



The Role of Immunohistochemistry and Molecular Analysis of Succinate Dehydrogenase in the Diagnosis of Endocrine and Non-Endocrine Tumors and Related Syndromes

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Abstract

Succinate dehydrogenase (SDH) is an enzyme complex, composed of four protein subunits, that plays a role in both the citric acid cycle and the electron transport chain. The genes for SDHA, SDHB, SDHC, and SDHD are located in the nuclear DNA, and mutations in these genes have initially been described in paragangliomas (PGL) and pheochromocytomas (PCC), which are relatively rare tumors derived from the autonomic nervous system and the adrenal medulla, respectively. Patients with SDH mutations, that are almost exclusively in the germline, are frequently affected by multiple PGL and/or PCC. In addition, other tumors have been associated with SDH mutations as well, including gastrointestinal stromal tumors, SDH-deficient renal cell carcinoma, and pituitary adenomas. Immunohistochemistry for SDHB and SDHA has been shown to be a valuable additional tool in the histopathological analysis of these tumors, and can be considered as a surrogate marker for molecular analysis. In addition, SDHB immunohistochemistry is relevant in the decision-making whether a genetic sequence variant represents a pathogenic mutation or not. In this review, we highlight the current knowledge of the physiologic and pathologic role of the SDH enzyme complex and its involvement in endocrine and non-endocrine tumors, with an emphasis on the applicability of immunohistochemistry.

Keywords Pheochromocytoma · Paraganglioma · Gastrointestinal stromal tumor · Histology · Genetics · Succinate dehydrogenase

SDH Complex: Genes, Proteins, Function

Structure and Assembly

Succinate dehydrogenase (SDH) is a unique protein complex, being part of the citric acid cycle and playing a role in the mitochondrial respiratory chain. SDH activity was first detected in 1909 [1]. SDH consists of two hydrophilic proteins and two hydrophobic proteins. The hydrophilic proteins are SDH subunit A (SDHA), a flavoprotein containing an FAD cofactor

and the binding site for succinate, and SDH subunit B (SDHB), an iron-sulfur protein with three FeS clusters. The two hydrophobic proteins SDH subunits C and D (SDHC and SDHD) are transmembrane proteins that contain a bound heme and a binding site for ubiquinone (Fig. 1) [2]. All SDH subunits are encoded by nuclear genes. This is in contrast to all other oxidative phosphorylation complexes, of which the proteins are encoded by the mitochondrial genome (<https://www.genecards.org>). The gene details of the four SDH genes are listed in Fig. 1.

The correct assembly of the SDH complex relies on so-called assembly factors. Currently, at least four of these have been described. Of these, SDH assembly factor 1 and 2 (SDHAF1 and SDHAF2) are the best studied ones. SDHAF1 is a soluble protein, but is not a stable component of the SDH complex. Data suggests that SDHAF1 is important for the insertion or retention of the Fe-S centers within SDH [3]. Yeast mutants lacking SDHAF1 showed only a 60–70% decrease in SDH activity and not a complete loss of function, rendering the possibility that other (unknown) assembly factors are involved as well [4].

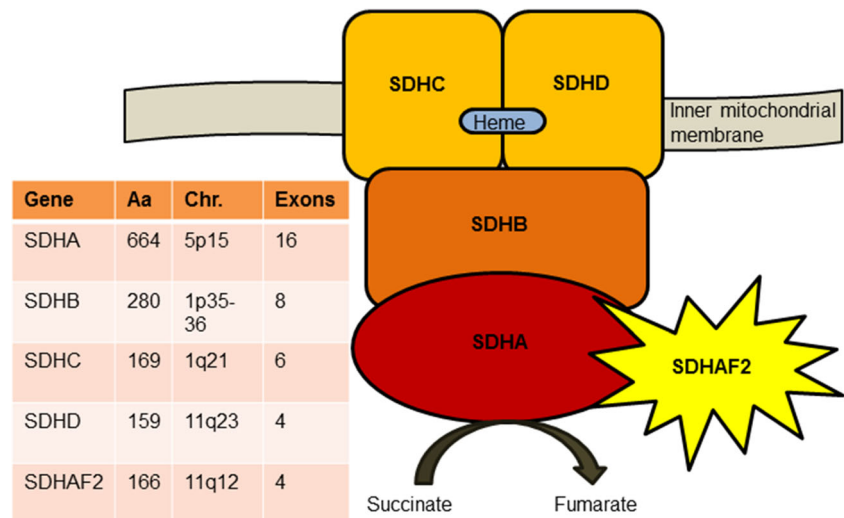
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Fig. 1 Schematic structure of the SDH complex localized in the inner mitochondrial membrane. The table on the left gives an overview of the number of amino acids (Aa), chromosome (Chr.) location, and number of exons of each of the SDH genes



