A new twist in neuroendocrine tumor research: Pacak-Zhuang syndrome, HIF-2 α as the major player in its pathogenesis and future therapeutic options

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Background. There is increasing evidence of the role of hypoxia or pseudohypoxia in tumorigenesis, including pheochromocytoma (PHEO) and paraganglioma (PGL). (Pseudo)hypoxia leads to activation of hypoxia-inducible transcription factors (HIFs) and thus, promotes the transcription of hypoxia-responsive genes which are involved in tumorigenesis. Recently identified is a new syndrome consisting of multiple and recurrent PGLs or PHEOs, somatostatinoma, and congenital polycythemia, due to somatic hypoxia-inducible factor 2 α gene (*HIF2A*) mutations.

Methods and Results. PubMed and Web of Science online databases were used to search reviews and original articles on the HIF, PHEO/PGL, and Pacak-Zhuang syndrome.

Conclusions. The novel somatic and germline gain-of-function *HIF2A* mutations described latterly emphasize the role of the HIF-2 α in the PHEO/PGL development and these findings designate HIF, especially HIF-2 α , as a promising treatment target.

Key words: hypoxia-inducible factor, tumorigenesis, pheochromocytoma, paraganglioma, Pacak-Zhuang syndrome

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INTRODUCTION

The last few years have provided novel information about the pathogenesis of neuroendocrine tumors (NETs), especially pheochromocytoma (PHEO) and paraganglioma (PGL). PHEOs are catecholamine-producing neuroendocrine tumors derived from the chromaffin cells of the adrenal medulla¹ and PGLs are tumors arising from extra-adrenal chromaffin tissues². PHEOs and PGLs share overlapping characteristics (histopathology, molecular pathobiology) but there are also many differences in terms of their behavior, aggressiveness, metastatic potential, etc. PHEOs and PGLs can occur as sporadic tumors or as a component of hereditary tumor syndromes, including multiple endocrine neoplasia syndromes (MEN2A and MEN2B), von Hippel-Lindau disease (VHL), and neurofibromatosis type 1 (NF1) resulting, respectively, from mutations of RET (Rearranged in Transfection) protooncogene, VHL tumor suppressor gene (*VHL*), or the NF1 tumor suppressor gene³⁻⁷. The other known PHEO susceptibility genes are germline mutations in succinate dehydrogenase subunits (SDHx): SDHB, SDHC, SDHD and SDHAF2 (also known as SDH5). Following identification of their loci familial paraganglioma they were classified into the four groups, PGL4, PGL3, PGL1 and PGL2 (ref.^{5,8-12}). The study of PHEO/PGL familial syndromes is a cornerstone in understanding the pathogenic mechanisms involved in the development of these tumors. In the last five years, excellent pioneering studies have identified new genetic candidates that have been found to predispose to the development of PHEO and PGL: SDHA (ref.¹³), transmembrane protein 127 (TMEM127)

(ref.^{14,15}), Myc-associated factor X (MAX) (ref.¹⁶), kinesin family member 1B, transcript variant beta (KIF1B β) (ref.¹⁷), prolyl hydroxylase domain 2 (PHD2) (ref.¹⁸), and recently hypoxia-inducible factor 2 α (HIF-2 α) (ref.¹⁹) genes. Currently gene mutations can be identified in more than one third of PHEOs/PGLs (ref.²⁰). It is obvious that in last five years important progress was made in the understanding of the pathophysiology of PHEO/PGL due to the discovery of these new genes and their involvement in PHEO/PGL development. Moreover, a new unique syndrome type disease carrying PHEO/PGL-somatostatinoma-polycythemia, also named the Pacak-Zhuang syndrome with the hypoxia sensing domain mutations at *HIF2A* has substantially improved our understanding of a critical molecular mechanism in PHEOs/PGLs²¹. This finding for the first time indicated that HIF α protein with its regulatory elements is a central player in the pathogenesis of PHEOs/PGLs with various genetic causes²².

HIF-2A AND TUMORIGENESIS

HIF transcription factors mediate adaptive responses of cells to hypoxia or pseudohypoxia²³⁻²⁶. HIFs form heterodimeric complexes comprising an oxygen-sensitive α subunit and a stable β subunit. The HIF α -subunit is ubiquitously expressed and consists of three isoforms, HIF-1 α , HIF-2 α , and HIF-3 α (ref.^{24,25}). The HIF β subunit is constitutively expressed²⁷. Heterodimers of HIFs bind to the promoter of multiple genes associated with angiogenesis, glycolysis, and cell growth.

