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## EDITED BY

Ichiro Abe,  
Fukuoka University Chikushi Hospital,  
Japan

## REVIEWED BY

Yuto Yamazaki,  
Tohoku University, Japan  
Alice Helena Dutra Violante,  
Federal University of Rio de Janeiro, Brazil

## \*CORRESPONDENCE

Yishu Wang  
✉ wangys@jlu.edu.cn  
Honglan Zhou  
✉ hlzhou@jlu.edu.cn

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# The connection between tricarboxylic acid cycle enzyme mutations and pseudohypoxic signaling in pheochromocytoma and paraganglioma

Yuxiong Wang<sup>1</sup>, Bin Liu<sup>1</sup>, Faping Li<sup>1</sup>, Yanghe Zhang<sup>2</sup>, Xin Gao<sup>1</sup>,  
Yishu Wang<sup>2\*</sup> and Honglan Zhou<sup>1\*</sup>

<sup>1</sup>Department of Urology, The First Hospital of Jilin University, Changchun, Jilin, China, <sup>2</sup>Key Laboratory of Pathobiology, Ministry of Education, Jilin University, Changchun, Jilin, China

Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors originating from chromaffin cells, holding significant clinical importance due to their capacity for excessive catecholamine secretion and associated cardiovascular complications. Roughly 80% of cases are associated with genetic mutations. Based on the functionality of these mutated genes, PPGLs can be categorized into distinct molecular clusters: the pseudohypoxia signaling cluster (Cluster-1), the kinase signaling cluster (Cluster-2), and the WNT signaling cluster (Cluster-3). A pivotal factor in the pathogenesis of PPGLs is hypoxia-inducible factor-2 $\alpha$  (HIF2 $\alpha$ ), which becomes upregulated even under normoxic conditions, activating downstream transcriptional processes associated with pseudohypoxia. This adaptation provides tumor cells with a growth advantage and enhances their ability to thrive in adverse microenvironments. Moreover, pseudohypoxia disrupts immune cell communication, leading to the development of an immunosuppressive tumor microenvironment. Within Cluster-1a, metabolic perturbations are particularly pronounced. Mutations in enzymes associated with the tricarboxylic acid (TCA) cycle, such as succinate dehydrogenase (SDHx), fumarate hydratase (FH), isocitrate dehydrogenase (IDH), and malate dehydrogenase type 2 (MDH2), result in the accumulation of critical oncogenic metabolic intermediates. Notable among these intermediates are succinate, fumarate, and 2-hydroxyglutarate (2-HG), which promote activation of the HIFs signaling pathway through various mechanisms, thus inducing pseudohypoxia and facilitating tumorigenesis. SDHx mutations are prevalent in PPGLs, disrupting mitochondrial function and causing succinate accumulation, which competitively inhibits  $\alpha$ -ketoglutarate-dependent dioxygenases. Consequently, this leads to global hypermethylation, epigenetic changes, and activation of HIFs. In FH-deficient cells, fumarate accumulation leads to protein succination, impacting cell function. FH mutations also trigger metabolic reprogramming towards glycolysis and lactate synthesis. IDH1/2 mutations generate D-2HG, inhibiting  $\alpha$ -ketoglutarate-dependent dioxygenases and stabilizing HIFs. Similarly, MDH2 mutations are associated with HIF stability and pseudohypoxic response. Understanding the intricate relationship between metabolic enzyme mutations in the TCA cycle and pseudohypoxic signaling is crucial for unraveling

the pathogenesis of PPGLs and developing targeted therapies. This knowledge enhances our comprehension of the pivotal role of cellular metabolism in PPGLs and holds implications for potential therapeutic advancements.

#### KEYWORDS

pheochromocytoma, paraganglioma, pseudohypoxia, VHL/HIF axis, genetic mutations, tricarboxylic acid cycle, metabolic reprogramming

## Introduction

Pheochromocytomas (pcc) and paragangliomas (PGL) (PPGLs) are infrequent neuroendocrine tumors deriving from chromaffin cells. Pheochromocytoma originates in the adrenal medulla, accounting for approximately 80%-85% of cases, while paraganglioma arises externally from sympathetic paraganglia dispersed throughout the body, constituting around 15%-20% of cases (1–3). Despite their low incidence rate (roughly 6.6 cases per million individuals annually) (4), these tumors bear noteworthy clinical significance due to their potential to induce excessive catecholamine secretion, precipitating perilous cardiovascular complications and myocardial degenerative alterations. In the latest World Health Organization guidelines, all PPGLs are now recommended for lifelong follow-up due to their metastatic potential, similar to epithelial neuroendocrine tumors (5–7).

