Abstract

Pheochromocytomas and paragangliomas (PPGL) are rare neuroendocrine tumors arising from the adrenal medulla or extra-adrenal paraganglia. Around 40% of all cases are caused by a germline mutation in a susceptibility gene, half of which being found in an SDHx gene (SDHA, SDHB, SDHC, SDHD or SDHAF2). They encode the four subunits and assembly factor of succinate dehydrogenase (SDH), a mitochondrial enzyme involved both in the tricarboxylic acid cycle and electron transport chain. SDHx mutations lead to the accumulation of succinate, which acts as an oncometabolite by inhibiting iron(II) and alpha-ketoglutarate-dependent dioxygenases thereby regulating the cell’s hypoxic response and epigenetic processes. Moreover, SDHx mutations induce cell metabolic reprogramming and redox imbalance. Major discoveries in PPGL pathophysiology have been made since the initial discovery of SDHD gene mutations in 2000, improving the understanding of their biology and patient management. It indeed provides new opportunities for diagnostic tools and innovative therapeutic targets in order to improve the prognosis of patients affected by these rare tumors, in particular in the context of metastatic diseases associated with SDHB mutations. This review first describes an overview of the pathophysiology and then focuses on clinical implications of the epigenetic and metabolic reprogramming of SDH-deficient PPGL.

Introduction

Pheochromocytomas and paragangliomas (PPGL) are rare neuroendocrine tumors arising from the adrenal medulla or extra-adrenal paraganglia, respectively. Paragangliomas can be found in the sympathetic nervous system of the thorax, abdomen, or pelvis or in the parasympathetic ganglia of the head or neck region. Pheochromocytomas and sympathetic paragangliomas commonly release catecholamines such as epinephrine, norepinephrine or dopamine, which can lead to cardio- or cerebro-vascular complications, whereas head and neck paragangliomas do not (Lenders et al. 2020). Most PPGL are curable by surgery when they are localized, with a recurrence rate estimated at 1% per year per patient (Amar et al. 2016). However, 10–15% of PPGL are metastatic, defined by the occurrence of metastatic lesions either in lymph nodes, bones, lungs or liver (Lam 2017), with an overall survival decreasing to 62% at 5 years, and treatments remaining mainly palliative with a limited efficacy (Hescot et al. 2019). Approximately 70% of PPGL have a genetic determinism with more than 20 susceptibility genes discovered so far, including germline mutations in about 40% of cases and somatic mutations in 30% of cases (Buffet et al. 2020).
Mutations in genes encoding the succinate dehydrogenase (SDH) complex are the most prevalent, occurring in SDHA, SDHB, SDHC, SDHD or SDHAF2 genes. Tumors carrying such mutations are classified in the pseudo-hypoxic expression cluster 1, together with tumors carrying mutations in VHL (von Hippel-Lindau), FH (fumarate hydratase), DLST (dihydrolipoamide S-succinyltransferase) genes and three genes encoding the malate-aspartate shuttle: GOT2 (glutamic-oxaloacetic transaminase), SLC25A11 (oxoglutarate carrier) and MDH2 (malate deshydrogenase) (Favier et al. 2015, Buffet et al. 2020). Cluster 2 tumors are associated with abnormal kinase signaling and activation of the mTOR pathway and include mutations in RET (multiple endocrine neoplasia type 2), NF1 (neurofibromatosis type 1), TMEM127, MAX, MET and HRAS. Cluster 3 is characterized by the activation of the Wnt-signaling pathway with MAML3 (mastermind-like transcriptional coactivator 3) anomalies (Fishbein et al. 2017).

SDHx genes encode the four subunits of SDH in the Tricarboxylic acid (TCA) cycle that also constitutes the mitochondrial complex II of the Electron transfer chain (ETC). It catalyses the oxidation of succinate to fumarate in the former and the transfer of electrons to the ubiquinone pool in the latter. SDHx genes are tumor suppressor genes and follow Knudson’s two-hit’ model (Knudson 1971), with a germline heterozygous mutation associated with somatic loss of heterozygosity (LOH), leading to a completely abolished (Gimenez-Roqueplo et al. 2001) or highly impaired (Kim et al. 2015) SDH activity. These mutations are transmitted in an autosomal dominant manner, with the exception of SDHD and SDHAF2, which are submitted to maternal imprinting, with only few cases of maternal transmission described so far for SDHD (Burnichon et al. 2017). SDHx mutations are associated with different phenotypes regarding penetrance, manifestations and rate of malignancy. SDHB mutations lead to metastatic evolution in 50% of cases, with a penetrance of 50% at age 50 while SDHD mutations mainly lead to multiple cervical paragangliomas with a greater penetrance of up to 80% (Benn et al. 2006, Amar et al. 2007, Burnichon et al. 2009, Buffet et al. 2020). SDHA, SDHC and SDHAF2 are scarcer. Around 40% of all PPGL patients carry a germline mutation in a susceptibility gene, half of which being found in an SDHx gene. Genetic counselling and testing are therefore recommended for all PPGL patients (Lenders et al. 2020), promoting the improvement of clinical outcome in patients carrying an SDHx or a VHL mutation (Buffet et al. 2019). Moreover, identification of one of these germline mutations allows to propose a genetic counselling and a pre-symptomatic screening to relatives, in order to include them in an appropriate follow-up protocol (Buffet et al. 2020).

