RABL6A-dependent regulation of c-Myc expression and activity is essential for cell cycle progression and survival of pancreatic neuroendocrine cells

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SUPPORTED BY THE IOWA NEUROENDOCRINE TUMOR SPORE

Background

Introduction

- Understanding of molecular mechanisms underlying neuroendocrine tumor (NET) pathogenesis is needed to improve treatment of NET patients.
- RABL6A is a novel GTPase is 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 contributed to RABL6A-mediated cell growth.
- Microarray data suggested Myc activation might be involved. Given the central role of Myc signaling in cancer, we investigated its role in RABL6A driven PNET proliferation and survival.
- These studies reveal RABL6A is a new essential regulator of c-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

RABL6A
- Oncogenic GTPase
- Marker of poor survival in PDAC (Muniz, 2013) and breast cancer (Li, 2013)
- Gene is amplified in >50% of primary human PNETs
- Promotes G1-S progression in PNET cells thru Rb1 inactivation

PNETs
- Growing clinical challenge
- Mechanisms underlying PNET development are poorly understood, biomarkers needed
- Akt amplified and mTOR signaling activated in PNETs, targeted clinically

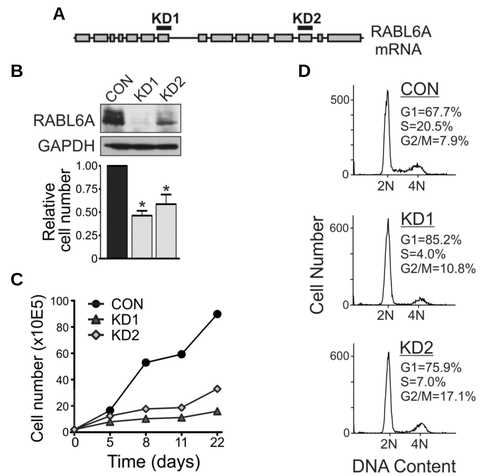

Figure 1: RABL6A is essential for PNET cell proliferation. A) Schematic of RABL6A mRNA regions targeted by the shRNAs KD1 and KD2. B) Western blotting shows effective RABL6A knockdown in BON-1 PNET cells compared with control (CON). Graph, cell numbers are reduced after RABL6A knockdown relative to CON cells. (*, $p < 0.05$) C) Long term analysis of cell number after RABL6A knockdown. D) Flow cytometric analyses of DNA content show RABL6A knockdown causes a predominant G_1 phase arrest.

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Results

A

Ingenuity Canonical Pathways	Genes Regulated		
	2-3 fold	*3-4 fold*	*>4 fold*
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	KAT2B, CDC25C, TOP2A, PKMYT1, PLK1, CHEK2, SKP2, CHEK1, CCNB1	CKS1B	
Mitotic Roles of Polo-Like Kinase	KIF23, CDC25C, PLK4, PTTG1, PRC1, PKMYT1, FBXO5, PLK1, CHEK2, KIF11, CDC25A, CCNB1		
Cell Cycle Control of Chromosomal Replication	MCM5, MCM2, CDK4, CDK6, CHEK2, MCM7	CDK2	CDC45
Role of CHK Proteins in Cell Cycle Checkpoint Control	CDC25C, RFC4, PLK1, RFC5, CHEK2, CDC25A, CHEK1, RFC3	CDK2	E2F2
Mismatch Repair in Eukaryotes	RFC4, RFC5, EXO1, RFC3	FEN1, POLD1	
Molecular Mechanisms of Cancer	GAB2, ADCY4, GNA11, CHEK1, MYC, NLK, FANCD2, RHOD, ARHGEF2, CHEK2, CDC25A, CAMK2B, CCNE2, PMAIP1, CDC25C, ADCY3, SMAD6, CDK6, TCF3, CDK4, GNAO1, TGFB3, ELK1	AKT1, SMAD7, CDK2	E2F2
Hereditary Breast Cancer Signaling	CDC25C, FANCD2, H2AFX, CHEK2, CDC25A, CHEK1, CCNB1	FANCG, AKT1, BLM	
ATM Signaling	CDC25C, FANCD2, H2AFX, CHEK2, CDC25A, CHEK1, CCNB1	BLM, CDK2	
Cell Cycle Regulation	Myc, CCNE2, CDK4, CDK6, TGFB3, SKP2, CDC25A, CCNB1	CCNA2, CDK2	E2F2

B

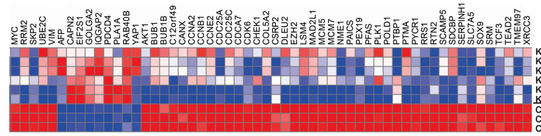


Figure 2: Microarray analyses predict RABL6A loss impairs Myc pathways. A) Gene expression alterations in Ingenuity Canonical Pathways. $p<0.05$, ≥ 2-fold change in mRNA, underlined genes are up regulated whereas all other genes are down regulated. B) Heat map of microarray data shows that RABL6A depletion 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.

