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REVIEW
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The VHL/HIF Axis in the Development and Treatment of Pheochromocytoma/Paraganglioma

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Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors originating from chromaffin cells in the adrenal medulla (PCCs) or extra-adrenal sympathetic or parasympathetic paraganglia (PGLs). About 40% of PPGLs result from germline mutations and therefore they are highly inheritable. Although dysfunction of any one of a panel of more than 20 genes can lead to PPGLs, mutations in genes involved in the VHL/HIF axis including *PHD*, *VHL*, *HIF-2A (EPAS1)*, and *SDHx* are more frequently found in PPGLs. Multiple lines of evidence indicate that pseudohypoxia plays a crucial role in the tumorigenesis of PPGLs, and therefore PPGLs are also known as metabolic diseases. However, the interplay between VHL/HIF-mediated pseudohypoxia and metabolic disorder in PPGLs cells is not well-defined. In this review, we will first discuss the VHL/HIF axis and genetic alterations in this axis. Then, we will dissect the underlying mechanisms in VHL/HIF axis-driven PPGL pathogenesis, with special attention paid to the interplay between the VHL/HIF axis and cancer cell metabolism. Finally, we will summarize the currently available compounds/drugs targeting this axis which could be potentially used as PPGLs treatment, as well as their underlying pharmacological mechanisms. The overall goal of this review is to better understand the role of VHL/HIF axis in PPGLs development, to establish more accurate tools in PPGLs diagnosis, and to pave the road toward efficacious therapeutics against metastatic PPGLs.

Keywords: pheochromocytomas, paragangliomas, VHL, HIF, metabolism, inhibitor

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INTRODUCTION

Pheochromocytomas (PCCs) are catecholamine-secreting tumors that originated from the chromaffin cells in the adrenal medulla. Paragangliomas (PGLs) are neural crest-derived neuroendocrine neoplasms originating from extra-adrenal sympathetic or parasympathetic ganglia (1). Both PCCs and PGLs are collectively known as PPGLs. PPGLs are rare tumors with the incidence rate between 0.2 and 0.8 per 100,000 (2–4) with great clinical manifestations (5). Due to elevated levels of catecholamines in the circulation, the common clinical presentations of PPGLs include episodes of headache, sweating, palpitation, and hypertension. In addition, about 10% of PCCs are metastatic (6) and 40% of PGLs are considered as metastatic disease (7, 8).

Etiologically, about 70%–80% of PPGLs are caused by genetic abnormalities which affect different signaling pathways (9). Approximately, 40% of PPGLs result from germline mutations, and therefore they are highly inheritable (10). Although dysfunction of any of these related susceptible gene products can lead to PPGLs, mutations in the genes encoding the VHL/HIF axis such as *VHL*, *HIF*, and *PHD* are more commonly found in PPGLs (11). Moreover, multiple lines of evidence suggest that pseudohypoxia plays a crucial role in the tumorigenesis of PPGLs. In this review, we will discuss the genetic alterations affecting the VHL/HIF axis and dissect the underlying molecular mechanisms in pseudohypoxia signaling and PPGLs. We will also summarize the currently available compounds or drugs targeting VHL/HIF axis, their specific targets, and pharmacological mechanisms.

THE VHL/HIF AXIS

The Von Hippel-Lindau (*VHL*) gene located on 3p25.5 encodes an ancient tumor suppressor, pVHL. Although pVHL functions in both physiology and pathology, as a component of an E3 ubiquitin-ligase complex, pVHL plays a determinant role in the degradation of hypoxia-induced factors (HIFs) including HIF-1α, HIF-2α, and HIF-3α. The roles of HIF-1α and HIF-2α in sensing and facilitating cellular adaptation to hypoxic conditions as well as their underlying molecular mechanisms are well-established (12). However, much less is known about HIF-3α. Functionally, HIF-1α and HIF-2α heterodimerize with HIF-β by HLH domain, which is also known as ARNT, to transcriptionally regulate a wide spectrum of HIF target genes. Both HIF-α and HIF-β belong to the basic helix-loop-helix-Per-ARNT-Sim (bHLH-PAS) family. They contain a basic DNA binding domain, a conserved NH_2-terminal domain (N-TAD), and two specialized transactivation domains located in their variable COOH-terminal domains (C-TAD) (13) (**Figure 1**). The asparagine residue (N803) in the C-TAD of HIF-α can be hydroxylated by factor-inhibiting HIF (FIH) to interrupt its interaction with CREB-binding protein (CBP)/p300, an essential coactivator of HIF (14–16). The N-TAD also contains an oxygen-dependent domain (ODDD), in which a few prolyl residues (Pro-402 and Pro-564 in HIF-1α; Pro-405 and Pro-531 in HIF-2α) are selectively hydroxylated under normoxic condition and hydroxylated HIFs are subsequently degraded (17–20). The enzymes responsible for HIF-α hydroxylation belong to the egg-laying-defective nine (*EGLN*) family known as PHD1, PHD2, and PHD3 because they all contain a prolyl-4-hydroxylase domain. These enzymes are dioxygenases and use both molecular oxygen and Fe^{2+} as their co-substrates to catalyze HIF-α hydroxylation.

