

# Hypoxia-inducible Factor 2 $\alpha$ : A Key Player in Tumorigenesis and Metastasis of Pheochromocytoma and Paraganglioma?

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## ABSTRACT

Germline or somatic driver mutations linked to specific phenotypic features are identified in approximately 70 % of all catecholamine-producing pheochromocytomas and paragangliomas (PPGLs). Mutations leading to stabilization of hypoxia-inducible factor 2 $\alpha$  (HIF2 $\alpha$ ) and downstream pseudohypoxic signaling are associated with a higher risk of metastatic disease. Patients with metastatic PPGLs have a variable prognosis and treatment options are limited. In most patients with PPGLs, germline mutations lead to the stabilization of HIF2 $\alpha$ . Mutations in *HIF2 $\alpha$*  itself are associated with adrenal pheochromocytomas and/or extra-adrenal paragangliomas and about 30 % of these patients develop metastatic disease; nevertheless, the frequency of these specific mutations is low (1.6–6.2 %). Generally, mutations that lead to stabilization of HIF2 $\alpha$  result in distinct catecholamine phenotype through blockade of glucocorticoid-mediated induction of phenylethanolamine N-methyltransferase, leading to the formation of tumors that lack epinephrine. HIF2 $\alpha$ , among other factors, also contributes importantly to the initiation of a motile and invasive phenotype. Specifically, the expression of HIF2 $\alpha$  supports a neuroendocrine-to-mesenchymal transition and the associated invasion-metastasis cascade, which includes the formation of pseudopodia to facilitate penetration into adjacent vasculature. The HIF2 $\alpha$ -mediated expression of adhesion and extracellular matrix genes also promotes the establishment of PPGL cells in distant tissues. The involvement of HIF2 $\alpha$  in tumorigenesis and in multiple steps of invasion-metastasis cascade underscores the therapeutic relevance of targeting HIF2 $\alpha$  signaling pathways in PPGLs. However, due to emerging resistance to current HIF2 $\alpha$  inhibitors that target HIF2 $\alpha$  binding to specific partners, alternative HIF2 $\alpha$  signaling pathways and downstream actions should also be considered for therapeutic intervention.

## ABBREVIATIONS

|               |  |
|---------------|--|
| ARNT          | aryl hydrocarbon receptor nuclear translocator |
| ATRX          | ATRX chromatin remodeler                       |
| ccRCC         | clear cell renal cell carcinoma                |
| DLST          | dihydrolipoamide S-succinyltransferase         |
| EMT           | epithelial-mesenchymal transition              |
| EPAS1         | endothelial PAS protein 1                      |
| FH            | fumarate hydratase                             |
| FGFR1         | fibroblast growth factor receptor 1            |
| GOT2          | glutamic-oxaloacetic transaminase 2            |
| HIF2 $\alpha$ | hypoxia-inducible factor 2 $\alpha$            |
| HREs          | hypoxia-responsive elements                    |
| IDH           | isocitrate dehydrogenases                      |
| NF1           | neurofibromin 1                                |
| MAX           | MYC associated factor X                        |
| MDH2          | malate dehydrogenase 2                         |
| neuroendoMT   | neuroendocrine-to-mesenchymal transition       |
| PNMT          | phenylethanolamine N-methyltransferase         |
| PPGLs         | pheochromocytomas and paragangliomas           |
| PHDs          | prolyl hydroxylases                            |
| RET           | Ret proto-oncogene                             |
| SDH           | succinate dehydrogenase                        |
| SLC25A11      | mitochondrial 2-oxoglutarate/malate carrier    |
| TET           | ten-eleven translocation                       |
| TERT          | telomerase reverse transcriptase               |
| TH            | tyrosine hydroxylase                           |
| TMEM127       | transmembrane protein 127                      |
| VHL           | von Hippel-Lindau                              |

