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Review

SDH mutations in cancer

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ABSTRACT

The *SDHA, SDHB, SDHC, SDHD* genes encode the four subunits of succinate dehydrogenase (SDH; mitochondrial complex II), a mitochondrial enzyme involved in two essential energy-producing metabolic processes of the cell, the Krebs cycle and the electron transport chain. Germline loss-of-function mutations in any of the *SDH* genes or assembly factor (*SDHAF2*) cause hereditary paraganglioma/phaeochromocytoma syndrome (HPGL/PCC) through a mechanism which is largely unknown. Owing to the central function of SDH in cellular energy metabolism it is important to understand its role in tumor suppression. Here is reported an overview of genetics, clinical and molecular progress recently performed in understanding the basis of HPGL/PCC tumorigenesis.

1. Introduction

The dysregulation of metabolism in malignant cells has been established for over 80 years. It has been known as the "Warburg effect", from the scientist who first observed an increase of glycolysis in cancer cells, even in the presence of oxygen, without an accompanying increase in respiratory chain production of energy [1,2]. So far the underlying reasons for aerobic glycolysis are unknown, but may be related to the different behavior of malignant cells, so that survival, growth and division are favored over functions. The switch from respiration to glycolysis has usually been considered a consequence rather than a cause of cancer. However, the discovery in the last ten years that inherited alterations in mitochondrial enzymes cause hereditary tumors has changed this viewpoint. These alterations comprise germline mutations in the genes encoding succinate dehydrogenase (SDH) enzyme subunits [3–6], succinate dehydrogenase complex assembly factors 2 (SDHAF2) [7] and fumarate hydratase (FH) [8]. Moreover, recently, somatic mutations in *IDH1* and *IDH2* genes, encoding isocitrate dehydrogenases 1 and 2 respectively, have been identified in a high proportion of glioblastomas [9,10].

The succinate dehydrogenase enzyme (also known as succinate-ubiquinone oxydoreductase) is a highly conserved heterotetrameric protein, with SDHA and SDHB as catalytic subunits, which protude into the mitochondrial matrix and are anchored to the inner membrane by SDHC and SDHD. These latter subunits provide also the binding site for the ubiquinone (Fig. 1). All the subunits are encoded by nuclear genes and then imported into the mitochondria where they are modified, folded and assembled. Unlike most of the Krebs cycle enzymes, SDH has no cytosolic counterpart. This enzyme comprises mitochondrial complex II, which is involved in the Krebs cycle and in electron transport chain (ETC) [11]. Complex II couples the oxidation of succinate to fumarate in the Krebs cycle with the electron transfer to the terminal acceptor ubiquinone in the ETC.

Germline mutations in *SDHD*, *-B* and *-C*, were observed in patients with hereditary paragangliomas and phaeochromocytomas [3–5] and (rare) somatic mutations were detected in the corresponding non-syndromic lesions [12–14]. Recently, mutations in genes encoding the subunit A of SDH (SDHA) and the SDH assembly factor 2 (SDHAF2), were found to be associated with hereditary paraganglioma and phaechromocytoma syndrome (HPGL/PCC) [6,7]. The genetic lesions in the *SDH* genes predisposing to the HPGL/PCC syndrome are germline heterozygous mutations, which cause inactivation of the protein function. The neoplastic transformation occurs when there is the loss of the remaining wild type allele in the somatic cells, i.e. loss of heterozygosity, leading to the complete loss of the enzyme function. Thus, *SDH* act as a classical tumor suppressor genes [12,15,16]. This article will review the latest current research in this field.

2. Hereditary paraganglioma-phaeochromocytoma syndrome (HPGL/PCC)

Paragangliomas (PGLs) are rare tumors, deriving from paraganglia, neuroendrocrine tissues symmetrically distributed along the para-vertebral axis from the base of the skull and the neck to the pelvis. The two major paraganglionic organs in the adult are represented by the carotid body and the adrenal medulla. The carotid body detects oxygen and carbon dioxide partial pressure changes in the arterial blood, but is also sensitive to pH and temperature. The adrenal

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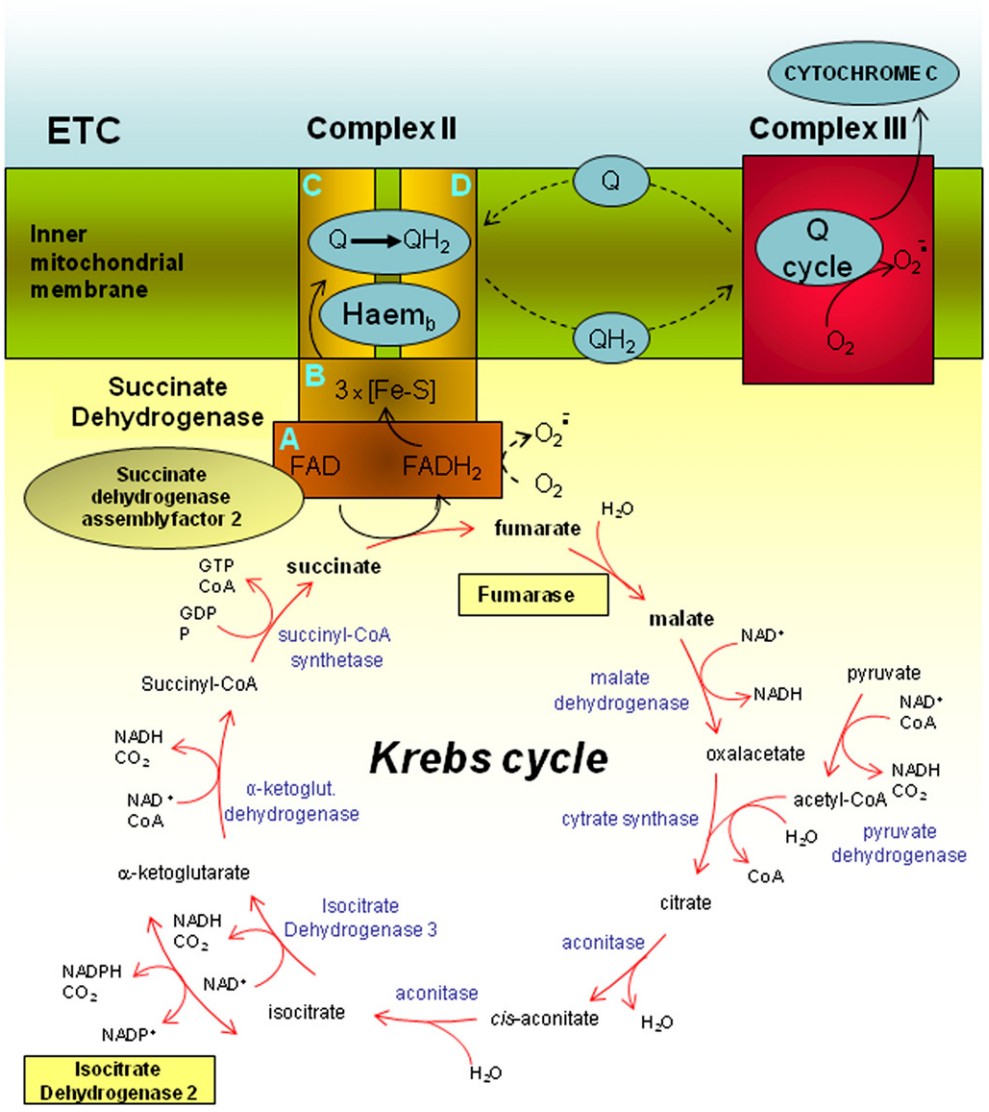

