Integrated CDK5 - AMPK phosphorylation network in Pheochromocytoma

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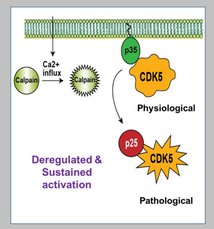
BACKGROUND

- Cyclin dependent kinase 5 (CDK5), a neuronal kinase and its cofactors p35/p25 has shown to be implicated in promoting proliferation and metastasis in Neuroendocrine (NE) tumors.
- The broader downstream signalling events mediated by CDK5 kinase activity has not been documented in Pheochromocytomas (PHEOs), NE tumors derived from chromaffin cells of the adrenal medulla.
- High throughput characterization of site specific phosphorylation events has allowed us to take a closer look at CDK5/AMPK (5'-AMP activated protein kinase) signalling cascade, a cellular energy sensor that could potentially modulate malignancy in PHEOs.



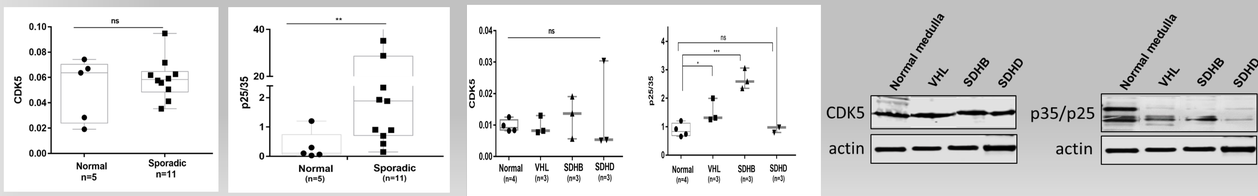
METHODS

- Expression analysis of CDK5 signaling components in Human PHEO tissue samples and cells.
- We combined quantitative phosphoproteomics (Phosphopeptide immunoaffinity purification and analysis by LC-MS/MS Mass Spectrometry) with aberrantly activated CDK5/p25 in growing vs arrested tumors to identify alternations of phosphorylation events downstream of CDK5.
- Generation of novel phosphorylation state specific antibodies and expression profiling in human PHEOs. Assessment of novel CDK5 inhibitors on metastatic PHEO model.

RESULTS

- CDK5, p35/p25 levels were consistently elevated in sporadic, VHL and SDHB mutant PHEO specimens.
- Overexpression of p25 (p25OE) in chromaffin cells under the control of neuroendocrine cell promoter developed chromograninA positive PHEOs in bitransgenic mouse model.
- Novel CDK5 inhibitor showed promising growth inhibitory effects in Pheo cell lines and metastatic mice model.

