Hypoxia Pathway Mutations in Pheochromocytomas and Paragangliomas

Diana Amorim-Pires^a Joana Peixoto^{a–c} Jorge Lima^{a–c}

^aMedical Faculty, ^bInstituto de Investigação e Inovação em Saúde, and ^cInstitute of Molecular Pathology and Immunology (Ipatimup), University of Porto, Porto, Portugal

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Abstract

Pheochromocytomas (PCC) and sympathetic paragangliomas (PGL) are rare neuroendocrine tumors, which derive from chromaffin cells occurring in the adrenal medulla and extra-adrenal sympathetic paraganglia. PCC and PGL are often benign, catecholamine-producing tumors, responsible for a myriad of symptoms that may be potentially hazardous to the patient. In contrast, nonsecreting parasympathetic PGL, derived from chief cells, develop mainly in the head and neck region. Although PCC/PGL are more commonly sporadic tumors, germline mutations are present in up to 40% of the patients, ranking these tumors among those with the highest degree of heritability. PCC/PGL are associated with a variety of hereditary syndromes, comprising genetic alterations in *RET*, *NF1*, *VHL*, and *SDHx* genes, the last 2 being involved in regulating the hypoxia pathway. Additional hypoxia pathway-related genes have been recently associated with PCC/PGL development, namely *EGLN1* and *EPAS1*. Thus, dysregulation of the hypoxia pathway seems to play a major role in PCC/PGL development, in particular through the sta-

bilization of hypoxia-inducible factors and the appearance of a pseudohypoxia signature. This article is focused on reviewing the tumorigenic mechanisms resultant from *VHL*, *SDHx*, *EGLN1*, and *EPAS1* mutations, as well as the associated tumors, namely PCC/PGL, and extra manifestations such as polycythemia. In the light of the recent discoveries, hypoxia pathway molecules appear as key players in PCC/PGL development.

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The peripheral nervous system is composed of a large variety of cell types, and thus, it can be the origin of multiple types of benign or malignant tumors. These include tumors arising from nerve cells, nerve-sheath cells (Schwann cells), and neuroendocrine cells, which give rise to pheochromocytomas (PCC) and paragangliomas (PGL) (Fig. 1) [Ariel, 1983].

PCC and sympathetic PGL are highly vascularized tumors that are derived from neuroendocrine chromaffin cells, which originate from the neural crest, and are present in the adrenal medulla and in the extra-adrenal thoracic and abdominal sympathetic paraganglia [Dahia, 2014]. PCC and PGL are rare tumors, having an estimated incidence of 1 case per 300,000 inhabitants per year

Fig. 1. Peripheral nervous system tumors and their cells of origin. Adapted from Ariei [1983].

[Pinato et al., 2013]. Accounting for 80% of PCC/PGL, PCC arise from the adrenal medulla, while thoracic and abdominal PGL have their origin in the sympathetic paraganglia [Pinato et al., 2013]. Being of neuroendocrine nature, chromaffin cells are responsible for the production of catecholamines, such as norepinephrine, epinephrine, and dopamine; therefore, PCC/PGL are often catecholamine-producing tumors, in their majority benign. However, malignant behavior occurs in 10–15% of the cases, the most common places for metastasis being the bone, liver, lungs, and lymph nodes [Pinato et al., 2013; Dahia, 2014; Jochmanová et al., 2014; Welander et al., 2014]. Metastasis appears either at first presentation or at recurrence, while tumor relapse may occur months to years after the initial diagnosis and treatment [Favier et al., 2002].

In addition to the sympathetic paraganglia, PGL can also develop in the parasympathetic paraganglia, being predominantly found in the head and neck region due to the presence of neuroendocrine chief cells in the vagal and glossopharyngeal nerves, including the carotid body [Merlo et al., 2013; Pacak et al., 2013]. Head and neck paragangliomas (HNPG) are extremely rare, representing 0.6% of the head and neck tumors and 0.03% of all neoplasms, with malignancy rates of approximately 15% [Lopez-Vazquez et al., 2014]. Contrary to PGL of sympathetic origin, these tumors are usually unable to secrete catecholamines [Dahia, 2014].

The catecholamines produced by chromaffin cells, especially norepinephrine and epinephrine, are involved in the regulation of the cardiovascular system and metabolism [Kolackov et al., 2012]. They are released into the bloodstream, stimulating cardiac inotropy and causing

the rise of blood pressure. Catecholamines also interfere with the secretion of insulin and lipid metabolism, leading to an augmentation of the blood glucose and lipid levels [Kolackov et al., 2012]. Thus, in the presence of catecholamine-producing tumors, these signs and symptoms may occur in an exacerbated fashion, due to the excessive secretion of catecholamines. The most common presentation of PCC/PGL refers to endocrine-related manifestations such as palpitations, tachycardia, hypertension, headaches, diaphoresis, heat intolerance, and anxiety, among other symptoms, although silent tumors may also exist. In some patients, the symptomatology can be potentially life-threatening, leading to events such as acute myocardial infarction, arrhythmias, stroke, or heart failure [Pacak et al., 2013; Pinato et al., 2013]. Local compressive symptoms may also occur in these patients as tumor mass grows [Tsang et al., 2014].

In their most common presentation, PCC/PGL are sporadic, appearing as isolated tumors, without evident familial background. In these cases, somatic mutations of the genes involved in the hereditary forms may be present, but the great majority of them remain unexplained [Dahia, 2014; Welander et al., 2014].

Strikingly, around 40% of PCC/PGL carry a germline mutation, meaning that these are one of the human tumor entities with the highest explained heritability worldwide, being associated with a large number of hereditary syndromes, such as multiple endocrine neoplasia type 2, which involves mutations in the *RET* proto-oncogene; von Hippel-Lindau disease, which involves mutations in the *VHL* gene; neurofibromatosis type 1, which involves mutations in the *NF1* gene; and familial pheochromocytoma-paraganglioma syndrome (FPPS) that is related to

mutations in the gene class *SDHx*, all of which code for proteins of the succinate dehydrogenase complex [Comino-Mendez et al., 2013; Dahia, 2014; Welander et al., 2014].

In addition, novel susceptibility genes have been associated with the development of these neuroendocrine tumors, including transmembrane protein 127 (*TMEM127*), MYC associated factor X (*MAX*), fumarate hydratase (*FH*), kinesin family member 1B (*KIF1B*), Egl-9 family hypoxia-inducible factor 1/prolyl hydroxylase domain-containing protein 2 (*EGLN1/PHD2*) and, more recently, endothelial PAS domain protein 1/hypoxia-inducible factor 2-alpha (*EPAS1/HIF2A*) [Pinato et al., 2013; Shankavaram et al., 2013; Dahia, 2014; Welander et al., 2014].

