New HIF2 α inhibitors: potential implications as therapeutics for advanced pheochromocytomas and paragangliomas

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Abstract

Two recent independent studies published in *Nature* show robust responses of clear cell renal cell carcinoma (ccRCC) cell lines, preclinical ccRCC xenograft models and, remarkably, a patient with progressive ccRCC despite receiving multiple lines of treatment, to the long-awaited, recently developed inhibitors of hypoxia-inducible factor 2- α (HIF2 α). This commentary published in *Endocrine-Related Cancer* is based on the recognition of similar molecular drivers in ccRCC and the endocrine neoplasias pheochromocytomas and paragangliomas (PPGLs), ultimately leading to stabilization of HIFs. HIF-stabilizing mutations have been detected in the von Hippel–Lindau (VHL) gene, as well as in other genes, such as succinate dehydrogenase (SDHx), fumarate hydratase (FH) and transcription elongation factor B subunit 1 (TCEB1), as well as the gene that encodes HIF2 α itself: *EPAS1*^{HIF2 α} . Importantly, the recent discovery of *EPAS1* mutations in PPGLs and the results of comprehensive *in vitro* and *in vivo* studies revealing their oncogenic roles characterized a hitherto unknown direct mechanism of HIF2 α activation in human cancer. The now available therapeutic opportunity to successfully inhibit HIF2 α pharmacologically with PT2385 and PT2399 will certainly spearhead a series of investigations in several types of cancers, including patients with *SDHB*-related metastatic PPGL for whom limited therapeutic options are currently available. Future studies will determine the efficacy of these promising drugs against the hotspot *EPAS1* mutations affecting HIF2 α amino acids 529–532 (in PPGLs) and amino acids 533–540 (in erythrocytosis type 4), as well as against HIF2 α protein activated by *VHL*, *SDHx* and *FH* mutations in PPGL-derived chromatin cells.

Key Words

- ▶ *EPAS1*
- ▶ hypoxia
- ▶ hypoxia-inducible factor
- ▶ HIF
- ▶ HIF2
- ▶ renal carcinoma
- ▶ kidney carcinoma
- ▶ pheochromocytoma
- ▶ paraganglioma
- ▶ PT2385
- ▶ PT2399

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Tumor hypoxia and anti-angiogenesis cancer therapy

The molecular mechanisms used by cells to adapt and respond to hypoxia have mainly been elucidated in a series of elegant studies conducted by Gregg L Semenza

(Johns Hopkins University School of Medicine, Baltimore, Maryland), William G Kaelin (Harvard Medical School, Boston, Massachusetts, USA) and Peter J Ratcliffe

(University of Oxford, London, United Kingdom). These researchers were awarded the 2016 Albert Lasker Basic Medical Research Award for these fundamental contributions (Kaelin *et al.* 2016).

