University of Iowa Neuroendocrine Tumor Program

University of Iowa Holden Comprehensive Cancer Center — University of Iowa Health Care

Investigating the role of RABL6A in pancreatic neuroendocrine tumor progression in vivo

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SUPPORTED BY THE IOWA NEUROENDOCRINE TUMOR SPORE

Background and Rationale

- **Pancreatic neuroendocrine tumors (PNETs)**
  - Rare, indolent malignancies that have risen greatly in incidence
  - Driven by activated Akt/mTOR pathway, inactivated Rb1 tumor suppressor, as well as other poorly defined pathways
  - Reliable prognostic markers are lacking, and new therapeutic targets are needed
- **RABL6A**: upregulated in PNETs, activates Akt/mTOR, inhibits Rb1, and drives PNET cell proliferation and survival *in vitro*
  - Data suggest it controls many other clinically relevant pathways (e.g., VEGFR) in PNETs

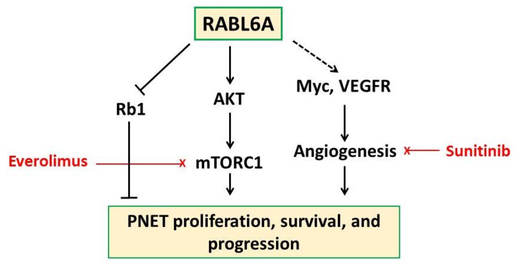

Figure 1: Schematic depicting RABL6A as an upstream regulator of Rb1, Akt/mTOR pathway, and HIF1α/VEGFR signaling in PNET cells. Perpendicular bars- inhibitory event, arrows- activating events, and dashed lines, predicted associations. Shown in red are FDA approved drugs targeting specific pathways in PNET pathogenesis**. [Hagen et al., Cancer Research, 2014; Umesalma et al., JCI, 2019]**

Hypothesis

RABL6A promotes PNET angiogenesis and development *in vivo*

Approach

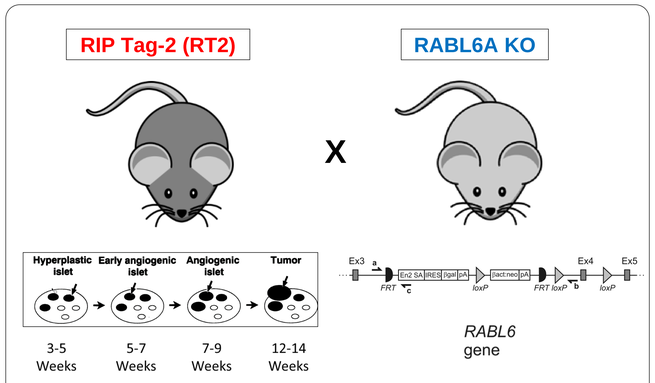

Figure 2: RT2 mice, a robust PNET genetic model, express oncogenic SV-40 large T-antigen under the rat insulin promoter. This transforms β cells into hyperplastic islets, angiogenic islets, and insulinomas in a time-dependent fashion. RT2 mice are crossed with RABL6A KO mice (generated by insertion of a gene-trap sequence between exon 3 and 4 of the *RABL6* gene).

Experimental groups:

A. **WT** B. **RT2** C. **RABL6A KO** D. **RT2, RABL6A KO**

Results

Loss of RABL6A promotes survival of RT2 females

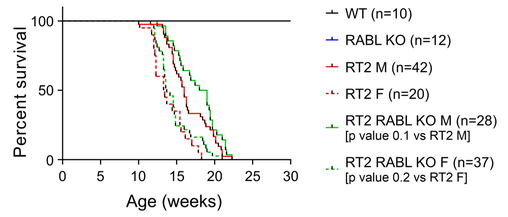

Figure 3: Kaplan-Meier survival curve showing RT2 females died earlier than the males. The loss of RABL6A in RT2 females improves survival. Differences between survival curves were compared using the log-rank test.