## Introduction

Pheochromocytomas and paragangliomas (PPGLs) are rare neural crest-derived tumors (2–8 cases per million per year) that respectively originate from chromaffin cells of the adrenal medulla or the extra-adrenal sympathetic paraganglia [1]. Paragangliomas of the head and neck on the other hand are derived mainly from non-chromaffin cells associated with parasympathetic ganglia. Most PPGLs do not metastasize, but up to 20% of patients with PPGLs present with distant metastases at sites where chromaffin cells are normally absent, such as lymph nodes, lung, liver, and bones [2–4]. Metastatic disease may be identified at either initial diagnosis (synchronous disease) or only become apparent at follow-up (metachronous disease). This article reviews the role of hypoxia-inducible factor 2 $\alpha$  (HIF2 $\alpha$ ), also referred to as endothelial PAS protein 1 (EPAS1), in tumorigenesis and metastasis of PPGLs. In this context, we also highlight the influence of HIF2 $\alpha$  on catecholamine phenotype and its effects on different steps of the invasion-metastasis cascade to clarify the therapeutic relevance of stabilizing an altered HIF2 $\alpha$  for treatment of patients with metastatic PPGLs.

Whether the metastatic disease is synchronous or metachronous, treatment options are limited and the prognosis is poor, though highly variable. An early diagnosis or even prediction of metastatic disease in metachronous cases or patients screened due to hereditary risk may nevertheless be useful for earlier intervention and an

improved outcome for any affected patient; to this end, several risk factors have been well established to be associated with metastatic disease. These include germline mutations in succinate dehydrogenase (SDH) subunit B (SDHB), large tumor size, extra-adrenal location, and elevated plasma methoxytyramine concentrations [2, 5, 6]. Telomerase activation and ATRX chromatin remodeler (ATRX) mutations are also described as independent risk factors for metastatic PPGLs [7]. Our recent findings further demonstrate that patients with PPGLs characterized by activation of pseudohypoxic pathways due to an increased expression and stabilization of HIF2 $\alpha$  are also at high risk of metastatic disease [8]. This association also appears to relate to some other established risk factors.

Hypoxia signaling has a far-reaching impact on cellular differentiation and tumorigenesis mainly driven by the two main HIF $\alpha$  isoforms: HIF1 $\alpha$  and HIF2 $\alpha$ , which are structurally comparable and mostly have complementary functions [9, 10]. In presence of oxygen, proline residues within HIF $\alpha$  subunits are hydroxylated by  $\alpha$ -ketoglutarate- and oxygen-dependent prolyl hydroxylases (PHDs); this then allows for von Hippel-Lindau (VHL) tumor suppressor-mediated proteasomal degradation of the HIF proteins (► **Fig. 1a**). In absence of oxygen (hypoxia), HIF $\alpha$  subunits form transcriptionally active complexes with aryl hydrocarbon receptor nuclear translocator (ARNT, also known as HIF $\beta$ ) and co-factors (e. g., CREB-binding protein and p300) followed by C-terminal transactivation of genes possessing hypoxia-responsive elements (HREs) (► **Fig. 1b**). In addition to HRE-mediated mechanisms, both HIF $\alpha$  subunits show interactions with NOTCH, WNT, and MYC pathways, thereby regulating gene expression through these additional pathways [11]. During cellular adaptation to hypoxia, the activity of HIF $\alpha$  differs temporally; while HIF1 $\alpha$  primarily mediates the acute response to severe hypoxia, HIF2 $\alpha$  mediates the response to chronic, even mild hypoxia [12]. This differential regulation is mediated by different hypoxia-associated factors and involves distinct cellular functions [13]. Moreover, several studies have indicated an oncogenic activity of HIF2 $\alpha$ , and tumor suppressor role of HIF1 $\alpha$  [14, 15]. HIF1 $\alpha$  is ubiquitously expressed in many cell types, while HIF2 $\alpha$  expression is restricted to specific cellular populations, including endothelial cells, neural crest cells, and glial cells [16].