Approximately 80% of PPGLs are linked to genetic mutations, often indicating a genetic predisposition (2, 8). Gene expression profiling enables the classification of PPGLs into three distinct clusters based on their molecular characteristics: the pseudohypoxia signaling cluster (Cluster-1), the kinase signaling cluster (Cluster-2), and the WNT signaling cluster (Cluster-3). Cluster-1 further divides into subclusters 1a and 1b. Cluster-1a comprises mutations in genes associated with the tricarboxylic acid cycle (SDHx, FH, MDH2, GOT2, SLC25A11, IDH, DLST, SUCLG2), while Cluster-1b encompasses mutations in genes constituting the hypoxia signaling pathway (PHD1/2, VHL, HIF2A/EPAS1) (9–11). Cluster-1 notably exhibits a significant enhancement of pseudohypoxic signaling pathways, resulting in heightened angiogenesis and metabolic anomalies during tumor development. Cluster-1a represents approximately 10%-15% of cases, while Cluster-1b accounts for approximately 15%-20% of PPGLs. Importantly, Cluster-2 within the PPGLs group demonstrates a marked increase in gene expression related to kinases. This involves critical mutations in genes associated with the PI3K/mTORC1 pathway and receptor kinase signaling, such as RET, NF1, H-RAS, K-RAS, TMEM127, and MAX, implying their potential significance in regulating tumor growth and metastasis. Cluster-2 constitutes approximately 50%-60% of PPGLs cases. Cluster-3 is characterized by the activation of the WNT signaling pathway, potentially contributing to increased cellular proliferation and invasiveness. Notably, Cluster-3 accounts for approximately 5%-10% of PPGLs cases and also includes tumors with mutations in the CSDE1 and the MAML3 fusion genes (2, 12, 13). In the three

clusters, Cluster 1 exhibits the most prominent risk of metastasis (9). Additionally, among the cases in Cluster 1, there is typically an elevation in norepinephrine levels, which is associated with a deficiency in phenylethanolamine N-methyltransferase (13). This results in a tendency for such patients to have persistent high blood pressure (14). These findings underscore our continued focus on Cluster 1.

Roughly 40% of PPGLs patients carry a germline mutation in one of over 20 known susceptibility genes. In an additional 30-40% of sporadic disease patients, somatic mutations in the same genes or other genes that drive tumorigenesis can be identified (14, 15). Multiple studies indicate that Cluster 1a is almost exclusively germline-mutated (100%), while Cluster-1b has 25% of cases with germline mutations (13, 16). Among PPGLs, the top three genes with germline mutations are SDHB (10.3%), SDHD (8.9%), and VHL (7-10%), while the genes with the highest somatic mutation frequencies are VHL (10%) and HIF2A (5-7%), with germline or somatic mutations in other genes being less than 2% (15, 17–19). This highlights the significant role of germline mutations in the pathogenesis of Cluster-1 PPGLs. Clinical studies on sporadic PPGLs show a significantly higher occurrence of germline mutations associated with multiple PPGLs compared to isolated PPGLs (54% vs. 11.5%). Moreover, the research suggests that the risk of germline mutations in PPGLs located outside the adrenal gland is significantly higher than those within the adrenal gland (14, 20). Specifically for Cluster 1, PPGLs associated with mutations in TCA metabolic enzyme genes (Cluster-1a) are mainly located outside the adrenal gland. For example, SDHA mutations are linked to sympathetic and parasympathetic PGLs (21). SDHB and SDHC mutations are primarily observed in sympathetic/parasympathetic PGLs, but are less common in PCC (22). SDHD and SDHAF2 mutation-related PGLs are predominantly found in the head and neck region, with lower incidence in other extrarenal locations or within the adrenal gland (23, 24). Tumors associated with FH mutations are found both inside and outside the adrenal gland. In Cluster-1b, VHL mutations causing PCC result in 50% of cases being bilateral and occasionally occurring outside the adrenal gland. HIF2A/EPAS1 mutations may lead to tumors in both intra- and extra-adrenal locations (14).

The hypoxia-inducible factor-2 $\alpha$  (HIF2 $\alpha$ ) protein gets upregulated in cells under normal oxygen conditions, culminating in the activation of downstream transcriptional processes, recognized as pseudohypoxia. Activation of the hypoxia response pathway confers advantages to tumor cell growth and adaptation to

adverse microenvironments (25). Studies have elucidated heightened pseudohypoxic signaling in hereditary renal cell carcinoma, giving rise to the upregulation of downstream signaling molecules such as glucose transporter 1 and vascular endothelial growth factor (VEGF), thereby fostering augmented energy metabolism and proliferative capacity in tumor cells (26). Pseudohypoxic signaling prompted by tumor cells modifies the communication patterns of immune cells, instigating alterations in immune cell metabolism that incline towards the attenuation of surveillance function in innate immune cells and the fostering of an immunosuppressive microenvironment (25, 27). Specimens of neuroblastoma and glioma have evinced the prevalence of elevated HIF2 $\alpha$  expression and a concomitant display of nascent characteristics, signifying the presence of a pseudohypoxic niche in these tumors that correlates with malignancy (28). The instigation of pseudohypoxia through HIFs signaling also plays a pivotal role in non-neoplastic ailments. The buildup of reactive oxygen species (ROS) in lung tissue engenders HIFs activation in fibroblasts, disrupting the structural integrity of the extracellular matrix and exacerbating the progression of pulmonary fibrosis (29). In the course of the aging process, diminished nuclear NAD<sup>+</sup> levels impede the activity of nuclear SIRT1, thus suppressing the pVHL ubiquitin-proteasome degradation pathway. This engenders heightened stability of HIF1 $\alpha$  within the cell and the concomitant emergence of HIF1 $\alpha$ -induced pseudohypoxia. The pseudohypoxic state engendered by HIF1 $\alpha$  disrupts intracellular signaling between the nucleus and mitochondria, thereby impairing mitochondrial function and accelerating cellular senescence and demise (30).