CDK5/p25 OVEREXPRESSES IN HUMAN PHEOCHROMOCYTOMA



ANTI-TUMOR RESPONSE OF CDK5 INHIBITORS

MODELLING PHEOCHROMOCYTOMA

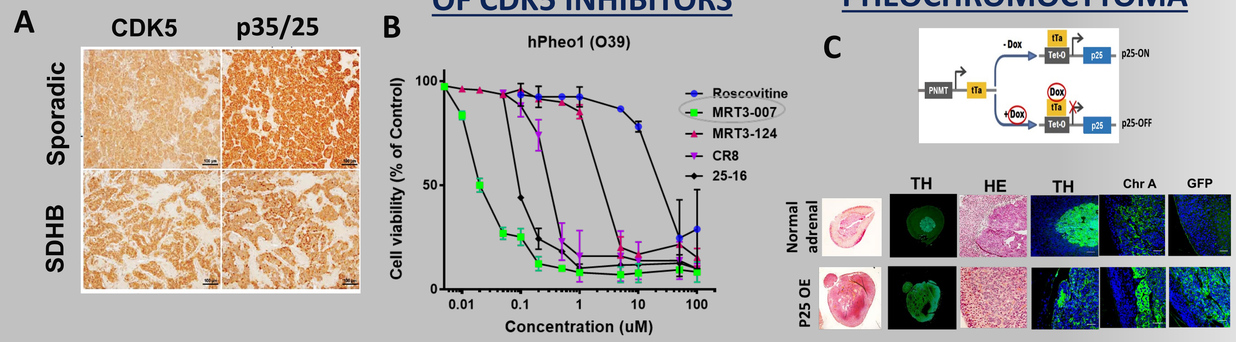


Figure 1: (A) Immunohistochemical detection of Cdk5 and p35/p25 in human PHEOs. (B) Screening of novel inhibitors of CDK5 in Human PHEO cells. Schematic of bi-transgenic system showing activation of TetOp X p25-GFP expression under PNMT promoter. (C) Gross appearance of PHEO tumors compared to normal mouse adrenals. Increased ChromograninA and p25-GFP immunostaining in tumors vs normal adrenals.

CDK5 inhibition suppress liver metastasis on Pheochromocytoma

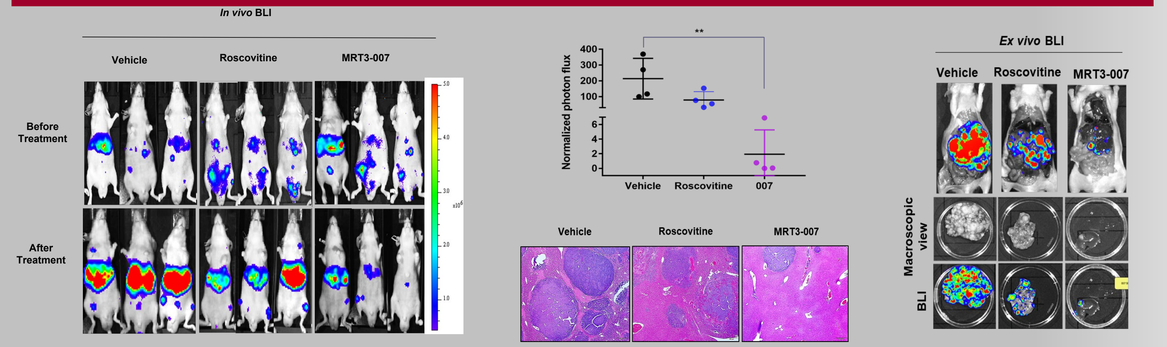


Figure 2: (A) CDK5 inhibition suppresses liver metastasis in murine model of metastatic Pheochromocytoma. (C) Quantification of metastatic lesions by bioluminescence (photons/sec). Graph indicating fold change in photon flux before and after respective treatments.

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Interrogation of CDK5 signaling in Pheochromocytoma

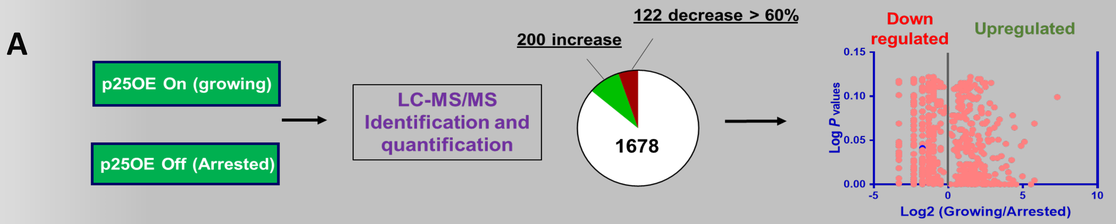


Figure 3: (A) Phosphoproteomic tool utilized to derive library of posttranslationally modified sites on signaling proteins representing putative CDK5 downstream targets.