## HIF2 $\alpha$ -dependent genotype-phenotype relationships in PPGLs

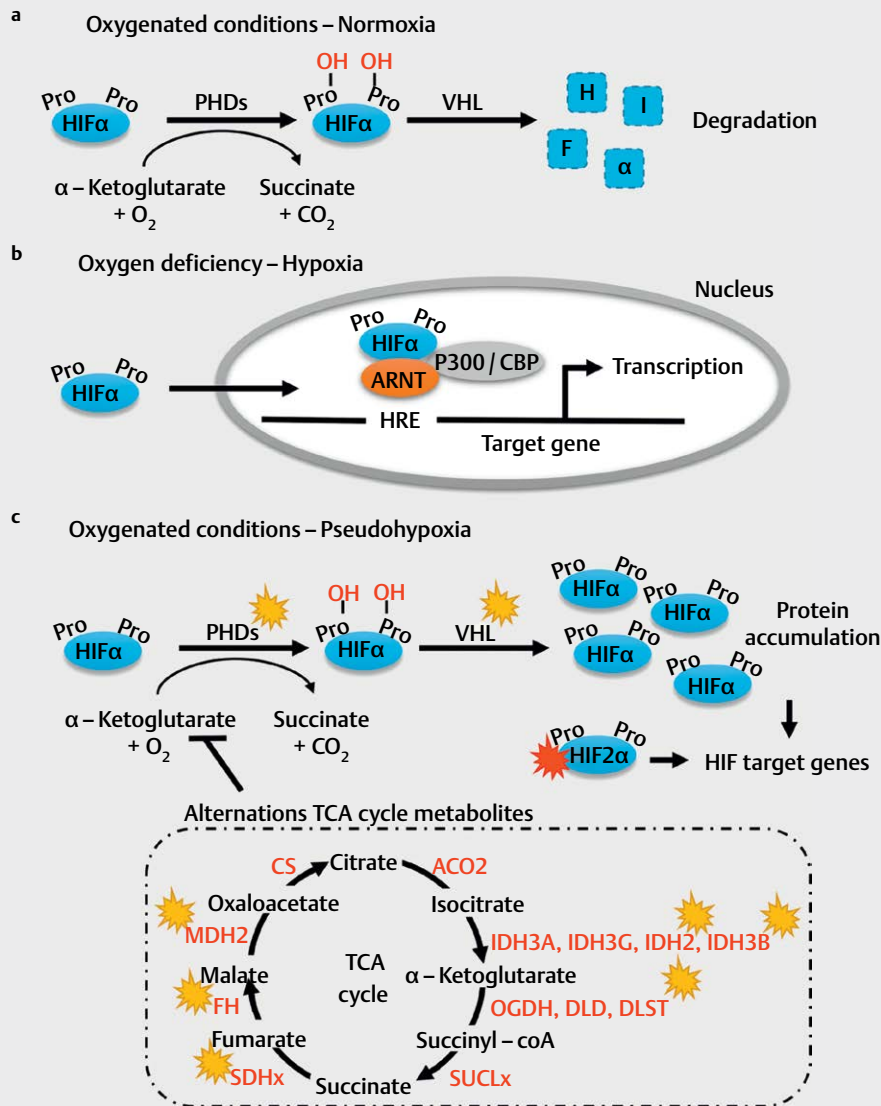
Currently, germline or somatic driver mutations can be identified in approximately 70% of catecholamine-producing PPGLs [17, 18]. Close links between genotype and phenotype including the risk of metastatic disease, age of onset, syndromic presentation, and a predominant anatomic site have been recognized [19, 20]. PPGLs are most broadly classified into two main clusters in accordance with their transcriptional profiles [21, 22]. Cluster 1 PPGLs comprise those with mutations encoding two groups of genes; these are either directly involved in the stabilization of HIFs or encode components of the tricarboxylic acid (TCA) cycle. The former include VHL, EGLN1/2 (encoding PHD1/2), and HIF2 $\alpha$ , whereas the latter include SDH subunits (SDHA, SDHB, SDHC, SDHD), fumarate hydratase (FH), malate dehydrogenase 2 (MDH2), mitochondrial 2-oxoglutarate/malate carrier (SLC25A11), isocitrate dehydrogenases

(*IDH1/IDH2/IDH3B*), glutamic-oxaloacetic transaminase 2 (*GOT2*), and dihydrolopoamide *S*-succinyltransferase (*DLST*) (► **Fig. 1c**) [18].

All cluster 1 mutations result in the stabilization of HIF $\alpha$  and lead to activation of hypoxia pathways even in the presence of oxygen (pseudohypoxia) [23]. Cluster 1 PPGLs are more prone to metastasize (18.6% excluding patients with *SDHB* mutation) compared to cluster 2 PPGLs (4.3%) caused by mutations of genes including (Ret protooncogene (*RET*), neurofibromin 1 (*NF1*), transmembrane protein 127 (*TMEM127*), H-Ras (*HRAS*), fibroblast growth factor receptor 1

(*FGFR1*), MYC associated factor X (*MAX*) that lead to activation of kinase signaling pathways [8]. However, due to mutations in *SDHB*, cluster 1 PPGLs are particularly prone to metastasize (75.6% in [8]) [24]. Both higher expression and stabilization of HIF2 $\alpha$  are characteristics of cluster 1 compared to cluster 2 PPGLs [21, 25, 26] and this seems to contribute to the pro-metastatic behavior of cluster 1 tumors [8, 27].