Metabolic reprogramming is one of the eight hallmarks of cancers defined by Weinberg and Hanahan (Hanahan & Weinberg 2011), as first suggested by Otto Warburg in 1926 with his hypothesis that a direct link existed between mitochondrial dysfunction and cancer (Warburg 1956). Since the initial discovery of SDHD gene mutations in 2000 (Baysal et al. 2000), studies on the pathophysiology of SDH-related PPGL have led to major discoveries, improving the understanding of their biology and patient management and contributing to the extension of the field of oncometabolism. Comprehension of these pathophysiological characteristics provides new opportunities for diagnostic and innovative therapeutic strategies in order to improve the prognosis of patients affected by these rare diseases, in particular in the context of metastatic forms associated with SDHB mutations. This review provides an overview of the pathophysiology and then focuses on clinical implications of the epigenetic and metabolic reprogramming of SDH-deficient PPGL.

Metabolic consequences of SDH deficiency and oncogenesis

Mutations in genes encoding SDH lead to the loss of SDH enzymatic activity and therefore to the interruption of the TCA cycle. To sustain cell proliferation, in this highly compromised metabolic context, SDH-deficient cells consume extracellular pyruvate and activate pyruvate carboxylation to re-supply the depleted pool of aspartate that is further used to provide amino and nucleic acids (Cardaci et al. 2015, Lussey-Lepoutre et al. 2015). Recently, several studies evaluating the respiration and mitochondrial complexes activity of different SDH-deficient cell types revealed some intriguing data that might be a first step in the understanding of the tissue specificity of tumor development in SDH-mutated patients. Indeed, while both respiration of intact cells and complex I-specific activity were found to be lower in SDH-deficient cells compared with WT adrenal fibroblast (Křučková et al. 2020), renal (Cardaci et al. 2015, Lorendeau et al. 2017) and breast epithelial cells (Bezawork-Geleta et al. 2018), it is not the case in a chromaffin cell-derived model. This model retains complex I function and a respiratory activity comparable to WT cells, thereby maintaining the ability to oxidise NADH within the ETC (Křučková et al. 2020). These data showed that in contrast to models of
SDH deficiency based on epithelial cells, a chromaffin cell model preserves some aspects of metabolic ‘health’, which could form the basis of cell specificity of this rare tumor type.

The main consequence of the truncated TCA cycle is the accumulation of SDH substrate, succinate (Pollard et al. 2005), which is thought to be the major actor of SDH-related oncogenic processes (Fig. 1). Indeed, it has been demonstrated that succinate can act as an oncometabolite by inhibiting iron(II) and alpha-ketoglutarate-dependent (α-KG) dioxygenases, a large family of enzymes that catalyze the hydroxylation of a wide range of organic targets (Islam et al. 2018). Although it is still debated, ROS accumulation together with iron overload is also suspected to participate to this tumorigenic pathway and further inhibit αKG-dioxygenases. Discrepant data have been published with some studies showing ROS increase (Guzy et al. 2008, Liu et al. 2020) and others not (Selak et al. 2006, Guzy et al. 2008). One hypothesis is that such differences may actually reflect the heterogeneity of the phenotype linked to the different subtypes of SDH subunits, with a general consensus that SDHB-mutated cells do show increased oxidative stress. Members of the α-KG-dioxygenases family that regulate the cell’s hypoxic response and epigenetic processes, particularly the demethylation of histones and DNA, seem to play a particularly important role in SDH-related tumorigenesis. These enzymes are dependent on molecular oxygen and α-KG as necessary substrates, and use iron and ascorbate as cofactors, making them critical in the processes of metabolic sensing.