In physiological hypoxia, the molecular pathways controlled by hypoxia-inducible factors (HIFs) cooperate to restore tissue homeostasis. In contrast, the same molecular pathways are hijacked by cancer cells localized in hypoxic tumor regions that are generated during rapid oncogenic clonal expansion (revised by Bertout *et al.* 2008, Kaelin 2008). Tumor progression depends on neo-angiogenesis through the secretion of vascular endothelial growth factor A (VEGFA). The process of tumor neo-angiogenesis was initially described by Moses Judah Folkman (1936–2008) at the beginning of the 1970s, and has since been investigated extensively (Folkman 1971, 1995, 2007, Weidner *et al.* 1991, Hanahan *et al.* 1996). Neo-angiogenesis is currently considered a hallmark of cancers (Hanahan & Weinberg 2011) and is briefly illustrated below. (a) the HIF2 α -target VEGFA gene is *de novo* transcribed in hypoxic (or pseudohypoxic, see below) cancer cells. (b) The translated VEGFA ligand is secreted and then binds and activates type-2 VEGF receptors (VEGFR2) expressed on the membranes of endothelial cells surrounding the tumor, stimulating the sprouting of blood vessels toward the tumor. (c) Cancer cells then gain access to greater amounts of nutrients and oxygen, sustaining and enhancing tumorigenesis (Bertout *et al.* 2008, Kaelin 2008). With a better understanding of this mechanism, the development of a new category of cancer drugs capable of impeding neo-angiogenesis with the aim of starving cancer was envisioned (Folkman 1972, Cherrington *et al.* 2000). Excitingly, the anti-VEGF monoclonal antibody bevacizumab, which is sold under the trade name Avastin (Roche, Swiss and Genentech, Roche's U.S. Biotech Unit), was made available for clinical trials and showed clinical benefits for patients with cancer (Willett *et al.* 2004, Jain *et al.* 2006). In 2004, the use of bevacizumab in combination with chemotherapy was approved by the Food and Drug Administration (FDA) to treat metastatic colorectal cancer (Ferrara *et al.* 2004, Hurwitz *et al.* 2004, Saltz *et al.* 2008). Bevacizumab has also been approved as a treatment for other types of cancer, including metastatic kidney cancer. In the subsequent years, several tyrosine kinase inhibitors targeting VEGFRs and, therefore, also functioning as anti-angiogenic drugs were developed and clinically approved as cancer therapies. Axitinib, sunitinib, sorafenib, pazopanib, lenvatinib and cabozantinib are small-molecule tyrosine kinase inhibitors (TKIs) that target VEGFR2 (among other

tyrosine kinases) and have been approved in different combinations as treatments for advanced kidney cancer. Despite the great initial excitement for anti-neo-angiogenic therapies, overall, both anti-VEGF and TKIs only benefit some patients for whom the drugs are recommended. Unfortunately, clinical responses are observed during a limited period until the development of resistance, which occurs in nearly all cases (Shojaei *et al.* 2012).

In normoxia, HIF1 α and HIF2 α are hydroxylated by the prolyl hydroxylase enzymes, ubiquitinated by the E3 ubiquitin ligase von Hippel–Lindau (VHL) and continually degraded via proteasomes (Fig. 1). When tissue environments are provided with low levels of oxygen, HIFs are no longer hydroxylated/degraded, becoming stable and promptly detectable. HIFs are transcription factors that control the *de novo* expression of dozens of genes involved in angiogenic signaling, cell migration and energy metabolism, among other pathways (Semenza *et al.* 1992, Maxwell *et al.* 1993, Wang *et al.* 1995, Iliopoulos *et al.* 1996, Epstein *et al.* 2001, Ivan *et al.* 2001, Jaakkola *et al.* 2001). Although the expression of several HIF target genes is regulated by both HIF1 α and HIF2 α , HIF1 α - and HIF2 α -exclusive gene targets have been identified and confer divergent and particular functions to each subunit (Rankin *et al.* 2007).

Pseudohypoxia caused by mutations in *VHL* and other genes

In addition to *real hypoxia* caused by accelerated clonal expansion, tumors also present oxygen-independent activation of HIFs due to genetic causes, a condition called *pseudohypoxia* (Bratslavsky *et al.* 2007). For example, the activation of HIF2 α caused by somatic inactivating mutations of the VHL gene is observed in nearly all ccRCCs (Gnarra *et al.* 1994, Herman *et al.* 1994). Based on the results from recent genomic studies, infrequent ccRCCs that express wild-type VHL carry recurrent hotspot mutations of transcription elongation factor B subunit 1 (*TCEB1*) that abolish Elongin C–VHL binding (Sato *et al.* 2013). *VHL*-null and *TCEB1*-mutated cells are unable to degrade HIF, leading to HIF2 α overexpression, which is considered the hallmark molecular feature of this cancer. Strengthening the role of HIF2 α as the main driver in ccRCC, knockdown of *EPAS1* hinders growth of these tumors in animal model studies (Kondo *et al.* 2003, Zimmer *et al.* 2004).