HIF2 $\alpha$  blocks glucocorticoid-mediated induction of phenylethanolamine N-methyltransferase (PNMT), the enzyme that converts



► **Fig. 1** Oxygen-dependent and independent regulation of HIF $\alpha$ . (a) Under normoxic conditions, HIF $\alpha$  subunits are hydroxylated by  $\alpha$ -ketoglutarate- and O<sub>2</sub>-dependent prolyl hydroxylases (PHDs) enabling von Hippel-Lindau (VHL)-mediated degradation of the HIF $\alpha$  protein. (b) Under hypoxic conditions, HIF $\alpha$  subunits form transcriptionally active complexes with ARNT (also known as HIF $\beta$ ) and co-factors followed by C-terminal transactivation of genes possessing hypoxia-responsive elements (HREs). (c) Pseudohypoxic conditions are characterized by increased stabilization of HIF $\alpha$ , especially HIF2 $\alpha$ , although oxygen is present. In PPGLs, pseudohypoxia can be caused by mutations in hypoxia-related genes encoding PHDs, VHL, and HIF2 $\alpha$  themselves, or by mutations in tricarboxylic acid (TCA) cycle-related genes leading to an alteration in TCA cycle metabolites, leading to an inhibition of  $\alpha$ -ketoglutarate-dependent PHDs. Genes reported as altered in PPGLs are highlighted by stars (yellow: loss-of-function mutation, red: gain-of-function mutation). CBP: CREB-binding protein; FH: fumarate hydratase; MDH2: mitochondrial malate dehydrogenase; CS: citrate synthase; ACO2: mitochondrial aconitase; IDH2: isocitrate dehydrogenase 2; IDH3A/IDH3B/IDH3G: subunits of isocitrate dehydrogenase 3; OGDH/DLD/DLST: subunits of the alpha-ketoglutarate dehydrogenase complex; SUCLx: subunits of succinyl-CoA synthetase; SDHx: subunits of the succinate dehydrogenase complex.

norepinephrine to epinephrine, providing a direct link between genotype and biochemical phenotype [26]. This, therefore, explains the expression of PNMT and epinephrine production by cluster 2 PPGLs, but not by cluster 1 PPGLs. HIF2 $\alpha$  also influences phosphorylation of tyrosine hydroxylase (TH), the rate-limiting step in catecholamine synthesis, thereby affecting cellular dopamine and norepinephrine production [28]. Cluster 1 PPGLs that produce predominantly norepinephrine with varying amounts of dopamine, have lower total tissue catecholamine content than cluster 2 PPGLs; nevertheless, rates of catecholamine secretion and urinary excretion are higher in cluster 1 than cluster 2 PPGLs [29]. This may reflect a more completely developed secretory system in cluster 2 than in cluster 1 PPGLs that acts to restrain otherwise continuous or constitutive secretion [30]. Thus, although cluster 1 tumors tend to secrete catecholamines more actively than cluster 2 tumors, they have lower catecholamine contents per unit of tissue and produce lower amounts of O-methylated metabolites than cluster 2 tumors. The role of HIF2 $\alpha$  in these differences remains unclear.

The predisposing role of HIF2 $\alpha$  expression may also explain the reason for tumorigenesis of certain neural crest derivatives according to mutations that involve stabilization of HIF2 $\alpha$  protein. As mentioned earlier, while HIF1 $\alpha$  is ubiquitously expressed, HIF2 $\alpha$  exhibits a much more restricted expression pattern [16]. In particular, HIF2 $\alpha$  is expressed transiently during the migration of trunk neural crest cells to sympathetic paraganglia and the adrenal medulla, and before further differentiation [31]. This expression impacts migration and proliferation and has also been associated with the development of neural-crest derived sympathoblast precursors to neuroblastoma subtypes with more aggressive and less differentiated features than other subtypes [32]. Furthermore, using single-cell transcriptomics, human embryos were shown to have different neural crest cell derivatives that populate both the sympathetic paraganglia and adrenal medulla and may be responsible for the heterogeneity of associated tumors [33]. It, therefore, seems possible that cluster 1 tumors may be derived from more primitive neural crest derivatives compared to cluster 2 tumors and that this is associated with expression of HIF2 $\alpha$  and different phenotypic features including the propensity for metastasis. Similarly, the younger age of patients with cluster 1 noradrenergic than cluster 2 adrenergic tumors, along with findings of higher proportions of multifocal, extra-adrenal, and metastatic tumors in younger than older patients with PPGLs, has also been proposed to reflect the development of cluster 1 tumors from different populations of chromaffin cell precursors [34, 35].