Pseudohypoxia has been meticulously scrutinized and observed in the context of PPGLs. Notwithstanding their typical highly vascularized nature, PPGLs are characterized by a conspicuous upregulation of hypoxia signaling pathways, particularly within Cluster-1, thereby fueling the onward progression of tumors (25, 31–33). The principalstay treatment for PPGLs remains surgical intervention, as effective therapeutic modalities for metastatic tumors are presently scarce (34). A profound comprehension of the hypoxia-related signaling mechanisms underpinning PPGLs pathogenesis is imperative for the development and assessment of molecularly targeted therapies tailored to the diverse subtypes of PPGLs, encompassing the intricacies of the tumor microenvironment (TME). This comprehensive review seeks to illuminate how mutations in metabolic enzymes within Cluster 1a of the tricarboxylic acid cycle impinge upon the VHL/HIF signaling pathway, consequently contributing to the genesis of PPGLs. This dissection facilitates an enhanced understanding of the pivotal role of cellular metabolism in the realm of PPGLs, thereby engendering substantial implications for the refinement of extant therapeutic modalities.

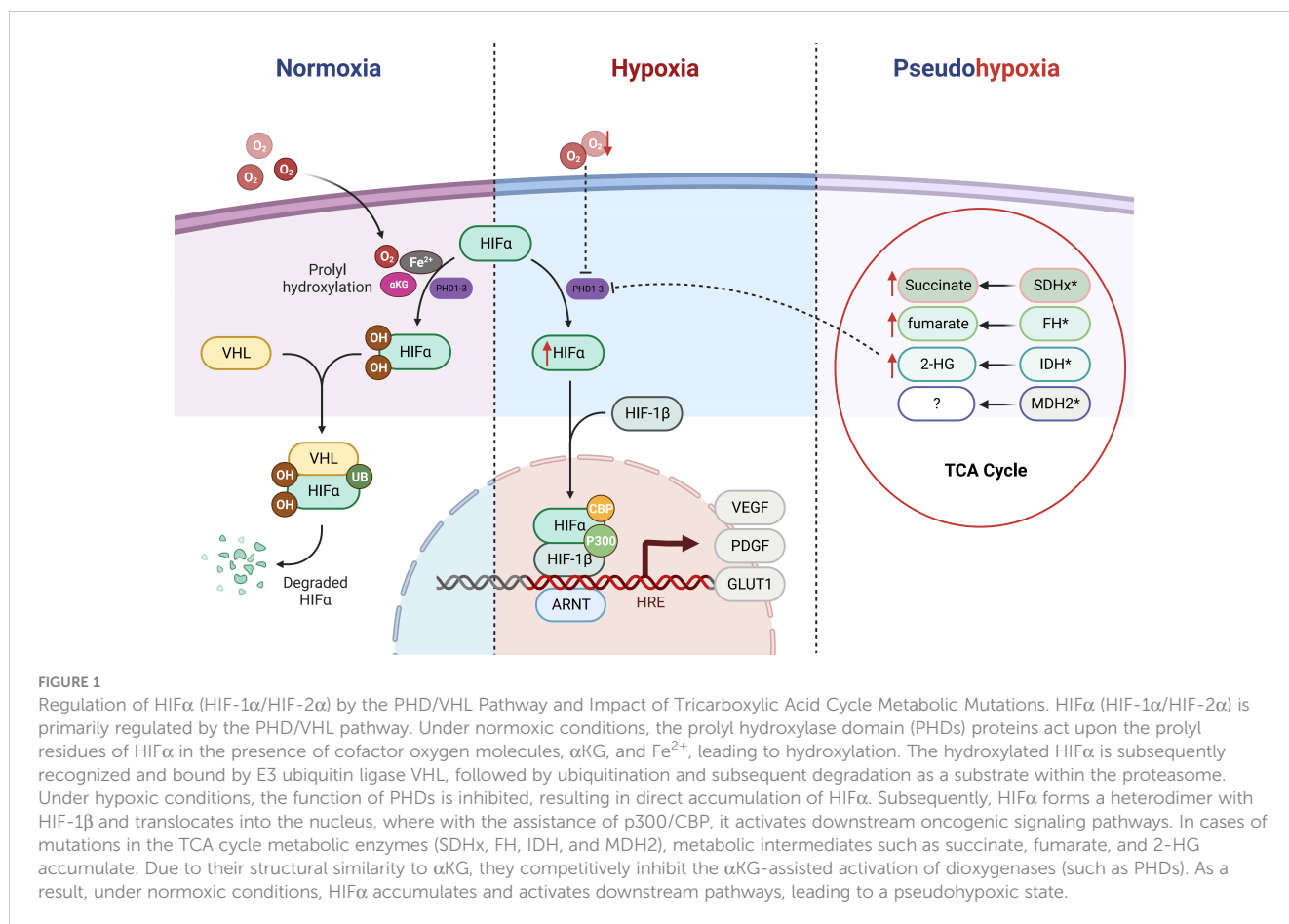
## Dysregulation of the VHL/HIF signaling axis in PPGLs