Fig. 1. Succinate dehydrogenase complex (SDH) in the electron transport chain and Krebs cycle. SDH is the only membrane-bound enzyme of the Krebs cycle and is also a functional member (complex II) of the electron transport chain (ETC). SDH is a complex of four different polypeptides (SDHA, SDHB, SDHC and SDHD) together with several prosthetic groups that include FAD, non haem iron ubiquinone and haem$_b$.

medulla secretes catecholamines in response to stress stimulation by preganglionic neurons.

Paragangliomas associated with the parasympathetic nervous system arise mainly in the head and neck region (HNPGLs), particularly in the carotid bodies (carotid body tumor), along the vagus nerve, in the jugular foramen and in the middle ear space, and generally do not hypersecrete catecholamines or other hormones. Paragangliomas associated with the sympathetic nervous system arise in the adrenal or extra-adrenal locations. Those arising from chromaffin cells of the adrenal medulla are defined as phaeochromocytomas (PCCs) [17], while paragangliomas deriving from extra-adrenal sympathetic tissue confined to the abdomen, thorax and pelvis are referred as extra-adrenal paragangliomas (extra-adrenal PGLs). Phaeochromocytomas (also known as adrenal chromaffin tumors) and extra-adrenal paragangliomas typically hypersecrete catecholamines such as epinephrine (adrenaline), noraepinephrine (noradrenaline) or dopamine.

HNPGLs are slow growing tumors, generally benign with an incidence of 1:30,000–100,000 in the general population [18]. Despite their benign nature, HNPGLs can cause untoward consequences due to compression of vital organs by the tumor mass. PCCs and extra-adrenal PGLs give rise to symptoms associated with catecholamine hypersecretion, such as uncontrolled hypertension. Since no accepted pathological or immunohistochemical marker distinguishes malignant from benign paraganglial tumors, malignant PGLs are defined by the presence of metastatic lesions at sites where neuroendocrine tissue is normally absent, e.g. lymph nodes, bone, lung, liver [19]. Thus, patients who have initially been assumed to have non malignant PGL, may only later present with unequivocal malignant disease. Extra-adrenal PGLs have a high risk of malignant progression. Malignancy is much less likely in adrenal PCCs and HNPGLs, but occurs. Thus depending on the localization, malignancy has been reported in 2% to 19% [20,21].

Patients with HPGL/PCC syndrome can present with head and neck paragangliomas only, adrenal and/or extra-adrenal tumors only, or a combination of the two types of tumors [22,23].

Paragangliomas occur either sporadically or as a part of hereditary syndrome. Patients with inherited predisposition often develop multiple, bilateral and early onset paragangliomas, as a result of germline mutations in the predisposing genes.

Hereditary susceptibility to familial form of head and neck PGLs was first recognized in 1933 by Chase [24]. However, it was about a decade ago, when the first predisposing gene to HPGL/PCC syndrome

was identified [3]. Besides the well-known familial cancer syndromes, associated with a susceptibility to develop adrenal phaeochromocytomas, such as multiple endocrine neoplasia type 2 (MEN2), von Hippel Lindau disease and neurofibromatosis type I, caused respectively by germline mutations in *RET*, *VHL* and *NF1* genes, the study of inherited predisposition to head and neck paragangliomas led to the discovery of the novel paraganglioma–phaechromocytoma syndrome. This syndrome was found to be caused by germline mutations in *SDHD*, *SDHB* and *SDHC* genes. Initially linkage analysis identified on chromosomes 11 and 1 three PGL susceptibility loci, which were labeled respectively 'paraganglioma locus 1' (PGL1) on 11q23 [25–28], PGL2 on 11q13.1 [29,30], PGL3 on 1q21 [31,32]. Subsequently, gene mapping studies led to the discovery of *SDHD* as the gene responsible for PGL1 in familial HNPGLs [3]. Following studies then revealed that mutations in the *SDHC* (PGL3) and *SDHB* (PGL4, 1p36.1–p35) genes can also cause familial PGLs and PCCs [4,5]. Recently, the susceptibility gene for PGL2 was identified as *SDHAF2* gene (succinate dehydrogenase complex assembly factor 2) [7]. In addition, a germline mutation in the *SDHA* gene was identified in a patient suffering of catecholamine-secreting abdominal PGL, suggesting that *SDHA* is an additional paraganglioma/phaeochromocytoma susceptibility gene [6].

An updated database of all reported *SDH* allelic variants, which cause familial PGL syndromes is available online: http://chromium.liacs.nl/lovd_sdh/ [33]. This database is based on the Leiden Variation Database (LOVD) system.

Susceptibility to HPGL/PCC is transmitted in an autosomal dominant manner with age-dependent and incomplete penetrance. Although the incidence of PGLs in the general population is low, it is noteworthy that 38% to 60% of individuals with underlying *SDHD*, *-B*, *-C* mutations will develop PGLs by 35 years old [34]. *SDHD* mutations conferred 50% penetrance by 40 years of age and 80% by 60 years of age. *SDHB* mutation carriers were shown to have a penetrance of 40% for paraganglial tumors by 40 years age, which increased to 70% by the age of 60 [35]. The prevalence of underlying *SDH* mutations is 10% to 30% among patients with apparently sporadic PGLs and 10% to 70% among patients with familial PGLs [22,23,34,36–45]. In addition, it has been reported that 8–12.5% of non-syndromic, non-familial extra-adrenal PGLs and PCCs carry occult germline mutations in *SDHB* or *SDHD* genes [42]. Interestingly PGLs, which develop in susceptible families, are more aggressive and with an increased risk of metastasis and mortality [23,34,43,44].

2.1. SDHD (PGL1)

The hereditary syndrome PGL1 (OMIM ID: 168000) is caused by mutations in the *SDHD* gene (RefSeq: NM_003002.2; 11q23.1; *SDHD* Leiden Open Variation Database http://chromium.liacs.nl/LOVD2/SDH/home.php?select_db=SDHD). This gene encodes the integral membrane-anchoring protein cybS, which together with cybL encoded by *SDHC*, comprise respectively the small and the large subunits of the heme-protein cytochrome b in the mitochondrial complex II.