Gross anatomy of PNETs, angiogenic-, and hyperplastic-islets

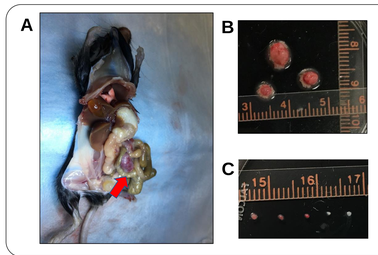

Figure 4: A) A dissected 16-week-old RT2 male with multiple angiogenic PNETs. **B)** PNETs from a 16-week-old RT2 male. **C)** Photograph of 3 angiogenic islets (left) and 2 hyperplastic islets (right).

Tumor burden of RT2 vs RT2; RABL KO mice

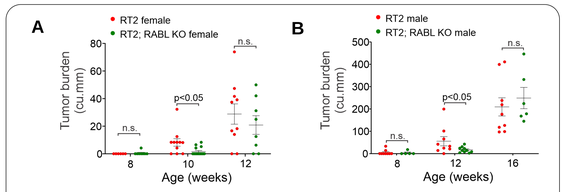

Figure 5: Tumor burden of **(A)** female and **(B)** male RT2 mice vs RT2; RABL6A KO mice were calculated by addition of tumor volumes (i.e. 0.52 x width x width x length). Data are presented as mean ± SEM.

Plasma insulin levels of RT2 vs RT2; RABL KO mice

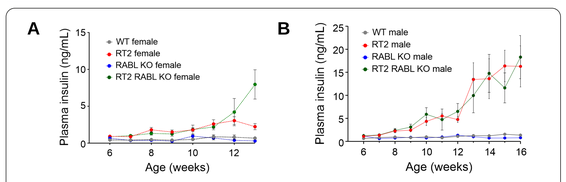

Figure 6: ELISA analyses of plasma insulin in **(A)** females and **(B)** males of the indicated genotypes. Values are presented as mean ± SEM.

Conclusions

- RABL6A loss reduces tumor burden in RT2 mice, which correlated with a trend towards improved survival
- In RT2 females, RABL6A loss reduced PNET tumor burden (endocrine area), number of angiogenic islets, and number of mitoses within islets
- In vivo results are consistent with decreased mRNA expression of established pNET oncogenes and angiogenic factors, *c-Myc* and *Vegfa*, in RABL6A-deficient mouse pancreatic islets

RABL6A loss reduces the pancreatic endocrine area in RT2 females

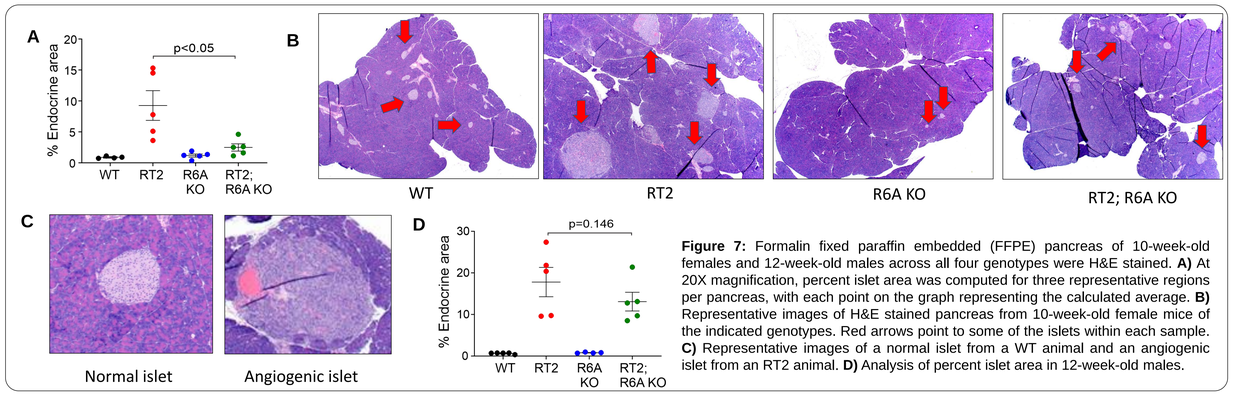

Figure 7: Formalin fixed paraffin embedded (FFPE) pancreas of 10-week-old females and 12-week-old males across all four genotypes were H&E stained. **A)** At 20X magnification, percent islet area was computed for three representative regions per pancreas, with each point on the graph representing the calculated average. **B)** Representative images of H&E stained pancreas from 10-week-old female mice of the indicated genotypes. Red arrows point to some of the islets within each sample. **C)** Representative images of a normal islet from a WT animal and an angiogenic islet from an RT2 animal. **D)** Analysis of percent islet area in 12-week-old males.