As a result of multiple gene expression studies, some authors proposed the division of hereditary and sporadic PCC/PGL in 2 main clusters: Cluster 1 includes tumors with *VHL* and *SDHx* gene mutations, displaying a transcription profile characterized by the activation of a pseudohypoxia signaling pathway, whereas Cluster 2 comprises tumors with *RET/NF1/TMEM127/MAX* mutations, displaying a transcription profile characterized by the activation of kinase signaling pathways [Szabó et al., 2012; Shankavaram et al., 2013; Welander et al., 2014].

This review will focus on the pseudohypoxia-related Cluster 1 of PCC/PGL. In this cluster, loss-of-function mutations in *VHL* and *SDHx* genes lead to an increased stability of the hypoxia-inducible factor (HIF) proteins, which are the main components of the response to low oxygen levels [Toledo et al., 2013]. HIF proteins are transcription factors that, in their active form, are composed of a heterodimer: a constitutively expressed β subunit, and an inducible and highly regulated α subunit. HIF α , which exists in 3 different forms (HIF1 α , HIF2 α , and HIF3 α), is regulated by oxygen-dependent prolyl hydroxylation in 2 proline residues, a reaction catalyzed by prolyl hydroxylases (PHDs) [Welander et al., 2014]. Proline hydroxylation by PHDs allows for HIF α recognition by VHL protein (pVHL), resulting in HIF α ubiquitination and, consequently, proteasomal degradation. Thus, at high oxygen levels, HIF α is continuously degraded; however, under hypoxia, the low oxygen levels hinder the correct prolyl hydroxylation of HIF α , which becomes stabilized and leads to the transcription of several target genes involved in multiple processes, such as angiogenesis, glycolysis, and cell growth [Welander et al., 2014]. Mutations in the components of this cascade – VHL, PHDs, HIF – can cause stabilization and accumulation of HIF α , even in the presence of high oxygen levels, leading to a phenomenon known as pseudohypoxia, which contrib-

utes to neuroendocrine cell tumorigenesis [Toledo et al., 2013; Welander et al., 2014].

The purpose of this work is to elucidate the importance of the hypoxia pathway in the development of PCC/PGL by discussing how the genetic alterations interfere with the signaling cascades and how they can lead to tumorigenesis. To achieve this goal, an article search and retrieval was performed in the database PubMed during July 2015, using as keywords “pheochromocytoma,” “paraganglioma,” “hypoxia,” and “HIF.” The final pool was composed of original works and case reports based on human tumor tissue analysis.

Angiogenesis in PCC and PGL

The importance of angiogenesis in the origin and progression of PCC/PGL is highlighted by the abundance and density of blood vessels within the tumor bed [Favier et al., 2002]. Angiogenesis corresponds to the emergence and development of new blood vessels from established vasculature, and its induction is a crucial step in tumor growth, invasion, and metastatic spread [Jyung et al., 2000; Eleno et al., 2010]. This process, essential to guarantee the input of oxygen and nutrients to cancer cells, can be stimulated by extrinsic factors (hypoxia) or intrinsic triggers (genetic mutations) [Jyung et al., 2000; Favier et al., 2002; Eleno et al., 2010]. Thus, genes/proteins involved in the hypoxia pathway seem to play a significant role in the development of these hypervascularized tumors by generating a pseudohypoxia signature [Taieb et al., 2009].

As previously mentioned, PCC/PGL can be divided into 2 clusters: Cluster 1, which includes tumors characterized by the activation of a pseudohypoxia signaling pathway, and Cluster 2, which comprises tumors with activation of kinase signaling pathways, where pathogenic dysregulation of Ras and mTOR pathways occurs as a result of mutations in *RET/NF1/TMEM127/MAX* [Szabó et al., 2012; Shankavaram et al., 2013; Welander et al., 2014].

Cluster 1, the focus of this review, is characterized by pathogenic alterations in genes involved in the hypoxia pathway. The most well characterized ones are *VHL* and *SDHx*, but additional genes, such as *EPAS1/HIF2A*, which encodes hypoxia-inducible factor 2 α (HIF2 α) [Qin et al., 2014], and *PHDs* also appear to play a relevant role [Welander et al., 2014]. In the following sections, we describe the genetic alterations associated with Cluster 1 PCC/PGL, as well as the different mechanisms by which they are capable of modulating the hypoxia pathway and response.

Fig. 2. Schematic illustration of the wild-type (WT, upper panel) and mutant (lower panel) pVHL function. Under normoxia conditions, pVHL recognizes the hydroxylated HIFα proteins and targets them to degradation in the proteasome; mutant pVHL, however, is no longer able to recognize HIFα proteins, which become stabilized in the cytoplasm and nucleus, where they act as transcription factors for a number of genes involved in proliferation, angiogenesis, and glycolytic metabolism.

VHL Gene

The *VHL* gene is originally known for causing von Hippel-Lindau disease, a rare autosomal dominant cancer syndrome, responsible for the appearance of incapacitating and life-threatening tumors in children and adults [Sorrell et al., 2011]. This disorder, caused by *VHL* germline mutations, includes tumors such as central nervous system hemangioblastoma, clear-cell renal cell carcinoma (RCC), PCC, and additional neuroendocrine tumors, endolymphatic sac tumors, cystadenomas in multiple locations, and renal and pancreatic cysts [Sorrell et al., 2011]. Depending on the specific *VHL* alteration, VHL disease has a considerable degree of heterogeneity, particularly in the age-related penetrance and in the types of tumors developed by the patients. Hence, genotype-phenotype correlations are crucial for predicting the risk of PCC: while *VHL* deletions or truncating mutations are associated with a high risk of RCC and low risk of PCC

(VHL disease type 1), *VHL* missense mutations are linked to a higher risk of developing PCC (VHL disease type 2) [Favier et al., 2009; Sorrell et al., 2011; Ladroue et al., 2012; Lanikova et al., 2013]. Besides the cases associated with familial cancer syndromes, sporadic PCC/PGL may also carry somatic *VHL* mutations [Dahia, 2014].

The *VHL* tumor suppressor gene, which codes for pVHL, is located on the short arm of chromosome 3 and is composed of 3 exons [Sorrell et al., 2011]. pVHL forms an E3 ubiquitin ligase complex with elongin B, elongin C, Rbx1, and Cul2, which is able to recognize and bind to prolyl-hydroxylated HIFα under normoxia conditions. After pVHL binding, HIFα is polyubiquitinated and targeted for proteasomal degradation. Under hypoxic conditions, or in the presence of a mutated (nonfunctional) pVHL, HIFα subunits become stabilized in the cytoplasm and are able to translocate to the nucleus, which leads to the transcription of genes involved in angiogenesis, glucose metabolism, erythropoiesis, cell proliferation, and

apoptosis (Fig. 2) [Pollard et al., 2006; Forman et al., 2009; Szabó et al., 2012]. Noteworthy, *VHL* loss-of-function mutations appear to be particularly associated with the stabilization of HIF2 α , which is the main isoform expressed in catecholamine-producing cells [Sorrell et al., 2011; Comino-Mendez et al., 2013]. In addition, *VHL*-associated PCC present higher plasma levels of normetanephrine (a norepinephrine metabolite), which results from the lower levels of the norepinephrine-to-epinephrine converting enzyme phenylethanolamine *N*-methyltransferase (PNMT) and the lower levels of tyrosine hydroxylase, the rate-limiting enzyme involved in catecholamine biosynthesis. This characteristic demonstrates the immature phenotype of tumors associated with *VHL* mutations, a phenomenon sustained by HIF2 α overexpression and stabilization [Vogel et al., 2005; Qin et al., 2014].