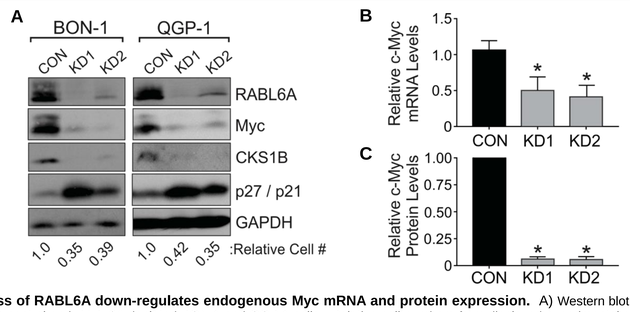


Figure 3: Loss of RABL6A down-regulates endogenous Myc mRNA and protein expression. A) Western blot analyses of indicated proteins in both control and RABL6A depleted BON-1 and QGP-1 cells. Relative cell numbers from displayed experimental samples shown below. B) Quantitative RT-PCR of c-Myc mRNA levels in control and RABL6A depleted BON-1 cells. n=3 C) Densitometry analysis of c-Myc protein expression in BON-1 vector control cells. n=3

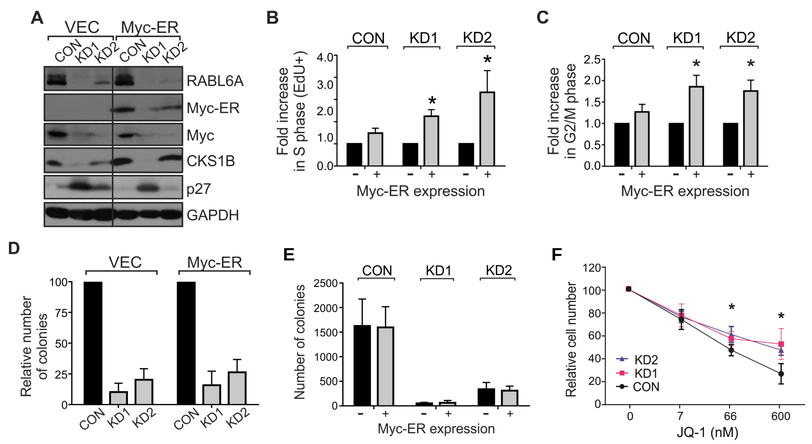
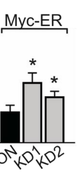
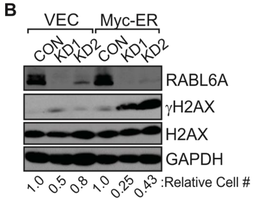


Figure 4: Myc expression rescues the cell cycle arrest phenotype caused by RABL6A loss and dictates sensitivity to JQ-1. Analyses performed in control and RABL6A depleted BON-1 cells expressing vector (VEC) or the tamoxifen inducible MYC-ER (Myc-ER) fusion protein. A) Western blot analyses of indicated proteins in VEC and Myc-ER cells. B) Cell cycle analysis by incorporation of 5-ethynyl-2-deoxyuridine (EdU) into VEC and Myc-ER cells. n=3; *, $p<0.05$. C) Flow cytometric analyses of cell cycle populations by propidium iodine DNA staining in VEC and Myc-ER cells. n=3; *, $p<0.05$. D) Relative colony number in low density colony formation assays using VEC and Myc-ER cells. n=3. E) Quantitation of colony number in soft agar / anchorage independent growth assays using VEC and Myc-ER cells. n=3. F) JQ-1, a BRD4 bromodomain inhibitor was exposed for 3 days to CON and KD cells, and relative cell number quantified. Expression of RABL6A (and Myc) sensitized cells to JQ-1 inhibitory effects. n=3; *, $p<0.05$.





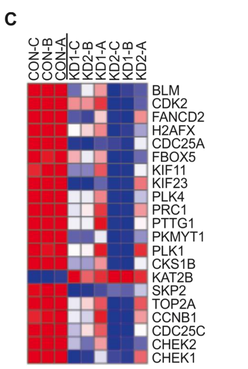


Figure 5: RABL6A affects DNA damage checkpoint signaling. A) Cell death as measured by trypan dye exclusion. n=3; *, $P<0.05$. B) Western blot analyses of BON-1 Vector and BON-1 Myc-ER cells following RABL6A knockdown (KD1, KD2). C) Heat map of BON cell microarray data showing impaired expression of genes in ATM, Polo-like kinase and G2/M DNA damage checkpoint pathways. **Red**, relatively increased expression; **blue**, relatively decreased expression.

Conclusions

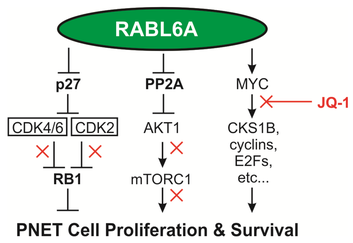
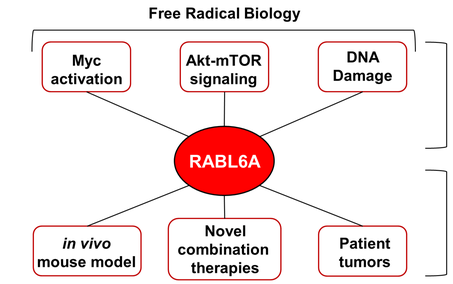


Figure 6: RABL6A is a global regulator of clinically relevant cancer pathways in PNETs. JQ-1 is a bromodomain inhibitor that suppresses Myc transcription. **X**, other clinically targeted pathways in cancer.

Future Directions



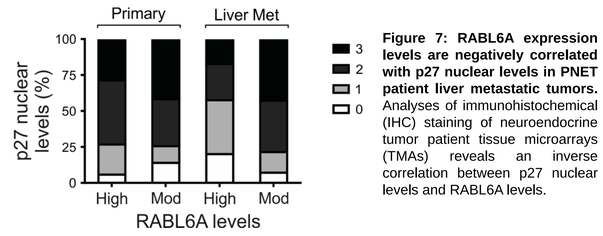


Figure 7: RABL6A expression levels are negatively correlated with p27 nuclear levels in PNET patient liver metastatic tumors. Analyses of immunohistochemical (IHC) staining of neuroendocrine tumor patient tissue microarrays (TMAs) reveals an inverse correlation between p27 nuclear levels and RABL6A levels.

Acknowledgements

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