SDHAF2 is also a soluble protein of 166 amino acids, encoded by the *SDHAF2* gene. Yeast mutants for *SDHAF2* showed undetectable SDH activity, while the activity of other citric acid cycle enzymes and electron transport chain complexes was normal. SDHAF2 seems to be required for the covalent insertion of FAD into SDHA [5]. The role of SDHAF2 in relation to pheochromocytoma (PCC) and paraganglioma (PGL) is discussed below, in the paragraph on SDH genes in PCC and PGL.

Very recently, a variant in *SDHAF3* (c.157 T>C [p.Phe53Leu]) has been associated with an increased prevalence of PCC and/or PGL. However, the exact meaning for the genesis of PCC and PGL is still unclear and further research is needed [6].

For SDHAF4, no direct link with tumorigenesis has been described so far. However, in *SDHAF4* knockout cells, a decreased SDH enzymatic activity is seen and it was concluded that SDHAF4 is required for the proper assembly and activity of the SDH complex. In *Drosophila*, a proper functioning of SDHAF4 is critical for preventing motility defects and neurodegeneration [7].

Function

The SDH complex is situated at the crossroads of the mitochondrial respiratory chain and the citric acid cycle (which is also known as the tricarboxylic acid cycle or Krebs cycle). The citric acid cycle which was identified in 1937 by Hans Adolf Krebs is a process that oxidizes glucose derivatives through a series of enzyme-controlled steps [8]. During these steps, high-energy electrons are collected, which are transported by the activated carriers NADH and FADH₂ to the electron transport chain. The first step is not really part of the citric acid cycle, but it links glycolysis to the citric acid cycle. In step 6 of the citric acid cycle, SDH catalyzes the

oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol.

In the mitochondrial respiratory chain, there are four large membrane protein complexes in the inner mitochondrial membrane, including Complex I (NADH: ubiquinone oxidoreductase), complex II (SDH), complex III (cytochrome c reductase), and complex IV (cytochrome c oxidase). The SDH complex is the only of the four complexes that does not function as a proton pump. Instead, SDH contributes additional electrons to ubiquinone that originates from succinate [9]. During these steps, an electrochemical proton gradient is created that drives the synthesis of ATP, of which huge amounts are needed in the eukaryotic cell for proper cell functioning. The functional role of the SDH complex is relevant in case of its disturbance by mutations in any of the composing proteins as will be discussed later.

Detection of SDH Gene Mutations in Human Disease

SDH in Neurodegenerative Disorders

As can be inferred from the previous paragraph, SDH mutations cause mitochondrial dysfunction. Tissues with high energy demand (heart, skeletal muscle, and the central nervous system) are most vulnerable to this [10]. *SDHA* mutations have been described as a rare cause of Leigh syndrome or subacute necrotizing encephalomyelopathy [11]. Leigh syndrome is a lethal, autosomal recessive progressive neurodegenerative disorder of childhood. The syndrome consists of focal, bilateral lesions in the basal ganglia, thalamus, cerebellum, and/or spinal cord [10]. Partial SDHA deficiency (residual activity > 50%) has been described in two sisters and caused late onset optic atrophy, ataxia, and myopathy [12]. In addition, autosomal recessive *SDHA* mutations have been described in a family with neonatal isolated dilated

cardiomyopathy [13]. In 2009, homozygous *SDHAF1* mutations were found in children of a Turkish and an Italian family with SDH-defective infantile leukoencephalopathy [4]. Also, mutations in *SDHB* and *SDHD* have been found to cause Leigh syndrome [14, 15].

