Investigating the Role of Pseudohypoxia-related Metabolites in GEP-NET

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Introduction

Pseudohypoxia and pancreatic NET

Von Hippel-Lindau (VHL) disease is a multi-neoplasm inherited disorder caused by a germline mutation in the *VHL* tumor suppressor gene. Among other neoplasms, patients with VHL are at high risk of developing pancreatic neuroendocrine tumors (PNET). VHL-related PNETs (vPNET) have different genetic and clinical phenotypes compared to sporadic PNET (sPNET).

VHL protein (pVHL) is critical for cellular oxygen sensing. In pVHL absence, hypoxia-inducible factor 1 (HIF1) accumulates, resulting in a metabolic shift towards glycolysis. Considering the literature supporting pseudohypoxic state in a subset of neuroendocrine tumors, we use vPNET as an extreme model of NET characterized by pseudohypoxia, aiming to study pro-tumoral factors and possible targeted interventions.

We compared VHL-related and sporadic PNET. We found a higher adenosine 3´-monophosphate (3´-AMP) level in vPNET vs. sPNET, with a consistently elevated level of 3´-AMP across all VHL-related neoplasms. Hence, we propose to study the potential oncogenic impact of 3´-AMP in vPNET compared to sPNET.

The adenosine pathway

Adenosine concentration in biological fluids and extracellular spaces (eADO) is low but increases dramatically in hypoxic, injured, or inflamed tissues and in neoplasms. eADO is metabolized through several pathways. In the canonical pathway, adenosine triphosphate is metabolized to adenosine 5' monophosphate (5'-AMP) eADO is also metabolized via an alternative pathway: Initially, intracellular adenine to 2',3'- cyclic adenosine monophosphate, to intracellular and extracellular 3´-AMP and 2´-AMP.

Adenosine receptors

eADO acts through four G-protein coupled receptors, particularly the $A2_A$ and $A2_B$ receptors that trigger intracellular cAMP accumulation and its dependent pathways. $A2_A$ and $A2_B$ receptors ($A2_AR$, $A2_BR$) are overexpressed in a hypoxic environment, and their stimulation activates the phosphatidylinositol 3-kinase/mammalian target of rapamycin (PI3K/mTOR) pathway.

Hence, we study the role of $A2_AR$ and $A2_BR$ activation as a pro-neoplastic factor in Gastroenteropancreatic neuroendocrine tumors (GEP-NET) and the expression of adenosine receptors on the various cells in various cell subtypes in NET tissue samples.

Methods

Metabolomic processing of tumor tissues was performed at an external facility, focusing on polar metabolites.

Polar metabolites raw data were processed using MetaboAnalyst for normalization and scaling. Statistical analysis for comparing sporadic pancreatic NET vs. small-intestine NET and both compared to VHL-related NET, included Volcano plot, PatternHunter analysis, principal component analysis and unsupervised hierarchical clustering visualized by heatmap (**Figures 1 & 2**).

Pathway analysis of polar metabolites will be performed using MetaboAnalyst, based on the KEGG (Kyoto Encyclopedia for Genes and Genomes) database.

Data and statistical analysis for both datasets was executed using R Studio.

Objectives

- To assess a possible pro-neoplastic role for oncometabolites in GEP-NET
- To compare metabolic alterations between sPNET and vPNET and to explore metabolites that may drive tumor progression
- To investigate the role of the adenosine pathway and its components in pseudohypoxic PNETs