It is known that HIF1 α and HIF2 α regulate distinct collections of target genes and, thus, may have different tumorigenic effects in the context of PCC/PGL [Pollard et al., 2006]. In *VHL*-related tumors, HIF2 α accumulation is a major phenomenon, when compared with HIF1 α , and results in the overexpression of hypoxia-induced angiogenic genes, such as erythropoietin (*EPO*) and its receptor (a possible marker of delayed differentiation), vascular endothelial growth factor (*VEGF*), cyclin D1 (*CCND1*), and other mitogens [Eisenhofer et al., 2004; Vogel et al., 2005; Pollard et al., 2006; Qin et al., 2014].

Activation of the HIF pathway in *VHL*-associated tumors may mediate the molecular and biochemical features of the Warburg effect by overexpressing genes involved in glucose metabolism, such as the glucose transporters 1 (*GLUT1/SLC2A1*) and 3 (*GLUT3/SLC2A3*), the glycolytic enzyme hexokinase II, and the pivotal enzyme pyruvate dehydrogenase, in a mechanism apparently mediated by HIF1 α dysregulation [Favier et al., 2009; Lopez-Jimenez et al., 2010]. *VHL*-mutated tumors also show overexpression of the cell surface receptors VEGFR2 and neuropilin 1 that play an important role in facilitating endothelial cell migratory responses [Eisenhofer et al., 2004]. Genes that regulate the production and function of several extracellular proteins involved in angiogenic extracellular matrix remodeling are also overexpressed in *VHL* tumors. These include matrix metalloproteinase 2 and members of the integrin family that can induce improper assembly of the extracellular fibronectin matrix as well as disorganized growth [Eisenhofer et al., 2004]. Thus, an important role is imposed to HIF2 α during the development of *VHL*-associated PCC, which consists of activation of angiogenic processes and extracellular matrix reorganization [Eisenhofer et al., 2004].

Genotype-phenotype correlations in *VHL* disease have suggested that HIF dysregulation alone is not sufficient to cause PCC susceptibility; indeed, rare *VHL* missense mutations that are associated with PCC susceptibility alone (*VHL* disease type 2C) retain the ability to regulate HIF α [Pollard et al., 2006]. It has been suggested that a HIF-independent developmental apoptosis pathway, involving JunB-mediated inhibition of cJun and EGLN3/PHD3, may be defective in all familial PCC disorders [Pollard et al., 2006; Lopez-Jimenez et al., 2010; Astuti et al., 2011]. Related to this pathway, the *VHL* X214L mutation in exon 3 appears to be deleterious by extending the length of the normal pVHL for 14 amino acids, resulting in failure to downregulate JunB, attenuation of apoptosis, and neoplastic transformation. Interestingly, the pVHL X214L mutant protein is able to downregulate HIF α expression in a canonical way. The pattern of high JunB expression is consistent with the high PCC risk in the context of von Hippel-Lindau disease type 2A, a phenotype that has a high risk of developing PCC and hemangioblastoma, but low risk of RCC [Astuti et al., 2011; Sorrell et al., 2011].

VHL missense mutations related with PCC may also occur at conserved sites responsible for pVHL interaction with elongin C, thereby reducing elongin C binding and, consequently, destabilizing pVHL [Forman et al., 2009]. Noteworthy, this event seems not to interfere with pVHL ability to downregulate HIF α , but rather to inhibit p53 binding to the elongin C interface, thus preventing p53 stabilization and cellular apoptosis [Forman et al., 2009]. Loss of p53 has also been associated with altered mitochondrial and glycolytic metabolism, suggesting that in *VHL*-mutated tumors, p53 loss may contribute to the activation of the glycolytic pathway [Favier et al., 2009].

In conclusion, *VHL*-related PCC/PGL show activation of the hypoxia pathway, a phenomenon that is intimately related with HIF α stabilization, predominantly HIF2 α , and activation of multiple pathways capable of inducing tumor development. Nevertheless, additional proteins and pathways may also be dysregulated in *VHL*-mutated tumors, such as developmental neuronal apoptosis pathway and p53-related networks, exemplifying the complex mechanisms involved in PCC/PGL tumorigenesis.

***SDHx* Genes**

SDHx genes have been classically associated with PCC/PGL development, in particular those belonging to the pseudohypoxia-related Cluster 1 [Shankavaram et al.,

Fig. 3. Schematic illustration of the wild-type (WT, upper panel) and mutant (lower panel) SDH function. In the Krebs cycle, SDH converts succinate (S) into fumarate (F), with the concomitant release of electrons into the quinone pool of the electron transport chain. In the presence of *SDHx* inactivating mutations, succinate accumulates in the mitochondrial matrix and leaks out to the cytosol where it prevents prolyl hydroxylation of HIF α by inhibiting PHDs. This process ultimately results in HIF α stabilization.

2013]. The *SDHx* family comprises 5 genes: *SDHA* (succinate dehydrogenase subunit A), *SDHB* (succinate dehydrogenase subunit B), *SDHC* (succinate dehydrogenase subunit C), *SDHD* (succinate dehydrogenase subunit D), and *SDHAF2* (succinate dehydrogenase complex assembly factor 2) [Shankavaram et al., 2013]. *SDHA–D* genes code for a group of proteins that form the mitochondrial succinate dehydrogenase enzyme (SDH), a component of the citric acid cycle and the aerobic respiratory chain (where it corresponds to complex II); the *SDHAF2* gene encodes a protein that is essential in the assembly of the SDH complex [Burnichon et al., 2010]. SDH participates in the Krebs cycle by oxidizing succinate to fumarate, resulting from this process the liberation of 2 hydrogen atoms that are delivered to the electron transport chain, contributing to energy production (Fig. 3) [Pollard et al., 2005; Ghayee et al., 2013].