SDH Genes in PCC and PGL, SDH Syndrome

It is now known that in about 40% of hereditary PCC/PGL SDH germline mutations can be found. In sporadic cases, the prevalence of SDH germline mutations is up to 15% [16–18]. In 2000, *SDHD* was the first SDH gene demonstrated to be associated with familial PGL type 1 (PGL1) [19]. Subsequently, other SDH genes were found to be PCC/PGL susceptibility genes, and germline mutations of *SDHAF2*, *SDHC*, *SDHB*, and *SDHA* are associated with the familial syndromes PGL2 to PGL5, respectively [5, 20–22]. These syndromes are autosomal dominant, with maternal imprinting effects for *SDHD* and *SDHAF2* [23, 24]. In patients with germline *SDHD* or *SDHAF2* mutations, tumors develop almost exclusively if mutations are inherited via the paternal line. Both genes are located on the long arm of chromosome 11. It has been shown that in *SDHD*-mutated PGL, loss of the entire maternal copy of chromosome 11 occurs. Since neither *SDHD* nor *SDHAF2* is imprinted, other genes that are exclusively maternally expressed should play a role in tumor development. This phenomenon is now known as the Hensen Model [25, 26].

The penetrance of tumor development in patients with SDH mutations is highly variable, being high for *SDHD* (87–100%) and much lower for *SDHB* (25–40%) [27]. In 2018, Rijken et al. found an estimated *SDHB*-linked penetrance of 21% at age 50 [28]. *SDHD* mutations predispose to multifocal head and neck PGL and less commonly to non-metastatic abdominal PGL or PCC [29]. *SDHAF2* mutations are rare; only four PGL2 families have been described, often with multifocal, but non-metastatic head and neck PGL [30]. Germline *SDHC* mutations are found in around 4% of head and neck PGL, usually non-metastatic carotid body PGL [31]. *SDHB*-mutated tumors are mostly extra-adrenal PGL and are associated with a high metastatic potential of up to 50% [32]. Head and neck PGL occur in 20–30% of *SDHB*-mutation carriers [33]. *SDHA* mutations are rare and demonstrate a low penetrance, accounting for only 3% of PCC/PGL [34]. Importantly, SDH mutations have been associated with other disorders and solid tumors, which will be discussed hereafter. An overview of SDH genes and tumor associations of PCC/PGL is shown in Table 1.

SDH Genes in Other Tumors

SDH-Deficient Gastrointestinal Stromal Tumor

Most gastrointestinal stromal tumors (GIST) occurring in adults result from gain of function gene mutations of *KIT*

(75–80% of the cases) or *PDGFRA* (5–25%) [35]. The remaining 10% of adult GIST, and about 85% of pediatric GIST are non-*KIT*/*PDGFRA*-mutated tumors, so-called wild-type GIST. The latter group can be classified into SDH-deficient and non-SDH-deficient cases (including tumors with mutations in *NF1*, *BRAF*, *KRAS*, *PIK3CA*, or containing the *ETV6-NTRK3* fusion) [36]. SDH-deficient GIST are now being recognized as a distinct class of tumors with specific clinico-pathological features compared to non-SDH-deficient GIST. These tumors arise in the stomach, are more common in children and young adults and show a female predisposition. Histopathologically, they are multifocal, demonstrating a multinodular, lobulated growth pattern composed of predominantly epithelioid cells [37]. Even though lymph node metastases are frequently present, their behavior is commonly indolent. In contrast to *KIT*/*PDGRA*-mutated GIST, SDH-deficient GIST do not respond to therapy with Imatinib [37, 38]. About 5–10% of all gastric GIST are SDH-deficient, in which *SDHA* mutations are most commonly identified (30%), while up to 20% are associated with *SDHB*, *SDHC*, or *SDHD* mutations. The remaining 50% is associated with *SDHC* epimutations resulting in promoter hypermethylation [38]. *SDHC* epimutation has also been reported in patients with Carney triad [39, 40]. In this very rare, non-familial syndrome mostly affecting young females, SDH-deficient GIST are associated with PGL and pulmonary chondromas [41]. Tumors from patients with Carney triad do not harbor SDH gene mutations, but SDH deficiency results from *SDHC* promoter-specific CpG island hypermethylation and subsequent gene silencing. A related disease is Carney-Stratakis syndrome, the familial dyad of PGL and GIST affecting both young males and females. Carney-Stratakis syndrome is inherited as an autosomal dominant trait in which germline mutations in *SDHB*, *SDHC*, and *SDHD* can be identified. PGL are the predominant manifestation in Carney-Stratakis syndrome, while GIST are predominant in Carney Triad [41, 42].

