Vitamin D and VDR as possible biomarkers of Pancreatic Cancer

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INTRODUCTION
Pancreatic cancer is hard to detect at its early stages and due to the lack of symptoms (4). Most people would not notice the symptoms which include jaundice, pain in the abdomen and back, weight loss and fatigue (4). The pancreas is a gland behind the stomach and hidden behind other organs, making it hard for health professionals to detect abnormalities during health exams (4). Blood tests, imaging tests, and a biopsy can be used to diagnose pancreatic cancer (4). Because of the nature of the pancreatic cancers’ lack of symptoms, better treatments and prevention are critical.

The purpose of our research was to search for genes that would use the Vitamin D Receptor levels in the body as a biomarker for pancreatic cancer. VDR – vitamin D (1,25- dihydroxyvitamin D3) receptor is a protein coding gene (2). VDR gene encodes the nuclear hormone receptor for vitamin D3 (2). In Pancreatic cancer, stellate cells react to pancreatic cancer cells by surrounding them. The vitamin D receptor acts as a transcriptional regulator of pancreatic stellate cells (PSC) and returns the activated PSC back to their dormant state (3). This induces stromal reprogramming and decreases the tumor volume (3).

For most people, more than 90% of their vitamin D status comes from exposure to sunlight (5). Exposure of the skin to solar ultra-violet B light (280–320 nm) induces cutaneous production of precursors to vitamin D. In addition to vitamin D synthesized endogenously from sunlight, dietary sources of vitamin D include cholecalciferol (D3) that occurs naturally in some animal foods (i.e. fatty salt-water fish, liver, and egg), ergocalciferol (D2) from plants, in vegetable preparations, and fortified foods such as milk and margarine (D2) and D3 (5, 6). 25-hydroxy(OH) vitamin D (D) is the major vitamin D metabolite in humans and is also considered the best indicator of vitamin D status as determined by the sun and diet.

METHODS
• Vitamin D levels and VDR expression and localization in different pancreatic regions such as endocrine islets, in acinar, ductal, and stromal cells can serve as potential biomarkers for pancreatic cancer.
• We searched the NCBI SRA database using the search terms pancreatic cancer with the following parameters: Illumina, RNA, and gene.
• Using the NCBI SRA, RNA sequences of four MisPaCa-2 pancreatic cancer cells were selected; two were untreated and two were treated with conditioned media (Mia-CM) to see what genes were differentially expressed in pancreatic cancer tumors.
• RNA sequences were entered into FastQ for formatting and run in DNA Subway. When DNA Subway finishes the run through Manage Data, FastX, TopHat and cufflinks, we will have a table of differentially expressed genes.
• We can research selected genes from this table to find the genes that have been shown in the work of others to be regulated by Vitamin D and the VDR to be additional genes for inclusion in our research proposal.

RESEARCH PROPOSAL
• VDR is a key regulator of pancreatic stellate cells. Upregulation of VDR expression may be responsible for switching the stellate cells into a barrier phenotype. Therefore, down-regulating VDR expression may change the stellate cells back to the dormant state, ultimately decreasing tumor size.
• We propose to conduct a study using mice given either water (control), water with vitamin D or mice exposed to UVB irradiation to see the effect on VDR protein levels.
• We hypothesize that exposure of mice to UVB light (290–320 nm) or vitamin D supplement will increase vitamin D level (1). We hypothesize this increase in vitamin D would in turn enhance the VDR activity in the mice, resulting in a decrease in pancreatic tumor size.
• The mouse model we will use Pancreatic Cancer PDX tumor bearing mice (J000077960) from Jackson Labs. These mice develop primary malignancy of the pancreatic ductal epithelium (5).
• The mice will be irradiated with UVB range (290–320 nm) for either 13 min (2.5 kJ/m2) or 26 min (5.0 kJ/m2), 40 cm away from the UV lamp (1).
• Tumor volume of the mice will be monitored to verify if returning PSC to a dormant state alters tumor size.
• The pancreas including the tumors and the stromal cells surrounding the tumors will be removed from the mice. The pancreatic tissue will be sectioned and stained to check for histological changes.
• Immunohistochemistry staining using antibodies for the pancreatic cancer marker (CA19-9) and the VDR will be used on the mouse pancreatic tissue sections (8, 9). We will examine the CA19-9 and VDR protein expression patterns to analyze whether UVB and Vitamin D supplement treatments impact tumor volume.

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REFERENCES
5. The Jackson Laboratory. [n.d.]. Retrieved April 21, 2016, from https://www.jax.org/record/pdbDetailsDetailsModelID=000077960