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Functional Genetic Screen to Identify Drivers of Pancreatic Neuroendocrine Tumor Pathogenesis

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BACKGROUND: RABL6A is an oncogenic driver of pancreatic neuroendocrine tumor (pNET) cell proliferation, survival and tumor growth in vivo. RABL6A acts through inhibition of the Rb1 tumor suppressor and activation of Akt-mTOR signaling. However, rescue experiments suggest that other, currently undefined pathways are also required for RABL6A's role in pNETs. To identify those novel RABL6A-regulated drivers of pNET pathogenesis, we used a cell-based Sleeping Beauty (SB) genetic screen wherein a mutagenic DNA segment (transposon) is randomly integrated into the pNET genome by a SB transposase. This system allows the identification of genes whose alteration functionally enables pNET cell growth in the absence of RABL6A.

METHODS: BON-1 pNET cells stably expressing the SB100 transposase were generated and transiently transfected with the "T2Onc3' transposon or vector control. RABL6A was silenced using lentiviral shRNA vs control, and rescue of colony formation in T2Onc3/RABL6A deficient cells relative to controls was measured. Pooled, bar-coded genomic DNA from T2Onc3/control and T2Onc3/RABL6A deficient cells was sequenced (Illumina) to identify common insertion sites (CIS) in the genome of rescued colonies. Genes whose activation (likely oncogenes) or inactivation (likely tumor suppressors) occurred frequently in the rescued cells were shortlisted by bioinformatic analyses for validation in biological assays.

RESULTS: Reduced growth of BON-1 SB100 cells following RABL6A loss was partially rescued by T2Onc3 expression, demonstrating success of the screen. A pilot run of Illumina sequencing identified several candidate pNET driver genes: 4 were overexpressed in rescued cells (NCAPD2, MYO1E, SPON1, LCOR) while 3 were inactivated (APBA2, LRMDA, TSC22D1).

CONCLUSION: SB transposition is a powerful mutagenesis system commonly used in mouse models. Its successful application here in a cell-based context provides a simple and relatively inexpensive functional screen for drivers of RABL6A-mediated pNET pathogenesis. Initial data are promising and will be strengthened by ongoing bioinformatics and validation studies.

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