Inherited mutations of *VHL*, which are also associated with the stabilization of HIFs, are the cause of VHL familial syndrome, which is characterized by blood-formed

Figure 1

Activation of HIF2 α by frequent somatic mutations in the *VHL* gene is the hallmark of clear cell renal cell carcinoma (ccRCC). *VHL* is also a major PPGL susceptibility gene. In addition to *VHL*, mutations in *SDHx*, *FH* and *MET* genes are also shared by both ccRCC and PPGLs. According to the results of tumor profiling studies, *VHL*-mutated ccRCCs and *EPAS1* (HIF2 α)-mutated PPGLs present elevated levels of the HIF2 α target genes *VEGFA*, *CCND1* and *GLUT1*, which are involved in angiogenesis, the cell cycle and cell metabolism. Newly developed HIF2 α antagonists downregulate the expression of these cancer-related genes in ccRCC 786-0 *VHL*-null cell lines and in 786-0 xenograft tumors (Chen *et al.* 2016, Cho *et al.* 2016). A full color version of this figure is available at <http://dx.doi.org/10.1530/ERC-16-0479>.

tumors (hemangioblastomas), ccRCC and pancreatic neuroendocrine tumors, including pheochromocytomas (Latif *et al.* 1993). Notably, specific germline *VHL* mutations are associated with distinct clinical features, i.e., renal carcinoma alone, renal carcinoma associated with pheochromocytoma, and pheochromocytoma alone (Crossey *et al.* 1995, Zbar *et al.* 1996).

Pheochromocytomas and paragangliomas (PPGLs) are neural-crest-derived catecholamine-secreting neoplasias with remarkable genomic features. PPGLs are the most heritable tumors occurring in humans, and approximately 40% of these tumors are caused by a germline mutation in one of the more than fifteen different PPGL susceptibility genes reported to date (Jochmanová *et al.* 2013, Dahia 2014, Favier *et al.* 2015, Toledo *et al.* 2016). In addition to *VHL*, inactivating mutations in the *SDHA/B/C/D* genes, which encode the respective subunits of the succinate dehydrogenases, are also involved in PPGL predisposition and formation (Baysal *et al.* 2000, Niemann *et al.* 2000, Astuti *et al.* 2001, Milunsky *et al.* 2001, Burnichon *et al.* 2010). *SDHs* are enzymes involved in the tricarboxylic acid (TCA) cycle, and their genetic disruption triggers the accumulation of the TCA cycle metabolite succinate in the cytosol, where it inhibits prolyl hydroxylases

(PHDs), leading to HIF stabilization (Selak *et al.* 2005). Similar mechanisms of HIF stabilization are caused by rare mutations in the *SDHAF2* gene, which encodes a HIF complex assembly protein (Bayley *et al.* 2010); the *EGLN1* and *EGLN2* genes, which encode the prolyl hydroxylase domain proteins responsible for signaling the *VHL*-dependent degradation of HIF (Ladroue *et al.* 2008, Yang *et al.* 2015a); and the fumarate hydratase (*FH*) gene, which disrupts the Krebs cycle similar to *SDH*, leading to the stabilization of HIFs (Letouzé *et al.* 2013, Clark *et al.* 2014). Finally, non-heritable mutations in the *EPAS1* gene are molecular drivers of PPGLs, as described below.

Somatic or mosaic *EPAS1* mutations occur in PPGLs

Recently, mutations in the *EPAS1* gene that encodes HIF2 α were independently discovered in PPGLs by two groups using candidate gene (Zhuang *et al.* 2012) and whole exome sequencing (Toledo *et al.* 2013) approaches. These discoveries represented the first reports of *EPAS1* mutations in human cancers. PPGL cohorts have been screened worldwide for *EPAS1* mutations, and to date, approximately forty PPGL-mutated cases have been

reported (Table 1 lists all PPGL *EPAS1* mutations reported to date by Favier *et al.* 2012, Zhuang *et al.* 2012, Comino-Mendez *et al.* 2013, Lorenzo *et al.* 2013, Taieb *et al.* 2013, Toledo *et al.* 2013, Yang *et al.* 2013, Buffet *et al.* 2014, Crona *et al.* 2014, Toyoda *et al.* 2014, Welander *et al.* 2014a,b, Darr *et al.* 2016, Zhuang *et al.* 2016). Frequencies between 6% and 12% were observed, and thus *EPAS1* mutations are one of the most frequent genetic defects occurring in PPGLs.