The VHL/HIF axis responses to reduced oxygen concentration or hypoxia. Although HIF-1α and HIF-2α have about 48% sequence similarity, they regulate two different groups of target genes with limited overlap mainly due to their dissimilar transactivation domains (21, 22). In addition, HIF-1α is widely expressed, while HIF-2α is only expressed in certain cell types (23, 24). For example, the genes involved in glucose metabolism are mainly regulated by HIF-1α. HIF-2α plays a more important role in the adjustment to high altitudes and the regulation of EPO expression (25, 26). As mentioned above that compared to HIF-1α and HIF-2α, much less is known about HIF-3α. Since it lacks the transactivation domain (27), HIF-3α likely does not

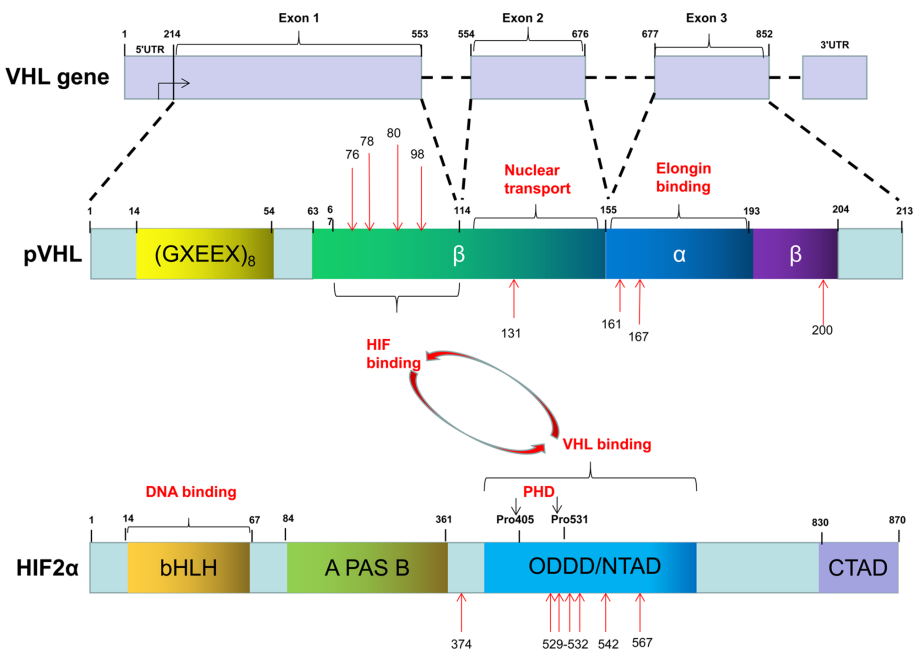

FIGURE 1 | The common mutation sites of VHL and EPAS1 genes in PPGLs.

transcriptionally regulate its target genes. Overall, the levels and functions of both HIF-1α and HIF-2α are oxygen-concentration dependent. Specific proline residues of HIF-1α and HIF-2α are hydroxylated by PHD under normoxic conditions. With the involvement of the molecules such as elongin B, elongin C, cul2, the hydroxylated HIFs are recognized by the pVHL (28–30), subsequently ubiquitinated and ultimately degraded (31, 32). Under hypoxic conditions, the non-hydroxylated HIFs are dissociated from pVHL, accumulated in the cells, and subsequently upregulate their target genes transcriptionally. However, failure in the degradation of HIFs due to either deletion or mutation of either *VHL*, *HIFs*, or *PHDs* can lead to dysregulation of HIFs-regulated genes in a variety of diseases including PPGLs (**Figure 2**).

DYSREGULATION OF THE VHL/HIF AXIS AND PPGLs

As mentioned above that mutation in either the three genes encoding pVHL, HIFs and PHDs can lead to abnormal accumulation of HIFs. Minor alteration of this axis usually causes erythrocytosis; whereas major dysregulation of the axis is associated with tumorigenesis (33). Although a wide spectrum of tumors including hemangioblastomas, renal cell carcinoma (RCC), pancreatic neuroendocrine tumor, and PPGLs can result from dysregulation of the VHL/HIF axis (34–37), this review will only focus on the relationship between aberrations of these genes and PPGLs.

