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Characterization of Genes in Common to Pancreatic Cancer Metastasis and Neurosciences

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This study focused on genes with established roles in neurobiology / neurodegenerative disease in pancreatic ductal adenocarcinoma (PDAC). These genes had been identified as highly modulated from analyzing a microarray of PDAC cells re-isolated from a pancreatic cancer liver metastasis rat model. Fifteen genes were selected using IPA software and gel electrophoresis to show the basal expression of these genes. A detailed study was performed with two genes from these to further portray their characteristic roles in PDAC. These were Ly6/neurotoxin (Lynx1), which has a role in modulating nAChRs functions and optineurin (OPTN), which is linked to several neurodegenerative diseases including glaucoma and amyotrophic lateral sclerosis.

A systematic approach was taken to study the two genes, starting with down-regulation by specific siRNAs and confirming the KD at both, mRNA and protein levels. The consequences of the gene's altered expression on cellular phenotype were identified in BXPC3, Miapaca and Suit2-007 PDAC cells by cellular assays for proliferation, migration and colony formation. In addition, microarrays were performed to study the genetic alterations following knockdown of the two genes, and finally, the effects of some drugs were studied, which influence the expression of the two genes.

OPTN was second highest expressed among autophagy genes in PDAC in publically available data, topped only by SQSTM1. Knockdown of this gene was associated with insignificant changes of cell proliferation, but with increased migration and severely reduced clonogenicity of respective PDAC cells. A microarray analysis, confirmed later for some genes by WB, revealed altered expression of 52 common genes in both Miapaca and Suit2-007 cells. These included reduced expression of CDK6, HSP90 and PRMT6 as well as up-regulation of LAMP2 and ATF4. The reduced clonogenicity may be attributed to the reduction of PRMT6.

It was hypothesized that OPTN KD causes a state of autophagy inhibition that the cells tried to counteract by different mechanisms. OPTN KD was associated with activation of ER stress response governed by up-regulation of the PERK/pEIF2α/ATF4 pathway as well as down-regulation of heat shock proteins as HSP90, up-regulation of chaperone mediated autophagy through overexpression of LAMP2, the main controller of this autophagy pathway; and slight activation of ROS. All these mechanism contribute to the induction of autophagy and hence the

observed mild effect following knockdown. PARP cleavage was induced following OPTN modulation, which can be mediated through enhanced ER stress.

In comparison, effects to Lynx1 KD by either siRNA or CRISPR-Cas9 genome editing were more pronounced with reduced proliferation, colony formation and increased migration. The nAChRs antagonist benzethonium chloride caused reduced survival of PDAC cells as well as reduced expression of Lynx1 at protein level. Lynx1 KD was associated with inhibition of the PI3K/Akt/mTOR -signaling pathway as well as down-regulation of many proteins as PRAS40, FAK, pAkt-ser473 that explain the reduced proliferation and colony formation but not the increased migration. This widespread degradation of molecules at protein level was associated with increased ROS production and induced autophagy as observed by overexpression of LC3b and increased numbers of autophagic vacuoles. mTOR pathway inhibition contributed to the induction of autophagy as well as apoptosis, observed with apoptotic features by Hoechst staining and increased percentage of cells stained with Annexin V- FITC, as well as reduced transcription of several molecules such as MYC, recorded by microarray analysis. Disease and function analysis of the microarray revealed reduced DNA repair mechanisms, reduced proliferation as well as increased chromosomal aberrations and increased apoptosis of the cells following KD.

Increased ROS production and mTOR inhibition was estimated as a factor triggering the changes observed following KD as DNA damage, induction of autophagy and apoptosis. Induction of autophagy may lead to the observed increased migration. Lynx1 knockout clones failed to establish a tumor in vivo or regressed over time when compared with respective controls following their intraportal injection, owing to many defective proteins. A TCGA study revealed low average expression of nicotinic receptors, and above average expression of Lynx1 and mTOR molecules in PDAC.

In summary, the area in common between neurology and cancer is not yet clearly defined, but shows a growing list of convincing connections. Neurologic genes may have some controlling roles on the growth and development of cancer in general and of PDAC in particular that can expose an interesting scope in understanding the biology and open new avenues for cancer treatment. Deregulation of autophagy as a mechanism common between neurodegenerative disease and cancer may represent a way through which some neurologic genes control and affect cancer behavior.