SDHD mutations are typically associated with multifocal HNPGLs and less frequently with adrenal PCCs and extra-adrenal PGLs, which are usually benign [23,34,46]. Rare cases of metastatic HNPGLs have been described within *SDHD* mutation carriers and their estimated prevalence is 0–10% [23,34,35,46–52].

Recurrent mutations with a founder effect have been identified in Dutch (p.Asp92Tyr, p.Leu95Pro, p.Leu139Pro) [36,53], American (p.Pro81Leu) [22], Italian (p.Gln109X) [54], Chinese (p.Met1Ile) [36,55] and Spanish (p.Trp43X) [56] families. Furthermore a large *SDHD* founder deletion (4944-base pair) between Alu elements was recently indentified in two Austrian families with hereditary head and neck PGLs [57].

Although PCCs and extra-adrenal PGLs are relatively rare in patients with *SDHD* germline mutations, recently Ricketts and co-authors described that *SDHD* mutations predicted to result in an absent or unstable SDHD protein were associated with an increased risk of PCCs and extra-adrenal PGLs, compared to missense mutations or in-frame deletions, which were not predicted to impair protein stability [35].

SDHD-related disease has been characterized by a parent-of-origin effect, as it is transmitted only when the mutated allele is inherited from the father [22,23,46]. This pattern of inheritance suggested maternal genomic imprinting of this gene. In support of this idea Badenhop and colleagues showed expression of only the mutant paternal *SDHD* allele in tumor samples [58]. However, subsequent studies did not support this hypothesis, but demonstrated bi-allelic expression of *SDHD* in different normal tissues and in neural crest-derived tumors, with no promoter hypermethylation in normal adrenal tissues or phaeochromocytomas [3,49,59–61]. Moreover, further studies demonstrated that *SDHD* does not belong to an imprinted DNA region [62]. Since allelic loss in *SDHD*-associated paragangliomas always involves the entire wild type maternal allele [3,63], it was proposed that the observed pattern of inheritance resulted from the effects of a gene cluster on the same chromosome as *SDHD*, but at a distant site (11p15). Thus a growth advantage could be gained when the wild type maternal *SDHD* allele on 11q23 and a maternally active, but paternally imprinted tumor suppressor gene (TSG) mapped on 11p15, is lost simultaneously [63]. Interestingly, loss of 11p was shown in 33–50% of HNPGLs, in 27% of abdominal PGLs, in 17–48% of sporadic PCCs and in 40% and 86% of PCCs from MEN2 and von Hippel-Lindau (VHL) patients, respectively [18,64–67]. Further evidence supporting the idea that a locus located on the same chromosome as *SDHD* could be involved in the parent-of-origin effect of this gene, came from the study of Pigny and colleagues. In this work the authors described for the first time the occurrence of PGL in a case of maternal transmission of a *SDHD*-mutated allele. In this report genetic analysis of a patient suffering of a jugulo-tympanic PGL, showed a gain of imprinting in the region upstream of the maternally expressed *H19* gene. Interestingly, this gene was known to be paternally imprinted. The patient, who inherited the mutated allele from his mother, carried hypermethylation of two CpGs within the differentially methylated region 1 (DMR1) upstream of *H19* [68]. To date this is the only reported case of maternal transmission of a *SDHD*-linked PGL. Since the patient has not been operated no material was available for histological and molecular studies of the tumor mass [69], which would add further and important information of this case.

To test the hypothesis that a maternally expressed imprinted locus on chromosome 11, could modulate *SDHD* tumorigenesis, Bayley and co-authors generated a conventional *Sdhd* knockout mouse model, which was crossed with a mouse knockout of a candidate modifier gene *h19*, in order to generate a double knockout. Thus to evaluate the possibility that the loss of these genes together would lead to tumor development, mice were observed for their entire lifespan. However, both *Sdhd* and *Sdhd*/*H19* knockout mice, showed no signs of paraganglioma or phaeochromocytoma development at any age [70].

2.2. SDHAF2 (PGL2)

The *SDHAF2* gene (RefSeq: NM_017841.2; 11q12.2; *SDHAF2* Leiden Open Variation Database http://chromium.liacs.nl/LOVD2/SDH/home.php?select_db=SDHAF2), also known as *SDH5*, was recently identified as the susceptibility gene for the PGL2 syndrome (OMIM ID: 601650). This gene encodes a highly conserved protein, necessary for the incorporation of FAD cofactor in the subunit A of the succinate dehydrogenase complex. Correct flavination of the SDHA subunit is essential for the SDH enzyme activity [7].

To date *SDHAF2* mutations have been associated to benign, often multifocal head and neck paragangliomas, with young age of onset [7,71,72].

Functional studies in the yeast demonstrated that loss of SDHAF2 resulted in decreased stability and impaired functionality of the entire SDH enzyme complex [7]. A germline loss-of-function mutation in a conserved region of *SDHAF2* (c.232 G>A in exon 2, p.Gly78Arg) was identified in a Dutch PGL2 family, affected by head and neck paragangliomas. This mutation resulted in a decrease in flavination of SDHA subunits in the tumors of the affected patients. Expression of the mutant SDHAF2 *in vitro* demonstrated that the p.Gly78Arg mutation destabilized SDHAF2 protein and impaired its interaction with SDHA, resulting in a complete loss of SDH enzyme activity. Interestingly individual who inherited the mutation from the mother did not develop the disease, suggesting a *SDHD*-like parent-of-origin specific inheritance pattern for PGL2 syndrome [7].

Subsequently, mutation analysis in Spanish kindred affected by early onset head and neck paragangliomas, who tested negative for both mutations and deletions of succinate dehydrogenase genes, revealed the identification of a second family carrying the p.Gly78Arg mutation in *SDHAF2*. This mutation showed a high penetrant phenotype, inherited via the male line, as described in the Dutch PGL2 family. Haplotype analysis of the Spanish and Dutch patients seemed to exclude a common genetic origin between the two families, suggesting that p.Gly78Arg mutation has no founder role but is a recurrent variant, which affects an important residue for the function of SDHAF2 protein [71].

However, two different studies did not identify any germline or somatic mutations of *SDHAF2* in a large patients' cohorts with apparently sporadic paragangliomas and phaeochromocytomas, which have no mutations in the *SDHD*, *SDHC* or *SDHB* genes [71,73]. Neither were gross germline deletions, which might account for the tumors cases tested negative for *SDHAF2* point mutations, noted in the subset of patients analyzed [71]. The absence of additional p.Gly78Arg mutations carriers and of other mutations at the *SDHAF2* gene in the patients' series examined implies that *SDHAF2* mutations are rare in head and neck paragangliomas. Moreover no extra-adrenal PGLs and PCCs have been linked to mutations in this gene so far, suggesting that *SDHAF2* mutations may not be relevant for the development of these types of tumors.