SDH-Deficient Renal Cell Carcinoma

SDH-deficient renal cell carcinomas (RCC) account for 0.05–0.2% of all RCCs [43]. *SDHB*-mutated patients affected by RCC were reported for the first time in 2004 [44]. Similar to SDH-deficient GIST, SDH-deficient RCC show distinctive clinico-pathological characteristics. Therefore, they are currently being recognized as a distinct type of RCC in the 2016 WHO classification of renal tumors [45]. The mean age at presentation is 38 years, with a slight male predominance. Multifocal or bilateral tumors are detected in 30% of patients at long-term follow-up, and the risk of metastasis is low (estimated at 11%) [45]. Most SDH-deficient RCC show distinctive histologic features with the presence of cytoplasmic vacuoles and inclusion-like spaces containing eosinophilic or flocculent material [43]. However, variant morphologies

Table 1 SDH genes and tumor associations of PCC and PGL

Gene	PCC	sPGL	HNPGL	Penetrance	Metastatic potential	Other tumors
SDHD	x	x	xx	87–100%	Up to 4%	GIST, RCC, PA
SDHB	x	xx	x	25–40%	Up to 50%	GIST, RCC, PA
SDHC		x	xx	Low	0%	GIST, RCC, PA
SDHAF2			x	Not known	0%	PA
SDHA	x	x	xx	Very low	Not known	GIST, PA, RCC

PCC pheochromocytoma, *sPGL* sympathetic paraganglioma, *HNPGL* head and neck paraganglioma, *GIST* gastrointestinal stromal tumor, *RCC* renal cell carcinoma, *PA* pituitary adenoma

have been reported. SDH-deficient RCC are most commonly due to *SDHB* mutations. In *SDHB* mutation carriers, the risk of RCC by age 60 years is 4.7% [33]. However, RCC can also occur in *SDHC* and *SDHD* mutation carriers (in 14% and 8% of patients, respectively), in addition to one case of *SDHA*-deficient RCC [38, 46].

SDH-Deficient Pituitary Adenoma

The combination of pituitary adenomas (PA) with PCC or PGL (termed “3Pas”) is very rare and can either be coincidental or the result of a common pathogenesis [47]. In 2012, an SDH-related PA was described for the first time in an *SDHD* mutation carrier with multiple head and neck, thoracic and abdominal PGL [48]. In the subsequent year, another *SDHA*-related PA was described [49]. To date, about 25 patients with SDH-related PA have been described with *SDHA*, *SDHB*, *SDHC*, *SDHD*, or *SDHAF2* mutations [50]. These tumors are most often prolactin- or growth hormone-producing macroadenomas with varying ages at diagnosis, ranging from 15 to 84 years [36]. Some authors report vacuolated, clear cytoplasm as a hallmark of SDH-mutated PA, reminiscent of that seen in SDH-deficient RCC [47]. However, others could not identify distinctive morphologic features [38, 49]. Recently, a pituitary carcinoma has been described in a female patient with an *SDHB* mutation and a history of glomus PGL [51].