**Pseudohypoxia**

Their role as oxygen sensor is mediated by the hypoxia-inducible factors (HIF) prolyl hydroxylases (PHD 1–3) that regulate HIFs stability. In normoxic conditions, PHD enzymes hydroxylate the α subunits of hypoxia-inducible factors (HIF1 and HIF2) on two highly conserved proline residues, making their recognition by von Hippel Lindau protein (pVHL) possible. pVHL is a E3 ubiquitin ligase that will then promote their ubiquitination and subsequent degradation by the proteasome. Being dependent on oxygen to be fully active, PHDs are inhibited when oxygen concentrations fall in hypoxic conditions; HIFs can therefore escape from their proteasome destiny and translocate to the nucleus where they form an active transcription factor following their heterodimerization with their β counterpart, namely the aryl hydrocarbon receptor nuclear translocator (ARNT or HIF1 β). In hereditary PPGL, as in clear cell renal cell carcinoma (ccRCC), activation of HIFs occurs even in the presence of oxygen, through the process of pseudohypoxia. This molecular or metabolic activation of the hypoxic response can be mediated either directly by loss-of-function mutations (such as tumors caused by germline or somatic inactivating mutations of the VHL gene) or through their metabolic consequences. In SDH- and FH-mutated PPGL,

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**Figure 1**

Epigenetic and metabolic reprogramming of SDH-deficient pheochromocytomas and paragangliomas and their potential treatments. AA6, alpha-ketoglutarate-dependent dehydrogenase inhibitor; 2-OG, alpha-ketoglutarate; C-MET, hepatocyte growth factor receptor; CVD, cyclophosphamide-dacarbazine-vincristine; FGFR, Fibroblast growth factor receptor; HDAC, histone deacetylase; HIF, hypoxia-inducible factors; HIF-PHD, hypoxia-inducible factors prolyl hydroxylase; HRE, hypoxia-inducible factors response elements; MGMT, O(6)-methylguanine-DNA-methyltransferase; OXPHOS, oxidative phosphorylation; PARP, Poly(ADP)-ribose polymerase; PDGFR, platelet-derived growth factor receptor; RET, rearranged during transfection; ROS, reactive oxygen species; SDH, succinate dehydrogenase; TET, ten-eleven translocation enzyme; VEGFR, vascular endothelial growth factor receptor.
the accumulation of succinate and fumarate respectively promotes the inhibition of PHDs and a pseudo-hypoxic signaling (Briere et al. 2005, Pollard et al. 2005, Selak et al. 2005, Letouze et al. 2013). The wide range of HIF target genes make this response critical for many cellular functions and participate to the occurrence of tumors through many aspects such as metabolic adaptation (glycolytic switch), proliferation, regulation of cell death, angiogenesis, modification of the extracellular matrix components, acquisition of mesenchymal hallmarks (Favier & Gimenez-Roqueplo 2010).

Epigenetic reprogramming

Another class of α-KG-dependent dioxygenases that were demonstrated to be inhibited by oncometabolites are the DNA and histone demethylases: TET dioxygenases that hydroxylate DNA methylated cytosines (5mC) into 5hmC and JmjC-domain containing histone lysine demethylases (KDM) (Cervera et al. 2009). In PPGL as in GIST, SDHx mutations were shown to promote a massive hypermethylator phenotype. Several groups, including ours, studied the metylome of these oncometabolite-driven tumors and reported enhanced DNA methylation in and outside CpG islands (Killian et al. 2013, Letouze et al. 2013). Interestingly, SDHB-deficient PPGL displayed higher global methylation levels than other subtypes of SDH-deficient tumors (with mutations in SDHA, C or D), suggesting that epigenetic reprogramming may participate to the increased metastatic capacities of SDHB-mutated tumors. Recently, we were able, using oxidative reduced representation bisulfite sequencing (oxRRBS), to demonstrate that SDHB-mutated PPGL did display lower levels of 5hmC than NF1-mutated tumors (Morin et al. 2020). Moreover, we showed that hypermethylated DNA sequences were enriched in polycomb repressor complex 2 (PRC2) target genes, albeit independently of PRC2 activity. These observations revealed a tropism of TET enzymes for PRC2 targeted chromatin regions, thereby linking the hypermethylator phenotype of a de-differentiation process. Using functional assays in an Sdhb-deficient immortalized mouse chromaffin cell line, this study provided the first demonstration that TET-mediated DNA hypermethylation indeed promoted a massive transcriptomic reprogramming, but that the synergy between these epigenetic modifications and a HIF2α-mediated pseudo-hypoxic response was required to promote the invasive and the mesenchymal phenotypes of Sdhb-deficient cells.

Metabolic and epigenetic biomarkers

Rapid identification of SDH deficiency is important at various levels, by accelerating genetic testing or facilitating the interpretation of genetic variations in SDHx genes provided by next-generation sequencing (NGS) (Toledo & Dahia 2015). Moreover, it can impact patient management by considering the higher risk of metastasis of SDHB-related tumors, the high rate of multiple cervical PGL for SDHD-mutated patients and driving therapeutic choices for inoperable and metastatic patients. Thus, several biomarkers have been developed based on assessment of protein expression, functionality and its metabolic and/or epigenetic consequences (Table 1).