EPAS1 mutations are missense or compound missense mutations, with very rare cases of indels (5%), and have been detected in patients with PPGL and different clinical features. Phenotypes included nonsyndromic PPGL, as well as patients with Pacak-Zhuang syndrome, which is characterized by polycythemia-PPGL with or without the co-occurrence of somatostatinoma (Table 1). All *EPAS1* mutations are somatic or mosaic (Yang *et al.* 2015b), except for the germline variant F374Y (Lorenzo *et al.* 2013), which has been described as a rare polymorphism (NCBI dbSNP rs150797491). Although no genotype-phenotype correlations have been described within PPGLs, a superposition of the PPGL-related and familial erythrocytosis type 4 (MIM #611783)-related *EPAS1* mutations has not been observed. Mutations occurring in both disorders are gain-of-function and occur within the oxygen-dependent degradation (ODD) domain; however, they affect near but distinct amino acids. PPGL mutations mainly affect amino acids 529–532, and erythrocytosis type 4 mutations occur exclusively within amino acids 533–540 (Percy *et al.* 2008a,b). A detailed assessment of the clinical features associated with *EPAS1* mutations is beyond the scope of this manuscript and has been described elsewhere (Darr *et al.* 2016).

In contrast to the frequent *EPAS1* mutations detected in PPGLs, these mutations are extremely rare in non-PPGL tumors. The visualization of genomic data from more than 31,000 tumor samples included in 145 projects of The Cancer Genome Atlas (TCGA) via CbioPortal for Cancer Genomics (www.cbioportal.org) reveals that a very small number of non-PPGL tumors carry *EPAS1* mutations: only 221 samples were mutated (0.007%) (Supplementary Table 1, see section on supplementary data given at the end of this article). These mutations occur throughout the gene and have no clear impact on tumorigenesis. Importantly, only three non-PPGL TCGA tumors harbor mutations between the L529 and Y532 PPGL mutation hotspot mutation residues (Supplementary Tables 1 and 2).

Supplementary Figures 1 and 2 show the mRNA expression levels and somatic mutation status of *VHL* and *EPAS1*, respectively, among twenty-six TCGA projects. The highly frequent rates of *VHL* mutations in ccRCC are associated with very low *VHL* mRNA levels. PPGLs also present decreased levels of the *VHL* mRNA, despite the presence of few mutations (Supplementary Fig. 1). In contrast, ccRCC and PPGLs present the highest and second highest levels of the *EPAS1* mRNA among all tumor types (Supplementary Fig. 2). Notably, ccRCC samples with or without a *VHL* mutation present low levels of the *VHL* mRNA, consistent with the reported genetic and epigenetic inactivation of *VHL* in these tumors (Gnarra *et al.* 1994, Herman *et al.* 1994). Interestingly, all PPGLs harboring an *EPAS1* mutation present elevated levels of the *EPAS1* mRNA (Supplementary Fig. 3), whereas the *EPAS1* gene is expressed at very high levels in ccRCC, despite the extremely rare incidence of mutations in this gene (Supplementary Fig. 4).

PPGL *EPAS1* mutations affect the ODD domain of HIF2 α and are oncogenic

All *bona fide* *EPAS1* mutations observed in PPGLs are located in the ODD domain in the vicinity of the proline-531 hydroxylation site of HIF2 α . Two main hotspot mutations for *EPAS1* have been observed: proline-531 itself and its neighboring residue alanine-530 (Fig. 2). Together, Ala530/Pro531 residues account for 62% of *EPAS1* mutations described in PPGLs. Remarkably, up to 81% of all *EPAS1* PPGL mutations are located between L529 and Y532 (Table 1).

