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Abstract

Our R21 NIH application is focused on the preclinical evaluation of the efficacy and mechanism of action for an alkylated polyamine analogue diethylnorspermine (DENSPM) in treating pheochromocytoma/paraganglioma. In Aim 2b of our R21 application we proposed to explore suitable biomarkers to predict plausible clinical benefits, endpoints and combination treatments in either parental hPheo1 or DSDHB mutant hPheo1 cells and in animals carrying xenograft tumors derived from these cells.

Recently we have performed an RNAseq analysis that identified several informative candidates to explore, to delineate the mechanism of polyamine inhibitor action in treating neuroendocrine cancers, including pheochromocytoma. Specifically, it appears that polyamine inhibitors interfere with two key biochemical pathways critical for rapidly dividing cancer cells, mitochondrial stress response and lipid (fatty acid) metabolism. We have recently performed real-time PCR analysis, to unequivocally confirm RNAseq data. We identified genes that drastically change their expression as a result of polyamine treatment. Moreover, we also identified genes differentially expressed in parental and DSDHB mutant hPheo1 cells. This is important, since cancers with DSDHB mutation are a lot more likely to present as a metastatic uncurable disease in patients.

In the following months we plan to corroborate our results through several other biochemical and molecular biology approaches. Specifically, a FVSP student in the lab will perform Western Blotting analysis to examine protein expression and phosphorylation in parental hPheo1 or DSDHB mutant hPheo1 cells treated and untreated with DENSPM. To specifically define the role of lipid pathway, cells will be treated with specific inhibitors of fatty acid desaturases (SCD1 and FADS2) and their viability and proliferation will be compared.

Inducible transcription factors [hypoxia-inducible factor (HIF) α/β dimers] are key gatekeepers of the response to low oxygen. It has been noted that tumors harboring SDHB mutations have a strong hypoxic signature. Pheocromocytoma have been historically closely associated with hypoxia, because these highly vascularized tumors arise either in tissues known to be susceptible to oxygen deprivation (i.e., cells of the adrenal medulla and organ of Zuckerkandl) or in cells known to serve as oxygen sensors (i.e., neurons of the carotid body). Hence, elucidation of hypoxia and hypoxic pathways can shed light on the genetic and metabolic alterations observed in these tumors and connection from metabolic alterations to tumorigenic process. Pseudohypoxia and HIFs are known to be involved in other hallmark cancer pathways that sustain tumor cell growth, vascularization, and proliferation.

To verify induction of the hypoxia response program, the FVSP student will verify hypoxia marker gene expression in untreated and DENSPM treated hPheo1 and hPheo1_DSDHB cells. S/he will examine the expression of genes encoding the E1B 19K/Bcl-2-binding protein Nip3 (BNIP3) and fructose-bisphosphate aldolase (ALDOA), two established hypoxia marker genes by qRT-PCR and differential expression will be calculated using the standard ΔΔCt method. S/he will also assess HIF1a RNA expression in parallel. RNA concentrations will be compared between hPheo1 and DSDHB cells with and without treatment. Differences in RNA concentrations will be analyzed by using Student's t-test on 5 or more independent cell or tumor samples. If the result from this experiment are not conclusive (due to only a few markers examined), we will perform a more thorough qRT-PCR analysis of the 14 genes that constitute the hypoxia metagene signature (ALDOA, MIF, TUBB6, P4HA1, SLC2A1, PGAM1, ENO1, LDHA, CDKN3, TPI1, NDRG1, VEGFA, ACOT7 and ADM) as previously described.