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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 been 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), 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/GSK3/AMPK (5'-AMP activated protein kinase) signalling, a cellular energy sensor that could potentially modulate malignancy in PHEOs.

METHODS: We combined quantitative phosphoproteomics with aberrantly activated CDK5/p25 in growing and arrested tumors.

RESULTS: We observed that CDK5 and its co-activators p35/p25 were consistently elevated in human PHEO specimens. Interestingly, overexpression of p25 (p25OE) in chromaffin cells of adrenal medulla developed chromogranin A positive PHEOs in mouse model. We further obtained comprehensive map of CDK5 led protein phosphorylations and examined novel phosphosites downregulated by >60% in growing tumors. These phosphosites represent potential tumor suppressive mechanisms regulated by CDK5. PRKAG2 is a noncatalytic regulatory gamma subunit of a larger enzyme called AMPK. Here we demonstrate that novel phosphosite Ser65 on PRKAG2 is downregulated in

human PHEOs that overexpresses CDK5/p25 activity. Constitutive expression of phosphomimetic mutants of PRKAG2 reduces the cellular proliferation in PHEO lines with simultaneous activation of AMPK, indicated by increased phosphorylation at α-Thr172 site located on enzyme activation domain. in vitro phosphorylation results reveal that Glycogen synthase kinase (GSK3) is key mediator of PRKAG2 phosphorylation at the stoichiometry of 0.81 mol PO4/ mol,whereas CDK5 inhibition alleviates inhibitory regulation on GSK3 resulting in concomitant increase of phospho-PRKAG2/AMPK signalling stimulating anti-tumorigenic effects on cellular and in vivo metastatic PHEO models.

Conclusion: Our results speculate that the regulatory cross talk between GSK3 and CDK5 may underlie PRKAG2/AMPK hyperactivation and thus may constitute an important mechanism for therapeutic intervention in Pheochromocytomas.

Table 1:
Basal phosphorylations levels of target molecules in human Pheochromocytoma compared to its normal counterparts. Phopsho-(PRKAG2 / AMPK) is negatively regulated by hyperactive CDK5/p25 kinase activity.

Human tissues/cell line >	**Normal adrenal medulla**	**Sporadic PHEO**	**hPHEO1 (Cell line)**
Phosphoproteins in Study			
CDK5/p25 (Kinase activity)	+	+++	+++
Phopsho-GSK3 (Ser21/9);Inhibitory Phosphorylation	++	+++	+++
Phospho-PRKAG2 (Ser65)	+++	-	-
Phospho-AMPK (α-Thr172)	+++	-	-

Levels of expression indicated by (+ Basal); (+++High); (-very low)