Importantly, these mutations were carefully validated by studies conducted at three levels: (a) ectopic expression of HIF2 α mutants in *VHL*-wt and *VHL*-null cell lines (Zhuang *et al.* 2012, Toledo *et al.* 2013, Yang *et al.* 2013); (b) molecular characterization of gene expression and/or methylation signatures in the tumor (Favier *et al.* 2012, Zhuang *et al.* 2012, Toledo *et al.* 2013, Yang *et al.* 2013); and, finally, (c) analyses of the tumor growth capacity in xenografts generated from cell lines stably expressing HIF2 α mutants (Toledo *et al.* 2013). According to these studies, *EPAS1* mutations are undoubtedly pathogenic, revealing a previously unrecognized mechanism of activation of the HIF2 α transcription program and tumor promotion via direct gain-of-function mutations in its gene.

Protein structure studies led to the development of HIF2 α inhibitors

For many years, a number of transcription factors, including HIF2 α , were considered undruggable, and pharmaceutical companies focused on the downstream pathway of HIFs, including VEGF and VEGFR2. In 2003, studies lead by Kevin Gardner and Rick Bruick from the University of Texas Southwestern Medical Center, Dallas, Texas, used nuclear magnetic resonance spectroscopy to define the structure of the HIF2 α protein and its binding with the aryl hydrocarbon receptor nuclear translocator (ARNT), also called HIF1B (Erbel *et al.* 2003). In 2009, the same group used crystallography to obtain structures of the heterodimer formed by HIF2 α PAS domains and ARNT. These analyses enabled the authors to refine the HIF2 α structure to a high quality and to discover a previously unrecognized cavity within the PAS-B domain. The HIF2 α cavity is located within the HIF2 α PAS-B domain and has large extensions measuring $7 \times 14 \times 15 \text{ \AA}$, yielding a total volume of $290 \text{ \AA}^3$ (Scheuermann *et al.* 2009, 2013, 2015, Rogers *et al.* 2013). This type of large cavity is extremely rare in proteins. This finding was therefore unexpected and very promising, as this cavity was likely capable of accommodating small molecules and being successfully targeted. Importantly, despite the great similarity in the protein sequences, this specific $290 \text{ \AA}^3$ PAS-B cavity was exclusively observed in HIF2 α , whereas the two other HIF α subunits (HIF1 α and HIF3 α) present PAS-B cavities with distinctive features (Cardoso *et al.* 2012, Fala *et al.* 2015, Wu *et al.* 2015). Based on evidence from the protein structure, HIF2 α inhibitors were confirmed to be selective for HIF2 α and did not bind to HIF1 α and HIF3 α subunits (Scheuermann *et al.* 2009, 2013).

Screening and selection of HIF2 α inhibitors

Based on the structure of the HIF2 α :ARNT protein dimer, Peloton Therapeutics (Dallas, Texas) performed an extensive screening of small-molecule libraries and generated 130 HIF2 α /inhibitor crystal structures. After analyzing the potency, selectivity and oral viability, compound PT2385 was chosen for clinical development. Compound PT2399 has been developed as a tool HIF2 α inhibitor used in *in vivo* studies and exhibits pharmacological properties that are equal to those of PT2385 (Scheuermann *et al.* 2013, 2015).

PT2399 and PT2385 inhibitors downregulate the hypoxia transcriptional program and inhibit tumor growth in ccRCC models