SDHx are tumor suppressor genes [Pollard et al., 2005]. In the event of a germline *SDHx* mutation, associated with loss-of-heterozygosity of the corresponding

wild-type allele, SDH enzymatic activity is abrogated, resulting in loss of both its catalytic activity and the electron flow to the ubiquinone pool [Pollard et al., 2005; Burnichon et al., 2010]. The consequent accumulation of succinate (the substrate of SDH) will competitively inhibit the prolyl hydroxylation of HIF α , thus enabling its stabilization and heterodimerization [Burnichon et al., 2010]. This promotes the expression of target genes, such as *VEGF* and *GLUT1*, and enables the emergence of the pseudohypoxia signature [Ghayee et al., 2013].

Germline mutations in *SDHx* genes predispose to FPPS, an autosomal dominant disorder with age-related incomplete penetrance [Gimenez-Roqueplo et al., 2001; Shankavaram et al., 2013]. There are 4 types of FPPS, which are essentially distinguished by the underlying gene: FPPS type 1 harbors *SDHD* mutations, type 2 *SDHAF2* mutations, type 3 *SDHC* mutations, and type 4 *SDHB* mutations [Shankavaram et al., 2013]. Distinct genotype-phenotype correlations exist in this disease: *SDHD* mutations are predominantly associated with

multiple, but mostly benign HNPGL, while *SDHB* mutations are linked with a higher malignancy potential, poor prognosis, and a greater risk of PCC [Pollard et al., 2005]. *SDHC* and *SDHAF2* mutations are less common events [Pollard et al., 2005; Burnichon et al., 2010]. Additionally, *SDHB*, *SDHC*, and *SDHD* mutations are associated with the PGL and gastrointestinal stromal tumor dyad named Carney-Stratakis syndrome. *SDHB* mutations are also associated with the development of clear-cell RCC [Shankavaram et al., 2013; Dahia, 2014].

SDHA was the gene most recently found to be involved in the development of PCC/PGL [Burnichon et al., 2010]. This relationship was detected in a patient with an extra-adrenal PGL that harbored an *SDHA* germline mutation in the highly conserved R589 residue. It was also observed that this loss-of-function *SDHA* mutation resulted in loss of *SDHB* protein and induced a pseudohypoxia signature, 2 typical features of *SDHx*-related tumors [Burnichon et al., 2010]. Interestingly, germline mutations in *SDHA* had been previously established as a cause for the development of Leigh syndrome [Burnichon et al., 2010]. The fundamental difference is that Leigh syndrome patients carry germline homozygous or compound heterozygous mutations, while PCC/PGL patients carry germline heterozygous mutations. Therefore, although a rare event in chromaffin-cell tumorigenesis, *SDHA* mutations should be suspected in patients with negative *SDHA* immunohistochemistry in the tumor tissue, loss of *SDH* activity, or loss of chromosome 5p15 in the tumor [Burnichon et al., 2010]. Additionally, *SDHA* mutations have also been associated with gastrointestinal stromal tumors [Dahia, 2014].

The mitochondrial dysfunction and hypoxia pathway activation associated with *SDHx* mutations are probably also interlinked with additional mechanisms of tumorigenesis, namely those involving oxidative stress, apoptosis resistance, and hypermethylation [Dahia, 2014]. Reactive oxygen species were shown to inhibit prolyl hydroxylase action, thereby increasing HIF1 α availability and VEGF expression [Span et al., 2011]. In addition, PCC from individuals with *SDHB/SDHD* germline mutations exhibit, besides HIF stabilization and activation of hypoxia-inducible target genes, inactivation of the developmental neuronal apoptosis pathway involving JunB, cJun, and EGLN3/PHD3 [Pollard et al., 2006; Lehtonen et al., 2007; Blank et al., 2010].

A mechanism recently proposed to be involved in PCC/PGL development is the epithelial-to-mesenchymal transition (EMT) [Loriot et al., 2012]. This is a phenomenon that typically occurs during embryonic develop-

ment, but that is reactivated in cancer cells, where it stimulates migratory and invasive properties. During EMT, differential expression of specific transcription factors leads to the loss of epithelial markers and to the acquisition of hallmarks of mesenchymal phenotype [Loriot et al., 2012]. In the context of PCC/PGL, EMT was associated with increased malignancy, being specifically induced in *SDHB*-related metastatic tumors, where it occurs in the context of pseudohypoxia, and contributes to the particular metastatic properties of this subset of tumors. Thus, markers involved in EMT, such as SNAI1/2, may be histological markers of malignancy in this set of tumors and may constitute important parameters for the clinical management of these patients [Loriot et al., 2012].

In conclusion, *SDHx*-related PCC/PGL show hypoxia pathway dysregulation, similar to *VHL*-mutated tumors, having as final consequence the stabilization of HIF and activation of multiple pathways capable of inducing tumor development. However, additional proteins and pathways are also dysregulated in *SDHx*-mutated tumors, such as reactive oxygen species-mediated prolyl hydroxylase inhibition, developmental neuronal apoptosis pathway and EMT, underlining the complex mechanisms involved in PCC/PGL tumorigenesis.

EGLN1/PHD2 Gene

Prolyl-4-hydroxylase domain (PHD) proteins are dioxygenases that convert α -ketoglutarate to succinate and hydroxylate 2 key proline residues located in the oxygen-dependent degradation domain of HIF α subunits, in an oxygen- and iron-dependent way (Fig. 4) [Ladroue et al., 2008, 2012]. As described previously, this prolyl hydroxylation is fundamental for the correct recognition of HIF α by pVHL and subsequent targeting to degradation in the proteasome. The PHD family of proteins includes PHD1, PHD2, and PHD3, among which PHD2, encoded by *EGLN1*, seems to be a crucial oxygen sensor regulating HIF α levels [Ladroue et al., 2008]. Being a crucial regulator of erythropoiesis, the dysregulation of the PHD2-pVHL-HIF2 α pathway, with concomitant HIF2 α stabilization and accumulation, leads to a pseudohypoxic state that may underlie pathologic conditions such as polycythemia or PCC/PGL [Ladroue et al., 2008; Eltzschig et al., 2009].

Heterozygous germline mutations of the *EGLN1* gene were first described in patients with familial polycythemia associated with elevated EPO levels [Ladroue et al., 2012]. Ladroue et al. [2008] further reported a case of an

Fig. 4. Schematic illustration of the wild-type (WT, upper panel) and mutant (lower panel) PHD2 function. PHD2 hydroxylates HIF α in a reaction that requires oxygen, iron, and α -ketoglutarate (K), while producing succinate (S). PHD2 loss-of-function mutations prevent HIF α hydroxylation, thereby disabling pVHL-mediated HIF α degradation.

individual, in follow-up for polycythemia, who posteriorly developed a recurrent PGL. This patient was found to carry a heterozygous germline *EGLN1* mutation in the H374 residue, a highly conserved residue that is involved in the binding of Fe²⁺ ions to PHD2. Hence, the H374R mutation detected may predispose to instability and loss of activity of PHD2, consequently leading to upregulation of HIF2 α . In addition, the finding that the H374R mutation was associated with loss of heterozygosity in the DNA from the recurrent PGL indicates that *EGLN1* can act as a tumor suppressor gene [Ladroue et al., 2008]. This case could be compared with the *VHL* missense mutations seen in *VHL* disease type 2, which are associated with a high risk of PCC/PGL [Ladroue et al., 2012].