SDHAF2 mutation analysis should be suggested in young patients with head and neck paraganglioma, who tested negative for mutations in *SDHD*, *SDHC* or *SDHB* genes.

2.3. SDHC (PGL3)

The PGL3 syndrome (OMIM ID: 605373) is caused by mutations in the *SDHC* gene (RefSeq: NM_003001.3; 1q23.3; *SDHC* Leiden Open Variation Database http://chromium.liacs.nl/LOVD2/SDH/home.php?select_db=SDHC). SDHC constitutes the large subunit (cybL) of cytochrome b in the mitochondrial complex II.

SDHC mutations were originally believed to be associated only with HNPGLs, but recently rare cases of adrenal PCCs and extra-adrenal PGLs were reported [34,74–77].

Germline *SDHC* mutations appear to be less frequent than *SDHB* and *SDHD* mutations and a limited number of *SDHC* mutation carriers have been identified worldwide [4,34,39,40,44,45,56,74,75,78–80].

In general, the clinical features of *SDHC*-associated cases are similar to those found in patients with sporadic HNPGLs. Mutation carriers typically present with solitary head and neck tumors with incomplete penetrance and a very low tendency to malignant transformation [39]. Only a single case of malignant catecholamine-producing carotid body tumor has been reported in a patient with IVS5 + 1 G>T *SDHC* mutation [79].

An Alu-mediated genomic deletion of 8.4 kb involving exon 6 has been detected in the *SDHC* gene. The common haplotype found in the family and in an unrelated sporadic case, in which the large Alu-mediated *SDHC* deletion was identified, supported a common ancestral origin for these cases. Moreover, it has been reported that this large deletion caused PLG3 following both maternal and paternal transmission, suggesting that *SDHC* is not characterized by parent-of-origin effect [80].

Since *SDHC*-associated mutations are so rare, molecular genetic testing of *SDHC* is done only after the screening of *SDHD* and *SDHB*.

2.4. SDHB (PGL4)

The PGL4 syndrome (OMIM ID: 115310) is due to mutations in the *SDHB* gene (RefSeq: NM_003000.2; 1p36.13; *SDHB* Leiden Open Variation Database http://chromium.liacs.nl/LOVD2/SDH/home.php?select_db=SDHB), which encodes an iron-sulfur protein that together with SDHA constitutes the catalytic domain of SDH.

SDHB mutations mainly predispose to extra-adrenal PGLs with high malignant potential and to a lesser extent to adrenal PCCs and head and neck PGLs [23,46,81–83].

In contrast to the predominantly benign nature of *SDHC*- and *SDHD*-associated tumors, *SDHB*-related extra-adrenal PGLs can develop into highly aggressive tumors, which are associated with poor prognosis and can occur at very young age [16,34,48,83–85]. In fact, although the mean age of tumor presentation in *SDHB* mutations carriers is ~30 years [23,83], there are cases in which the index cases were diagnosed before 10 years of age [35,46,86,87]. This suggests that tumor screening of asymptomatic *SDHB* carriers should start as early as 10 years of age.

A high frequency of *SDHB* germline mutations, identified in malignant extra-adrenal PGLs, were reported in different cohorts of patients examined: 83% [84] 34.3% [23], 71.4% [48], 30% [82], 37.5% [46], 28% at initial presentation, then 97% of patients developed metastasis 2.7 ± 4.1 years after diagnosis [83], 31% [88], 37.5% [34] 20.8% [77]. In addition to malignant PGLs, *SDHB* mutations have been suggested also to be associated with malignant tumors of the extra-paraganglial system, i.e. renal cell carcinoma [23,35,88–90] and thyroid carcinoma [23,35,91].

Although to date a clear genotype-phenotype correlation for *SDHB* mutations does not exist, Ricketts and co-authors recently detected an association between *SDHB* missense mutations and an increased risk of HNPGL, compared to truncating mutations [35].

In the last few years, an increasing number of reports have shown that gross deletions in the *SDHB* gene might account for a considerable number of both familial and apparently sporadic PGL cases, which were previously tested negative for point mutations [34,45,76,85,87,92–98]. Although the clinical manifestations of cases associated with large deletions in *SDHB* are not well known due to the small number of cases described, it seems that they have similar phenotypes and penetrance to patients with point mutations. The large *SDHB* deletion-associated cases described so far presented with adrenal PCCs or extra-adrenal PGLs, which were frequently malignant and also with HNPGLs. Some cases of either extra-adrenal or head and neck PGLs due to large *SDHB* deletions were also associated to tumors of the extra-paraganglial system [87,94,97]. *SDHB* large deletion testing should be considered in patients with familial PGLs, who lack evidence of point mutations.

Large germline founder deletions in *SDHB* were characterized at the sequence level in multiple unrelated subjects from Netherlands (7905 bp deletion in the exon 3, c.201-4429_287-933del, p.C68HfsX21) [96], and Spain (16 kb deletion involving the exon 1, c.1-10413_73-3866del) [94]. A second example of a *SDHB* gene deletion with a founder effect in the Spanish population was reported recently (c.166_170delCCTCA, p.P56delYfsX5) [56]. In addition, the *SDHB* splice site mutation IVS1 + 1 G>T was found in 4 apparently unrelated Scottish ancestry families, indicating a possible founder effect [46].

In contrast with *SDHD*, no parent-of-origin effect was described in *SDHB* positive families as both paternal and maternal inheritance has been observed [99].

2.5. SDHA

The *SDHA* gene (RefSeq: NM_004168.2; 5p15.33; *SDHA* Leiden Open Variation Database http://chromium.liacs.nl/LOVD2/SDH/home.

php?select_db=SDHA) encodes the major catalytic subunit of the succinate dehydrogenase enzyme. This subunit contains a covalently-attached flavin adenine dinucleotide (FAD) prosthetic group and binds enzyme substrates (succinate and fumarate) and physiological regulators (oxaloacetate and ATP).

Germline mutations in *SDHA*, which result in loss or reduced enzymatic activity, have been shown to cause neurodegenerative diseases such as an early-onset encephalopathy, known as Leigh syndrome [100–103] and a late-onset optic atrophy, ataxia and myopathy [104]. A single case of a pathogenic *SDHA* mutation (c.1664 G>A, p.Gly555Glu) not associated to Leigh syndrome has been described in a patient with a lethal infantile presentation, which leaded to death due to respiratory infection and acute hypoglycemia, before any sign of the syndrome could develop [105]. Recently, the same *SDHA* missense mutation, which was reported to cause a multisystemic failure leading to neonatal death [105] and a relatively mild Leigh syndrome [103], was also described in a familial neonatal isolated cardiomyopathy [106]. Interestingly, although SDHA constitutes the mitochondrial complex II enzyme together with the other SDH subunits, no experimental evidence have linked mutations in the *SDHB, -C, -D* genes to metabolic neurodegenerative disorders or cardiomyopathies. Recently, however homozygous germline mutations in the *SDHAF1* gene (succinate dehydrogenase complex assembly factor 1), were observed in patients with SDH-defective infantile leukoencephalopathy syndrome [107].

