

# B-17

## A New Critical Regulator of Akt-mTOR Signaling in Pancreatic Neuroendocrine Tumor Cells

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**BACKGROUND:** A better molecular understanding of pancreatic neuroendocrine tumors (PNETs) is needed to improve patient diagnosis and treatment. PI3K/ Akt/mTOR signaling is aberrantly activated in PNETs, leading to therapies targeting the pathway but tumor resistance invariably develops. We discovered that RABL6A, a novel oncoprotein amplified in PNETs, is a key regulator of this clinically relevant pathway.

**METHODS:** RABL6A was silenced while Nek2 or Akt were overexpressed in PNET cells. Transcripts were assayed by microarray and qRT-PCR, proteins by immunoblotting, and cell proliferation/survival by cell counts, trypan blue exclusion and EdU positivity.

**RESULTS:** RABL6A loss caused PNET cell cycle arrest that coincided with Akt pathway inactivation. Immunoblotting revealed loss of Akt Ser-473 phosphorylation following RABL6A depletion along with impaired S6K phosphorylation, a downstream target of Akt-mTOR signaling. Multiple mechanisms control Akt-S473 phosphorylation. We demonstrated mTORC2, the kinase that phosphorylates Akt at Ser-473, remains intact and active in RABL6A deficient cells. Moreover, overexpression of Nek2 kinase, which promotes Akt-S473 phosphorylation and is downregulated by RABL6A loss, was unable to rescue the RABL6A knockdown phenotype. Our findings suggest protein phosphatases are activated by RABL6A loss and promote

Akt-S473 dephosphorylation. Given the central role of Akt in tumorigenesis, we hypothesized that reinstating its activity may rescue the arrest phenotype caused by RABL6A loss. Akt restoration in RABL6A-depleted cells partially rescued the G1 phase arrest and induced S phase entry but was insufficient for mitosis, suggesting RABL6A regulates other factors required for cell division. Importantly, as predicted, RABL6A expression sensitized PNET cells to inhibitors of Akt and mTOR (MK-2206 and rapamycin).

**CONCLUSION:** This work demonstrates that RABL6A is a new essential activator of Akt-mTOR signaling in PNET cells whose expression dictates response to clinical drugs targeting the pathway. RABL6A may be a new predictive biomarker and target for anticancer therapy in PNET patients.