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Identification and Characterization of Candidate Genes Regulating Tumor-initiating Cell Activity in Human Pancreatic Cancer

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers due to its rapid progression, frequent local recurrence and distant metastases. It has been shown that pancreatic cancer progression is driven and maintained by a succession of transiently active tumor-initiating cell (TIC) clones. How TIC activity is regulated in PDAC is completely unknown. To gain more insights into the mechanisms of TIC activation and its key regulators, an *in vivo* mutagenesis screen using a gene-activating lentiviral vector (trapping vector) was performed The potent promoter-enhancer vector elements can lead to the overexpression of neighboring genes, while the strong splice donor and weak acceptor enables *trans*-splicing of vector parts into nearby genes, leading to overexpression of truncated genes in the vicinity of the vector integration site (IS). It was hypothesized that overexpression or truncation of genes regulating pancreatic TIC activity could induce self-renewal and thereby long-term activity of TIC clones.

Initially, patient-derived primary PC1 cells were transduced with the trapping or control vector and then subjected to three generations of serial xenotransplantation. Subsequently, linear amplification-mediated PCR (LAM-PCR) combined with Illumina MiSeq sequencing was conducted to trace the vector integration sites, assess the TIC clonal activity, and identify PTAR candidate genes. Strikingly, the trapping vector showed 4 shared integration sites (SISs), which were stable through *in vivo* passaging, a hallmark never observed in the control group. Next, qPCR and RNA sequencing were performed to check expression of SIS neighboring genes. Remarkably, overexpression of GNB1 and RABL2A in the trapping vector group was confirmed, compared to control, which were selected for further investigation.

To validate if GNB1 overexpression leads to long-term TIC activation, GNB1 overexpressing cells were serially transplanted and then LAM-PCR was further conducted. Strikingly, LAM-PCR results confirmed the gene-activating screening results, as overexpression of GNB1 maintained long-term activity of contributing TIC clones in serial transplantation. Currently, RABL2A is still under investigation.

To address the influence of GNB1 on cell proliferation, *in vitro* cell proliferation assay, cell cycle and apoptosis analysis, as well as *in vivo* tumor growth assay were performed. Strikingly, knockdown of GNB1 significantly impaired *in vitro* cell proliferation and tumor growth *in vivo.* Furthermore, GNB1 knockdown led to an increase in apoptosis and cell cycle arrest. Rescue experiments inverted the phenotype, functionally confirming the knockdown specificity. Also, overexpression of RABL2A increased cell proliferation and tumor growth *in vitro* and *in vivo*; however, the effect was not significant.

Since previous work has shown that RAS-RAF-MEK-ERK pathway plays a central role in initiation and maintenance of pancreatic cancer, ERK1/2 phosphorylation after overexpression or knockdown of GNB1 was checked. It was observed that increased after overexpression and correspondingly phosphorylation decreased after knockdown of GNB1. As ERK phosphorylation is known to contribute to the progression of many cancer entities, GNB1-mediated increase in ERK phosphorylation may underlie our findings that knockdown of GNB1 impaired pancreatic cancer cell proliferation and tumor growth.

This work identified candidate genes potentially regulate pancreatic cancer TIC activity through gene activation *in vivo* screen. Further investigation of candidate gene GNB1 demonstrated that overexpression of GNB1 maintained long-term TIC activation and knockdown of GNB1 impaired pancreatic cancer cell proliferation and tumor growth. Future work should focus on investigating the molecular mechanisms that underlie the observed phenotypic change caused by deregulated GNB1 to better understand the mechanism of the regulation of pancreatic cancer TIC activity.