The intricate interplay between the VHL tumor suppressor and HIFs signaling axis bears a profound relevance to the emergence of PPGLs (35, 36). Central to this dynamic is the orchestration of

HIFs' stability by oxygen concentration within the microenvironment (see Figure 1). Under normoxic conditions, prolyl hydroxylation of HIF1 $\alpha$ /HIF2 $\alpha$  by oxygen-dependent prolyl hydroxylases (PHDs) triggers subsequent recognition by the E3 ubiquitin ligase VHL protein. This culminates in selective ubiquitination and proteasomal degradation of hydroxylated HIF $\alpha$  subunits. Conversely, under hypoxia, PHDs activity inhibition leads to non-hydroxylated HIF $\alpha$  subunit accumulation. Following nuclear translocation, heterodimerization with HIF1 $\beta$  ensues, facilitating transcriptional complex formation with coactivators p300/CBP (25, 37). This complex binds to hypoxia response elements, activating transcription of genes such as VEGF, platelet-derived growth factor (PDGF), and glucose transporter (GLUT) (11, 38, 39). Such adaptations promote metabolic reprogramming in hypoxic cells, characterized by elevated glucose uptake, anaerobic glycolysis, diminished mitochondrial mass, and heightened energy provisioning, thus fostering tumorigenic progression. Tumors frequently exhibit an intriguing phenomenon whereby normoxic conditions lead to an anomalous stabilization of HIFs within tumor cells. This multifaceted phenomenon results from diverse influences, ultimately culminating in the accumulation or heightened functional activity of HIFs. Consequently, this dynamic process upregulates the expression of HIFs target genes, remarkably mimicking the pathophysiological response seen in hypoxic states. Coined as “pseudo-hypoxia,” this intriguing state prompts notable cellular transitions, including epithelial-mesenchymal transition, augmented tumor cell stemness, thereby effectively fueling the cascade of tumorigenic initiation, progression, and the potentiation of malignant attributes (40, 41).

The regulatory apparatus governing the VHL/HIF axis is intricate, encompassing a myriad of factors. Within the landscape of sporadic PPGLs, a significant subset, approximately 14%, manifests somatic VHL mutations (42). While the precise mechanistic underpinnings characterizing VHL dysfunction and its structural aberrations in the pathogenesis of PPGLs remain enigmatic, a discernible association between missense mutations in the VHL gene, particularly at positions 167 and 238, and the occurrence of PPGLs is evident (43). The implications of such missense mutations or reduced VHL expression are profound, manifesting in the impediment of HIF $\alpha$  ubiquitination and degradation. Consequently, a stabilization of HIFs transpires, subsequently engendering an augmented susceptibility to the development of PPGLs, specifically implicating Type 2 VHL disease (44–46). The landscape of HIFs genetic alterations is further nuanced, with HIF2 $\alpha$  mutations assuming a more pronounced presence. Notably, germline mutations in HIF2A exon 9 (c.1121T>A, p.F374Y) significantly enhance the propensity for PPGLs (47). Comparatively, PHD mutations within the realm of PPGL patients present as a relatively infrequent occurrence, a notable distinction in contrast to the prominent roles of VHL and HIF $\alpha$  (48).

Intriguingly, PPGLs are increasingly recognized as metabolic disorders, particularly within the spectrum of cluster 1a manifestations. The manifestation of mutations within tricarboxylic acid (TCA) cycle enzymes precipitates an accumulation of pivotal metabolic intermediates. Subsequently, a



multipronged cascade is set in motion, activating the HIFs signaling pathway via diverse mechanisms, thus engendering a pseudo-hypoxic milieu. Elevated levels of metabolites such as succinate, fumarate, or 2-hydroxyglutarate (2-HG) are indicative hallmarks within PPGLs, closely mirroring mutations inherent to TCA cycle enzymes (49–51). These metabolites, intricately interwoven with the tumorigenic context, proceed to exert their influence by modulating HIFs activity or by influencing the regulatory cascade upstream, thereby orchestrating the transduction of oncogenic signals. Within this framework, our review assumes a focal orientation, dedicated to the elucidation of the nuanced impact instigated by mutations within four pivotal TCA cycle enzymes—namely, Succinate dehydrogenase (SDHx), fumarate hydratase (FH), isocitrate dehydrogenase (IDH), and malate-dehydrogenase type 2 (MDH2)—upon the intricate VHL/HIF axis in PPGLs.