VHL Mutations

After the *VHL* mutations were first described in an ophthalmic disease (34), multiple studies subsequently confirmed that *VHL* mutations can cause a variety of diseases including cancers (35–37). To honor the contributions of the German ophthalmologist Eugen von Hippel and the Swedish pathologist Arvid Lindau, the gene responsible for these diseases is, therefore, named as *VHL*. Of note, VHL disease caused by heterozygous germline mutations is autosomal dominant and almost completely penetrant (97%) (38). VHL diseases are generally classified into two types, type 1 (without PCCs) and type 2 (with PCCs). The type 2 disease is manifested as RCCs, PCCs, central nervous system, retinal hemangioblastomas, pancreatic neuroendocrine tumors and pancreatic and renal cysts and can be further divided into three subtypes (34), PCCs with all types of VHL disease manifestations without RCC (Type 2A), PCC with all types of VHL disease including RCC (Type 2B), and isolated PCCs (Type 2C).

To date, more than 1,000 mutations in *VHL* gene have been identified. These mutations can be categorized as missense mutation (52%), frameshift mutation (13%), nonsense mutation (11%), in-frame deletion/insertion mutation (6%), large/complete deletion mutation (11%), and splicing mutation (7%) (39). The common germline mutations in *VHL* are delPhe76, Asn78Ser, Argl61Stop, Arg167Gln, Argl67Trp, and Leu178Pro (40) (**Figure 1**). Recently, we reported four missense mutations in five Chinese unrelated families c.239G>T (p.Ser80Ile), c.232A>T (p.Asn78Tyr), c.500G>A (p.Arg167Gln), c.293A>G (p.Try98Cys), and all four mutations

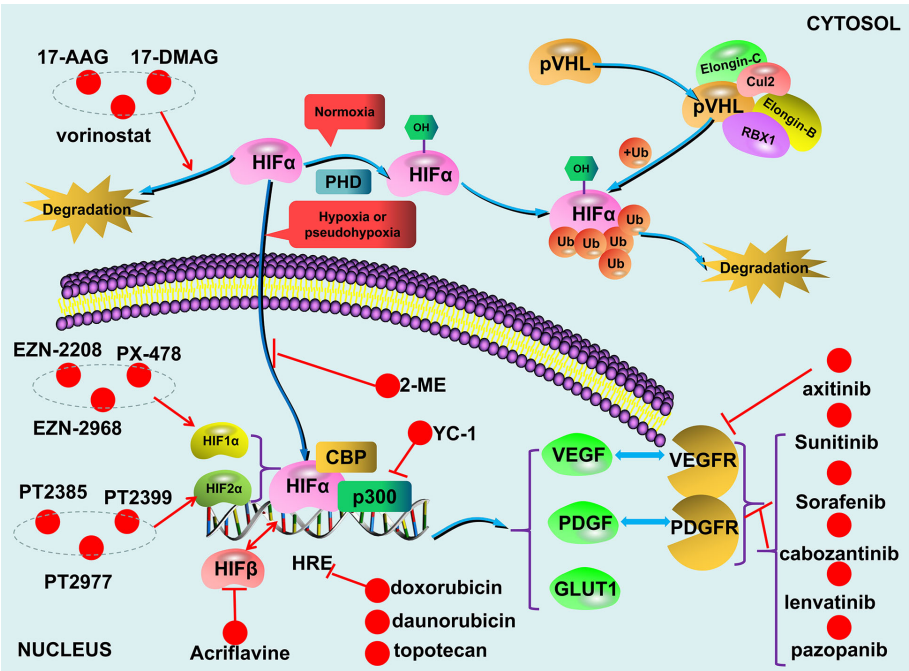

FIGURE 2 | The VHL/HIF axis and compounds targeting the axis.

predispose the patients to VHL disease (41). Notably, type 2 VHL disease mainly resulted from missense mutations (85%–92%) (40, 42), especially mutations in codons 167 and 238, are mainly associated with PPGLs (43, 44). In contrast, homozygous germline mutations are rare or barely cause tumors. Sonny et al. found a c.598C>T (p.Arg200Trp) homozygous missense germline mutation of *VHL* caused Chuvash polycythemia (45). In addition, somatic *VHL* mutations were found in majority (50%–70%) of clear-cell RCC cases (38).