Other SDH-Deficient Tumors

Even though other tumors than GIST, RCC, and PA have been reported in PCC/PGL patients with germline SDH mutations, very few of these have been proven to be related to SDH deficiency. Ni et al. investigated the occurrence of both germline and somatic SDH mutations/variants in 754 cases of differentiated thyroid carcinoma [52]. They found germline SDH missense variants in 48 (6%; 26 *SDHD* and 22 *SDHB*) patients. In addition, they found large somatic duplications within chromosome 1 encompassing *SDHC* in 27 of 513 tumors (5%) [52]. Furthermore, both papillary and follicular thyroid tumors showed a significant reduction in *SDHC* and

SDHD mRNA expression compared to normal thyroid tissues. Thyroid tumors with lower SDH gene expression were associated with an earlier age at diagnosis and a high pathological-TNM stage [52]. However, the link between pathogenic SDH mutations and thyroid carcinogenesis remains to be established [53, 54]. To the best of our knowledge, to date, no *SDHB* immunonegative thyroid carcinoma has been described.

In 2015, an SDH-deficient low-grade pancreatic neuroendocrine tumor has been described in a patient with a germline *SDHD* mutation with multiple head and neck PGL and an oligodendroglioma in the frontal lobe [50]. The tumor was immunonegative for *SDHB*, immunopositive for *SDHA*, and showed loss of the wild-type *SDHD* allele. However, others postulate that this single case may be better classified as an intrapancreatic PGL [38]. Scarpa et al. did not find any pathogenic SDH mutations in 102 primary pancreatic neuroendocrine tumors after whole genome sequencing [55].

Pópulo et al. analyzed *SDHD* promoter mutations in 86 cutaneous melanomas and found alterations in 2 (2%) of the cases [56]. They also evaluated *SDHD* protein expression in 107 cutaneous melanomas. A low staining score and a moderate/high staining score was observed in 41% and 59% of the tumors, respectively. Moreover, a significant association was found between lower mean *SDHD* protein expression and the presence of ulceration and higher pathological-T stage [56]. *SDHD* mutations might be associated with prognosis of cutaneous melanoma, but are not proven to be related to tumorigenesis.

Many other tumors including adrenal neuroblastoma, bronchial carcinoid, ependymoma, Hodgkin lymphoma, and colon cancer have been described in SDH mutation carriers or their relatives [57]. Up to now, no support has been found for a pathogenetic association of any of these tumors with the presence of SDH mutations.

SDH Immunohistochemistry

In the rapidly expanding field of molecular genetic testing, it is important to assess the pathogenicity of identified SDH “variants of unknown significance” [58]. *SDHB* and *SDHA* immunohistochemistry can be used as a quick, reliable, and

cheap tool to determine the functionality of such genetic variants. Normally, SDHB and SDHA staining is positive in all cells, with strong granular and cytoplasmic labeling representing mitochondrial localization of the protein [59]. In tumors, non-tumoral cells such as endothelial cells, fibroblasts, or lymphocytes can be used as an internal positive control. If any of the subunits of the SDH complex is lost due to (epi) mutation, the entire complex becomes unstable and the SDHB subunit is degraded in the cytoplasm [60]. This loss of the SDHB protein can be shown by SDHB immunohistochemistry (Figs. 2 and 3c–d) [59]. Tumors with inactivating *SDHA*, *SDHB*, *SDHC*, *SDHD*, or *SDHAF2* mutations demonstrate loss of cytoplasmic SDHB staining. If SDHB staining in the tumor cells is evidently less intense compared to non-tumoral cells, or if it shows a weak diffuse cytoplasmic blush instead of a granular staining pattern, SDHB immunohistochemistry should be regarded negative [38]. Of note, SDHB immunostaining might appear falsely negative in tumor areas with clear cytoplasm, for example in clear cell RCC. In such cases, the best approach is to look for areas with eosinophilic cytoplasm for interpretation of the staining. In a multicenter study, SDHB immunohistochemistry was shown to be a robust technique with almost 90% consensus in a group of seven reviewers. The positive and negative predictive value of the seven reviewers of SDHB immunohistochemistry to detect SDH mutations ranged from 67 to 93%, and from 90 to 99%, respectively [58].