RABL6A loss reduces angiogenic islet formation in RT2 females

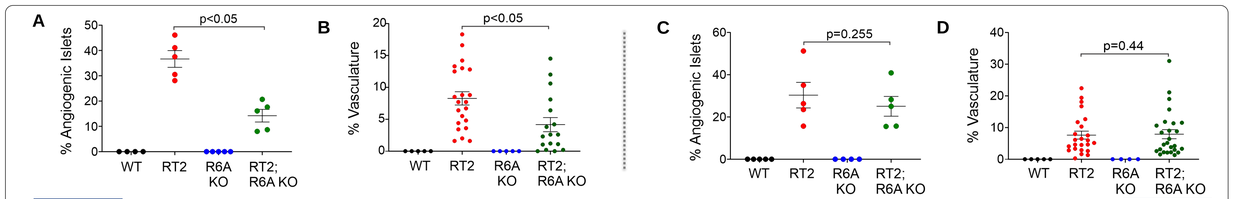

Figure 8: Percentage angiogenic islets and vasculature in 10-week-old female **(A,B)** and 12-week-old male **(C,D)** pancreas.

RABL6A promotes islet cell mitosis in RT2 females

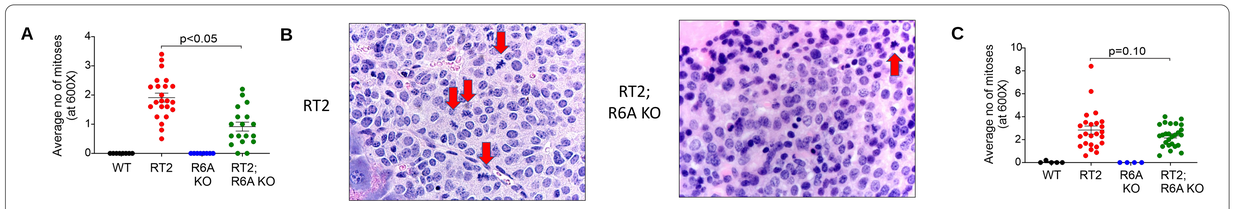

Figure 9: A) Average number of mitoses per field of view at 600X magnification per islet was quantified in 10-week-old female mice. **B)** Representative islet images in RT2 and RT2; RABL6A KO females showing mitotic figures (highlighted with red arrows). **C)** Comparative evaluation of islet mitoses in 12-week-old males.

RABL6A promotes Myc and VEGF transactivation *in vivo*

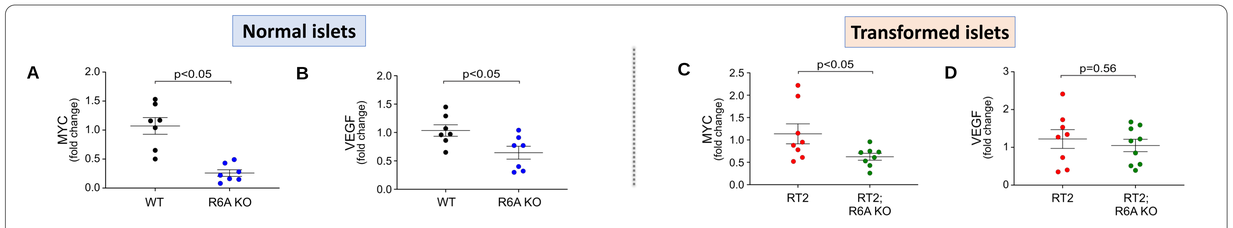

Figure 10: (A,B) Comparison of Myc and VEGF mRNA levels in the normal islets of 8-10-week-old WT vs RABL KO female mice. **(C,D)** Comparison of Myc and VEGF mRNA levels in the transformed islets of 10-week-old RT2 vs RT2; RABL KO female mice

Future Directions

- Immunohistochemical analysis of RABL6A, Ki-67, CC3, Myc, Akt/mTOR, Rb1, and angiogenic markers in pancreas
- Western analysis of isolated islets and tumor samples

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