**Background**

**RABL6A regulation of Myc expression and activity promotes cell cycle progression and survival of PNET cells.**

- **RABL6A** is a novel GTPase required for pancreatic NET (PNET) cell proliferation and survival.
- RABL6A promotes G1-S progression in PNETs through retinoblastoma (Rb1) tumor suppressor inactivation, but other unidentified pathways also contribute to RABL6A-mediated cell growth.
- Microarray data suggested Myc activation might be involved. Given the central role of Myc in cancer signaling, this was investigated in role of RABL6A-driven PNET cell proliferation and survival.
- These studies reveal RABL6A is a new essential regulator of Myc expression and activity, advancing our understanding of Myc regulation and strengthening the potential value of therapeutically inhibiting RABL6A function in PNET patients.

**Hypothesis**

RABL6A regulation of Myc expression and activity promotes cell cycle progression and survival of PNET cells.

**Methods**

- **Introduction**
  - Understanding of molecular mechanisms underlying neuroendocrine tumor (NET) pathogenesis is needed to improve treatment of NET patients.
  - RABL6A is a novel GTPase required for pancreatic NET (PNET) cell proliferation and survival. We found that RABL6A promotes G1-S progression in PNETs through retinoblastoma (Rb1) tumor suppressor inactivation, but other unidentified pathways also contribute to RABL6A-mediated cell growth.
  - Microarray data suggested Myc activation might be involved. Given the central role of Myc in cancer signaling, this was investigated in role of RABL6A-driven PNET cell proliferation and survival.
  - These studies reveal RABL6A is a new essential regulator of Myc expression and activity, advancing our understanding of Myc regulation and strengthening the potential value of therapeutically inhibiting RABL6A function in PNET patients.

- **Results**
  - **Figure 2:** Microarray analyses predict RABL6A loss impairs Myc pathways. A) Gene expression alterations in hyperplasia, Castrated, and ER-treated xenografts in tissues vs. control xenografts. B-D) Heat map of microarray data shows that RABL6A depletion in BON cells results in altered expression of many genes involved in Myc signaling, including Myc itself. Red, relatively increased expression; blue, relatively decreased expression.

- **Figure 3:** Loss of RABL6A downregulates endogenous Myc mRNA and protein expression. A) Western blot analysis of indicated proteins in total control and RABL6A-depleted BON-1 and G2P1 cells. Relative cell numbers from experimental samples shown below. B) Quantitative RT-PCR of Myc mRNA levels in control and RABL6A-depleted BON-1 cells. n=3 C) Dot blot analysis of indicated proteins in BON-1 and control cells. n=3 D) Immunoblot analysis of indicated proteins in BON-1 and control cells. n=3

- **Figure 4:** Myc expression rescues the cell cycle arrest phenotype caused by RABL6A loss and dictates sensitivity to JQ-1. Analyses performed in control and RABL6A-depleted BON-1 cells expressing vector (VEG) or the shRNA inducible Myc-ER (Myc-ER) cDNA. A) Flow cytometric analyses of cell cycle populations by propidium iodide (PI) staining in control and Myc-ER cells. B) Flow cytometric analysis of cell cycle populations by propidium iodide (PI) staining in control and Myc-ER cells. C) Flow cytometric analysis of cell cycle populations by propidium iodide (PI) staining in control and Myc-ER cells. D) Flow cytometric analysis of cell cycle populations by propidium iodide (PI) staining in control and Myc-ER cells. E) Flow cytometric analysis of cell cycle populations by propidium iodide (PI) staining in control and Myc-ER cells. F) Flow cytometric analysis of cell cycle populations by propidium iodide (PI) staining in control and Myc-ER cells.

- **Figure 5:** RABL6A affects DNA damage checkpoint signaling. A) Cell cycle arrest measured by 5-bromo-2-deoxyuridine incorporation (EdU) in BON-1 cells following RABL6A knockdown (KD1, KD2). B) Flow cytometric analyses of cell cycle populations by propidium iodide (PI) staining in control and Myc-ER cells. C) Flow cytometric analysis of cell cycle populations by propidium iodide (PI) staining in control and Myc-ER cells. D) Flow cytometric analysis of cell cycle populations by propidium iodide (PI) staining in control and Myc-ER cells. E) Flow cytometric analysis of cell cycle populations by propidium iodide (PI) staining in control and Myc-ER cells. F) Flow cytometric analysis of cell cycle populations by propidium iodide (PI) staining in control and Myc-ER cells.