Mutations in all the other *SDH* genes and in *SDHAF2* have been associated with paraganglioma-phaeochromocytoma syndrome. It has been proposed that the absence of *SDHA* mutations in tumors related to HPGL/PCC syndrome might be due to the identification of two distinct genetic loci for *SDHA*, which encoded two different isoforms with similar enzymatic activity [108,109]. Thus, in order to obtain inactivation of *SDHA* in the PGL tumors tetrallelic mutations should be required [108,110], making *SDHA* an improbable tumor suppressor gene. However, this theory was not supported by further studies, which demonstrated that SDHA is encoded by a highly polymorphic single gene [111].

Recently, however, Burnichon and co-authors identified a heterozygous germline *SDHA* mutation (c.1765 C>T, p.Arg589Trp), in a woman suffering from catecholamine-secreting abdominal paraganglioma. *In vivo* and *in vitro* functional studies demonstrated that the p.Arg589Trp mutation abolished SDH enzymatic activity in the yeast model and in the patient's tumor tissue. The authors showed that the mutation was associated with somatic loss of heterozygosity at the *SDHA* locus within the tumors, demonstrating that *SDHA*, like the others *SDH* genes, can act as a tumor suppressor gene [6].

This finding suggests that also *SDHA* should be considered as a susceptibility gene for paraganglioma/phaeochromocytoma syndrome. However, SDHA-related tumors are rare, as demonstrated by the relatively low frequency of LOH at the chromosomal region containing the *SDHA* locus (5p15) in the PGL tumors, compared with the 1p36 (*SDHB*) and 11q23 (*SDHD*) loci that often undergo losses in tumor tissues [6].

Thus, *SDHA* genetic screening should perhaps be added to paraganglioma or phaeochromocytoma affected patients, who show loss of SDH enzymatic activity but tested negative for *SDH* genes mutations or if loss of 5p15 chromosome is found in the tumor.

3. *SDH* mutations in other tumor types

In addition to head and neck PGLs, extra-adrenal PGLs and PCCs, a number of other neuroendocrine or non-neuroendocrine neoplasms have been associated with mutations in *SDH* genes. These include gastrointestinal stromal tumors (GISTs), renal tumors, thyroid tumors, testicular seminoma and neuroblastomas.

The best known association between *SDH* germline mutations and other tumors is represented by the Carney–Stratakis syndrome (or dyad). The patients affected by this syndrome develop GISTs and PGLs, which have been associated with germline point mutations or large deletions of the genes encoding the subunits B, C or D of SDH. The tumor suppressor function of *SDH* in the GIST neoplasms, was demonstrated by the allelic losses around the *SDHB* and *SDHC* chromosomal loci in the samples of patients carrying the respectively germline mutations. Interestingly, none of these patients had germline mutations in *cKIT* or *PDGFRA* genes, which were frequently mutated in the sporadic and familial GISTs [76,112].

In contrast with the Carney–Stratakis syndrome, in the Carney triad (CT), which describes the association of paragangliomas with gastrointestinal stromal tumors and pulmonary chondromas, none of the affected patients have been found to possess mutations in the genes encoding the subunits A, B, C or D of SDH or in the *cKIT* or *PDGFRA* genes. Although the tumors related to this syndrome show a similar pattern of genetic changes, the genetic defects associated with this condition are still elusive [113].

Recently, inactivating germline mutations in *SDHB* or *SDHC* were also identified in sporadic GISTs occurring in patients without a personal or family history of paraganglioma. These tumors lacked *cKIT* or *PDGFRA* mutations [114].

Renal tumors, which have been described in association or not with paraganglial tumors, have also been described in patients with germline *SDHB* mutations. Recently, Ricketts and co-authors have reported that the risk of developing renal tumors in *SDHB* mutation carriers is 14% at the age of 70 years [35]. These tumors, which generally occur at young age, present various histological subtypes, including clear cell renal carcinoma [23,35,89,90,115], eosinophilic chromophobe renal cell carcinoma, [90], oncocytoma [116] and malignant type II papillary renal cell carcinoma [88]. Moreover, three different cases of renal angiomiolipoma [93], renal oncocytoma [94] and hybrid renal cell carcinoma chromophobe/oncocytoma tumor have been observed in carriers of large *SDHB* deletions. In the latter cases the causative role of the *SDHB* large deletion was suggested by loss of heterozygosity at the *SDHB* locus within the tumors [97]. Recently a case of renal cancer has been also described in a *SDHD* mutation carrier [35].

In addition both papillary and medullary thyroid carcinoma have also been associated with *SDHB* and *SDHD* mutations [23,35,91]. A unique case of testicular seminoma has been reported in a carrier of germline *SDHD* mutations, which showed loss of the wild type allele in tumor cells [117].

The common neural crest embryonal origin of both phaeochromocytoma and neuroblastoma (NBL) and the frequent loss of the locus 1p35-36 in the latter tumors, a region where *SDHB* gene resides, suggested that genetic alterations in *SDHB* might be implicated in the development or progression neuroblastoma tumors. Previous studies have provided no association between *SDHB* point mutations and sporadic neuroblastomas [118,119]. However, recently, two different cases of patients affected by neuroblastomas have been both associated to germline *SDHB* deletions. The first case reported was of a patient affected by a familial phaeochromocytoma, who was diagnosed with a malignant adrenal neuroblastoma at the age of 5 years. Both phaeochromocytomas and the neuroblastoma from this patient with *SDHB* deletion showed 1p36 loss, suggesting a possible correlation between *SDHB* mutation and neuroblastoma susceptibility [94]. The second case described a composite paraganglioma/neuroblastoma in a 13 years old patient, who had no family history of familial PGL tumors [87]. Recently, a further case of malignant neuroblastoma, was identified in a index case of a PGL susceptible family, carrying a *SDHB* mutation. This patient synchronously developed a malignant neuroblastoma, phaeochromocytoma and renal cell carcinoma [115].

4. Mechanism of tumorigenesis caused by *SDH* mutations

Although a role of mitochondria in tumorigenesis has been suggested by the identification of many somatic mutations in the

mitochondrial DNA of different types of neoplasias, the contribution of these mutations to tumor initiation or progression is unclear. The discovery that germline mutations in nuclear genes encoding SDH subunits lead to development of HPGL/PCC cancer syndrome, represented the first unequivocal link between a genetic mitochondrial defect and tumor development.