It has been reported that different mutations in *VHL* lead to diverse clinical symptoms (41, 46–49), and sometimes even the same mutation can lead to different phenotypes (50–53). Since pVHL has multiple functional domains, one of the potential explanations for this phenomenon is that a specific mutation causes particular dysfunction. It appears that missense mutations are more likely linked with type 2 disease and truncating mutations are responsible for type 1 disease (54). However, Liu et al. further stratified the missense mutations as HIF-α binding site missense mutations (HM) group and non-HIF-α binding site missense mutations (nHM) group, and found that the missense mutations in HM group had similar risks of most tumors with truncating mutations with the exception that the HM group had a lower risk of RCC. Moreover, compared to nHM, missense mutations in HM had a higher risk of pancreatic cyst or tumor and a lower risk of PCCs (55). Secondly, some functions of pVHL are O_2-independent (56, 57) or unrelated to HIF regulation, these functions may also be involved in PPGLs pathogenesis. Michael et al. found that RCCs with deficient pVHL exhibited deficiency in fibronectin matrix assembly (58). Intriguingly, Clifford et al. reported that mutations associated with type 2C phenotype could even promote, rather than inhibit, HIF-α ubiquitylation and degradation (39). These findings altogether supported the notion that disturbing the functions of pVHL contributes to the development of PPGLs. Additionally, based on Knudson's Two-Hit model (59), it is understandable that the diverse phenotypes of VHL diseases could be the result of two different "hits".

The VHL/HIF axis also can be affected by dysregulated epigenetic modifications such as gene silencing by methylation of the CpG islands in the promoter of related genes. Indeed, promoter hypermethylation occurs in about 3%–42% of clear-cell RCC (60). Adam Andreasson found that the promoter methylation of the *VHL* gene is not only elevated in PPGLs compared with normal tissue (57% vs. 27%) but also significantly higher in malignancies than that in tumors (63% vs. 55%) (61). However, the precise molecular mechanisms in the pathogenesis of PPGLs related to loss-of-function of pVHL are still largely unknown and therefore need further investigation.

HIF-A Mutations

As mentioned above that HIF-α family composed three members, HIF-1α, HIF-2α, and HIF-3α. But little is known about HIF-3α. Compared with *HIF-2A*, *HIF-1A* has relatively few mutations, ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) only collects 30 records. Morris et al. reported a somatic mutation (p.Val116Glu) and a germline missense mutation (p.Ala475Ser) of *HIF-1A* in a clear-cell RCC with *VHL* inactivation. Of note, the germline mutation (p.Ala475Ser) was likely to be a benign variant (62). Furthermore, Gladek et al. found that *HIF-1A* Single-Nucleotide Polymorphisms (SNPs) are association with the phenotypes of many tumors (63). In PPGLs patients, only copy number aberration (TCGA-QT-A5XP, https://portal.gdc.cancer.gov/), not *HIF-1A* mutation, have been found. On the other hand, both germline and somatic mutation in *HIF-2A* have been identified in patients with polycythemia and/or PPGLs. However, it appears that germline mutations of *HIF-2A* including p.Met535Val, p.Gly537Arg, p.Gly537Trp only leads to polycythemias, not tumors (64, 65). A gain-of-function germline mutation in *HIF-2A* alone is not sufficient for tumorigenesis presumably that simultaneous loss-of-function in some tumor suppressors is needed. In fact, we recently reported that germline mutations in *HIF-2A* (c.1609G>A, p.Gly537Arg) are responsible for polycythemia formation and additional somatic *VHL* mutations are needed for the development of clear-cell RCC (66). Similarly, a germline mutation in *HIF-2A* exon 9 (c.1121T>A, p.F374Y) leads to polycythemia and predisposes the patients for PPGLs development (67). In addition, somatic mutations in *HIF-2A* appear to be more frequent genetic events in PPGLs (68). For example, Zhang et al. reported two gain-of-function somatic mutations (c.1588G>A, p.Ala530Thr and c.1589C>T, p.Ala530Val) in exon 12 of *HIF-2A* resulting in paraganglioma and polycythemia, respectively. Further analyses suggest that mutations in the vicinity of the hydroxylation site Pro-531 affect the catalytic activity of PHD and then lead to the interrupted interaction between HIF-2α and pVHL (69). Moreover, Karel Pacak et al. reported two somatic mutations of *HIF-2A* (c.1595A>G p.Y532C and c.1586T>C p.L529P) in patients with either congenital polycythemia, multiple recurrent PPGLs, or somatostatinoma (70). We recently found that a gain-of-function mutation of *HIF-2A* (c.1589C>T) leads to PPGLs with polycythemia simultaneously (26) and a mutation in *HIF-2A* immediately distal to its DNA binding domain (p.Ser71Tyr) has been identified in sporadic PPGLs (71) (**Figure 1**). Germline or somatic mutations of *HIF-2A* can be mosaic. Buffet et al. reported two cases of *HIF-2A*-related Polycythemia-Paraganglioma Syndrome resulted from mosaicism mutations. They found that these patients could present with young age and multiplicity; and also the mutations could be transmitted to the offspring (72). In addition, *HIF-2A* mosaic mutation might be involved in high secretion of catecholamines and cyanotic congenital heart disease (73).