In addition, SDHA immunohistochemistry was proven to be a useful marker to detect tumors with *SDHA* mutations, as

is the case in tumors with pathogenic *SDHA* mutations together with second hit inactivation [34]. SDHA cytoplasmic staining is negative in *SDHA*-mutated tumors, while tumors with mutations in any of the other SDH subunits show preserved SDHA staining (Fig. 3a–b). These findings suggest that the SDHB protein is degraded when the SDH complex is disrupted, whereas the SDHA protein remains intact [34]. Of note, *SDHA* mutations may be present in up to 0.3% of the general population, as these mutations have a low, incomplete penetrance pattern [34, 38].

Molecular genetic testing is labor intensive and requires clinical molecular diagnostic laboratories, so SDHB and SDHA immunohistochemistry can be used as a supplementary tool to identify patients with SDH-deficient tumors. As has been mentioned above, some tumors show a weak diffuse cytoplasmic SDHB immunostain, particularly PCC/PGL with *SDHD* mutations [38]. Therefore, Menara et al. evaluated in 170 PCC/PGL if SDHB immunohistochemistry could be used to detect *SDHD* mutations [60]. Interestingly, they showed that SDHB immunostaining is positive in SDH-deficient tumors and negative in SDH wild-type tumors. A possible explanation for this paradoxical result is that the SDHB epitope is masked in the transmembrane domain of the protein in the normal state, but that disruption of the SDH complex releases the epitope, making it accessible for immunostaining [61]. Even though SDHB immunohistochemistry cannot be used to specifically detect *SDHD*-mutated tumors, the staining provides a complement to difficult to interpret SDHB immunostainings. Weak

Fig. 2 **a** Positive SDHB immunostaining in a non-*SDH*-related PGL demonstrating strong granular (mitochondrial) cytoplasmic staining, **b** negative SDHB immunostaining in an *SDH*-mutated PGL demonstrating absent SDHB staining in tumor cells. Endothelial cells serve as internal positive controls and show positive SDHB staining, **c & d** weak diffuse (in contrast to granular) cytoplasmic SDHB staining in two *SDH*-related PGL. The staining of the endothelial cells is more intense dark brown and granular, meaning that the tumor cells should be scored as SDHB negative

