CMLS, Cell. Mol. Life Sci. 62 (2005)

DOI 10.1007/s00018-005-5237-6
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CMLS Cellular and Molecular Life Sciences

Review

Succinate dehydrogenase deficiency in human

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Received 31 May 2005; received after revision 28 June 2005; accepted 11 July 2005

Abstract. Mitochondrial succinate dehydrogenase (SDH) consists merely of four nuclearly encoded subunits. It participates in the electron transfer in the respiratory chain and insuccinate catabolism in the Krebs cycle. Mutations in the four genes, SDHA, B, C and D, have been reported, resulting in strikingly diverse clinical presentations. So far, SDHA mutations have been reported to cause an encephalomyopathy in childhood, while mutations in the genes encoding the other three subunits have been associated only with tumour formation. Following a brief description of SDH genes and subunits, we examine the properties and roles of SDH in the mitochondria. This allows further discussion of the several hypotheses proposed to account for the different clinical presentations resulting from impaired activity of the enzyme. Finally we stress the importance of SDH as a target and/or marker in a number of diseases and the need to better delineate the consequences of SDH deficiency in humans.

Key words. Mitochondria; tumour; encephalomyopathy; succinate dehydrogenase; Krebs cycle.

Introduction

Succinate dehydrogenase (SDH) plays such an important role in the mitochondria, being both part of the respiratory chain and the Krebs cycle, that for a long time any severe deficiency of this enzyme was regarded as being incompatible with life [1]. However, in 1995, inherited SDH deficiency was recognized as a rare cause of encephalomyopathy in children [2]. Any ambiguity was excluded by the report of the underlying deleterious mutation in the gene encoding the flavoprotein, one of the four SDH subunits. This was indeed the very first mutation reported in a nuclear gene encoding a respiratory chain component. Five years later, totally unanticipated results from linkage analyses show that the SDH-encoding genes are tumour suppressors, responsible for paraganglioma (PGL) formation (reviewed in [3-5]). These findings have triggered renewed interest in the enzyme and its function, with the hope of understanding the striking difference between the phenotypes associated with SDH deficiency.

Human SDH: from genes to active complex

The SDH enzyme, also known as respiratory chain complex II, faces the mitochondrial matrix and is bound to the inner membrane [6]. Unlike most of the Krebs cycle enzymes, it has no cytosolic counterpart. It consists of four subunits, A–D, with one hydrophilic domain, made of the subunits A and B, protruding into the mitochondrial matrix (fig. 1). The two other subunits, C and D, an-

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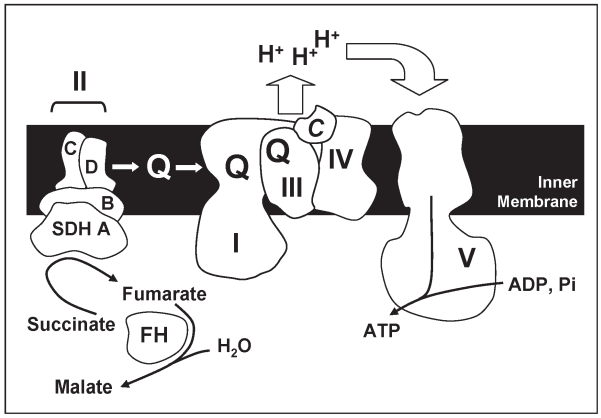

Figure 1. A schematic view of the respiratory chain. Notice that respiratory chain complexes do form a supercomplex, i.e. the respirasome, sparing succinate dehydrogenase (SDH) and ATPase (complex V). SDH (four subunits A–D) feeds electrons to the quinone pool and oxidizes succinate to fumarate, further metabolized into malate by fumarase (FH). I, II, III, IV, V: complex I, II, III, IV and V of the respiratory chain, respectively.

chor the complex to the mitochondrial inner membrane. Four nuclear genes encode the four subunits, *SDHA* (15 exons), *SDHB* (8 exons), *SDHC* (6 exons) and *SDHD* (4 exons), mapping on to chromosomes 5p15, 1p35-p36.1, 1q21 and 11q23, respectively [7]. While the *SDHA* and *SDHB* genes code for proteins well conserved among species, the amino acid sequences of the human SDH C and D subunits show little similarity to the subunits from other species. In some species, only one unique protein anchors the enzyme to the membrane [8]. Recognition sites for mitochondrial transcription, NRF-1, NRF-2 and Sp1, are common to most nuclear genes encoding the respiratory chain components, as well as some of the antioxidant enzymes, several of the heme biosynthetic enzymes and of the components of the mitochondrial protein import machinery. While NRF-1 binding sites are found in the promoter region of all four *SDH* genes, NRF-2 and Sp1 binding sites are not present in the *SDHA* and *SDHC* promoters, respectively. Mitochondrial transcription factor (mt2, mt3, mt4) binding sites are found in the *SDHA* and *SDHB* promoters, but not in the *SDHC* and the *SDHD* promoters [9]. Both *SDHA* and *SDHB* also harbour IREs (iron responsive elements) in their promoter region, denoting their potential implication in cellular/mitochondrial iron homeostasis [10].