To explain how loss-of-function mutations of *SDH* lead to tumor formation, two leading biochemical mechanisms have been proposed. These are represented by the metabolic signaling role of succinate, as an intracellular messenger between mitochondria to cytosol and by redox stress resulting from increased reactive oxygen species (ROS) production in mitochondria (Fig. 2). These mechanisms may be not mutually exclusive.

The first model implies that, due to SDH dysfunction, accumulated succinate leaves the mitochondria via the dicarboxylate carrier and inhibits the activity of enzymes such as HIFα prolyl hydroxylases (PHDs) in the cytosol, leading to the induction of a hypoxic response under normoxic conditions (pseudo-hypoxia) (Fig. 2) [120]. This response is mediated by the oxygen regulated HIF transcription factor, the physiological function of which is to promote adaptation of cells to low oxygen tension (hypoxia) [121]. In normoxic conditions, HIFα is labile due to proteasomal degradation, following the oxygen-dependent ubiquitination by an ubiquitin ligase complex targeted to HIFα by the von Hippel-Lindau (VHL) protein. VHL recognition of HIF requires hydroxylation of two proline residues on HIFα by the PHD enzymes, which use oxygen and α-ketoglutarate as substrate, and iron and ascorbate as co-factors [121]. Thus, in normoxic conditions, HIFα after the modification given by PHD enzymes, can be bound by VHL protein, polyubiquitylated and degradated. However, if PHDs are inhibited by the accumulated succinate, HIFα is not hydroxylated and can escape degradation. HIFα then migrates from the cytosol into the nucleus, where it can heterodimerize with HIFβ to forms an active complex that induces the expression of genes involved in angiogenesis, proliferation, cell survival and glycolysis [121].

In support to this model Gimenez-Roqueplo and co-authors studied the biological effect of *SDHD* and *SDHB* mutations in tumors from PGL families, showing a complete loss of complex II functions in the respiratory chain of these tumor tissues, with an activation of the HIF pathway and the consequent angiogenic response, in agreement with the high vascularization of these types of tumor [15,16]. Pollard

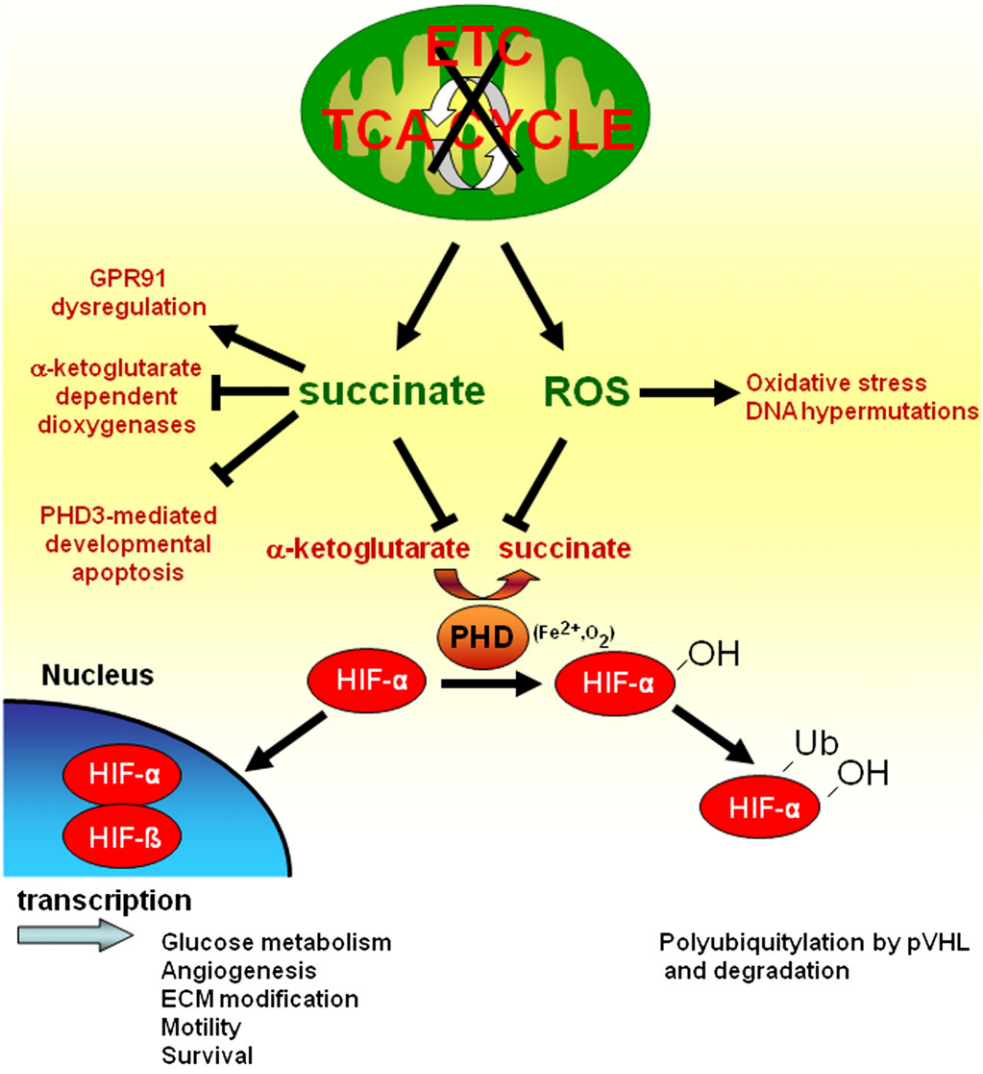

Fig. 2. Mechanisms of tumorigenesis due to SDH inactivation. Different mechanisms have been proposed to explain the link between *SDH* mutations and tumorigenesis. Loss of function of SDH could cause accumulation of succinate and the production of reactive oxygen species (ROS). Both succinate and ROS could independently or in a sinergetic way, lead to the induction of hypoxic response under normoxic conditions (pseudo-hypoxia). In addition to pseudohypoxia, succinate might inhibit other α-ketoglutarate-dependent dioxygenases or PHD3-mediated developmental apoptosis of neuronal cells or it could lead to dysregulation of the G-protein-coupled receptor (GPCRs). ROS accumulation might instead result in oxidative damage to DNA and genomic instability.

and co-authors demonstrated that compared to sporadic tumors, SDH-deficient paragangliomas accumulated succinate and displayed increased expression of HIF1α and VEGF, with high density of microvessels [122]. Interestingly, tumors deficient for the FH enzyme, which catalyzes the subsequent step in the Krebs cycle after SDH, also displayed high vascularity, increased HIFα levels and activity and accumulation of both succinate and fumarate [122,123]. High levels of succinate and HIF1α accumulation and nuclear translocation were demonstrated in SDHA-deficient cells by Briere and co-authors [110]. Moreover, a gene expression micro-arrays analysis of 76 sporadic and hereditary phaeochromocytomas confirmed a hypoxic-angiogenic gene expression profile that was similar in tumors from patients carrying *SDHB*, *SDHD* or *VHL* mutations [124]. A hypoxic transcriptional signature, that was common between SDH and VHL tumors was also described subsequently in a recent study performed by López-Jiménez and colleagues: both HIF1α and HIF2α target genes were found over-expressed in the SDH/VHL cluster, suggesting that a global HIF deregulation describes the common profile these tumors. Despite this common transcriptional profile, a high number of HIF target genes were also found differentially expressed between SDHB and VHL cluster, suggesting that specific HIF target genes could influence the different clinical features between these two types of tumor (extra-adrenal PGLs usually malignant in the SDHB-associated cases and adrenal PCCs typically benign in the VHL-associated cases) [125]. Another study also showed an analogous angiogenic profile in SDH and VHL tumors and although both specimens demonstrated a decrease in electron transport protein expression and activity, the stimulation of glycolysis was only found in VHL tumors [126].