Mutation in PHD and Other Related Factors

Heterozygous germline mutations in *PHD2* gene were first reported in familial erythrocytosis (74, 75). Later, Ladroue et al. reported a heterozygous loss-of-function mutation of *PHD2* (c.1121A>G, p.His374Arg) with the development of both erythrocytosis and recurrent paraganglioma. Functional analysis indicates that His374 is important in the binding of cofactor Fe2+, and mutation of this residue is expected to impair the catalytic function of PHDs (76). Yang et al. reported heterozygous germline mutations in *PHD1* (c.188T>A,

p.Ser61Arg and c.682G>T, p.Ala228Ser) in patients with polycythemia and PPGLs, respectively. Further research found that the half-lives of both PHD1 and PHD2 are reduced with these *PHD1* mutants (77). These findings collectively demonstrated that mutant *PHDs* are indeed associated with susceptibility to PPGLs. However, compared to *VHL* and *HIF-A*, mutations in *PHDs* are relatively rare in patients with PPGLs (78). Additionally, mutations of enzymes in the TCA cycle can affect VHL/HIF axis indirectly. For example, elevated levels of HIFs can be caused by the mutations in *SDHx*, *FH*, *MDH*, and *IDH* with subsequent accumulation of specific metabolites and reactive oxygen species (31, 79–89). In addition, multiple lines of evidence indicated that mutations in cluster 2 (Kinase Signaling Cluster) genes, including *NF1*, *RET*, *TMEM127*, *ERK*, *MAX*, and *H-RAS* could affect the VHL/HIF axis indirectly (90–94), although these mutations were initially thought to drive PPGLs through the oxygen-independent kinase signaling pathway, such as mTOR axis.

THE MECHANISMS IN DYSREGULATED VHL/HIF AXIS AND PPGLs

Under normal physiological conditions, HIFs are degraded during normoxic condition and HIFs accumulation only occur during hypoxia. The undegraded HIF-α translocates to the nucleus and dimerizes with HIF-β (95). Together with p300/CBP, the HIF-α/HIF-β heterodimer is recruited to the hypoxia-responsive elements (HREs) located on the promoter regions of HIF-regulated targets to transcriptionally upregulate the expression of the genes including vascular endothelial growth factor (*VEGF*), platelet-derived growth factor (*PDGF*), and glucose transporter (*GLUT*) (93, 96–98) (**Figure 2**). The combined effects of these upregulated gene products result in an increased supply of blood and nutrients to the hypoxic tissues and switch glucose metabolism from aerobic to anaerobic glycolysis. Due to the fast growth of tumor tissues, this process occurs in all solid tumors (99, 100), and dysregulated VHL/HIF axis further exacerbate the development of certain tumors such as PPGLs.

Aerobic glycolysis, also known as Warburg Effect (9, 101, 102), occurs in all solid tumor cells. However, dysregulated VHL/HIF axis plays a more important role in certain cancer types such as clear-cell RCC and PPGLs. Pseudohypoxia, mimicking the hypoxic condition, can affect different cancer processes including tumorigenesis and malignant transformation by promoting epithelial-mesenchymal transition and enhancing stem cell-like property. Of note, metabolic reprogramming can affect each of these processes and the role of VHL/HIF axis in cancer metabolic reprogramming has been well defined. HIF-1 aberrant activation due to either *VHL* or *PHD* mutations increases glucose uptake and glycolysis with a concomitant decrease in mitochondrial mass (103). HIF-α, especially HIF-1α, controls a wide spectrum of enzymes including GLUT1, GLUT3, hexokinase 1/2, lactate dehydrogenase-A (LDH-A), and pyruvate dehydrogenase kinase 1 (PDK1) (104–108). Upregulating these enzymes collectively shifts glycolysis from aerobic to anaerobic (109).

PPGLs are also considered as metabolic diseases due to the increased secretion of one or more catecholamines (epinephrine, norepinephrine, and dopamine). Catecholamines play a crucial role in the regulation of multiple metabolic pathways. Patients with PPGLs usually manifest with impaired insulin secretion, increased insulin resistance, elevated lipolysis, and the bone resorption marker C-terminal telopeptide of type I collagen (110). Many studies have revealed that oncometabolite such as succinate, fumarate, and 2-hydroxyglutarate (2HG) are increased in PPGLs (83, 111, 112). Another study found that compared to PPGLs without *SDHx* mutation, PPGLs with a deficient SDH have 25-fold higher succinate and 80% lower levels of fumarate, cis-aconitate, and isocitrate (113). Mutation in *FH* and *IDH* lead to the accumulation of fumarate and (R)-2-hydroxyglutarate, respectively (88, 114). Mechanistically, these oncometabolite modulate the activity of α-ketoglutarate-dependent dioxygenases such as PDH, which are involved in the induction of the pseudohypoxia pathway and activation of HIF axis (10, 31, 115). In addition, PPGLs with a germline mutation in genes encoding enzymes in the TCA cycle belong to Cluster I tumors, characterized by a pseudohypoxia signature (31). Together with the other intermediate metabolites of the TCA cycle, succinate can increase the chance of tumor development and progression through an ill-defined mechanism (83).