Under normoxic conditions, HIF-1 α and HIF-2 α are degraded via the ubiquitin-proteasome pathway. This degradation is controlled mostly by the hydroxylation of the two specific prolyl residues by prolyl hydroxylase domain proteins (PHDs – PHD1, PHD2, and PHD3) (ref.^{24,25}). This hydroxylation is crucial for recognition of HIF-1 α and HIF-2 α by the VHL protein (pVHL) (ref.²⁸) and subsequent proteasomal degradation²⁵. The negative regulation of HIF is mediated via the inhibitory PAS domain protein (IPAS), which is the HIF-3 α 2 splice variant. IPAS forms inactive complexes with HIF-1 α and thus, it is a negative regulator of HIF-1 α (ref.²⁹⁻³¹). The other HIF-1 α inhibitor is factor-inhibiting HIF-1 α (FIH1) which hydroxylates HIF-1 α and blocks its interaction with the coactivators³² and thus inhibits the transactivation of HIF target genes.

Under hypoxic or pseudohypoxic conditions, HIF α becomes stabilized, forms heterodimers with HIF β , recruits coactivators and binds to the core DNA at hypoxia-responsive elements (HREs) in target genes and activates their transcription^{25,33,34}. Short periods of severe hypoxia lead to HIF-1 α activation, whereas HIF-2 α is activated under mild or prolonged hypoxia³⁵. Different regulation of HIF-1 α and HIF-2 α is mediated by hypoxia associated factor (HAF) and this difference leads to distinct cellular functions³⁶. For a review, see Richter et al.³⁷. The HIF target genes include genes involved in angiogenesis, glucose metabolism, extracellular matrix formation, cell proliferation and survival, pH regulation, epithelial to mesenchymal transition, red blood cell production and iron metabolism and many others³⁸⁻⁴³. Many of these genes are involved in cancer development, progression, and metastasis. HIF α expression or stabilization can also be regulated by mechanisms other than hypoxia, including growth factors signaling pathway and a loss of tumor suppressor genes⁴⁴. This allows crosstalk between different signaling pathways resulting in cell transformation and tumor development^{25,45,46}.

Deregulation of HIF α has long been implicated in tumorigenesis since there is an interaction of HIF with many pivotal signaling pathways and (pseudo)hypoxia is found in almost all cancers^{25,34,46-49}. Both HIF-1 α and HIF-2 α seem to act as master regulators in adaptation of cancer progenitor/stem cells and their differentiated progenies to oxygen and nutrient deprivation (for a review, see Mimeault et al.⁵⁰). HIF-1 α and HIF-2 α were found to be overexpressed in almost all human cancers. HIF-2 α is mostly overexpressed in advanced lesions and is associated with poor prognosis⁵¹⁻⁵⁴. Moreover, HIF-2 α has been shown to regulate HIF-1 α target gene expression in the absence of HIF-1 α and vice versa^{55,56}, reviewed by Keith et al.²⁶

HIF-2 α has also been proposed as a tumor suppressor in *Kras*-driven lung tumor mouse model, since *HIF2A* deletion leads to tumor growth and progression⁵⁷. But the overexpression of HIF-2 α in the same mouse model was associated with increased tumor formation⁵⁸. These findings suggest that both HIF-2 α upregulation and downregulation can promote tumor formation and progression and that a balance of HIF-2 α production is needed for its proper function.

In renal cell carcinoma (RCC) decreased HIF α degradation was observed due to VHL mutations and HIF-2 α inhibition was shown to be sufficient to suppress tumor growth^{59,60}. It was also shown that protumorigenic genes encoding vascular endothelial growth factor (VEGF), cyclin D, and transforming growth factor α (TGF α), are predominantly regulated by HIF-2 α , while proapoptotic gene encoding BNip3 responds to HIF-1 α in RCC (ref.⁶¹).

HIF-2 α also plays an important role in angiogenesis, hematopoiesis and iron metabolism. Under hypoxic conditions HIF-2 α is able to induce the expression of angiogenesis related genes (e.g. VEGF) in endothelial cells⁶². HIF-2 α regulates erythropoietin expression and thus, is critical for hemopoiesis^{55,63-65}. HIF-2 α was shown to play a crucial role in maintaining of iron balance by the regulation of iron absorption in the intestine⁶⁶ and HIF signaling may contribute to altered iron metabolism in cancer⁶⁷.

The other tumorigenic processes, such as cell migration, matrix vascular remodeling, invasion are also affected by HIF-2 α upregulation, and in process of tumorigenesis the stromal microenvironment plays also an important role. Further information can be found in references^{37,51,68-72}.

***HIF2A* MUTATIONS IN PACAK-ZHUANG SYNDROME AS WELL AS IN SPORADIC PHEO/PGL**

In the first report, novel somatic gain-of-function *HIF2A* mutations in two patients with polycythemia and multiple PGLs with duodenal somatostatinomas (in 2nd patient somatostatinoma was identified after the report was published) were identified by Zhuang et al.¹⁹. Later on the same group and other investigators found somatic *HIF2A* mutations in patients with PGL/PHEO with and without association with polycythemia and somatostatinoma^{21,73,74}. The association of congenital polycythemia with two distinct types of NETs – PGL/PHEO and duodenal somatostatinoma found world-wide, strongly indicated the existence of a unique disease cluster that shared same gene mutation. This was reported as a new syndrome (Pacak-Zhuang syndrome) (ref.²¹). Subsequently, a novel germline *HIF2A* mutation in a patient with congenital polycythemia with multiple PGLs was described by Lorenzo et al.⁷⁵ and more patients presenting with *HIF2A* mutations and PGLs/PHEOs with or without polycythemia were reported⁷⁶⁻⁷⁹. Based on the multiple organs and multiple and distant tumor sites involved in a same patient, one could speculate that the mutation in *HIF2A* must occur in early life or during embryogenesis similar to the McCune-Albright syndrome. It is interesting to point out that this syndrome has been so far found mostly in female patients.