Enzyme functionality and metabolomics

For SDH expression, the routinely method employed is SDHB immunohistochemistry (IHC) performed on formalin-fixed paraffin-embedded (FFPE) tumors that predicts SDHx mutations with a good sensitivity, specificity and reproducibility among expert endocrine pathologists in PPGL (van Nederveen et al. 2009, Gill et al. 2010, Papathomas et al. 2015) and in other tumors as GIST (Gaal et al. 2011, Oudijk et al. 2013) and kidney cancers (Gill et al. 2011). The mitochondrial-specific granular staining is lost in tumor cells for all subtypes of SDHx mutations (SDHA, SDHB, SDHC, SDHD and SDHAF2) while it is maintained in stromal cells such as endothelial cells, adipocytes or sustentacular cells, providing internal positive controls (van Nederveen et al. 2009, Gill et al. 2010). SDHA IHC is also a valuable method in tumors with a negative SDHB immunostaining to specifically identify SDHA-related tumors, as it is negative for SDHA mutations but positive for all other SDH-related tumors (Korpershoek et al. 2011). In some cases, especially SDHD-related PGL, SDHB IHC may fail because of a weak diffuse cytoplasmic blush that should be considered as negative but can be misinterpreted (Gill et al. 2010, Papathomas et al. 2015). In that context, SDHD IHC can be useful, with a mirror result, that is negative in non-SDHx PGL and a positive labeling characterized by a cytoplasmic diffuse signal with patchy accumulation in SDH-mutated PGL (Menara et al. 2015).

The direct method to assess SDH functionality is the quantification of SDH activity on fresh frozen tumor samples using a spectrophotometric assay (Rustin et al. 1994). Measurement of Krebs cycles metabolites by liquid chromatography-mass spectrometry (LC-MS/MS) is
Table 1  Metabolic and epigenetic biomarkers of SDH-deficient pheochromocytomas and paragangliomas.

<table>
<thead>
<tr>
<th>Type</th>
<th>Interpretation</th>
<th>Sn/Sp</th>
<th>Current use</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Immunohistochemistry</td>
<td>Mitochondrial-specific granular staining lost in tumor cells of non-SDHx mutated tumors. Negative in tumors cells with all types of SDHx mutations (SDHA, SDHB, SDHC, SDHD and SDHAF2). Weak diffuse cytosolic staining should be considered as negative.</td>
<td>94%/84% (Papathomas et al. 2015)</td>
<td>Clinical</td>
<td>van Nederveen et al. 2009, Gill et al. 2010, Papathomas et al. 2015</td>
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<td>SDHB</td>
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<td>SDHA</td>
<td>Negative in SDHA-mutated tumors but positive in all other SDHx- or non-SDHx-mutated tumors</td>
<td>75%/93% (Papathomas et al. 2015)</td>
<td>Clinical</td>
<td>Kopershoek et al. 2011, Papathomas et al. 2015</td>
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<tr>
<td>SDHD</td>
<td>Used in case of SDHB IHC interpretation (such as weak diffuse staining); negative in non-n-SDHx PGL and positive labeling (cytoplasmic diffuse signal with patchy accumulation) in SDHx-mutated PGL.</td>
<td>96%/97% (Menara et al. 2015)</td>
<td>Research</td>
<td>Menara et al. 2015</td>
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<td>DNA methylation: 5hmC</td>
<td>5hmC staining inferior (or absent) in the nuclei of SDHx-mutated tumor cells compared with endothelial or sustentacular cells. Increase in H3K9me3 in SDHx-related tumors (no change in H3K4me3 and H3K27me3)</td>
<td>Not available</td>
<td>Research</td>
<td>Letouze et al. 2013, Hoekstra et al. 2015</td>
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<td>Histone methylation:</td>
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<tr>
<td>H3K4me3, H3K9me3,</td>
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<td>H3K27me3</td>
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<tr>
<td>(LC-MS/MS)</td>
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<tr>
<td>Succinate in vivo</td>
<td>Accumulation of succinate in tumor in case of an SDHx mutation</td>
<td>87%/100% (Lussey-Lepoutre et al. 2020)</td>
<td>Research/clinical</td>
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<tr>
<td>(13H-MRS)</td>
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Shmc, 5-methylcytosine; 13H-MRS, proton magnetic resonance spectroscopy; IHC, immunohistochemistry; LC-MS/MS, liquid chromatography with mass spectrometry; SDH, succinate deshydrogenase; Sn, sensitivity; Sp, specificity.