Results from more recent researches indicate that HIFs can regulate non-coding RNA (ncRNA) either directly or indirectly. Direct regulation is achieved by the recruiting HIFs to the HREs located on the promoter regions of ncRNAs. Whereas indirect regulation of ncRNA is achieved by epigenetic modification (116). One of the HIFs targets microRNA 210 (miR-210) (117) participates in a variety of biological processes including carcinogenesis, cancer cell proliferation, apoptosis, angiogenesis, and metastasis (118–120). On the other hand, miRNA can also activate HIF *via* mTOR indirectly. Calsina et al. reported miR-21-3p can regulate TSC2/mTOR axis in metastatic PPGLs and proposed that miR-21-3p can be the predictive markers of metastases (121). In addition, some lncRNA such as H19, MALAT1, HOTAIR, and lncRNA-SARCC play important roles in the activity of VHL/HIF axis (122).

INHIBITORS TARGETING THE VHL/HIF AXIS

Since the VHL/HIF axis plays a critical role in the development of PPGLs, targeting this axis could be a promising therapeutic strategy. Multiple reagents targeting the VHL/HIF axis have been explored and some of them have been applied clinically (123–127). Among them, the tyrosine kinase inhibitors (TKIs) are most widely used because TKIs can repress angiogenesis by inhibiting the VEGF pathway (128–130). Some compounds targeting the VHL/HIF axis can inhibit tumor growth in both animal models and clinical trials (**Table 1**).

TABLE 1 | The inhibitors targeting the VHL/HIF axis.

	Drugs or compounds	Targets or mechanisms	Clinical trials for PPGLs (www.clinicaltrials.gov)
Tyrosine kinase inhibitors	Sunitinib	Targeting VEGFR-1,2, PDGFR-β,RET, FGFR	NCT01371201, NCT00843037
	Sorafenib	Targeting RAF kinase, c-KIT, FLT-3, RET, VEGFR1-3, and PDGFR-β	None
	Cabozantinib	Targeting VEGFR, MET, RET	NCT02592356, NCT04400474, NCT02302833
	Axitinib	Targeting VEGFR	NCT01967576, NCT03839498
	Lenvatinib	Targeting VEGFR, FGFR, RET, c-Kit, PDGFα	NCT03008369, NCT02592356
	Pazopanib	Targeting VEGFR1-3, PDGFR-α,β, c-Kit	NCT01340794
Non-selective HIFs inhibitors	17-AAG	Promoting protein degradation	None
	17-DMAG	Promoting protein degradation	None
	Vorinostat	Promoting protein degradation	None
	Topotecan	Inhibiting translation and transcription activity	None
	Acriflavine	Inhibiting heterodimerization	None
	2-Methoxyestradiol	Inhibiting nuclear translocation and transcriptional activity	None
	YC-1	Inhibiting protein accumulation and transcription activity	None
	Doxorubicin/ daunorubicin	Inhibiting DNA binding	NCT00002764, NCT00002608, NCT00002641
HIF-1α inhibitors	PX-478	Inhibiting mRNA expression and translation	None
	EZN-2208	Inhibiting mRNA expression	None
	Chetomin	Disrupting binding to p300	None
	Echinomycin	Inhibiting DNA binding	None
	KC7F2	Inhibiting protein synthesis	None
	Glyceollins	Inhibiting protein synthesis and stability	None
	Bisphenol A	Promoting protein degradation	None
	LW6	Promoting protein degradation	None
	PX-12	Promoting protein degradation	None
	Cryptotanshinone	Blocking nuclear translocation	None
	cyclo-CLLFVY	Inhibiting heterodimerization	None
	Indenopyrazole 21	Inhibiting transcriptional activity	None
	EZN-2968	Inhibiting mRNA expression and translation	None
HIF-2α inhibitors	PT2385	Inhibiting heterodimerization	None
	PT2399	Inhibiting heterodimerization	None
	PT2977	Inhibiting heterodimerization	None

Tyrosine Kinase Inhibitors

To date, more than 40 protein kinase inhibitors have been approved by the FDA for cancer treatment (131). Several TKIs including sunitinib, cabozantinib, axitinib, Lenvatinib, and pazopanib are currently being evaluated in phase II clinical trials (www.ClinicalTrials.gov). By repressing the tyrosine kinase receptors, these reagents can inhibit cancer cell growth, metastasis, and the development of therapeutic resistance (132). More recently, several case studies and/or clinical trials in small cohorts suggest that TKIs could be a promising treatment for metastatic PPGLs or the syndrome-associated PPGLs.