It has been shown that HIF plays an important role in neural crest development and differentiation, and in the function of adrenal medulla and paraganglia. HIF-1 α is essential in the development of neural tube and cardiovascular system and high HIF-2 α expression was observed in developing paraganglia and HIF-2 α is necessary for catecholamine synthesis⁸⁰⁻⁸², reviewed in Richter et al.³⁷.

HIF-2 α is also considered the key regulator of erythropoiesis and this association has been demonstrated initially in four patients with polycythemia who were found to have activating germline *HIF2A* mutations⁸³. HIF-2 α stabilization and PGL-associated EPO production have been also found in patients with *PHD2* and *VHL* mutations^{84,85}.

HIF2A gain-of-function mutations in patients with the Pacak-Zhuang syndrome lead to reduced HIF-2 α hydroxylation and binding to the pVHL resulting in 4-6 times higher stability of mutant HIF-2 α compared to a wild-type^{19,21}. The clinical presentations of patients were consistent with HIF-2 α dysregulation. PGLs are found to have a typical noradrenergic biochemical phenotype, which reflects the involvement of HIF-2 α in the preferential norepinephrine synthesis^{80,82,86} and the strong positive immunohistochemical staining for HIF-2 α in patient's tumor tissues and increased tumor mRNA for HIF-2 α downstream genes indicate HIF2 α upregulation.

Interestingly, all patients with polycythemia were diagnosed with PGL or PHEO at a young age, especially under 35, but patients without polycythemia presented with PHEO/PGL later and mostly without multifocal disease. These findings suggest the different timing during gestation when somatic *HIF2A* mutations occur, may affect the phenotypes of the syndrome in these patients³⁷. It cannot be excluded that in those patients not presenting with a polycythemia despite having *HIF2A* mutations such a somatic mutation occurred later on in the fetus development. The degree of hypoxia and microenvironmental changes, including nutrition, may also play an important role.

The HIF pathway has been found to be disrupted in some PHEOs and PGLs^{8,25,87-95}, depending on their genetic background and contributing to their development, recently reviewed by Jochmanova et al.²² and Richter et al.³⁷.

CONCLUSIONS AND FUTURE THERAPEUTIC OPTIONS

The finding of the Pacak-Zhuang syndrome led to an important discovery of HIF-2 α mutation in PHEO/PGL. HIF-2 α signaling pathway appears to play one of the most important roles in PHEO/PGL pathogenesis and other cancers development and this designates HIF-2 α as an attractive and promising therapeutic target. The most promising therapeutic strategies are HIF pathway targeted therapies, especially on HIF-2 α inhibition. Currently, there are several agents affecting the HIF-1 α signaling⁹⁶ (for a review, see Mellilo et al.^{97,98}). Drugs selectively targeting HIF-2 α signaling have not been fully developed yet but are under investigation^{99,100}. Moreover, it has been shown that HIF-1 α and HIF-2 α can activate target genes alternatively^{26,56}, thus development of drugs targeting both HIF-1 α and HIF-2 α is of a great interest. Another therapeutic approach could be changing the balance of HIF α isoforms by modulating HAF signaling in tumors, since it was shown that HAF switches cells from HIF-1 α to HIF-2 α signaling³⁶. Further investigations are needed to elucidate the other signaling pathways involved in PHEO/

PGL development and crosstalk within these pathways and HIF signaling pathway.

Finding novel diagnostic biomarkers associated with hypoxia and altered metabolic pathways should help to select patients who are likely to respond to a specific type of therapy (e.g. to the HIF signaling inhibitors) for personalized anti-cancer treatment. Based on this, multi-targeted therapeutic approaches, which should be more effective, can be used in PHEO/PGL treatment.

Furthermore, it is of a great interest to find out whether females found in Pacak-Zhuang syndrome are exclusively affected and if so, what pathogenic mechanism is involved in this sex selected process. In addition, erythropoietin is elevated in tumor tissue, however, it is not clear how this elevation occurs in early life and from which additional tissues, except tumor tissue, erythropoietin is derived from. Finally, it would be of interest to further study these patients whether they develop metastatic disease, other types of neuroendocrine and/or other abnormalities as the syndrome has been just discovered and more studies are needed to fully understand this disease.

AUTHORSHIP CONTRIBUTIONS

IJ: Literature search, data analysis and interpretation, manuscript writing; IL: Conception and design, critical revision of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors state that there are no conflicts of interest regarding the publication of this article.

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