Based on expression analyses performed using qPCR and microarrays, the HIF2 α target genes VEGFA, cyclin D1 (CCDN1) and SLC2A1 (glucose transported protein type 1, GLUT1) are upregulated in PPGL tumors carrying an *EPAS1* mutation. These findings were confirmed by ectopic transient or stable expression of HIF2 α mutants in human cell lines (Zhuang *et al.* 2012, Toledo *et al.* 2013, Yang *et al.* 2013), strengthening the gain-of-function character of these mutations. High levels of the mRNAs for these genes are also detected in *VHL*-mutated ccRCC cell lines or corresponding xenograft models. Treatment with PT2399 downregulates the expression of these genes and controls tumor growth induced by this transcriptional program both *in vitro* and *in vivo* (Chen *et al.* 2016, Cho *et al.* 2016, Wallace *et al.* 2016). Based on these results, PT2385 and PT2399 are expected to effectively control the hypoxia response gene signature in PPGLs. However, in addition to the classical hypoxia response genes, researchers must also consider *EPAS1* mutations that upregulate the expression of the insulin-like growth factor 2 (IGF2) and c-MYC oncogenes, as well as genes involved with chromaffin differentiation and stemness, such as neuropeptide Y (NPY), NANOG, octamer-binding transcription factor 4 (OCT4), JAGGED2, keratin 19 (KRT19), SRY-box 9 (SOX9) and cadherin 2 (CDH2) (Toledo *et al.* 2013).

A phase 1 clinical trial (ClinicalTrials.gov ID NCT02293980) has evaluated the maximum tolerated dose of oral PT2385 alone and in combination with nivolumab, a PD-1 immune checkpoint inhibitor, and cabozantinib, an anti-angiogenic VEGFR2 inhibitor in patients with advanced ccRCC. Importantly, a prolonged response has been observed in at least one heavily treated, resistant patient with ccRCC (Chen *et al.* 2016). Importantly, the initial results of the combinations of HIF2 α inhibitors with immunotherapy have been recently reported and showed potential therapeutic synergism (Han *et al.* 2016).

Prospective of testing HIF2 α inhibitors in PPGLs

In a recent issue of *Nature*, two studies reported exciting results showing that the recently developed

Table 1 List of thirty-nine somatic/mosaic mutations occurring in EPAS1/HIF2A gene in PPGLs patients.

Patient #	Clinical features	Mutation type	AA change	Residue	N mutations per residue	Protein domain	References
1	Paraganglioma(s)	Missense	S71Y	71	1	No domain defined	Toledo et al. (2013)
2	Paraganglioma(s)	Missense	M368I	368	1		Welander et al. (2014a)
3	Familial polycythemia and paraganglioma	Missense	F374Y	374	2		Lorenzo et al. (2013)
4	Familial polycythemia and paraganglioma	Missense	F374Y				Lorenzo et al. (2013)
5	Paraganglioma(s)	Missense	F374Y				Welander et al. (2014a)
6	Paraganglioma(s)	Compound missense	L403P (+ A530V)	403	1		Welander et al. (2014b)
7	Paraganglioma(s)	Compound missense	I412N (+ Y532C)	412	1		Welander et al. (2014a)
8	Gangliocytic paraganglioma	Missense	T519M	519	1	Oxygen-dependent degradation (ODD) domain	Zhuang et al. (2016)
9	Polycythemia-pheochromocytoma/paraganglioma-somatostatinoma syndrome (Pacak-Zhuang syndrome)	Missense	L529P	529	3		Yang et al. (2013)
10	Polycythemia-pheochromocytoma/paraganglioma-somatostatinoma syndrome (Pacak-Zhuang syndrome)	Missense	L529P				Buffet et al. (2014)
11	Paraganglioma(s)	Missense	L529P				Crona et al. (2014)
12	Paraganglioma(s)	Missense	A530E	530	10		Welander et al. (2014a)
13	Polycythemia-pheochromocytoma/paraganglioma syndrome (Pacak-Zhuang syndrome), no somatostatinoma up to the report date	Missense	A530E				Toyoda et al. (2014)
14	Polycythemia-pheochromocytoma/paraganglioma-somatostatinoma syndrome (Pacak-Zhuang syndrome)	Missense	A530T				Zhuang et al. (2012)
15	Polycythemia-pheochromocytoma/paraganglioma syndrome (Pacak-Zhuang syndrome), no somatostatinoma up to the report date	Missense	A530T				Comino-Mendez et al. (2013)
16	Polycythemia-pheochromocytoma/paraganglioma-somatostatinoma syndrome (Pacak-Zhuang syndrome)	Missense	A530V				Zhuang et al. (2012)
17	Paraganglioma(s)	Missense	A530V				Comino-Mendez et al. (2013)
18	Polycythemia-pheochromocytoma/paraganglioma syndrome (Pacak-Zhuang syndrome), no somatostatinoma up to the report date	Missense	A530V				Taieb et al. (2013)
19	Pheochromocytoma(s)	Missense	A530V				Crona et al. (2014)
6	Paraganglioma(s)	Compound missense	A530V (+ L403P)				Welander et al. (2014b)
20	Pheochromocytoma(s)	Missense	P531R	531	10		Welander et al. (2014b)
21	Paraganglioma(s)	Missense	P531A				Welander et al. (2014b)
22	Polycythemia-pheochromocytoma/paraganglioma syndrome (Pacak-Zhuang syndrome), no somatostatinoma up to the report date	Missense	P531L				Comino-Mendez et al. (2013)
23	Paraganglioma(s)	Missense	P531L				Welander et al. (2014a)
24	Paraganglioma(s)	Missense	P531L				Toledo et al. (2013)
25	Paraganglioma(s)	Missense	P531S				Favier et al. (2012)