The biochemical explanation for the pseudohypoxic drive induced by loss of function of SDH enzyme was reported by Selak and co-authors [120]. This work showed that succinate caused HIF stabilization, by interfering with the PHD2 (also known as HPH-2 or EglN1) activity. In fact PHD enzymes, catalyzing HIF-α prolyl hydroxylation, couple decarboxylation of α-ketoglutarate to succinate. Therefore the authors demonstrated that SDH downregulation *in vitro* increased levels of succinate, which by feedback inhibition of HIF-α PDH in the cytosol, led to a stabilization of HIF1α transcription factor in normoxic conditions. As a consequence HIF target genes, which lead to angiogenesis, glycolysis and metastasis, were activated [120]. It was subsequently shown that fumarate which accumulates in FH-deficient cells and tumors, was able to inhibit PHD activity, more effectively than succinate, and so to cause HIF accumulation and activation [127].

Besides metabolic signaling given by accumulated succinate and fumarate, other mitochondrial messenger molecules, which have been suggested to participate in tumorigenesis due to loss of function of SDH enzyme, are represented by reactive oxygen species (ROS). Important sites for ROS production in the electron transport chain are complex I (NADH-ubiquinone oxidoreductase) and complex III (ubiquinone-cytochrome c oxidoreductase) [128]. Complex II is not normally considered as a major site of ROS, but increasing experimental evidence has demonstrated that *SDH* mutations lead to oxidative stress, reduced lifespan in model organisms, genomic instability and tumorigenesis.

Structural and functional analysis of bacterial SDH suggested a mechanism for ROS production during the electron transport at the complex II, exactly at the FAD sites of the subunit A of SDH [129]. The first experimental evidence for a feasible ROS production by SDH, came from the study of *mev-1* mutant of *Caenorhabditis elegans*, which carried a homozygous inactivation mutation in the SdhC subunit. This mutation did not affect the SDH's ability to oxidize succinate to fumarate in the Krebs cycle, but compromised its ability to catalyze the electron transport from succinate to the final acceptor ubiquinone, leading to electrons leakage. Consequently this mutant was found to develop oxygen hypersensitivity and a premature aging phenotype [130]. The equivalent *SDHC* gene mutation studied in *C. elegans* mev-1, was then expressed in NIH3T3 mouse fibroblasts. In accordance with the worm model, transgenic *SDHC* mutant cells exhibited elevated oxidative stress, DNA hypermutation, an increased rate of transformation and tumor growth in a mouse xenograft model [131]. A further study, where a nonsense mutation of *SDHC* was expressed in hamster fibroblasts, evidenced increased levels of ROS production, oxidative stress and genomic instability of the mutant cells compared to the parental ones [132]. In addition, functional studies in the yeast model of *sdha* or *sdhb* gene deletion [133] and of *sdhb* [134], *sdhc* and *sdhd* point mutations [135], were associated to an increased production of ROS, showing that the dysfunction of all the SDH subunits in the yeast lead to the formation of reactive oxygen species. However, inhibition either pharmacologically or via RNA interference of SDHB or SDHA subunits in human cells showed that, while SDHB inhibition increases normoxic reactive oxygen species production and HIFα accumulation, complex II inhibition at SDHA does not increase normoxic ROS levels and HIFα [136]. Conversely, other studies reported no signs of ROS production and oxidative stress owing to *SDH* mutations, but HIF1α accumulation and activation, which depend on succinate-mediated PHD inhibition, as demonstrated in cells in which *SDHD* was downmodulated by means of RNA interference [120,137] or in *SDHA*-mutant fibroblasts [110]. In addition, it has been reported that the *SDHB* gene knock-down by RNA interference in human cells did not result in ROS production and the further expression of SDHB missense mutants in *SDHB* silenced cells did not affect ROS levels. However, SDHB inactivation resulted in an up-regulation of HIF1α and HIF2α and in a defective cellular proliferation and respiration with a corresponding shift to glycolysis [138]. Whether the contrasting observations regarding the ROS production consequent to *SDHB, SDHD* and especially to *SDHA* genes inactivation, are due to biological or technical reasons is to be determined.

In addition of mutagenesis, it has been proposed that reactive oxygen species might promote tumor formation in SDH-deficient cells by inducing a pseudo-hypoxic response. It has been reported in fact, that reactive oxygen species can inhibit HIF PHD activity under normoxic conditions, by promoting the oxidation of the PHD cofactors ferrous iron and ascorbate [139]. Conversely, in support of a predominant role of succinate and fumarate in inducing pseudohypoxia through the inhibition of PHD enzymes, it has been demonstrated that succinate- and fumarate-mediated PHD inhibition could be reversed by increasing the intracellular levels of α-ketoglutarate. MacKenzie and co-authors demonstrated in fact, that cell-permeable esters of α-ketoglutarate restored normal PHD activity and thus alleviated pseudohypoxia caused by the accumulation of these metabolites [140]. Briere and co-authors demonstrated that exogenous α-ketoglutarate prevented the nuclear translocation of HIF in *SDHA*-mutant cells [110].

Beyond to pseudohypoxia and ROS models, it has also been proposed that *SDH* mutations cause phaechromocytoma because, during embryogenesis, neuronal precursor cells which carry mutations in *SDH* fail to undergo apoptosis in response to growth factor withdrawal. This developmental apoptosis, which is c-Jun dependent, is mediated by prolyl hydroxylase PHD3 (also known as EglN3 or HPH-1), the activity of which is inhibited, as demonstrated for PHD2, by succinate accumulation [141]. This model was further supported by a recent study of PDH3 knock-out mice, which showed reduced apoptosis of sympathetic neurons [142].