Sunitinib, an orally administered TKI, can target both VEGFR and PDGFR (133), and therefore, it could potentially serve as a therapeutic reagent for PPGLs treatment. Early *in vitro* studies showed that sunitinib can repress the growth of PCCs (134), inhibit both synthesis and secretion of catecholamine (135). Several clinical trials have suggested that patients with metastatic PPGLs responded well to sunitinib (136–140). Results from one of our recent studies also suggested that sunitinib could be an optional therapy for patients with VHL disease-associated PCCs (141). Results from the SNIPP trial showed that sunitinib at 50mg daily benefited most patients with progressive PPGLs. Of 23 evaluable cases, the disease control rate (DCR) was 83% and median progression free survival (PFS) was 13.4 months, 3 (13%) patients with germline variants in *RET* or *SDHx* achieved a partial response (PR), 16 (70%) patients had stable disease (SD) (142). Currently, a phase II clinical trial (the First International Randomized Study in Malignant Progressive Pheochromocytoma and Paraganglioma, FIRSTMAPP) studying the effect of sunitinib on PPGLs is ongoing. In addition, results from sdhb knockout tumors bearing mice showed that sunitinib treatment can prevent tumor growth and vessel development in the first 2 weeks; thereafter, resistance will develop (143). Another study by using both *in vivo* and vitro models demonstrated that sunitinib and sorafenib can inhibit the growth of PCCs (144, 145). Previous study reported that a patient with recurrence and metastatic PPGLs responded well to 12 weeks of sorafenib treatment evidenced by regressed metastatic and decreased catecholamine level (146).

In addition, cabozantinib also appears to be a promising TKI for patients with PPGLs, especially for those with bone metastases. A trial (NCT02302833) enrolled 11 PPGLs patients with bone metastases is currently ongoing. Preliminary results identified 4 patients with PR (37%) and 6 patients with SD (55%); all patients with SD had tumor regression (18%–29%). The DCR was 92%, PFS was 16 months. None of the patients had any serious hypertension or cardiovascular events (147). A recent trial (NCT01967576) showed that 36% of patients with metastatic PPGLs achieved a PR when treated with axitinib

(148); while only one of seven patients with metastatic PPGLs who received pazopanib showed a PR (149). Finally, recruitment for a phase II clinical trial has just begun to test if lenvatinib can be used as an anti-angiogenic medication for metastatic PPGLs (www.ClinicalTrials.gov) (**Figure 2**).

Although the promising therapeutic effects of TKIs on PPGLs have been widely reported, the toxicity of TKIs should also be mentioned. The side effects of TKIs include fatigue, nausea, thrombocytopenia, hypertension, myocardial infarction, and restrictive cardiomyopathy and so on. O'Kane et al. reported that due to severe adverse events, several patients needed to reduce the dose of sunitinib, and even 20% patients discontinued trial participation (142). A phase III clinical trial compared the safety of pazopanib and sunitinib in metastatic RCC, the results showed that patients treated with sunitinib had a higher incidence of fatigue, the hand-foot syndrome and thrombocytopenia than patients treated with pazopanib. Although the rate of cardiovascular adverse events of pazopanib were similar to that of sunitinib, the abnormal liver tests leading to discontinuation in pazopanib-treatment patients should be noted (139). Furthermore, the tolerance of axitinib was similar to that of other VEGFR inhibitors. Rini et al. reported that axtinib more frequently causes hypertension than sorafenib (40% vs. 29%) (NCT00678392) (140). Similarly, Van Geel et al. reported that the incidence of hypertension in axtinib-treatment patients was higher than that in pazopanib-treatment patients (150). Burotto Pichun et al. reported that even 80% axtinib-treatment patients developed severe hypertension (148). Recently, a phase III randomized ATLAS trial assessed the safety of axitinib versus placebo, axitinib-treated patients had more grade 3/4 adverse events and discontinuations (151). Taken together, the safety of TKIs needs to be further evaluated in the future.

