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

Yuval Yossef¹,², Alona Telerman¹, Amit Tirosh¹,²

(1) ENTIRE - Endocrine Neoplasia Translational Research Center, Sheba Medical Center, Ramat Gan, Israel; (2) Tel Aviv University Faculty of Medicine, Tel Aviv, Israel

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 pseudohypoxia 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 adenosine 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 A2a and A2a receptors that trigger intracellular AMP accumulation and its dependent pathways. A2a and A2a receptors (A2aR, A2aR) 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 A2aR and A2aR 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 onco-metabolites in GEP-NET
- To compare metabolic alterations between vPNET and sPNET and to explore metabolites that may drive tumor progression
- To investigate the role of the adenosine pathway and its components in pseudohypoxic PNETs

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 A2aR/A2aR and may influence VNET development.
- Validation of A2aR 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.