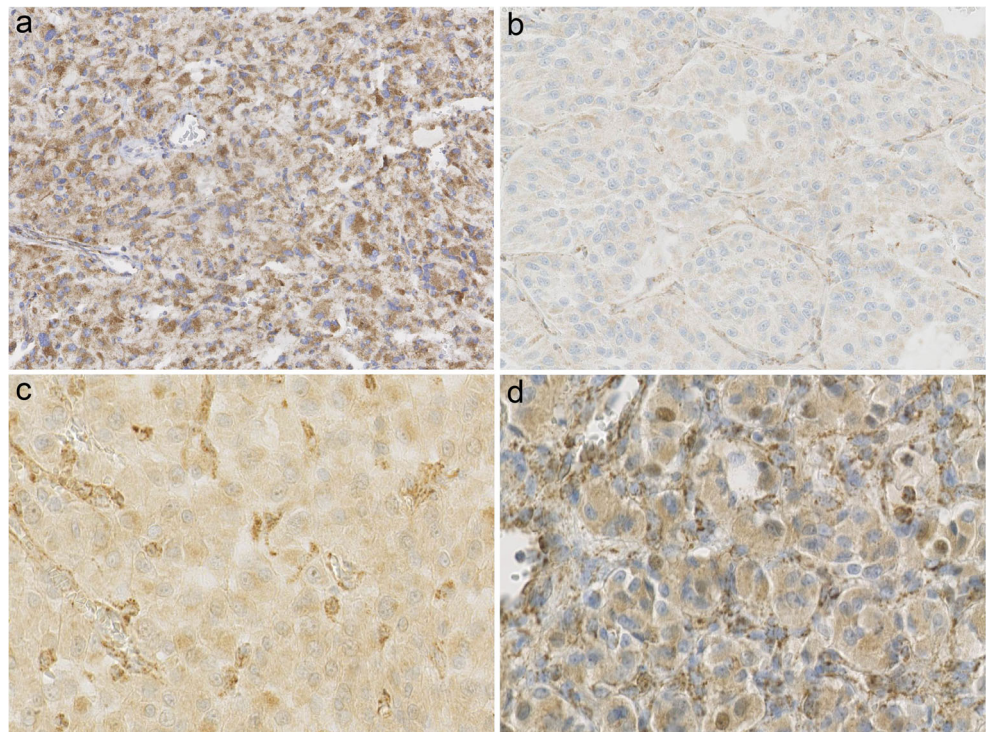
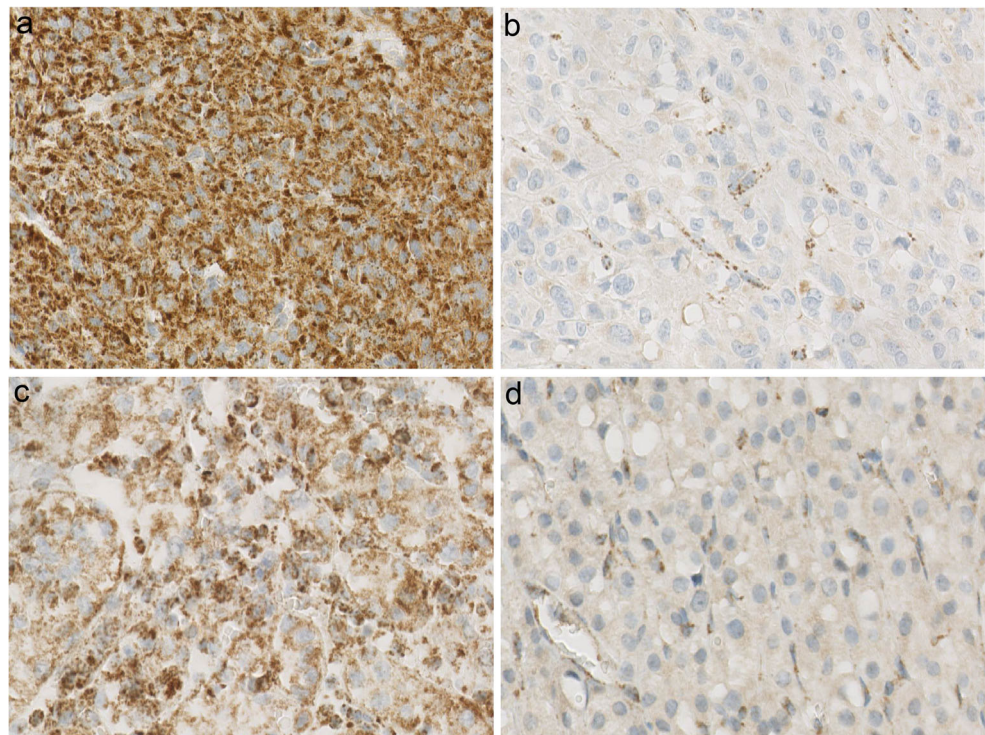


Fig. 3 **a** Positive granular SDHA immunostaining in a non-*SDH*-related GIST, **b** absence of SDHA immunostaining in the tumor cells of an *SDHA*-mutated GIST. In contrast, staining is positive in endothelial cells that serve as internal positive control, **c** positive SDHB immunostaining in a non-*SDH*-related clear cell RCC, **d** negative SDHB immunostaining in an *SDH*-deficient RCC



diffuse or discordant negative cytoplasmic SDHB staining has also been described in a minor subset of *VHL*- and *NF1*-mutated PCC/PGL. Therefore, if SDH genetic testing of an SDHB-immunonegative tumor does not show a mutation, SDHC promoter methylation, *VHL*, and/or *NF1* molecular testing is recommendable [58].

Tumorigenic Mechanism of SDH Abnormalities

As described above, SDH mutations are commonly observed in a number of hereditary and sporadic malignancies. Mutations in any of the four SDH subunits lead to the disintegration of the SDH complex and result in a complete loss of SDH enzymatic activity [62]. Dysfunction of SDH can lead to succinate accumulation and a low level of fumarate. The accumulation of succinate promotes angiogenesis by activation of the hypoxia-inducible factor (HIF) pathway, as has been shown in gene expression analysis. In that study, *SDHB*-, *SDHD*- or *VHL*-mutated PCC revealed a gene expression pattern with a “HIF signature” [63]. In addition, Selak et al. showed that SDH dysfunction increased succinate levels, first accumulating in the mitochondrial matrix and subsequently leaking into the cytosol where it inhibits the activity of prolyl hydroxylases, leading to HIF α stabilization and activation of the HIF pathway [64]. Another explanation for the stabilization of HIF-1 α in SDH-deficient tumors is the generation of reactive oxygen species (ROS). Although SDH is not considered a major site for ROS production in the electron transport chain under normal conditions, in PCC and PGL, evidence of

an association between SDH mutations and oxidative stress is present [65].