In line with the above considerations and as outlined by Flidner et al. [19], the cellular origins and the location of tumors can also influence tumor behavior in terms of catecholamine phenotype and metastatic behavior. The majority of chromaffin cell tumors arise from the adrenal medulla [35, 36]. About half of these produce norepinephrine exclusively and reflect cluster 1 tumors and the other half, the cluster 2 tumors, present with variable amounts of both norepinephrine and epinephrine [29, 37]. Sympathetic paragangliomas usually exclusively produce norepinephrine, in some cases additional variable amounts of dopamine, and in isolated cases exclusively dopamine [29]. Head and neck paragangliomas, which are derived from parasympathetic ganglia, show

only limited expression of TH [38, 39]; thus, most are biochemically non-functional, though about 30% produce dopamine as manifested by the increase in the dopamine metabolite, methoxytyramine [40].

A recent study focusing on Sino-European differences in the genetic landscape of patients with PPGLs initiated a paradigm shift in the understanding of genotype-phenotype relationships of these genetically heterogeneous tumors [41]. Epinephrine production in the adrenal medulla depends on the expression of PNMT, which is regulated by glucocorticoids produced in the surrounding adrenal cortex [42]. According to previous understanding, mainly based on findings in Caucasian populations, this explains why epinephrine production is confined to adrenal pheochromocytoma [37, 43, 44]. Among the Chinese population, a substantial proportion of extra-adrenal PGLs produce epinephrine mainly associated with somatic mutations in *HRAS* and *FGFR1*. These findings further clarify that the adrenergic phenotype of PPGLs primarily depends on the underlying genetic mutation rather than tumor location [41].

Nevertheless, the aforementioned revised understanding does not mean that glucocorticoids are not relevant to induction of PNMT, but only that they may act on chromaffin cells to impact phenotypic features more distantly than previously believed. As outlined earlier, failure to induce PNMT, whether at adrenal or extra-adrenal locations, is related to the activity of HIF2 $\alpha$  to block glucocorticoid-mediated induction of the enzyme. As also clarified by Qin et al. [26], these activities possibly involve interactions of HIF2 $\alpha$  with the MYC/MAX complex, actions that are independent of binding of HIF2 $\alpha$  to ARNT/HIF $\beta$ .

### HIF2 $\alpha$ mutations in PPGL tumorigenesis

Gain-of-function mutations of HIF2 $\alpha$  are associated with pheochromocytomas and extra-adrenal paragangliomas and are often multifocal and recurrent. HIF2 $\alpha$ -mutant PPGLs are more frequent in females than in males and are characterized by the production of norepinephrine [23]. Mutations in HIF2 $\alpha$  are predominantly somatic and postzygotic, and are closely located on the oxygen-dependent degradation domain of HIF2 $\alpha$ ; this hinders hydroxylation by PHDs and degradation by VHL [45, 46]. Germline mutations (germline variant F374Y) are rare, but mosaicism is more common [47, 48]. Such patients present with PPGL–somatostatinoma–polycythemia syndrome (Pacak–Zhuang syndrome), which occurs exclusively in females [46, 49, 50].

In Caucasian cohorts, frequency of HIF2 $\alpha$  mutations in PPGLs varies between 1.6–4.6% [8, 17, 45, 51], which is lower than in a Chinese cohort at 6.2% [41]. In our cohort, one-third of patients bearing HIF2 $\alpha$  mutations were diagnosed with metastatic disease [8]. This confirmed a previous study in which 29% of HIF2 $\alpha$  mutant PPGLs showed metastatic disease [52]. The comparatively high metastatic tendency of HIF2 $\alpha$  mutant PPGLs together with the generally increased metastatic risk of cluster 1 PPGLs, characterized by stabilization of HIF2 $\alpha$ , further supports the potential role of HIF2 $\alpha$  as a pro-metastatic factor in PPGLs.