## The TCA cycle and PPGLs

A century ago, Otto Warburg's seminal investigations unveiled a noteworthy phenomenon: even under aerobic conditions, tumor cells tend to favor glycolytic pathways for energy procurement over mitochondrial respiration, positing mitochondrial dysfunction as a plausible cause, known as the Warburg effect (52). Subsequent research has illuminated that mitochondrial dysfunction isn't an obligatory trigger for tumorigenesis. Certain tumor cells adeptly

generate energy through oxidative phosphorylation (OXPHOS) despite the mitochondrial milieu (53). Within this energetic landscape, the TCA cycle emerges as a pivotal conduit of mitochondrial energy metabolism. It orchestrates the oxidation of acetyl-CoA, transported to the mitochondria, into carbon dioxide, concomitantly releasing energy and reducing agents (NADH and FADH $_2$ ) to facilitate subsequent OXPHOS (see Figure 2). Beyond its energy-contributing role, the TCA cycle acts as a nexus for intracellular carbohydrate, lipid, and amino acid metabolism, endowing other metabolic pathways with acetyl-CoA or diverse intermediary substrates (54, 55). Collectively, the nexus of tumorigenesis and the TCA cycle is unequivocally evident. Further probing into the intricate relationship between the TCA cycle and tumorigenesis holds the potential to unravel unique facets of tumor metabolism and unveil novel therapeutic targets.

Within the context of certain PPGLs, genetic perturbations affecting enzymes integral to the TCA cycle, such as SDH and FH, emerge as salient features (56). Since the initial discovery in 2000 of the correlation between SDHD germline mutations and familial paragangliomatosis, a growing compendium has cataloged mutations within at least twelve TCA cycle-related genes in the landscape of PPGLs (SDHB, SDH, SDHC, SDHD, SDHAF2, FH, IDH1, MDH2, SUCLG2, DLST, SLC25A11, and GOT2) (8, 57, 58). Among these, SDHx, FH, IDH1, and MDH2 have garnered relatively more attention, with mutations within SDHx and FH, in particular, emerging as pivotal drivers in the genesis and

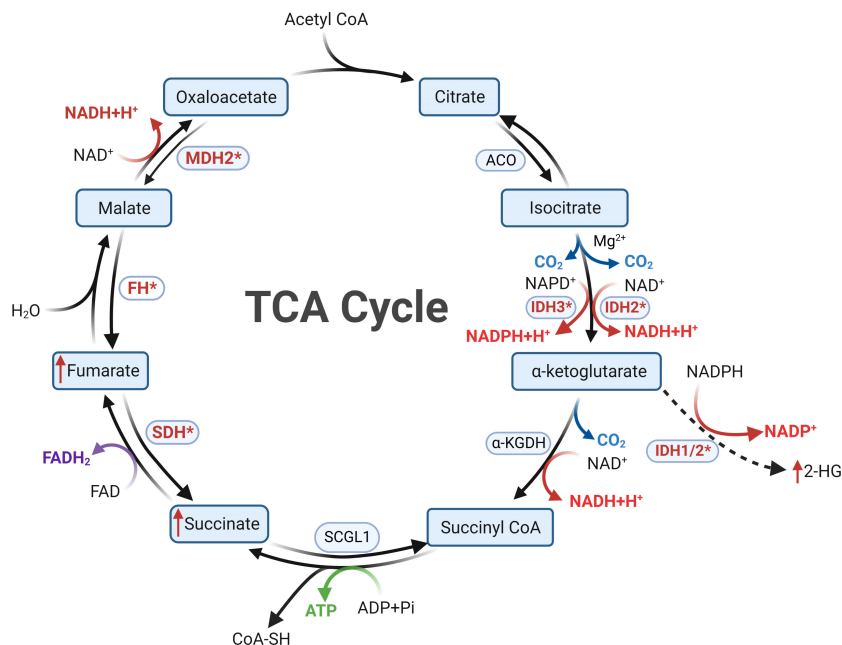


FIGURE 2

Metabolic reprogramming following mutations in tricarboxylic acid cycle enzymes is a key feature of Cluster 1a. In cells with normal mitochondrial function, the tricarboxylic acid cycle proceeds in a clockwise order. Mutations in SDHx and FH result in diminished substrate metabolism, leading to the accumulation of succinate and fumarate, respectively. Mutations in IDH confer the ability to convert  $\alpha$ KG into 2-HG, resulting in 2-HG accumulation. Succinate, fumarate, and 2-HG all possess the capability to inhibit PHDs, thereby inducing a pseudohypoxic state. The oncogenic mechanisms following MDH mutations in PPGLs remain currently unclear.

progression of PPGLs. Subsequent discourse delves into the myriad mutations within these four enzyme categories, expounding on their repercussions on intermediary metabolism.

