Functional Genomics of Anaplastic Wilms' Tumor

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Specific Aims

Wilms' tumor (also known as nephroblastoma) is the most common kidney cancer found in children, and most (over 90%) respond favorably to traditional cancer treatments (surgery, chemotherapy, and radiation). However, those with the anaplastic variant of the disease tend to respond poorly, with overall survival rates of less than 30 percent. Anaplastic Wilms' tumor is an unfavorable subtype of classic Wilms' tumor with relatively little resource development, basic science or translational research relative to other forms of childhood cancer. The typical treatment protocol for children with anaplastic histology is increased chemotherapy, often resulting in significant long term side effects for those few who do survive. Therefore, novel treatment strategies are greatly needed. This study seeks to characterize the genomic landscape of Wilms' tumor and explores novel drug combinations which might be more effective and less toxic for children with anaplastic disease.

AIM 1. Screen and Sequence a Series of Wilms' Tumor Primary Cell Cultures, Cell Lines, and Mouse Explants (Anaplastic and Non-anaplastic)

This aim takes advantage of Wilms' tumor resources already at cc-TDI; 4 primary cell cultures, 2 anaplastic cell lines, 7 non-anaplastic cell lines, 2 anaplastic mouse explants, and 2 blocks of FFPE tissue from anaplastic Wilms tumor patients. These resources will be cultured and outsourced to The Beijing Genomics Institute for whole exome and deep transcriptome sequencing. We will then perform a chemical screen of n=60 targeted therapy agents using our EpMotion5075 liquid handling robot. This drug screen was recently designed and built at cc-TDI and is comprised of 60 compounds known to inhibit a variety of biological pathways involved in cancer progression. Several of the above resources have been cultured, screened, and sequenced, and others remain in culture and await further testing.

AIM 2. Define the Classes of Drugs Effective against Anaplastic Wilms Tumor and Develop a Predictive Drug Combination as an Ideal Treatment Strategy

To accomplish this aim, we will integrate chemical (drug) sensitivity data with DNA mutations/amplifications and RNA expression data and will employ a probabilistic Boolean modeling approach which was developed at cc-TDI and recently published (BMC Bioinformatics, 2013. 14:239). A prediction of effective drug combinations against Anaplastic Wilms tumor will be developed based on collected data and application of our predictive Boolean model. Both anaplastic and non-anaplastic models will be compared.

AIM 3. In-vitro, In-ovo, and In-vivo Validations of Drug Combination Prediction

Next, we will validate the predicted drug combination against Anaplastic Wilms' tumors in-vitro, in-vivo, and ex-ovo. To accomplish these validations, we will perform the following experiments against both our anaplastic and non-anaplastic resources.

-Western blot analysis' will be performed to determine protein expression levels of important proteins before and after drug exposures to determine which proteins are key drivers of the disease. As a possible example, Brefeldin A is a compound within our drug screen which showed significant activity (cancer cell growth inhibition) against two anaplastic Wilms tumor cell lines (Wit49 and 17.94). Brefeldin A is an ATPase inhibitor which is known to interfere with protein transport from the Endoplasmic Reticulum to the Golgi and has been shown to both reduce VEGF expression and induce apoptosis in refractory CLL cells via p53-independent pathways. We could use western blots to examine VEGF levels following Brefeldin A exposure. We could also explore levels of other known anti-apoptotic proteins such as BCL-2, BCL-x, MCL-1, and XIAP to determine if Brefeldin A is an effective apoptosis-inducer despite the presence of these proteins, or if Brefeldin A exposure directly inhibits these proteins. Amount of VEGF secreted by the cells can also be explored using an Enzyme-linked immunosorbent assay (ELISA). Amount of apoptosis could be determined using our flow cytometer and an Active Caspase-3 Mab Apoptosis FITC kit coupled with annexin V-FITC/propidium iodide (PI) double-staining.

-Other possible in-vitro experiments could include…...

-The Chick Chorioallantoic Membrane assay can be used to explore angiogenesis, tumor invasion and metastasis potential. cc-TDI uses an ex-ovo quail egg CAM assay. The CAM assay provides a unique and inexpensive model and is a well-established xenograft system. Wilms' tumor cells will be vitally labeled with carboxyfluorescein succinimidyl ester (CFSE), and then grafted with Matrigel onto the CAM of fertilized quail eggs. These will then be incubated with our predicted drug combination or buffer (as a control) in both anaplastic and non-anaplastic xenografted CAMs. Tumor and vascular growth or reduction will be observed and recorded.

-Mouse studies using orthotopically injected NOD/SCID immunodeficient recipient mice will be performed to explore effects on tumor development or regression against the proposed combinational therapies.