HIFs Inhibitors

Transcription factors including HIFs have been historically considered undruggable. This is one of the reasons that research in the pharmaceutical field has been mainly focusing on HIF's downstream pathways, such as VEGF. However, based on the structure of HIF-2α (152), two compounds PT2385 and PT2399 targeting HIF-2α were successfully identified (145, 153). Subsequent *in vitro* and *in vivo* studies showed that these compounds can inhibit the growth of clear-cell RCC (154). A phase I trial found that for patients with progressive clear-cell RCC the complete response, partial response, and stabilized disease to PT2385 were 2%, 12%, and 52%, respectively (155). It has been proposed that HIF-2α inhibitors possess a great potential for the treatment of advanced PPGLs (156). These initial results could also spearhead a multitude of preclinical and clinical studies assessing the efficiency of the compounds in other tumor types. In fact, PT2385 has entered its phase II clinical trial (NCT03108066) evaluating its efficacy in patients with advanced cancers carrying a *VHL* germline mutation. Recently, second-generation allosteric inhibitor of HIF-2α PT2977 (MK-6482) was identified. Compared to PT2385, PT2977 have increased potency and improved pharmacokinetic profile (157). The result of phase I/II trial of PT2977 in 55 patients with advanced RCCs revealed that 24% patients experienced a confirmed PR and 54% had SD, with a clinical benefit rate of 78%. Moreover, a PT2977 monotherapy Phase III trial in patients with previously treated advanced RCC is planned (158). Notably, previous studies reported that HIF-2α was overexpressed in VHL and in SDH-related PPGLs compared to HIF-1α (159, 160). Therefore, inhibitors targeting HIF-2α appear to be more promising than inhibitors targeting HIF-1α.

Other Compounds Targeting VHL/HIF Axis

Theoretically, any compounds capable of inhibiting the VHL/HIF axis can potentially become therapeutic reagents for the treatment of metastatic PPGLs. For example, the HSP90 inhibitors, 17-N-allylamino-17-demethoxy geldanamycin (17-AAG) and 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) (161–163), and histone deacetylase inhibitor, vorinostat (164, 165), are capable of inducing HIF-α degradation. Topotecan can downregulate HIF-α by inhibiting topoisomerase I (TOP-I) (166–168). Of note, topotecan has already been used as a therapeutic reagent for the treatment of metastatic ovarian carcinoma, recurrent small cell lung cancer, and recurrent cervical cancer (169–171). Acriflavine can inhibit dimerization between HIF-α and HIF-β and subsequently repress the expression of HIFs target genes (172). 2-Methoxyestradiol (2-ME), an active metabolite of 17β-estradiol, can inhibit the synthesis, nuclear translocation, and transcriptional activity of HIF-α (173, 174). In addition, an antiplatelet aggregation agent YC-1 can not only suppress HIFs transcriptional activity by inhibiting p300 recruitment but also promote HIF-α degradation by enhancing FIH binding (175). Finally, two anthracyclines, doxorubicin and daunorubicin, have been demonstrated to inhibit the expression of HIFs targets efficiently by interrupting HIF-α recruitment (176).

There are also compounds inhibiting HIF-1α synthesis. For example, PX-478 is capable of downregulating both the mRNA and protein levels of HIF-1α (177–179). EZN-2208 (PEG-SN38) can downregulate the expression of HIF-1α in lymphocytic leukemia (180). By hybridizing with HIF-1α mRNA, EZN-2968, a 3rd generation antisense oligonucleotide, can specifically inhibit HIF-1α translation (181, 182). Chetomin is capable of repressing xenograft growth *in vivo* by disrupting HIF-1α and p300 interaction (183). Finally, there is a myriad of compounds including echinomycin, CAY10585, KC7F2, glyceollins, bisphenol A, LW6, PX-12, cryptotanshinone (CPT), cyclo-CLLFVY, and indenopyrazoles 21 that have all been validated as selective inhibitors of HIF-1α with different molecular mechanisms (184–196).

CONCLUSION

The VHL/HIF axis plays an important role in oxygen homeostasis and cellular metabolism in both physiology and pathology. Dysregulation of this axis due to either germline mutations, somatic mutations, and epigenetic dysregulation can be involved in tumorigenesis and progression of different cancer types

including PPGLs. Mechanistically, by reprogramming metabolic pathways the abnormally activated HIFs drive cancer cells toward aerobic glycolysis. Based on the underlying molecular mechanisms of VHL/HIF axis in PPGLs development, a wide spectrum of drugs specifically targeting this axis have been and will continue to be developed as PPGL therapeutics. With a better understanding of the relationship between VHL/HIF axis and PPGLs, more accurate diagnosis and prognosis of PPGLs, as well as efficacious therapeutics against PPGLs, are expected in the near future.

AUTHOR CONTRIBUTIONS

SP, QL, JZ, XT, JX, and YH contributed to the writing of the manuscript. NS, JJ, and DZ provided consultation and contributed to the revising of the manuscript. All authors contributed to the article and approved the submitted version.

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