## SDHx and succinate

Within the context of PPGLs, SDHx-related genetic mutations stand out as the most prevalent, accounting for approximately 15% (59). SDHx comprises four core subunits (SDHA, SDHB, SDHC, SDHD) and an assembly factor (SDHAF2) (8, 60). Among the five constituent molecules of SDHx, mutations associated with SDHD and SDHB are more commonly observed, while those affecting SDHA, SDHC, and SDHAF2 are relatively less frequent. In patients with SDHB mutations, approximately 50% experience metastatic progression, whereas patients with SDHD mutations typically manifest with multiple neck paragangliomas (8, 61–63).

SDHx plays a dual role by participating in both the TCA cycle, where it oxidizes succinate to fumarate, and serving as a constituent of mitochondrial complex II in the electron transport chain (ETC), catalyzing electron transfer to the ubiquinone pool (59). In the realm of PPGLs, SDHx serves as suppressors of tumorigenesis. When germline or somatic mutations occur, the functional integrity of succinate dehydrogenase is compromised, leading to interruptions in the TCA cycle and impairment of the electron transport chain. Following such disruption, the accumulation of succinate, a metabolic substrate of SDHx, occurs intracellularly. This accumulation is responsible for reduced metabolic product

generation, leading to diminished mitochondrial energy production. Concurrently, tumor cells undergo metabolic reprogramming to satisfy the demands for crucial synthesis processes. In cells with SDHx functional defects, heightened glycolysis and enhanced citric acid cycle flux are observed, primarily to sustain aspartate requirements, crucial for protein and nucleic acid synthesis (64, 65). Additionally, the terminal product of compensatory upregulated glycolysis, lactate, has been demonstrated to stimulate tumor cell growth (66).

The accrual of succinate in cells with SDHx functional defects is deemed a primary mediator of SDH-associated tumorigenesis (67). Pathological succinate accumulation leads to its leakage from the mitochondrial matrix to the cytoplasm. As a competitor of  $\alpha$ -ketoglutarate ( $\alpha$ KG), succinate broadly inhibits  $\alpha$ KG-dependent dioxygenases, including the Ten-Eleven Translocation (TET) DNA hydroxylases and Jumonji (JM) histone demethylases (KDMs). Consequently, a global hypermethylation characterized by the CpG island methylator phenotype (CIMP) emerges in tumor cells, inducing alterations in gene expression and facilitating tumorigenesis (60, 68). Furthermore, SDHx deficiency-induced succinate accumulation competitively inhibits the  $\alpha$ KG-dependent PHDs family (PHD1-3) in the cytoplasm. This leads to the stabilization of HIF $\alpha$  under normoxic conditions, thereby contributing to the activation of the pseudohypoxia pathway (25, 69, 70). Notably, succinate serves not only as a substrate for mitochondrial SDHx but also as a product of cytoplasmic PHDs (33, 69, 71–74). Consequently, the accumulation of succinate, through negative feedback regulation, inhibits the activity of

PHDs, resulting in further stabilization and activation of HIF complexes under normoxic conditions. Research by Celada et al. suggests that in SDHx mutation-driven PPGLs, diminished expression of PD-L1 and lower infiltration of cytotoxic T lymphocytes (CTLs) contribute to a 'cold' immunophenotype significantly associated with SDHx mutations (25). This hints at the potential of SDHx mutations to foster an immune-suppressive tumor microenvironment.

Furthermore, extracellular succinate secretion by tumor cells with SDH defects yields functional consequences (75). Extracellular succinate, upon binding to succinate receptor-1 (SUCNR-1), activates several oncogenic signaling molecules, such as ERK1/2, Akt, and AMPK, thereby enhancing tumor cell invasion and metastasis (75–77). Notably, Wu et al. found that the accumulated succinate in SDH-deficient tumor cells not only upregulates HIF-1A expression via the activation of the intracellular PI3K/Akt signaling pathway but also contributes to tumor invasion by activating macrophage surface SUCNR-1, subsequently inducing M1 polarization and promoting a malignant tumor phenotype (75, 78). Beyond immune cell interactions, secreted succinate from tumor cells binds to endothelial cell SUCNR-1, activating downstream STAT3-ERK1/2 in a HIF-independent manner and subsequently upregulating VEGF, thereby fostering angiogenesis.

## FH and fumarate

FH utilizes fumarate as a metabolic substrate, catalyzing its reversible hydration into malate (79). Mutations in FH result in reduced or loss of enzymatic activity, leading to the accumulation of high levels of fumarate (at millimolar levels). This accumulation profoundly alters mitochondrial function and cellular metabolism. Upon mitochondrial dysfunction due to FH mutations, cells undergo metabolic reprogramming, transitioning from mitochondrial oxidative respiration to cytoplasmic glycolysis. This metabolic shift also leads to a further reduction in carbon sources entering the mitochondria, which poses a risk of decreased membrane potential, potentially resulting in increased ROS and cell death. In this scenario, glutamine becomes an alternative carbon source to sustain the compromised TCA cycle, generating NADH for subsequent OXPHOS and ATP production to maintain mitochondrial membrane potential (80). To prevent the accumulation of other metabolic intermediates within this compensatory pathway, a portion of the glutamine-derived product is utilized for heme biosynthesis and degradation pathways, crucial for maintaining mitochondrial quality control and cell viability in FH-deficient cells (80, 81).