26	Polycythemia-pheochromocytoma/paraganglioma syndrome (Pacak-Zhuang syndrome), no somatostatinoma up to the report date	Missense	P531S	Comino-Mendez et al. (2013)
27	Pheochromocytoma(s)	Missense	P531S	Welander et al. (2014b)
28	Paraganglioma(s)	Missense	P531S	Toledo et al. (2013)
29	Paraganglioma(s)	Missense	P531T	Toledo et al. (2013)
30	Polycythemia-pheochromocytoma/paraganglioma-somatostatinoma syndrome (Pacak-Zhuang syndrome)	Missense	Y532C	Yang et al. (2013)
31	Paraganglioma(s)	Missense	Y532C	Welander et al. (2014b)
7	Paraganglioma(s)	Compound missense	Y532C (+I412N)	Welander et al. (2014a)
32	Paraganglioma(s)	Indel	I533P_534del	Comino-Mendez et al. (2013)
33	Paraganglioma(s)	Indel	P534D_536del	Comino-Mendez et al. (2013)
34	Paraganglioma(s)	Missense	D539Y	Comino-Mendez et al. (2013)
35	Polycythemia-pheochromocytoma/paraganglioma-somatostatinoma syndrome (Pacak-Zhuang syndrome)	Missense	D539N	Darr et al. (2016)
36	Polycythemia-pheochromocytoma/paraganglioma syndrome (Pacak-Zhuang syndrome), no somatostatinoma up to the report date	Missense	L542P	Buffet et al. (2014)
37	Gangliocytic paraganglioma	Missense	P544S	Zhuang et al. (2016)