Another valuable method (Pollard et al. 2005, Rao et al. 2013, Richter et al. 2014, Imperiale et al. 2015) to assess the consequences of loss of enzymatic activity. Metabolic profiling of SDH-mutated PGL show important accumulation of succinate (SDH substrate), with concentrations up to 100-fold higher than tumors without SDHx mutations and very low levels of its product, fumarate. Thus, the tissue-specific succinate to fumarate ratio (SFR) has a good sensitivity and a recent study showed a higher specificity than SDHB IHC to detect SDHx mutations on fresh frozen tumors and FFPE (Wallace et al. 2020). Interestingly, SFR appeared particularly high in SDHB-mutated and metastatic PGL but lower in head and neck PGL (Richter et al. 2014). Other TCA cycle metabolites are also significantly impacted in SDH-mutated PGL: a decrease in isocitrate, cis-aconitate (Richter et al. 2014), aspartate and glutamate (Imperiale et al. 2015) may also be used as biomarkers but above all can improve understanding of metabolic reprogramming. Indeed, decrease of aspartate has been elucidated by in vitro 13C-glucose fluxes experiments as a consequence of the high dependence of SDH-deficient cells upon mitochondrial aspartate for cellular anabolism, suggesting novel therapeutic perspectives (Lussey-Lepoutre et al. 2015).

Altogether, metabolomic profiling and IHC provide valuable biomarkers of SDHx mutation but rely on the availability of tissue from the resected tumor. Because of the high vascularization of these tumors (Favier et al. 2009) and the secretion of catecholamines, the recourse to a biopsy is a contraindication and the surgical removal of the lesion is not always feasible.

Data on whether circulating or urinary levels of succinate are efficiently measurable in patients with
germline SDHx variants are still scarce. Using gas chromatography-mass spectrometry (GC-MS), Hobert et al. reported high succinate levels in the plasma of patients with SDHB- (1/1) and SDHD-(2/5) associated PPGL (Hobert et al. 2012). Assessment of SFR in plasma samples of patients with PPGL was then reported by Lendvei et al. but again not fully conclusive (Lendvei et al. 2014). Therefore, there was a need for a specific tumor biomarker allowing the characterization of inoperable tumors and suspicious lesions.

Succinate shows a characteristic peak at 2.44 ppm in spectral analysis that can be detected noninvasively by in vivo proton magnetic resonance spectroscopy (1H-MRS). 1H-MRS is a MRI sequence specific for the observation of tissue compounds after elimination of water protons signal. 1H-MRS has been initially developed in the field of cerebral tumors, especially gliomas, that have recurrent somatic mutations in one of the isocitrate dehydrogenase genes (IDH1 and IDH2) leading to the overproduction of 2-hydroxyglutarate (2-HG), detectable in vivo by 1H-MRS (Andronesi et al. 2012, Choi et al. 2012). Detection of succinate accumulation in vivo as a biomarker for the presence of an SDHx mutation is also feasible and has been developed in recent years (Varoquaux et al. 2015, Lussey-Lepoutre et al. 2016, 2020, Casey et al. 2018).

We previously developed a pulse 1H-MRS sequence to measure succinate in an allografted mouse model of Sdhb-deficient tumors and in a pilot study performed in nine patients with PPGL (five with SDHx mutations and four sporadic cases). We demonstrated the feasibility of detecting succinate in vivo by 1H-MRS as a very specific biomarker of SDHx mutations (Lussey-Lepoutre et al. 2016). The diagnostic performance of 1H-MRS was subsequently validated on a prospective cohort of patients carrying 50 PPGL (Lussey-Lepoutre et al. 2020), validating the good sensitivity (87%) and the perfect specificity (100%) of this technique, in particular for PGL of the head and neck. The limits of 1H-MRS are mainly the size of the tumor (which determines the size of the voxel) and the presence of necrosis in the tumor. On the other hand, sensitivity and specificity remain identical whatever the subtype of mutation (SDHA, B, C or D). 1H-MRS can also be applied to other tumors with SDHx deficiency such as GIST, as well as metastases of PPGL (Casey et al. 2018). Moreover, animal experiments demonstrated that the area under the succinate peak of the 1H-MRS spectra in vivo was correlated with the concentrations of succinate measured in the resected tumors by GC-MS in vitro, allowing quantifying succinate levels in vivo (Lussey-Lepoutre et al. 2016). Future studies are needed to show whether this correlation holds also in patients. If it turned out to be the case, it could provide a quantifiable surrogate marker of early response to treatment in SDHx-related PPGL patients.