Pseudogenes have been reported in the human nuclear genome for *SDHA* (3q29), *SDHC* (17p13) and *SDHD* (up to six pseudogenes, e.g. 3q26.3, 3p21.3). Pseudogenes of some of the SDH subunits are even found in the mitochondrial genome of several plant species, showing the ancestral mitochondrial origin of these proteins [11]. Interestingly, two forms of SDHA proteins have been identified in human cells, but the putative gene encoding the additional form has not yet been mapped on the human genome.

The assembly of SDH in the mitochondrial membrane is far from beingunderstood, and to date only one protein, Tcm62, a chaperonin-like protein with a more general function [12], has been shown to be involved in this process [13].

Finally, while the other RC complexes involved in electron transfer participate in larger structures known as super complexes, possibly forming a respirasome, as yet SDH has no defined protein association [14]. This sets SDH apart and adds to its peculiar redox properties, which allow the enzyme to work in the respiratory chain under conditions of highly reduced redox status [15] and suggest a possible role in the mitochondrial inner membrane (see below).

What does SDH do?

The SDHA subunit, a 70-kDa protein (621 aa) with a covalently attached FAD prosthetic group, binds enzyme substrates (succinate and fumarate) and physiological regulators (oxaloacetate and ATP) (fig. 2). Oxaloacetate, a succinate competitive inhibitor arising from malate (a poor substrate of SDH), can be displaced upon ATP binding onto the enzyme [16]. The electrons fed to the SDHA subunit are next transferred to several iron-sulfur cluster-binding SDHB subunits (27 kDa, 280 aa; three clusters: [2Fe-2S], [4Fe-4S], [3FeS]; for details see [17], before reaching the cyt *b* type (b_{558})-binding SDHC and SDHD subunits, also known as cybL and cybS (15 and 12–13 kDa)). Electrons are further delivered to the quinone pool through two quinine binding sites [18], as depicted in figure 1. However, the exact role of the *b*-type cytochrome and the different iron-sulfur clusters associated with SDH needs to be further clarified [17, 19].

The human enzyme readily oxidizes succinate to fumarate, while the reverse reaction is hardly detectable in most human cells and tissues under standard conditions. A tunnel diode effect, over a broad range of potentials, opposes the backward direction that may represent an evolutionary adaptation to aerobic metabolism [20]. It has, however, been postulated that the fumarate reduction is significant enough to have a physiological function under hypoxic conditions, where it might play a role in reactive oxygen species (ROS) generation in the pulmonary vasculature [21]. Notably, the enzymes of a number of microorganisms catalyse this reverse reaction, i.e. reducing fumarate into succinate, thus driving fumarate-driven anaerobic respiration. Yet, surprisingly, analysis of the amino acid sequence does not allow prediction of the in vivo function of the enzyme [6].

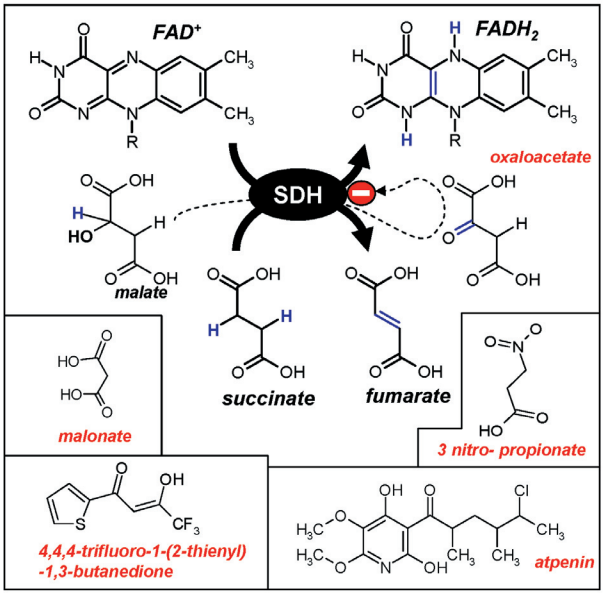

Figure 2. SDH substrates and inhibitors. Tight control of SDH by oxaloacetate is known to take place in vivo. Oxaloacetate, a competitive inhibitor of the enzyme, arises from the activity of mitochondrial NAD^+-dependent malate dehydrogenase or directly from malate, a poor substrate of SDH. Potent SDH inhibitors are featured such, as atpenin, 4,4,4,-trifluoro-1-(2-thienyl)1,3-butanedione (TTFA), malonate and 3-nitro-propionate (3-NP).