### HIF2 $\alpha$ -driven mesenchymal transition promotes a pro-metastatic phenotype in PPGLs

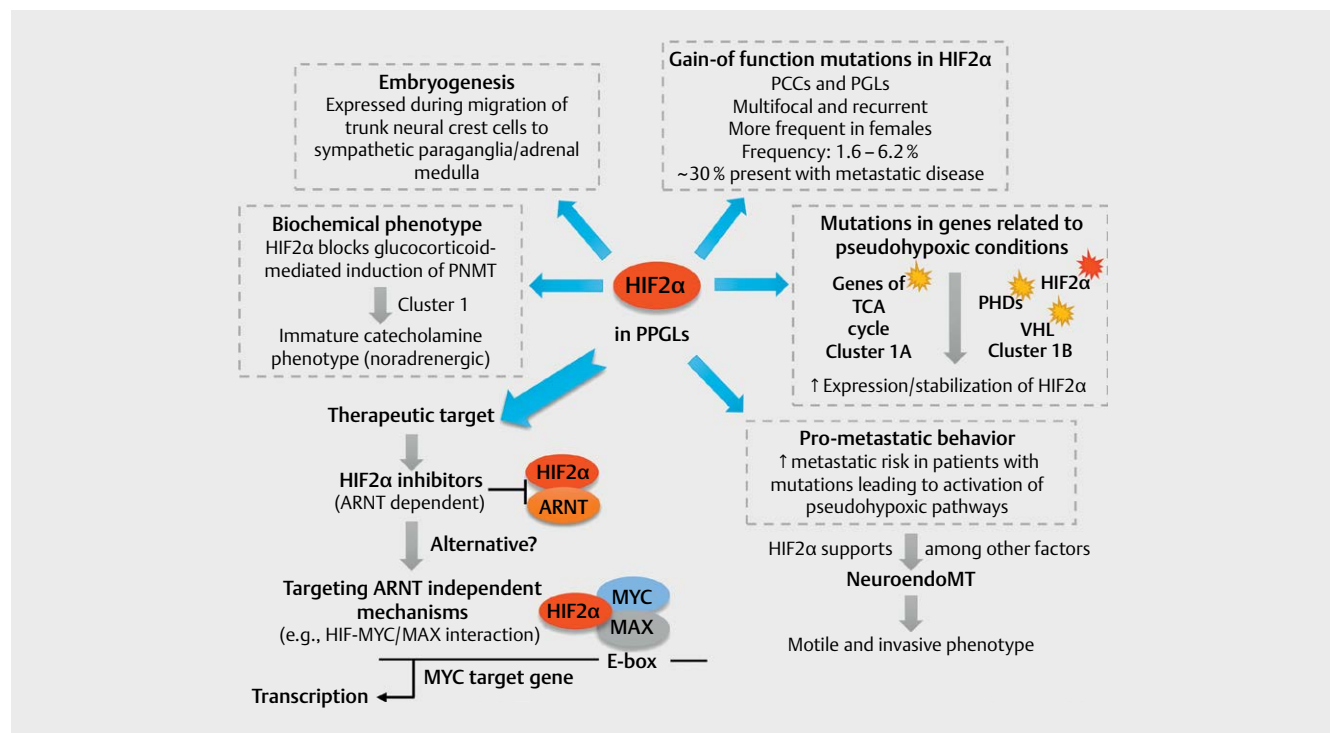
During the invasion-metastasis cascade, tumor cells must pass multiple steps to reach distant organs. Acquisition of a motile and in-

vasive phenotype (epithelial-mesenchymal transition, EMT) is the initiation step of this cascade and involves various changes in gene expression, including genes encoding proteins involved in cell adhesion and extracellular matrix interactions [53]. Due to the non-epithelial origin of chromaffin cells, PPGLs are assumed to undergo a neuroendocrine-to-mesenchymal transition (neuroendoMT) associated with activation of specific signaling pathways [54–56]. In pheochromocytoma cells, *SDHB* mutation-induced neuroendoMT is associated with a pro-metastatic phenotype [54]. Our data also confirmed the involvement of HIF2 $\alpha$  in this transition. The expression of *HIF2 $\alpha$*  leads to changes in focal adhesion and extracellular matrix-receptor interaction pathways [8].