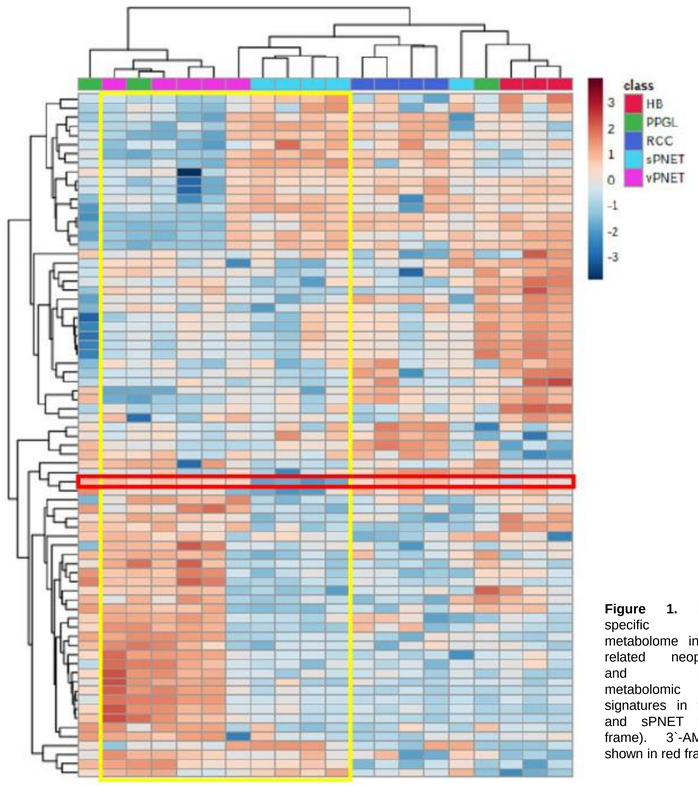

Figure 1. Organ-specific polar metabolome in VHL-related neoplasms, and distinct metabolomic signatures in vPNET and sPNET (yellow frame). 3`-AMP is shown in red frame.

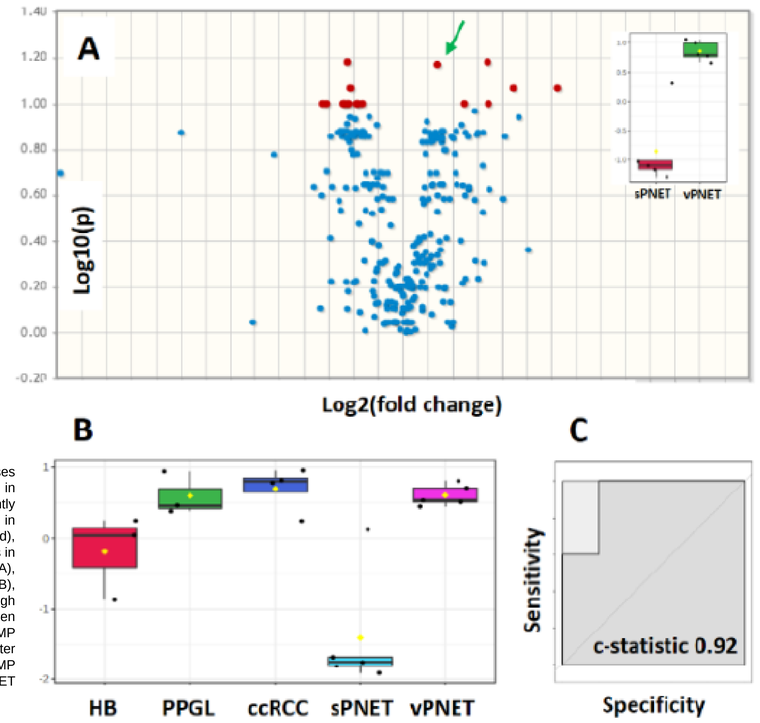

Figure 2. Independent analyses support key role for 3´-AMP in vPNETs. 3´-AMP is significantly different among all metabolites in Volcano plot (green arrowhead), frame shows higher 3´-AMP levels in vPNET (green) vs. sPNET (red, A), and across all VHL-neoplasms (B), ROC curve showing high discriminative efficacy between vPNET and sPNET by 3´-AMP levels (C) and PatternHunter homology analysis identified 3´-AMP as the leading metabolite in vPNET vs. sPNET(D).

Results

- Aiming to identify the driving mechanism for VHL-related pancreatic NET (vPNET) we performed an unbiased metabolomic analysis of VHL-related tumor tissues: 5 vPNET, 4 ccRCC, 3 (each) hemangioblastoma (HB) and pheochromocytoma (PPGL) and 5 sporadic PNET (sPNET).
- The samples were processed for polar and lipid metabolites. Both polar and lipid metabolic signatures were distinct between vPNET and sPNET (Figure 1, yellow frames) and between different VHL-related tumors.
- When we compared vPNET vs. sPNET, we identified six metabolites that were higher in vPNET (Figure 3A). Based on literature review, 3´-AMP had potential mechanism as pro-tumorigenic driver, via the PI3K/mTOR pathway activation, and by exerting pro-tumoral immunosuppressed microenvironment.

Conclusions

- The high levels of 3'-AMP and purine pathway in vPNET may drive tumorigenesis via activation of $A2_AR/A2_BR$ and may influence vPNET development
- Validation of $A2_AR$ expression on BON1 cells enables us to further test its role in tumor cells' neoplastic parameters
- Further accurate characterization of the tumor cells is in progress