### Molecular biomarkers: when epigenetics enters the game

Epigenetic reprogramming being a major consequence of SDH deficiency, it rapidly emerged as a hallmark of choice to be used to develop specific biomarkers, either of SDHx mutations or of metastatic disease. Specific antibodies have been developed to detect the different states of DNA cytosines (5mC or 5hmC) or the methylation marks of histones (H3K9me2, H3K9me3, H3K27me2, H3K27me3, H3K4me3). These antibodies have been used on paraffin embedded PPGL (Letouze et al. 2013, Hoekstra et al. 2015) or GIST (Killian et al. 2013) and correlated with the genetic status of the tumors. In PPGL tumors, we first evaluated the levels of 5mC, 5hmC, H3K9me3 and H3K27me3 in 40 tumor samples including 16 SDHx-mutated. We showed that 5mC staining was not different between the different tumors, while histone methylation was indeed increased in SDH-related tumors. However, such immunostainings were neither specific nor sensitive enough to be used as biomarkers. In contrast, low 5hmC levels in the nuclei of tumor cells were detected in all SDHx-mutated and in only 6/24 non-SDH samples. Following these initial observations, Hoekstra et al. evaluated these markers in a large collection of 134 PPGL (including 75 SDHx-mutated) and also concluded that 5hmC was significantly lower or even absent in the case of an SDHx gene mutation (Hoekstra et al. 2015). Interestingly, it is worth noting that low 5hmC is detected not only in SDH-deficient tumors but also in PPGL carrying other types of mutations affecting mitochondrial proteins: FH (Castro-Vega et al. 2014, Hoekstra et al. 2015), SLC25A11 (Buffet et al. 2018), and DLST (Buffet et al. personal communication).

Global DNA methylation was also evaluated in large collections of tumors to identify hypermethylated CpG that may be exploitable as predictors of genetic status or of the metastatic potential of a primary PPGL (Letouze et al. 2013, de Cubas et al. 2015). Hypermethylation of PMNT was reported in SDHx-related PPGLs while RET- and NF1-mutated PPGL showed hypomethylation and VHL-related one, hemimethylation. These observations are of particular interest because of the role of PMNT in catecholamine biosynthesis and explain the neurochemical phenotypes of these different subsets of tumors, with SDH and VHL-deficient PPGL showing a noradrenergic secretion (Eisenhofer et al. 2017). Hypermethylation of RDBP
Therapeutics perspectives

Around 10–15% of PPGLs will become metastatic and have a poor prognosis with a mortality rate of 37% at 5 years (Hamidi et al. 2017). Unfortunately, there are limited options for these patients, based on metabolic radiotherapy or chemotherapy, with imperfect efficacy (Baudin et al. 2014). Knowledge on PPGL biology has recently been turned upside down by genomics and metabolomics data, making it possible to envisage the use of targeted therapies and precision medicine (Favier et al. 2015) (Table 2). This review being focused on SDH-mutated PPGL based on new discoveries, especially those depending on epigenetic and metabolic reprogramming, we will not discuss several potentially promising therapies for PPGL such as radionuclide therapy, mTOR inhibitors, somatostatin analog or immunotherapy.

Targeting angiogenesis

SDH-mutated tumors being highly vascularized, the use of antiangiogenic therapies that block the VEGF pathway are an important hope for metastatic patients and in particular for the very aggressive forms linked to SDHB. In addition to their antiangiogenic properties, tyrosine kinase inhibitor (TKI) can also inhibit other receptors such as fibroblast growth factors receptor (FGFR) or c-met receptors, which may prevent tumor invasiveness and metastases. A recent and complete overview of antiangiogenic therapies can be found in Jimenez et al. (2020). Several retrospective studies, mostly with sunitinib (TKI targeting VEGF, PDGFR, RET and c-kit), seem promising and this drug is currently being evaluated in prospective trials. The phase II clinical SNIPP study evaluated sunitinib with 50 mg in 23 patients and found a disease control rate of 83% (95% CI: 56–93%), a progression-free survival (PFS) of 13.4 months (95% CI: 5.3–24.6 months) but with a very low objective response rate (O’Kane et al. 2019). The results of the first randomized trial of 78 metastatic PPGL patients with sunitinib 37.5 mg should be available soon (FIRSTMAPPP, NCT01371201). Others TKI have been or are currently evaluated such as axitinib (VEGFR), pazopanib (VEGFR, PDGFR, FGFR and the RET receptor) and lenvatinib (FGFR) but suffer from high toxicity, notably hypertension (Jimenez et al. 2020). The more promising one, cabozantinib (VEGFR and c-Met receptor), is currently evaluated in a phase II trial with encouraging preliminary results and unexpectedly, no severe hypertension or cardiovascular events (NCT02302833) (Jimenez et al. 2020). However, knowledge acquired in other types of cancers show that although TKI allows stabilizing tumor growth, anti-angiogenic agents rarely achieve complete responses, and it is common that after

Table 2  Therapeutic approaches for the treatment of SDH-mutated metastatic PPGL.
6–12 months of treatment, patients develop resistance. It requires the development of other alternative therapeutic strategies or the combination of antiangiogenic therapies with new treatments.

**Targeting pseudohypoxia**

Over the past decade, numerous data have suggested and then confirmed the oncogenic role of HIF2α in metastatic PPGLs (Toledo et al. 2013, Morin et al. 2020). HIF2α, although a crucial target, was long considered to be pharmacologically inaccessible. Based on the structure of the HIF2α-ARNT/HIF1β dimer, two antagonists of HIF2α (PT2385 and PT2977 (MK-6482)) were generated. These small molecules allosterically block the binding of HIF2α to ARNT/HIFβ, thereby inhibiting their transcriptional activity (Scheuermann et al. 2015).