Another appealing possibility is that accumulated succinate in SDH-deficient tumors might inhibit other components of the α-ketoglutarate-dependent dioxygenase family, besides the prolyl hydroxylases PHD2 and PHD3. This family comprises numerous enzymes involved in a wide range of biological roles, such as collagen biosynthesis, hypoxic signaling, fatty acid metabolism, histone and nucleic acid demethylation, hydroxylation of proteins associated with RNA splicing, carnitine metabolism and hydroxylation of 5-methyl-cytosine [143].

Some of the α-ketoglutarate-dependent enzymes might have a role in cell transformation, leading to different biochemical outcome that

link mitochondrial defect to tumorigenesis. Moreover, the succinate-inhibited dioxygenases, might have a tissue specific expression, which might contribute to the specific tumors spectrum of the inherited neoplasia syndromes given by *SDH* mutations.

Recently, the first evidence supporting this hypothesis was reported. It has been demonstrated that loss of Sdhb subunit in a yeast model led to succinate accumulation, which could cause the inhibition of two different α-ketoglutarate-dependent dioxygenases [133]. The first enzyme was Jlp1, involved in sulfur metabolism, while the second one was represented by the histone demethylases Jhd1, which belongs to the JmjC-domain-containing histone demethylases (JHDMs) enzymes class. Moreover, the authors showed that also the mammalian JmjC-domain histone demethylases were susceptible to succinate inhibition. They demonstrated that JMJD2D, a corresponding human JHDMs, as a purified enzyme or when expressed in mammalian cells, was inhibited by succinate. Therefore, it has been suggested that succinate accumulation by inhibiting the histone demethylases, might alter the expression of oncogenes and tumor suppressor genes, such as those responsible for DNA repair, growth inhibition or induction of apoptosis, thus leading to a possible transformed phenotype [133]. Because of their histone demethylase activity, JHDMs enzymes probably have a wide impact on gene expression, though they may regulate particular genes by specific recruitment of interacting proteins, thus defining an epigenetic signature which might be specific for the tumor spectrum of PGL inherited neoplasias.

Recent evidence corroborates the notion of a possible role for succinate in epigenetic dysregulation of chromatin remodeling, through the inhibition of histone demethylase JHDMs enzymes. It has been reported that SDH inactivation, either pharmacological and by RNA interference, led to an increased methylation of histone H3 in mammalian cells, which can be reversed by the over expression of the JMJD3 histone demethylase. This increased histone methylation in SDHB-silenced cells determined a decreased occupancy by H3K27me3 of the core promoter regions in the *IGFBP7* gene, a tumor related soluble factor, whose transcript was previously found up-regulated in a study of microarray analysis in SDHB-silenced cells [138]. Moreover, type I chief cells, which are considered the neoplastic component of paragangliomas, were the major immunoreactive cells type for both H3K27me3 and H3K36me2 in the paraganglial carotid tumors tested [144]. Interestingly, these findings demonstrated that succinate could act not only as a messenger between mitochondria to cytosol, but also as a signal between mitochondria to nucleus, in order to regulate chromatin structure and thus gene expression.

Another possibility to explain the role of the *SDH* mutations in HPGL/PCC tumorigenesis is represented by a non-physiological role of succinate through its cognate receptor GPR91. It is known that the intermediates of the citric acid cycle, which are regulated by respiration, metabolism and renal reabsorption/extrusion, are normally present in mitochondria, and are also found at micromolar concentrations in blood. It was shown in 2004 that the citric acid cycle intermediate succinate is the ligand for the G-protein-coupled receptors (GPCRs) GPR91 (also known as SUCRN1) [145]. Therefore by acting as ligand for GPR91, succinate was found to have unexpected signaling functions beyond its traditional role as a Krebs cycle metabolite. This suggests that accumulated succinate, dysregulating the physiological activity of the G-protein-coupled GP R91 receptor, could lead to sustained signaling pathway, which might play a role in the HPGL/PCC tumorigenesis. Interestingly, recent studies of gene expression by microarray analysis revealed that *GPR91* was significantly up-regulated in *SDHB*-silenced cells. Moreover, GPR91 mRNA was induced also in VHL negative cells and in HepG2 cells after overexpression of HIF2α [138]. All these findings suggest that GPR91 might be a HIF transcriptional target gene. Furthermore, in ischemic retina succinate, acting through GPR91, was found to mediate vessel growth through the release of proangiogenic factors vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1) and Ang-2 by the retinal ganglion neurons, in a HIF1α-independent manner [146]. This suggests that in *SDH*-associated tumors succinate could stimulate by a paracrine signaling, the proliferation of endothelial cells. These pro-angiogenic effects can synergize with the possible vascularization mediated by HIF signaling during pseudohypoxia. Thus, beyond the traditional role in energy production, succinate might exhibit through the activation of its receptor GPR91, additional biological functions, that when altered might promote tumor onset and progression.

5. Conclusions

SDH was the first nuclear-encoded mitochondrial tumor suppressor gene to be identified.

Despite the rapid and important progress achieved since the discovery of the first *SDHD* gene mutation a decade ago, many fundamental questions regarding the role of SDH in tumorigenesis remain to be answered. Mutations in each of the components of complex II have been shown to disturb complex formation and subsequently decrease the enzymatic activity of the remaining complex. Although the mechanism linking SDH deficiency to tumorigenesis remains poorly understood, compelling evidence showed that SDH inactivation leads to pseudohypoxia.

However it remains to be determined if succinate alone, or ROS, or combination of both are required to induce pseudohypoxia in SDH-deficient tumors. In addition, pseudo-hypoxia could not explain the different and restricted patterns of tumor predisposition that develop in HPGL/PCC tumorigenesis compared to HLRCC tumorigenesis, caused by mutations in *FH* gene. Pseudohypoxia alone is probably insufficient to induce HPGL/PCC tumors and it is feasible that the tumorigenic effect of SDH deficiency involves more than one mechanism.

The possibility that succinate could inhibit other α-ketoglutarate-dependent enzymes, is appealing. A recent study has indicated that the human genome encodes more than 60 known or predicted α-ketoglutarate-dependent dioxygenases [143]. Many of these, such as the Jumonji-domain histone demethylases, have credible roles in oncogenesis, and dysregulation might contribute to tumor predisposition [147,148]. Interestingly, a genomewide screening of renal cancer has identified mutation in histones modification enzymes [149]. Moreover, it has been reported that succinate and fumarate have different IC_{50} (half maximal inhibitory concentration) values for α-ketoglutarate-dependent enzymes [150], which might contribute to explain the specific tumors spectrum associated to *SDH* or *FH* mutations. However, it remains still unclear which α-ketoglutarate-dependent enzymes are effectively the relevant targets for succinate inhibition in the etiology of familial PGLs.

Further basic and therapeutic research is needed to answer these questions, which could be crucial for the discovery of new therapeutic targets capable of counteracting *SDH*-associated tumorigenesis.

Conflict of interest statement

The authors declare no conflict of interest.

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