Tumors in the Context of Other Citric Acid Cycle Gene Mutations

Apart from SDH, other citric acid cycle enzymes have been shown to be mutated or deregulated in various types of tumors. Isocitrate dehydrogenase (IDH), comprised of three isoforms (IDH1, IDH2, and IDH3), converts isocitrate to α -ketoglutarate. *IDH1* and *IDH2* mutations are frequently found in low-grade gliomas and in secondary glioblastomas. Mutations have also been described in acute myeloid leukemia and T cell lymphomas [66, 67]. *IDH* mutations may also occur in PGL, but are rare [68]. They result in increased conversion of α -ketoglutarate to 2-hydroxyglutarate (2-HG), leading to accumulation of 2-HG. This occurs in the brain of patients with 2-hydroxyglutarate dehydrogenase deficiency, who go on to develop leukoencephalopathy, and have an increased risk of developing brain tumors [69, 70].

Fumarate hydratase (FH) catalyzes the hydration of fumarate to L-malate. *FH* mutations are classically found in hereditary leiomyomatosis and renal cell cancer, where patients may present with multiple cutaneous leiomyomas and a specific RCC subtype. Similar to *SDH*-mutated tumors, *FH*-mutated tumors display upregulation of HIF [71]. *FH*-deficient cells show inhibition of HIF-1 α prolyl hydroxylation by accumulated intracellular fumarate. *FH* mutations have also been found in PCC and PGL, and *FH*-deficient tumors may

be detected by immunohistochemistry through their nuclear and cytoplasmic staining of 2SC and the absence of FH immunostaining [72, 73].

Malate dehydrogenase (MDH) is an enzyme that catalyzes the oxidation of malate to oxaloacetate using the reduction of NAD⁺ to NADH. There are two known isoforms. One isoform is a principal enzyme of the citric acid cycle operating within mitochondria. The other is found in the cytosol where it participates in the malate/aspartate shuttle. Cascon et al. described a PGL family carrying a germline mutation in *MDH2*. These tumors showed lower levels of MDH2 expression compared with control patients. In addition, knockdown of *MDH2* in HeLa cells showed accumulation of both malate and fumarate [74]. *MDH1* mutations are associated with retinitis pigmentosa, sideroblastic anemia, and spinocerebellar ataxia [75].

In addition to mutations detected in genes coding for citric acid cycle enzymes, several studies have demonstrated deregulated enzymes (e.g., α -ketoglutarate dehydrogenase, citrate synthase, Acetyl-coenzyme A carboxylase) in other cancer types, including colorectal, pancreatic, ovarian, renal, gastric, and prostate cancer [76–78].

Conclusions

The SDH complex is a biologically and biochemically fascinating enzyme complex, composed of four subunits, that has a pivotal role in both citric acid cycle and electron transport chain. Over the last two decades, we have discovered its crucial role in the pathogenesis of PCC, PGL, and several other tumor types, mostly in a hereditary setting. Immunohistochemistry for SDHB and SDHA has obtained an important place in diagnostic pathology for the recognition of any SDH-related tumor and has become a well-established surrogate marker for molecular analysis and for confirmation of genetic variants of unknown significance. The current challenges are as follows: (1) to elaborate on such immunohistochemical markers in other areas of pathology [79]; (2) to detect genetic abnormalities in related genetic sequences (promoter regions, intron/exon boundaries) or genes (SDH assembly factors, other citric acid cycle genes); (3) to use the increasing knowledge on the functional consequences of loss of enzymatic activity for the development of therapeutic interventions that may affect or prevent tumor development.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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