled: "Discoidin Domain Receptor 1 (DDR1) is Necessary for Tissue Homeostasis in Pancreatic Injury and Pathogenesis of Pancreatic Ductal Adenocarcinoma" has been published in the American Journal of Pathology. The journal chose a figure of the paper to use it as the monthly cover of the Journal. We have also submitted in April 2020, an R01 grant to the NIH entitled: "A novel DDR1/KIAA1199 axis in pancreatic cancer progression". Unfortunately, this grant was not funded. Our plan is to submit again next year with the addition of more data.

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