Recent reports have shown very promising results of these two molecules in ccRCC, with a clear decrease in tumor growth in vitro and in vivo and a reduction in the expression of HIF2α target genes (Chen et al. 2016, Cho et al. 2016, Xu et al. 2019). ccRCC is another example where tumorigenesis is mediated via a process of ‘pseudohypoxia’ through VHL mutations (Nickerson et al. 2008). These compounds were also found to be active in tumors progressing under sunitinib. A phase I study conducted with PT2385 in 51 patients with locally advanced or metastatic ccRCC previously treated with a TKI suggested its efficacy with a very good safety profile (Courtney et al. 2018). Several phase II studies are underway in ccRCC, alone (NCT03108066 or NCT03401788 only for VHL disease) or in combination with cabozantinib (NCT03634540) or everolimus (NCT04195750).

Given the very promising results of HIF2α antagonists in ccRCC and the tumor mechanisms of PPGLs known to date, anti-HIF2α appears to be good candidate for the treatment of metastatic PPGLs, in particular those mutated on the SDHB gene (Toledo 2017). In a currently recruiting basket trial of patients with advanced solid tumors treated with PT297, one patient with an SDHB mutant paraganglioma had stable disease >25 weeks with sustained decrease in normetanephrine levels (NCT02974738) (Papadopoulos et al. 2018). These data must however be counterbalanced by a recent in vitro study on a model of neuroblastosoma that also shows overexpression of HIF2α, which found no effects on HIF2α target genes expression and no major impact on cell survival in vitro or tumor growth in vivo upon treatment with PT2385 (Persson et al. 2020). In any case, the data are currently insufficient and other preclinical and clinical studies are necessary to evaluate this treatment, or the combination of the HIF2α inhibitors with another, in metastatic PPGLs.

**Targeting epigenetic alteration**

Cyclophosphamide-dacarbazine-vincristine (CVD) regimen is recommended as the standard chemotherapy for metastatic PPGL, with a partial response concerning tumor volume that can be achieved in about 37% in a meta-analysis (Niemiejer et al. 2014). However, temozolomide, the oral form of dacarbazine, seems to have higher response rates in patients with SDHB mutations, with a longer PFS compared with patients without SDHB mutation (19.7 vs 2.9 months). The efficiency of temozolomide, a DNA alkylating agent, is in fact dependent of the O(6)-methylguanine-DNA-methyltransferase (MGMT) enzyme which repairs the DNA adducts induced by this treatment. In SDHB-mutated tumors, the silencing of MGMT promoter region by hypermethylation explains why SDHB patients have better responses (Letouze et al. 2013, Hadoux et al. 2014).

The description of the hypermethylated phenotype of SDH-deficient tumors revealed the possibility of innovative epigenetic therapies involving DNA or histone demethylating agents. Decitabine, which acts as a hypomethylating agent through the inhibition of DNA-methyltransferase (DNMT), has been tested in Sdhb−/− mouse chromaffin cells and led to the reduction of their migratory capacities (Letouze et al. 2013). Histone Deacetylase (HDAC) inhibitors were found to enhance the amount of norepinephrine transporter and 123I-MIBG uptake in a mouse model of metastatic pheochromocytoma, leading to a possible combination of MIBG therapy and HDAC inhibitors (Martiniova et al. 2011). Preclinical studies with any of these treatments are still missing. However, a second-generation of DNA methylation inhibitor (guadecitabine, SGI-110), tested in acute myeloid leukemia (AML) and myelodysplastic syndrome, has been very recently evaluated by the NIH in a phase II study involving nine patients (seven with SDH-deficient GIST, one with SDH-deficient paraganglioma and one FH-mutated RCC (NCT03165721)). Results presented at the 2020 ASCO meeting unfortunately showed no complete or partial responses (Wedekind et al. 2020).

Another temptative option would be to restore α-KG-dependent enzymes activity, by inducing an excess of α-KG. In a mouse model of breast cancer-associated lung metastasis, an α-KG-dependent dehydrogenase inhibitor (AA6) increased the intracellular level of α-KG, thereby increasing TETs activity and reducing metastasis formation...
(Atlante et al. 2018). This promising treatment has never been tested in PPGLs so far.