In addition to the metabolic reprogramming caused by FH deficiency and fumarate accumulation, these changes can also activate pro-oncogenic signaling pathways in FH-deficient cells. Fumarate can stabilize the majority of proteins through succination, a modification in which fumarate reacts with exposed cysteine residues on protein surfaces to form stable thiols. This leads to functional inactivation of proteins within the dynamic intracellular environment of tumor cells (82, 83). In a cellular environment

characterized by elevated intracellular fumarate levels, succination of various oncoproteins promotes cell survival and proliferation. For example, fumarate succination of KEAP1 inactivates its ubiquitin ligase function, resulting in the stabilization and activation of its downstream target NRF2. As a transcription factor, NRF2 further upregulates the transcription of heme oxygenase 1 (HMOX1), a process crucial for maintaining heme metabolism. Additionally, iron-responsive element-binding protein 2 (IRP2) is a critical regulator of cellular iron metabolism, maintaining intracellular iron levels by inhibiting ferritin translation. Succination of IRP2 reduces its translational regulatory capacity on ferritin, leading to increased ferritin levels and decreased iron ions, disrupting intracellular iron homeostasis (84, 85). Furthermore, ferritin upregulates the expression of forkhead box protein M1 (FOXM1), promoting tumor cells to undergo mitosis (85).

Similar to succinate, fumarate also competitively inhibits PHDs, leading to increased stability of HIFs and inducing a pseudohypoxic state in tumorigenesis (86, 87). Thus, even in the context of mitochondrial defects in the energy metabolism chain, tumor cells maintain their malignant potential. Worth noting is that in FH-deficient tumor cells, activation of downstream pathways by HIFs results in significant upregulation of GLUT1, facilitating glucose uptake (86). Conversely, the expressions of pyruvate dehydrogenase kinase and lactate dehydrogenase are suppressed (88, 89). These changes in HIF-mediated metabolic genes lead to inhibition of mitochondrial oxidative metabolism, redirecting cells toward glycolysis and lactate synthesis, which hold essential significance in maintaining mitochondrial homeostasis.

## IDH and 2-HG

There are two forms of IDH in cells: IDH1/2 and IDH3, with IDH1/2 being NADP-dependent and IDH3 being NAD-dependent. While IDH1 and IDH2 share similarities in structure and function, they differ in subcellular localization. IDH1 is located in the cytoplasm and peroxisomes, whereas IDH2 is localized in the mitochondria. The function of IDH1/2 is to catalyze the oxidative decarboxylation of isocitrate to form  $\alpha$ KG, simultaneously reducing NAD(P) to NAD(P)H (90). Unlike IDH1/2, IDH3 is found in the mitochondria and functions in the respiratory chain by catalyzing the forward decarboxylation of isocitrate to produce  $\alpha$ KG (91). In 2010, Gaal and colleagues first identified a solitary IDH1 mutation in a case of sporadic carotid paraganglioma among 365 PPGLs specimens (92). Unfortunately, in another study by Yao and colleagues involving 104 PPGLs samples, no IDH1 mutations were detected (93). This suggests that pathogenic IDH mutations in PPGL are rare. Currently, there is a lack of authoritative research on the mechanisms by which IDH mutations contribute to PPGLs pathogenesis and progression, although the oncogenic mechanisms of IDH mutations have been extensively studied in diseases like gliomas and leukemias.

IDH1/2 mutations are considered gain-of-function oncogenic mutations. A single amino acid residue mutation in the IDH catalytic subunit prevents the conversion of isocitrate to  $\alpha$ -

ketoglutarate, while gaining new enzymatic activity to generate D-2HG from  $\alpha$ KG (94–96). This leads to a significant accumulation of this oncogenic metabolite in cells. Simultaneously, D-2HG disrupts the balance of  $\alpha$ KG-assisted dioxygenases, including histone lysine demethylases, TET DNA hydroxylases, and PHDs. For example, inhibition of histone lysine demethylase activity results in increased methylation levels of histone lysine residues in chromatin (91, 97, 98). Furthermore, D-2HG inhibits PHDs, thereby preventing the ubiquitination and degradation of HIFs, leading to their stabilization and accumulation within cells (99, 100). Consequently, this elevation in HIFs target gene transcription promotes the formation of a pro-tumorigenic immune microenvironment. Under physiological metabolic conditions, NADPH is a reduction product of IDH. After IDH mutation, reduced NADPH generation impairs the maintenance of the reduced glutathione (GSH) pool, causing a decrease in the ratio of GSH to GSSG and disrupting intracellular antioxidant system balance, resulting in increased ROS (98). However, in the context of elevated intracellular ROS due to IDH1 loss-of-function, HIF2 $\alpha$  stabilization can be induced in a ROS-dependent manner (101).