On the other hand, *EGLN1* truncating mutations, such as R398X, are associated with polycythemia but convey low risk for PCC/PGL development, in parallel with what is observed for *VHL* truncating mutations typical of *VHL* disease type 1 [Ladroue et al., 2012]. Thus, *EGLN1* mutations, like *VHL* mutations, may predispose to different

manifestations – either polycythemia or cancer disease – depending on the nature and specificity of the mutation [Ladroue et al., 2012]; this warrants further studies on large series of polycythemia, associated or not with PCC/PGL, in order to establish the genotype-phenotype associations.

In conclusion, *EGLN1* is a candidate gene for PCC/PGL development. Although infrequent, *EGLN1* mutations should be explored in families with unidentified genetic alterations, and carriers of *EGLN1* germline mutations should be carefully followed and screened due to the risk of PCC/PGL development [Ladroue et al., 2008, 2012].

***EPAS1/HIF2A* Gene**

In the previous sections, we have highlighted the heterogeneity of the genetic predisposition to PCC/PGL. Noteworthy, an important percentage of either hereditary or sporadic cases do not harbor mutations in the “classical” susceptibility genes [Taieb et al., 2009; Comino-Mendez et al., 2013]. Therefore, it is important to explore other genes and mechanisms that may take part in the development of PCC/PGL [Taieb et al., 2009].

The *EPAS1/HIF2A* gene encodes HIF2 α protein, a transcription factor that mediates the tumorigenic effects of *VHL*, *SDHx*, and *EGLN1* mutations [Comino-Mendez et al., 2013]. In these tumors, HIF2 α acts by inducing a pseudohypoxia state, due to abnormal stabilization and prolonged half-life of HIF proteins, being apparently a crucial step for chromaffin tumor development [Ladroue et al., 2008; Comino-Mendez et al., 2013].

Like other HIF α forms, HIF2 α is hydroxylated in 2 proline residues in an oxygen-dependent manner, to allow recognition by pVHL and subsequent proteasomal degradation [Toledo et al., 2013]. Additionally, prolyl hydroxylation is partly regulated by succinate levels and reactive oxygen species, which accumulate in the presence of dysfunctional SDH enzymes [Taieb et al., 2009].

EPAS1 has long been implicated in the development of aggressive, treatment-refractory tumors, being associated with poor prognosis of various human cancers [Toledo et al., 2013], and, in the recent years, *EPAS1* was added to the pool of genes associated with PCC/PGL, through activating mutations, mainly located in exons 9 and 12 [Weller et al., 2014].

Multiple studies reported the presence of exclusive somatic mutations of *EPAS1* in PCC/PGL, evidencing its oncogenic role. Comino-Méndez et al. [2013] found so-

matic *EPAS1* mutations in 3 patients with multiple PCC/PGL and also in 3 cases presenting with single tumors. Due to the high percentage in their series, *EPAS1* mutations appeared as one of the most relevant somatic postzygotic genetic events in the development of sporadic PCC/PGL, together with *NF1*, *VHL*, and *RET* alterations. These *EPAS1* mutations occur in the P531 residue, the primary prolyl hydroxylation site, and also in nearby amino acids, such as A530 and N539, resulting in the modification of the hydroxylation domain conformation and hampering the recognition by PHD2, thus leading to HIF2 α stability. Besides *EPAS1* mutations, the specific gain of the gene or the gain of chromosome 2p, where *EPAS1* is located, are genetic mechanisms that could trigger tumorigenesis [Comino-Mendez et al., 2013].

Welander et al. [2014] and Toledo et al. [2013] also found *EPAS1* somatic heterozygous mutations (P531 residue) in sporadic PCC/PGL and proposed a similar tumorigenic mechanism, having as a final endpoint the overexpression of HIF2 α target genes, such as *VEGFA*, *CCND1*, and *c-MYC* [Toledo et al., 2013; Welander et al., 2014]. Welander et al. [2014] also advanced that mutations involving A530 and Y532, both of them in close proximity to P531, promoted similar pathogenic mechanisms as P531 mutations. Additional *EPAS1* mutations were detected by these authors, although their oncogenic mechanism is still uncertain. Compound heterozygosity and combination of activating mutations with copy number gain of *EPAS1* were also reported as mechanisms that cause higher HIF2 α stability [Welander et al., 2014]. Besides, *EPAS1* mutations seem to inhibit the expression of chromaffin cell markers, such as norepinephrine-to-epinephrine converting enzyme PNMT, thus promoting an immature cell signature and contributing to a more aggressive tumor phenotype [Eisenhofer et al., 2004; Toledo et al., 2013; Welander et al., 2014].

In addition to somatic mutations, germline *EPAS1* mutations were also recently described [Lorenzo et al., 2013; Welander et al., 2014]. When present in the germline, *EPAS1* mutations are mostly associated with polycythemia, while PCC/PGL may appear in some patients, but less frequently. This implies that, similar to what happens in VHL disease, different mutations in *EPAS1* may predispose to different phenotypes, suggesting that additional genetic events or the timing of the mutation during development may also be factors influencing the phenotype [Comino-Mendez et al., 2013; Welander et al., 2014].

The first germline gain-of-function *EPAS1* mutation associated with congenital polycythemia and multiple PGL was reported by Lorenzo et al. [2013]. The F374Y

mutation, unlike other *EPAS1* mutations, occurs in a uniquely conserved site in exon 9, suggesting that it can lead to conformational changes at P103, G104, and T105 sites of the pVHL β domain, thus interfering with important functions such as impairment of VHL-elongin C interaction [Lorenzo et al., 2013]. In this way, the F374Y gain-of-function mutation allows a greater stabilization of the HIF2 α protein through a decreased binding to pVHL, causing a marked upregulation of *GLUT1* and *VEGFA*, 2 HIF2 α target genes [Lorenzo et al., 2013].

In conclusion, the somatic and germline *HIF2A* gain-of-function mutations exert tumor promoting effects, although additional events are probably needed for PCC/PGL development [Lorenzo et al., 2013].

Pacak-Zhuang Syndrome

The occurrence of 2 or more distinct types of neuroendocrine tumors in 1 individual is unusual, except in the presence of a hereditary syndrome [Pacak et al., 2013]. While germline *EPAS1* mutations in exon 12 were previously described in patients with hereditary polycythemia, the recent identification of patients carrying *EPAS1* mutations occurring at an early developmental stage, who develop PGL and somatostatinomas in a context of previously settled polycythemia, has led to the proposal of the Pacak-Zhuang syndrome [Toyoda et al., 2014].