**Targeting metabolic reprogramming**

Reprogrammed metabolic activities of PPGLs can also be exploited to treat them. Recently, IACS-010759, a clinical-grade small molecule inhibitor of oxidative phosphorylation (OXPHOS) through inhibition of mitochondrial complex I, has been tested with promising results in mouse models of AML and brain cancer (Molina et al. 2018). It inhibits proliferation and induces apoptosis through a combination of energy depletion and reduced aspartate production, leading to impaired nucleotide biosynthesis. IACS-010759 is currently evaluated in a phase I trial in solid tumor and relapsed/refractory AML and early results indicate that this treatment appears safe (NCT02882321 and NCT03291938). As explained before, upregulation of complex I and mitochondrial aspartate synthesis by pyruvate carboxylation are essential for Sdhb−/− chromaffin cells survival and proliferation, making IACS-010759 as potential drug for PPGL (Lussey-Lepoutre et al. 2015). Other OXPHOS inhibitors are under development, notably in glioblastoma (Shi et al. 2019).

Interestingly, metformin is another inhibitor of OXPHOS through complex I inhibition. Two *in vitro* studies performed on the cell lines derived from PPGL, mostly rat derived cells (PC12 cells), suggested an anti-proliferative potential of metformin (Li et al. 2017, Thakur et al. 2019). These effects have to be confirmed in animal models and human studies. In a model of head and neck PGL cells with SDH loss, dichloroacetate (DCA), a structural analog of pyruvate, reduced cell viability alone or in combination with metformin through mechanisms involving pyruvate dehydrogenase kinase inhibition. It results in reactivation of pyruvate dehydrogenase promoting oxidative metabolism, decreasing extracellular lactate and increasing intracellular ROS levels (Florio et al. 2018). These treatments promote both cell cycle arrest and apoptosis in these cells and have to be explored further in animal models.

Finally, alterations of complex I activity in Sdhb−/− cells induce an upregulation of NADH dehydrogenase activity which alters NAD+/NADH balance resulting in increased NAD+ availability and enhanced Poly(ADP)-ribose polymerase (PARP) DNA repair (Pang et al. 2018). Similar to MGMT, PARP is a highly conserved enzyme which repairs single strand DNA breaks and stabilizes DNA replication. In parallel, succinate has been found to suppress the homologous recombination DNA-repair pathway (that repairs double strand DNA breaks) through inhibition of the lysine demethylase KDM4B thus rendering renal cancer cells and tumor xenograft with SDHB-deficiency, all the more vulnerable to PARP inhibitors (Sulkowski et al. 2018, 2020). In mice with Sdhb−/− PPGL allografts, PARP inhibitors potentiated DNA damaging effects of temozolomide (but showed no effect alone) and reduced metastatic lesions, with prolonged overall survival of mice (Pang et al. 2018).

Thereby, a new phase II testing the addition of olaparib to temozolomide will start soon (NCT04394858).

**Targeting redox imbalance**

Sdhb−/− cells exhibit dysregulation of oxygen metabolic pathways, leading to increased formation of ROS concomitantly with iron overload, suggesting that targeting the redox imbalance pathway could be a potential therapeutic approach. Very recently, we and others have demonstrated that pharmacologic ascorbate induces ROS overload in Sdhb−/− cells leading to impaired cell survival. Moreover, ascorbate delays Sdhb-KD allograft *in vivo* (Liu et al. 2020). In addition to redox imbalance, ascorbate targets other vulnerabilities of SDHB tumors, that is the epigenetic reprogramming through TET activity and the oxygen-sensing regulation by inhibiting HIF activity (Ngo et al. 2019). Actually, several ongoing clinical trials are exploring the safety and efficacy of high-dose parenteral ascorbate in combination with chemotherapy or radiation, in various types of cancers such as glioblastoma, pancreatic, colorectal or non-small-cell lung cancers (Ngo et al. 2019). In all cases, high-dose i.v. ascorbate (generally >1g/kg) exhibits an excellent safety profile. Consequently, parenteral high-dose vitamin C, despite its controversial history, could to be a promising candidate for future treatment of SDH-related metastatic PPGL, probably in combination with others.

**Conclusion**

It has now been 20 years since the report by Baysal *et al.* of the first SDHD gene mutation in a family with hereditary paraganglioma (Baysal *et al.* 2000), immediately followed by the identification of SDHC (Niemann & Muller 2000) and SDHB (Aastuti *et al.* 2001) gene mutations and later by the demonstration of the similar tumor suppressor roles of SDHAF2 (Bayley *et al.* 2010) and SDHA (Burnichon *et al.* 2010). Twenty years of clinical and basic research...
on SDH-related tumors have highlighted to complexity of the processes that link mitochondrial dysfunction and tumorigenesis. They have revealed a central role of oncometabolites in the biology of these tumors and improved our understanding of oxygen sensing and epigenetic reprogramming in cancer development. They have changed the management of patients with these rare tumors and the counseling and follow-up of their family members, thereby improving the prognosis of affected subjects (Buffet et al. 2019). They revealed many innovative therapeutic strategies that are or will soon be tested in clinical trials or in isolated individual and that will hopefully provide an increasing hope for the treatment of metastatic patients.

### Declaration of interest

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

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