Biological relevance of down regulated phosphorylation sites

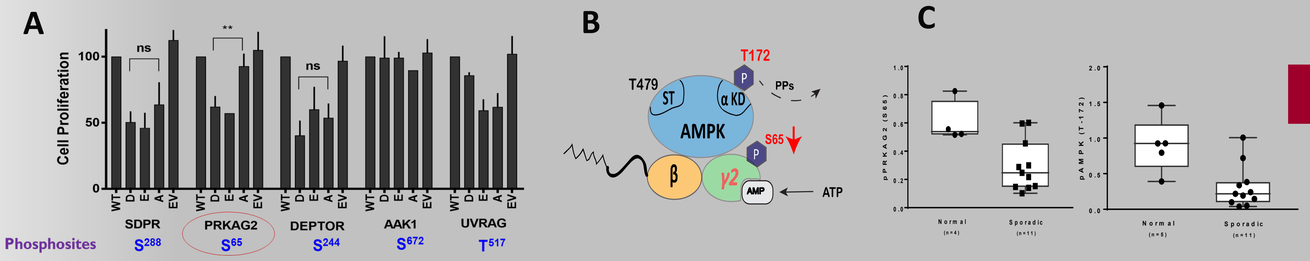


Figure 4: (A) Constitutive expression of phosphomimetic mutants of PRKAG2 reduces the cellular proliferation in PHEO lines(B) Schematic of Adenosine monophosphate–activated protein kinase complex. (C) Phospho-PRKAG2 downregulated in Sporadic human Pheo tissues consistent with lower activation phosphorylation of AMPK. Quantitation of fold change in phosphorylation states of PRKAG2/ AMPK in sporadic PHEOs compared to normal adrenal medulla.

CDK5 inhibition activates AMPK pathway

Metabolic consequence of AMPK activation

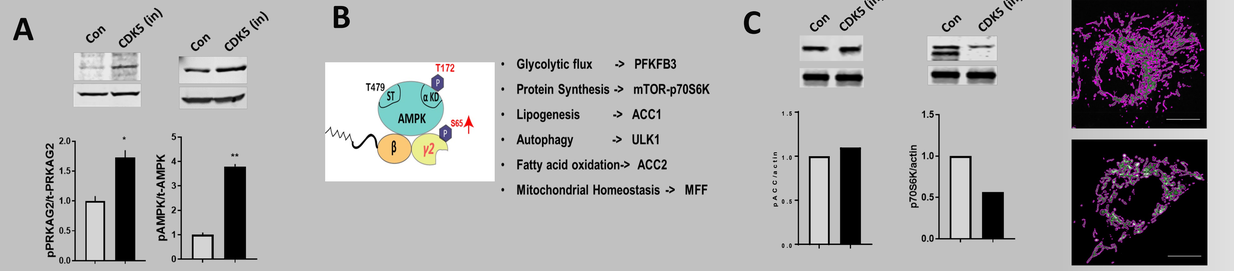
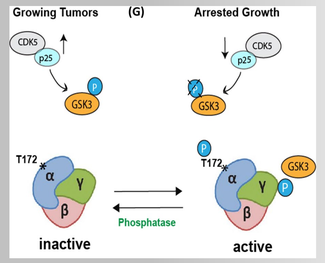


Figure 5: (A) CDK5 inhibition activates S65-PRKAG2 and T172-AMPK activating phosphorylations (B) Schematic representing functional consequences of activated AMPK complex. (C) CDK5 led AMPK activation appears to halt protein translation mechanism and reregulated mitochondrial dynamics.

CONCLUSIONS

- Presence of more p25/ p35 indicates aberrant CDK5 in human PHEOs.
- Anti- proliferative effects of novel CDK5 inhibitors in PHEOs.
- Novel phosphorylation Ser65 p-PRKAG2 site is downregulated in growing NE tumor.
- Ser65 PRKAG2 phosphomimetics reduces NE cell growth and invokes AMPK activation.
- Phosphorylation state of PRKAG2 and AMPK is downregulated in Human sporadic PHEOs compared to normal adrenals.
- Inhibition of CDK5 activates AMPK pathway in Pheochromocytoma.



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