Somatostatinomas are neuroendocrine tumors, derived from endoderm-derived, enteric endocrine cells [Pacak et al., 2013]. They are known for producing and secreting specific molecules, namely somatostatin, which may lead to somatostatinoma syndrome, characterized by diabetes mellitus, steatorrhea, and cholecystolithiasis [Pacak et al., 2013]. Somatostatinomas appear occasionally in patients with VHL disease, multiple endocrine neoplasia type 1 and 2, or neurofibromatosis type 1, syndromes that may also present with PCC/PGL [Zhuang et al., 2012; Pacak et al., 2013].

The Pacak-Zhuang syndrome is characterized by the presence of somatic gain-of-function *EPAS1* mutations, which occur in the early stages of embryonic development and are present in the tumor tissue of PGL and somatostatinomas, being clinically associated with polycythemia [Pacak et al., 2013]. Usually, these patients report polycythemia since early infancy or childhood, developing multiple PGL and somatostatinomas at a later stage [Pacak et al., 2013]. Interestingly, the hemoglobin levels of these patients decrease after tumor surgical removal, which may indicate that the tumors themselves promote polycythemia. The patients described by Zhuang et al., [2012], Pacak et al. [2013], and Yang et al. [2013] were all

females, developing multiple PGL and somatostatinomas in adolescence or early adulthood, without evidence of familial history for either polycythemia or PGL and somatostatinoma. The PGL show a noradrenergic phenotype, with diminished PNMT enzymatic activity, which denotes immature features and relation with hypoxia pathway alterations in these tumors [Pacak et al., 2013; Yang et al., 2013]. Pacak et al. [2013] added the presence of uncommon gallbladder disease in their 4 young patients, which accords with the possible symptomatology related to somatostatinoma.

The *EPAS1* mutations associated with Pacak-Zhuang syndrome occur in the A530, L529, and Y532 residues, at the vicinity of the primary hydroxylation site of the HIF2 α protein. They interfere with HIF2 α prolyl hydroxylation and pVHL binding, and lead to HIF2 α stabilization and prolonged half-life. This event allows for the upregulation of HIF target genes, such as *VEGFA*, *GLUT1*, *EDN1* (endothelin 1) and, importantly, *EPO*, which results in a high serum EPO level [Zhuang et al., 2012; Pacak et al., 2013; Yang et al., 2013].

The finding of identical somatic *EPAS1* mutations in different types of tumors (PGL and somatostatinoma) within the same patient prompted researchers to propose that such mutations may have occurred at early stages of embryogenesis, in a neural crest progenitor, leading to the distribution of the mutant cells throughout multiple neuroendocrine organs [Zhuang et al., 2012; Pacak et al., 2013]. Moreover, somatic mutations occurring in precursor cells that secrete excessive EPO may also explain the early onset of polycythemia, which usually manifests before gross tumor formation, and also the persistently high EPO levels often seen after PGL resection [Zhuang et al., 2012; Taieb et al., 2013; Qin et al., 2014]. However, subsequent embryologic studies suggested that chromaffin and enteroendocrine cells do not share the same progenitor cell, but rather a similar development and differentiation regulation, a hypothesis that may explain the common features of both types of tumors [Pacak et al., 2013].

Taieb et al. [2013] described the occurrence of bilateral PCC in the setting of Pacak-Zhuang syndrome, with concomitant multiple PGL and congenital polycythemia, also in a female patient. This patient harbored a somatic *EPAS1* mutation in the A530 residue, in agreement with the previous studies. In the light of this finding, the spectrum of manifestations in patients with somatic gain-of-function *EPAS1* mutations should also include PCC [Taieb et al., 2013].

Recently, Toyoda et al. [2014] described the Pacak-Zhuang syndrome in a male patient who presented with

polycythemia since infancy and, at the age of 15, developed multiple PGL; the *EPAS1* mutation at the A530 residue was found in the PGL tumor tissue. This is the first report of the Pacak-Zhuang syndrome in a male patient [Toyoda et al., 2014].

Other Alterations

miR-210

In the recent years, the role of microRNAs as important modulators of tumorigenesis has gained considerable interest [Tsang et al., 2014]. These are noncoding RNAs, 20–22 nucleotides in length, which participate in gene expression regulation by binding to 3' UTRs of their related parental mRNAs [Tsang et al., 2014]. Specifically, miR-210 is a recently discovered key regulator of hypoxia, which is upregulated by HIF1 α under low oxygen levels [Tsang et al., 2014]. Hence, miR-210 may promote tumorigenesis by regulating the expression of multiple genes and the activation of important oncogenic pathways, such as the hypoxia pathway [Tsang et al., 2014].

Tsang et al. [2014] observed that miR-210 is overexpressed in PCC/PGL carrying *VHL* and *SDHB* germline mutations, which fits with the pseudohypoxic signature of these tumors. miR-210 does not appear to convey a malignant potential for PCC/PGL, as it has been proposed for some cancer types, although larger studies are needed to confirm this observation. Current studies are underway to evaluate if miR-210-mediated gene dysregulation plays a direct role in the pathogenesis of PCC/PGL, or if its overexpression is only a direct consequence of *VHL* and *SDHx* mutations [Tsang et al., 2014].

The pseudohypoxia signature is particularly evident in HNPGL, which typically carry *SDHD* mutations [Tsang et al., 2014]. In a recent report, Merlo et al. [2012] linked miR-210 overexpression with HNPGL, independent of *SDHx* germline mutations, suggesting the axis HIF1 α /miR-210/ISCU/*SDHB* as a new possible pseudohypoxic pathway in the pathogenesis of HNPGL. This new mechanism links hypoxia with downregulation of the iron sulfur cluster unit, leading to the loss of SDH activity and mitochondrial dysfunction, in this way inducing the Warburg effect in the absence of *SDHx* mutations [Merlo et al., 2012]. The mechanisms involved in HIF1 α /miR-210 activation in non-*SDH*-mutant tumors are not yet fully understood; however, Merlo et al. [2013] subsequently reported the identification of the first somatic *VHL* mutation in sporadic HNPGL, which can be related with the activation of this new pathway. This dis-

covery underlines the necessity of new studies that confirm the association between the HIF1 α /miR-210 pathway and pVHL/HIF-related genes, which could explain the development of sporadic HNPGL [Merlo et al., 2013].