HIF2 α inhibitors PT2385 and PT2399 efficiently reduce the oncogenic growth of ccRCC *in vitro* and *in vivo* (Chen et al. 2016, Cho et al. 2016). As PT2385 is currently being tested in the clinic as a treatment for patients with advanced ccRCC, these initial results will spearhead a multitude of preclinical and clinical studies aiming to assess the efficiency of these compounds in other tumor types and in different combinations with immunotherapy and anti-VEGFR2 therapies. In the context of endocrine-related cancers, most PPGLs express high levels of HIFs (Dahia et al. 2005, Castro-Vega et al. 2015). These tumors are classified by the cluster 1 gene expression pattern and usually carry mutations in VHL, SDHx, and FH genes. Recently, mutations in *EPAS1* were discovered in PPGL cluster 1 tumors without a mutation in other PPGL-related genes. These patients presented diverse clinical features, including isolated disease, malignant, multiple and recurrent PPGLs, and syndromic features (Favier et al. 2012, Zhuang et al. 2012, Comino-Mendez et al. 2013, Lorenzo et al. 2013, Taieb et al. 2013, Toledo et al. 2013, Yang et al. 2013, Buffet et al. 2014, Crona et al. 2014, Toyoda et al. 2014, Welander et al. 2014a,b, Darr et al. 2016, Zhuang et al. 2016). Comprehensive functional and animal model studies have characterized *EPAS1* PPGL mutations as gain-of-function events with oncogenic capability (Toledo et al. 2013). Future studies are needed to determine the efficiency of PT2385 and PT2399 against HIF2 α mutants occurring in PPGLs (affecting amino acids 529–Y532), and erythrocytosis type 4 (affecting amino acids 533–540) (Percy et al. 2008a,b). Based on the results of expression analyses using microarray and qPCR, *EPAS1*-mutated PPGLs present a specific gene expression pattern compared to other cluster 1 PPGLs carrying *VHL*/*SDHx*/*FH* mutations, suggesting that direct activation of HIF2 α represents a subcluster of PPGL-HIF2 α among pseudohypoxic PPGL tumors (Favier et al. 2012, Zhuang et al. 2012, Toledo et al. 2013, Yang et al. 2013, Flidner et al. 2016). Importantly, no response was observed in some *VHL*-mutated ccRCC xenograft models treated with HIF2 α inhibitors, indicating that efficiency of these drugs is also regulated by other factors (Chen et al. 2016). Prolonged treatment with HIF2 α inhibitors generated resistance that was at least partially caused by acquired *EPAS1* mutations affecting the PAS-B binding domain of HIF2 α (Chen et al. 2016). Further studies are required to determine whether PT2385 and PT2399 will be effective against the HIF2 α protein activated by *EPAS1*, *VHL*, *SDHx* and/or *FH* mutations in PPGL-derived chromatin cells.

Figure 2

Mutations in the *EPAS1*^{HIF2 α} gene occur in the ODD domain. Mutations reported in pheochromocytomas and paragangliomas are depicted in black, mutations reported in familial erythrocytosis (type 4, OMIM 611783) are depicted in red and common polymorphisms detected at a high frequency in healthy controls are depicted in blue. Circles illustrate mutations that have been shown or are likely to be pathogenic, triangles indicate variants with an unknown clinical significance (VUS) and squares indicate common polymorphisms. Structures, protein domains and regions of binding and dimerization of HIF2 α and ARNT (HIF-B) are shown. A: PAS (Per-ARNT-Sim) A domain; B: PAS (Per-ARNT-Sim) B domain. bHLH, basic helix-loop-helix domain; HIF2 α antagonist, PT2399 and PT2385 (peloton therapeutics); ODD, oxygen-dependent degradation domain; TAD, transactivation domain. A full color version of this figure is available at <http://dx.doi.org/10.1530/ERC-16-0479>.

Clinical trials testing the efficacy of anti-angiogenic drugs in patients with PPGL

The robust molecular findings implicating the disruption of the genes involved in the hypoxia response in PPGL tumorigenesis and case reports of prolonged responses in patients with advanced PPGL who were treated with anti-angiogenic agents (Joshua *et al.* 2009) have spearheaded the initiation of further target therapeutic studies. Currently, several clinical trials are evaluating the efficacy of several VEGFR2 inhibitors in patients with advanced PPGL, including sunitinib (NCT00843037, NCT01371201), dovitinib (NCT01635907), axitinib (NCT01967576), lenvatinib (NCT03008369), cabozantinib (NCT02302833) and pazopanib (NCT01340794). Notably, the clinical study of cabozantinib is particularly interesting because, in addition to being the most potent VEGFR2 inhibitor (IC_{50} =0.035 nM), it also strongly inhibits c-MET (IC_{50} =1.3 nM), another oncogene that is frequently mutated in PPGLs (Castro-Vega *et al.* 2015, Toledo *et al.* 2016). Finally, the HIF2 α inhibitor PT2385 has now entered a phase II clinical trial (NCT03108066) evaluating its efficacy in advanced cancer patients carrying a *VHL* germline mutation, and hopefully, patients with PPGL will also be recruited.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/ERC-16-0479>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this commentary.

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