In relation to the above-mentioned findings, a study from Morin et al. showed that the neuroendoMT and pro-metastatic phenotype of *SDHB*-deficient cells results from synergistic effects of HIF2 $\alpha$  and ten-eleven translocation (TET) dioxygenase-mediated hypermethylation [27]. The 2-oxoglutarate-dependent TET hydroxylates DNA-methylated cytosine to form 5-hydroxymethylcytosine and is thereby directly involved in epigenetic regulation. Especially oncometabolite-driven tumors, such as PPGLs of the TCA cycle-related cluster 1, and here in particular tumors with *SDHB*

mutations show hypermethylation [57, 58]. A genome-wide DNA methylation analysis in metastatic PPGLs identified hypermethylation of negative elongation factor complex member E (RDBP) as a prognostic marker for stratifying patients according to the risk to develop metastatic disease [59]. This, therefore, provides a potential explanation for the much higher metastatic risk in patients with PPGLs due to mutations of *SDHB* compared to other cluster 1 genes. Thus, although HIF2 $\alpha$  may be pro-metastatic, other factors clearly play important roles in the development of metastatic disease. These factors can include secondary mutations, such as those in *ATRX*, *TERT*, and *p53*, which may occur after initiation of tumorigenesis and lead to an event that further contributes to the development and progression of metastatic disease [7, 60].

In addition to its participation in neuroendoMT and thus the initiation of tumor cell migration from a primary tumor, HIF2 $\alpha$  is also involved in the formation of pseudopodia in PPGLs [8]. This critical step of mesenchymal cell migration enables intravasation; this involves penetration by tumor cells of the endothelial basement membrane, thereby facilitating their entry into adjacent blood or lymph vessels [61]. The increased ability of HIF2 $\alpha$ -expressing cells to attach to extracellular matrixes, such as laminin, facilitates the



► **Fig. 2** Involvement of HIF2 $\alpha$  in tumorigenesis and metastasis of cluster 1 pheochromocytomas and paragangliomas (PPGLs). During embryogenesis, HIF2 $\alpha$  is involved in the migration of trunk neural crest cells to sympathetic paraganglia or adrenal medulla. Mutations in HIF2 $\alpha$  predispose to the development of pheochromocytomas (PCCs) and paragangliomas (PGLs). Moreover, mutations in genes related to activation of hypoxia pathways (pseudohypoxic cluster 1 PPGLs) are characterized by an increased expression and stabilization of HIF2 $\alpha$ . Patients with pseudohypoxic cluster 1 PPGLs bear a higher metastatic risk. HIF2 $\alpha$  supports, among other factors, the pro-metastatic behavior of these tumors by the acquisition of a motile and invasive phenotype (neuroendocrine-to-mesenchymal transition, neuroendoMT). HIF2 $\alpha$  blocks the glucocorticoid-mediated induction of phenylethanolamine N-methyltransferase (PNMT) and is thereby directly linked to the immature catecholamine phenotype of cluster 1 PPGLs. The versatile involvement of HIF2 $\alpha$  during tumorigenesis and metastasis in PPGLs makes it an ideal target for therapeutic interventions. Available HIF2 $\alpha$  inhibitors block dimerization with ARNT, but some tumors do not seem to respond to these kinds of inhibitors. Addressing ARNT-independent mechanisms of HIF2 $\alpha$ , such as interaction with the MYC/MAX complex, maybe an alternative strategy for these resistant tumors. Genes reported as altered in PPGLs are highlighted by stars (yellow: loss-of-function mutation, red: gain-of-function mutation). ARNT: aryl hydrocarbon receptor nuclear translocator; MAX: MYC associated factor X; PHDs: prolyl hydroxylases; VHL: von Hippel-Lindau tumor suppressor.

adhesion to the endothelial cell layer of the blood vessel, thereby encouraging subsequent extravasation [8]. The expression of genes involved in focal adhesion and the interaction of extracellular matrix-receptor promotes settlement of tumor cells in the pro-metastatic niche; the enhanced proliferation ability of HIF2 $\alpha$ -expressing cells allows further metastatic colonization [8]. The multifaceted involvement of HIF2 $\alpha$  in the invasion-metastasis cascade emphasizes the importance of this factor for potential therapeutic approaches in metastatic PPGLs.