Isocitrate Dehydrogenase

Somatic heterozygous mutations in isocitrate dehydrogenase 1/2 (*IDH1/IDH2*) are typically present in the vast majority of low-grade gliomas and secondary high-grade gliomas [Gaal et al., 2010]. Subsequently, Gaal et al. [2010] described a patient with a sporadic HNPGL that harbored a somatic heterozygous *IDH1* mutation, making *IDH* mutations a possible, but infrequent event in PCC/PGL. *IDH1* and *IDH2* are responsible for the oxidative decarboxylation of isocitrate to α -ketoglutarate, the latter a Krebs cycle metabolite that is essential for PHD activity [Gaal et al., 2010]. On the other hand, *IDH* mutations confer a neomorphic activity to the enzyme that consumes and converts α -ketoglutarate to the rare metabolite D-2-hydroxyglutarate. Thus, mutant *IDH* leads to diminished α -ketoglutarate levels, which in turn may hamper PHD activity, leading to HIF stabilization and to pseudohypoxia [Gaal et al., 2010].

Satellite Situations and Other Manifestations

Polycythemia

Polycythemia is defined as an elevated hemoglobin concentration because of increased red blood cell mass [Lanikova et al., 2013; Lorenzo et al., 2013]. It can arise due to congenital defects or as an acquired disease, and can present as a primary or secondary manifestation [Zhuang et al., 2012]. Primary polycythemias are caused by germline or somatic mutations in erythroid progenitors, which produce an increased response to EPO [Lanikova et al., 2013]. On the other hand, secondary polycythemias are caused by an appropriate, or inappropriate, increase in the red blood cell mass due to augmented levels of EPO [Ladroue et al., 2012; Lanikova et al., 2013]. Polycythemias may also be related to abnormalities of the hypoxia pathway, due to increased EPO production and increased sensitivity of progenitor cells to EPO [Zhuang et al., 2012].

Hypoxia, and its master regulator HIF2 α , is a crucial stimulus for EPO production [Lorenzo et al., 2013]. Hence, some congenital polycythemias may result from germline mutations of the hypoxia-sensing pathway, including gain-of-function mutations of *EPAS1* and inacti-

vating mutations of *VHL* and *PHD2*, all of which predispose patients to tumors and/or familial polycythemia [Ladroue et al., 2008, 2012; Zhuang et al., 2012; Lorenzo et al., 2013].

Chuvash Polycythemia

Chuvash polycythemia, an autosomal recessive disease and the first known congenital disorder of the hypoxia pathway, shares features with primary and secondary polycythemias and results from homozygous germline mutations of the *VHL* gene, typically the R200W, but also the H191D and the P138L mutation [Ladroue et al., 2012; Lanikova et al., 2013]. The R200W and H191D mutations cause polycythemia without the development of any other typical manifestation of VHL disease [Lanikova et al., 2013]. The recently discovered P138L *VHL* mutation also causes polycythemia, with both primary and secondary characteristics, without the appearance of VHL-related tumors, in contrast with heterozygous *VHL* mutations in the same amino acid residue that have been reported in VHL syndrome [Lanikova et al., 2013]. The absence of tumor development in this disorder may be due to a “weaker” effect on HIF α regulation, leading only to delayed ubiquitination [Ladroue et al., 2012]. Thus, the phenotypic heterogeneity of *VHL* mutations ranges from tumor development to increased erythropoiesis; nevertheless, the molecular basis of these differences remains unclear [Lanikova et al., 2013].

In Chuvash polycythemia, it is thought that the P138L, H191D, and R200W *VHL* mutations result, at least in part, in decreased stability of pVHL [Lanikova et al., 2013]. The altered pVHL structure interferes with the binding to HIF α , in this way delaying ubiquitination and degradation of HIF proteins; this assumption is supported by the enhanced expression of HIF-regulated genes [Lanikova et al., 2013].

Polycythemia Associated with EGLN1 Mutations

Ladroue et al. [2012] described 2 patients, who displayed features similar to Chuvash polycythemia (no increased risk of neoplasia) and harbored 2 *EGLN1* mutations, namely P200Q and R371H. Both mutations are located outside the catalytic domain of the enzyme, having a moderate impact on HIF α regulation, mimicking what is seen in Chuvash polycythemia.

Chronic Hypoxemia

As discussed previously, PCC/PGL are hypervascularized tumors whose development apparently relies on hypoxia pathway alterations [Jyung et al., 2000; Lopez-

Vazquez et al., 2014]. Thus, it is reasonable to assume that chronic hypoxic conditions, such as living in high altitudes, cyanotic heart disease, and chronic obstructive pulmonary disease – all related to chronic activation of the hypoxia pathway – may be risk factors for the development of PCC/PGL [Kita et al., 2003; van Nederveen et al., 2003].

The carotid body is the chief peripheral sensor of blood oxygen levels [Douwes Dekker et al., 2007]. Noteworthy, carotid body HNPGGL occur 10 times more frequently in populations living in high altitudes (low oxygen levels), than in those living at sea level; this is particularly evident when comparing Peruvians born and living in the Andes with those living at sea level [Jyung et al., 2000; Cerecer-Gil et al., 2010]. In this context, gene-environment interactions, involving mutations of the *SDHx* gene family and hypoxic environmental stimuli, may also be relevant for HNPGGL development [Cerecer-Gil et al., 2010]. An outstanding epidemiologic aspect of carotid body HNPGGL is their female preponderance, particularly in high altitude context. This may be explained by an estrogenic influence in tumor development, a reduced pulmonary capacity in females, or other factors such as periodic blood loss through menstruation [Jyung et al., 2000; Cerecer-Gil et al., 2010].

There are several reports in the literature describing patients with congenital heart defects, such as valvar atresia and Tetralogy of Fallot, who developed PCC/PGL later in life [Diez et al., 1999]. In this setting, PCC/PGL can be single or multiple, benign or malignant, and these patients may also present with acquired polycythemia, secondary to chronic hypoxia [Yoshihara et al., 2008; Subedi and Judson, 2014]. In addition to the chronic hypoxic stimulus, PCC/PGL may develop in these patients due to abnormalities in genes responsible for the function of neural crest cells, which could be involved in both PCC/PGL and the formation of the heart outflow tracts [Kita et al., 2003].

In summary, sustained severe hypoxia can lead to autonomous nervous system and endocrine hyperactivity, as well as mitogenic stimulation [Kita et al., 2003; Douwes Dekker et al., 2007; Yoshihara et al., 2008]. As a consequence, carotid body chief cell and chromaffin cell hyperplasia is promoted and HIF is abnormally stabilized, allowing the development of PCC/PGL [Diez et al., 1999; Subedi and Judson, 2014].

Therefore, clinicians should be aware of the PCC/PGL risk in this set of patients, prompting then a careful examination and screening for PCC/PGL [Cerecer-Gil et al., 2010; Subedi and Judson, 2014].