On the other hand, IDH mutation alters its original catalytic pathway, resulting in reduced metabolic flux of  $\alpha$ KG and NADPH in the cell, profoundly affecting cellular metabolic status (91). Interestingly, in contrast to the outcome of HIF signaling pathway activation observed after IDH mutation, studies suggest that tumor cells may release D-2HG into the tumor microenvironment, triggering instability of HIF-1 $\alpha$  in immune cells (regulatory T cells and Th17 cells) of the immune microenvironment (102). This subsequently modulates the energy metabolism and anti-tumor immune function of immune cells, although the precise mechanisms remain unclear. In a study focusing on enantiomer-specific mechanisms, it was found that IDH1/2 mutant variants can convert 2-oxoglutarate (2-OG) to (R)-2HG rather than (S)-2HG. (R)-2HG can activate PHD activity, thereby reducing the stability and levels of HIFs (103). Overall, this complex interplay contributes to malignant transformation of normal cells and enables tumor cells to survive energy reprogramming (97, 98, 104, 105).

## MDH2

Malate dehydrogenase 2 (encoded by the MDH2 gene) is downstream of fumarate and functions to oxidize malate into oxaloacetate. This enzyme is also involved in the malate-aspartate shuttle, which is essential for cellular respiration. In recent years, MDH2 has been considered a potential susceptibility gene for PPGLs (106). Reported cases of PPGLs associated with MDH2 mutations are extremely rare, with only a few cases retrievable in databases since the initial report by Alberto et al. in 2015 (106–108). MDH2 variants have been identified in metastatic cases, with approximately 50% of cases estimated to progress to metastasis (106), suggesting a potential association between MDH2 polymorphic variations and the metastatic nature of PPGLs. However, the mechanisms underlying the relationship between MDH2 mutations and the onset and progression of PPGLs remain unclear. In a larger-scale study, germline mutations in

MDH2 were found to account for about 0.6% of overall PPGLs, and the p.K314del variant's potential pathogenicity in PPGLs was identified, possibly due to its impact on amino acid stability, although the specific mechanism is not yet understood (106).

Research has indicated that MDH2 mutations can promote HIFs stability by inhibiting PHDs, thereby facilitating pseudohypoxic responses in PPGLs (109–111). Conversely, some pharmacological studies have shown that LW6, an aroxycetaminobenzoic acid analogue, can inhibit MDH1 activity. This leads to mitochondrial respiratory impairment, reducing cellular oxygen consumption and ATP production, resulting in elevated intracellular oxygen levels and triggering oxygen-dependent degradation of HIFs (112, 113).

## Conclusion and future perspectives

In summary, the intricate and complex interplay between metabolic enzyme mutations within the TCA cycle and the pseudohypoxic signaling has illuminated the potential mechanisms underlying the pathogenesis of PPGLs. The identification of key genetic mutations and their impact on critical metabolic intermediates such as succinate, fumarate, and 2-hydroxyglutarate underscores the significance of pseudo-hypoxia in promoting tumor initiation and progression. Under normoxic conditions, the activation of HIFs and their downstream effector pathways highlights the adaptive advantage of tumor cells within adverse microenvironments. Moreover, these mutations' influence on immune cell communication patterns and the establishment of an immune-suppressive tumor microenvironment further underscores the intricacy of PPGLs development. Insights gained from understanding alterations in PPGLs metabolism serve as the foundation for developing precise and effective therapeutic strategies. Additionally, the broader implications of these findings extend to the realm of cellular metabolism and its pivotal role in tumorigenesis.

Looking ahead, delving deeper into the intricate network of metabolic reprogramming and pseudo-hypoxic signaling in PPGLs holds great promise. Elucidating the detailed molecular mechanisms by which metabolic enzyme mutations drive HIFs activation and pseudohypoxia is paramount for designing targeted and efficacious therapeutic interventions. Furthermore, investigating the crosstalk between pseudo-hypoxic signaling and other molecular clusters such as the WNT and kinase pathways may reveal further complexities and potential therapeutic targets. The advancements in techniques such as high-throughput sequencing and metabolomics analysis offer exciting opportunities to uncover the genetic landscape and metabolic adaptations driving PPGLs development. Integrating these comprehensive datasets with functional studies will contribute to a profound understanding of the disease and aid in the discovery of novel biomarkers for early diagnosis and prognosis.

To sum up, delving into the role of TCA cycle metabolic enzyme mutations and pseudohypoxic signaling in PPGLs not only enhances our comprehension of these rare neuroendocrine tumors but also provides broader insights into cancer biology and the field of metabolism. These insights ignite the potential for

innovative therapeutic approaches and diagnostic methods, with the promise of significant clinical impact in the management of PPGLs and beyond.

## Author contributions

YXW: Writing – original draft, Writing – review & editing. BL: Conceptualization, Validation, Writing – review & editing. FL: Conceptualization, Validation, Writing – review & editing. YZ: Writing – review & editing. XG: Writing – review & editing. YSW: Funding acquisition, Supervision, Writing – review & editing. HZ: Funding acquisition, Supervision, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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