### Targeting HIF2 $\alpha$ as a therapeutic option for metastatic PPGLs

For metastatic PPGLs, treatment options are limited and could benefit from personalized considerations according to the nature of underlying disease-causing mutations [62]. The enlarging understanding of the genetic background and associated molecular alterations in metastatic PPGLs offers for the first time a promising approach for individualized treatment of these patients. HIF2 $\alpha$  appears to play an important role in tumorigenesis and metastatic spread of PPGLs (► Fig. 2), making it an ideal target for therapeutic approaches. Hypoxia/pseudohypoxia and derived signaling pathways are also associated with increased resistance to chemotherapy and radiotherapy [63, 64]. Therefore, targeting HIF2 $\alpha$  may provide a potential chemo-and/or radiosensitizing approach to employ alongside other therapies.

Initially, HIF2 $\alpha$  was considered to be undruggable, but the discovery of specific structural features led to the development of small molecule antagonists that can block the dimerization of HIF2 $\alpha$  with ARNT/HIF $\beta$  [65, 66]. In clear cell renal cell carcinoma (ccRCC), which is frequently characterized by inactivation of VHL causing an enhanced stabilization of HIF2 $\alpha$ , two specific HIF2 $\alpha$  inhibitors, PT2385 and Belzutifan (PT2977) showed promise in phase I clinical trials [67, 68]. Phase II clinical trials in glioblastoma [69] and ccRCC (e. g., NCT03108066, NCT04489771, NCT03401788) are ongoing and a phase I clinical trial is also in planning stages for patients with PPGLs (personal communication). However, some VHL-mutant ccRCC cell lines showed resistance towards HIF2 $\alpha$  inhibitors [70], which is also in line with recent clinical findings [71]. The ccRCC cell lines that were sensitive to HIF2 $\alpha$  inhibitors displayed a distinct HIF2 $\alpha$ -dependent gene signature and higher levels of HIF2 $\alpha$  than other cell lines [70]. Resistance can be induced by prolonged treatment with HIF2 $\alpha$  inhibitors [70, 71]. A gatekeeper mutation in HIF2 $\alpha$  (G323E) that interferes with drug binding further promotes the acquisition of resistance [71]. Our data showed a lack of efficiency of PT2385 in HIF2 $\alpha$ -dependent pheochromocytoma cell models [8]. Expression of HIF2 $\alpha$  induced a pro-metastatic phenotype in these cells, which could not be reversed by treatment with PT2385 [8]. Similar results were also obtained in HIF2 $\alpha$ -dependent neuroblastomas [72]. This raises the possibility of an ARNT/HIF $\beta$ -independent mechanism in these models, for example through interactions with the MYC/MAX complex [73, 74]. These ARNT/HIF $\beta$ -independent mechanisms of HIF2 $\alpha$ , already mentioned earlier, may offer alternative therapeutic approaches for patients who show resistance to HIF2 $\alpha$  inhibitors (► Fig. 2).

Further studies are needed to demonstrate the suitability of HIF2 $\alpha$  inhibitors for the treatment of metastatic disease. Due to the described resistance mechanisms and the radio- and chemothera-

py resistance associated with hypoxia/pseudohypoxia, suitable combination therapies should also be considered. There is also a need to identify markers that can predict sensitivity towards HIF2 $\alpha$  inhibitors in suitable patients for such therapy.

### Conclusion

Mutations in HIF2 $\alpha$  provide a direct link of HIF2 $\alpha$  to PPGL tumorigenesis. Increased expression and stabilization of HIF2 $\alpha$  are also associated with increased aggressiveness and phenotypic characteristics of these tumors. The involvement of HIF2 $\alpha$  in multiple steps of the invasion-metastasis cascade underscores the therapeutic relevance of targeting impaired HIF2 $\alpha$  signaling pathways in PPGLs. However, because of resistance to current HIF2 $\alpha$  inhibitors that has emerged for certain conditions, ARNT/HIF $\beta$ -independent mechanisms of HIF2 $\alpha$  might also be considered for therapeutic intervention.

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### Conflict of Interest

The authors declare that they have no conflict of interest.

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