Ocular Manifestations

Ocular disease has been recently identified as a novel manifestation of the Pacak-Zhuang syndrome [Pacak et al., 2014]. Three females and 1 male with features of Pacak-Zhuang syndrome and somatic gain-of-function *EPAS1* mutations presented with bilateral dilated capillaries and fibrosis overlying the optic disc in both eyes, among other manifestations such as exudation and consequent macular edema, retinal hard exudate with visual acuity decrease, and peripheral retinal neovascularization. It is postulated that the somatic gain-of-function *EPAS1* mutations are involved in the pathogenesis of these eye lesions, due to the importance of angiogenesis in the retina. This additional feature supports the relevance of recognizing the Pacak-Zhuang syndrome. Referral to an ophthalmologist for screening retinal abnormalities is advised [Pacak et al., 2014].

Discussion and Conclusion

PCC/PGL are neuroendocrine tumors whose development is closely related to alterations of the hypoxia pathway. The hypoxia pathway-related *VHL* and *SDHx* genes are of particular relevance, since they account for a high proportion of cases. *VHL* and *SDHx* mutations share multiple similarities at the genetic and phenotypic level, and, ultimately, lead to the stabilization and accumulation of HIF transcription factors, thereby enabling the expression of a large variety of genes involved in angiogenesis, glycolysis, and cell proliferation, among other features [Pollard et al., 2006]. Moreover, new pathways seem to be involved in the pathogenesis of these tumors, such as the developmental neuronal apoptosis pathway, exemplifying the complex mechanisms involved in PCC/PGL tumorigenesis. Alternative genetic and epigenetic mechanisms of pVHL and SDH inactivation, such as loss-of-heterozygosity or methylation, require further study [Tsang et al., 2014].

In addition to *VHL* and *SDHx*, other genes involved in the hypoxia pathway and their pathologic consequences should be examined in these tumors, namely the *PHD* genes, *EPAS1/HIF2A*, and *IDH* genes, among others [Comino-Mendez et al., 2013; Welander et al., 2014]. Additional studies are also required to better understand the functional interplay between HIF α (principally HIF2 α , but also HIF1 α), miR-210, and mitochondria, and its relevance in the pathogenesis of PCC/PGL [Merlo et al., 2012]. Table 1 summarizes some of the information about the main hypoxia pathway genes involved in PCC/PGL development.

Table 1. Summary of hypoxia pathway genes involved in pheochromocytoma and paraganglioma development, with associated syndromes and their manifestations

Gene	Gene type	Chromosome	Mutation type	Associated syndromes	Syndrome dominance	Associated tumors	Other manifestations
<i>VHL</i>	tumor suppressor	3p	inactivating	von Hippel-Lindau disease	autosomal dominant	CNS hemangioblastoma, clear-cell RCC, PCC, endolymphatic sac tumors, additional NET	renal/pancreatic cysts, cystadenomas
				Chuvash polycythemia	autosomal recessive	no associated tumors	–
<i>SDHA</i>	tumor suppressor	5p	inactivating	Leigh syndrome pheochromocytoma and paraganglioma syndrome	autosomal recessive autosomal dominant	– PGL, GIST	– –
<i>SDHB</i> <i>SDHC</i> <i>SDHD</i>	tumor suppressors	1p 1p 11q	inactivating	Carney-Stratakis syndrome pheochromocytoma and paraganglioma syndrome	autosomal dominant autosomal dominant	PGL, GIST multiple PCC/PGL, RCC, HNPGL, GIST	– –
<i>SDHAF2</i>	tumor suppressor	11q	inactivating	pheochromocytoma and paraganglioma syndrome	autosomal dominant	multiple PGL	–
<i>EGLN1/PDH2</i>	tumor suppressor	1q	inactivating	not documented	autosomal dominant?	recurrent PGL	familial polycythemia
<i>EPAS1/HIF2A</i>	proto-oncogene	2p	activating	Pacak-Zhuang syndrome	autosomal dominant?	PGL/PCC, somatostatinoma	early onset polycythemia, ocular manifestations

GIST, gastrointestinal stromal tumors; HNPGL, head and neck paragangliomas; NET, neuroendocrine tumors; PCC, pheochromocytoma; PGL, paraganglioma; RCC, renal cell carcinoma.

The recent discovery of *EPAS1/HIF2A* activating mutations in a particular set of patients harboring PCC/PGL, somatostatinomas, and polycythemia in a familial context, resulted in the proposal of a new syndrome, termed Pacak-Zhuang syndrome [Toyoda et al., 2014]. This opens new perspectives on cancer genetics, in particular the hypothesis that other tumors, especially those of neuroendocrine origin, have gain-of-function *EPAS1/HIF2A* mutations [Zhuang et al., 2012; Pacak et al., 2013]; the precise mechanism by which *EPAS1/HIF2A* mutations contribute to tumorigenesis also warrants additional studies [Pacak et al., 2013; Taieb et al., 2013]. These recent discoveries also underline the necessity of clarifying whether patients presenting with polycythemia should be screened for the presence of neuroendocrine tumors, and whether patients with PGL or somatostatinomas need to be screened for *EPAS1/HIF2A* mutations [Zhuang et al., 2012; Pacak et al., 2013]. Considering the female preponderance in this syndrome, it is also important to determine if unique female-related molecular mechanisms, such as hormone and gender-dependent copy number variations and signaling pathways, play a role in HIF2 α signaling and tumorigenesis [Taieb et al., 2013; Toyoda et al., 2014].

In respect of the malignant potential of PCC/PGL, other pathophysiological mechanisms besides hypoxia and angiogenesis, should be addressed. This is evidenced by the discrepancy in the metastatic potential of Cluster 1 tumors that harbor different genotypes: while *SDHB*-asso-

ciated tumors are strongly linked with a malignant course, *VHL* and *SDHD*-mutated ones are rarely malignant [Span et al., 2011]. Taking into consideration that predicting the malignant behavior of PCC/PGL is quite challenging, Blank et al. [2010] suggested that *SDHB* protein loss could be used as an adverse outcome marker, both in sporadic and in familial tumors; however, one should take into account that *SDHB* protein loss also occurs in *SDHC* and *SDHD*-related tumors, which are associated with a more benign course. Hence, the inclusion of this marker in the assessment of PCC/PGL prognosis should be primarily for directing the molecular genetic tests towards *SDH* genes (in the case of absent staining) [Blank et al., 2010].

Considering the high heritability of PCC/PGL and their difficult management when recurrent or malignant, it is important to detect hereditary conditions and advise patients to genetic counseling and screening, in an attempt to offer a closer follow-up and timely treatment [Gimenez-Roqueplo et al., 2001].

Currently, there is no cure for metastatic disease and the therapeutic options for patients with inoperable tumors or metastases are scarce. The involvement of hypoxia pathway elements in PCC/PGL opens new therapeutic perspectives, through pharmacological inhibition of angiogenesis, especially in patients with malignant PCC/PGL, where the available treatments are still limited [Jyung et al., 2000; Joshua et al., 2009]. HIF inhibitors may also be a future therapeutic option for these patients [Ghayee et al., 2013].

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Disclosure Statement

The authors declare no conflicts of interest.

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