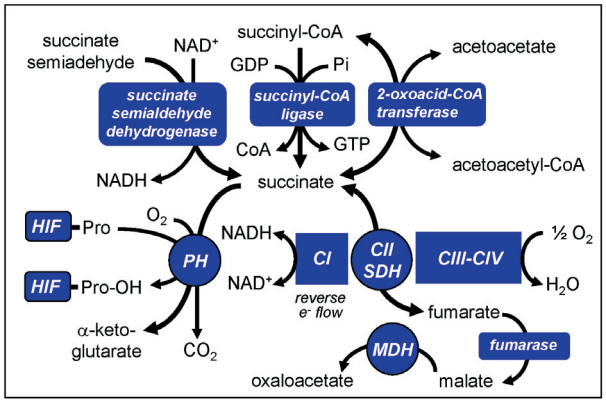

Figure 3. Succinate metabolism. The major enzymatic sources and uses of succinate in the cell. The scheme also features the HIF1α-prolyl hydroxylase (PH), which reaction controls triggering of the hypoxia-sensitive pathway observed in SDH-deficient tumours. CI, CIII; CIV, respiratory chain complexes I, III and IV, respectively; HIF, hypoxia-inducible factor; MDH, malate dehydrogenase.

SDH and succinate: a crossroads between the mitochondria and cell metabolism

Oxidizing succinate links SDH to the fast-cycling Krebs cycle portion [22] where it participates in the breakdown of acetyl-coenzyme A (CoA) throughout the whole Krebs cycle. The succinate can readily beimported into the mitochondrial matrix by the n-butylmalonate- (or phenylsuccinate-) sensitive dicarboxylate carrier in exchange with inorganic phosphate or another organic acid, e. g. malate [23]. In a number of species, mitochondrial carrier distribution varies among organs [24]. The mitochondrial carrier equipment controls the accumulation of extra/intra-mitochondrial substrate/product; the distribution of carriers in humans is only partially known. Notably, the distribution of carriers might participate in tissue-specific susceptibility to SDH defects. Thus, fumarate, the product of the SDH reaction, generally does not permeate the mitochondrial inner membrane, and its intra-mitochondrial accumulation has been shown to slow down the SDH reaction in skeletal muscle mitochondria in the case of fumarase deficiency [25]. Succinate can also be produced in the matrix by GTP-forming succinyl-CoA ligase, or by ketoacid-CoA transferase in the presence of acetoacetate [1, 23] (fig. 3). This latter enzyme, distributed in extra-hepatic tissues (heart, kidney and brain), is responsible for ketolysis and uses liver-synthesized ketone bodies (acetoacetate, hydroxybutyrate) during fasting and lipolytic stress. Acetoacetyl-CoA, derived from succinate acting as an insulin secretagogue, can also be utilized via combined 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase and HMG-CoA reductase reactions to produce mevalonate [26].

A tissue-specific function can be inferred from the significantly different activity of the enzyme among tissues as compared which other RC components [27]. In kidney and liver mitochondria, SDH activity and transcript level are particularly high compared which other mitochondrial enzymes, e. g. citrate synthase. In these tissues, SDH may deliver more electrons to the quinone pool than can be accommodated by the cytochrome segment of the chain, favouring overreduction of the quinone pool [15]. This overreduction may in turn trigger reversal of the electron flow through the RC complex I (fig. 3). In vivo, electrons from SDH could then be diverted to produce NADH. The rapid fumarate removal required for a sustained reverse electron flow may be favoured by the high fumarase activity found in these tissues as well. This redox control imposed by the reverse electron flow on the NAD^+ pool may favour catabolic pathways, e. g. formation of CoA and carnitine esters in liver mitochondria [28].

Very few extra-mitochondrial enzymes actually metabolize succinate compared with the many enzymes metabolizing other organic acids in the cell. Hence, succinate accumulation resulting from decreased SDH activity can hardly be buffered by any alternative enzyme activity, and even partial SDH defects should be rapidly detrimental to the cell. In keeping with this, a 50% residual activity would be sufficiently harmful to came late onset neurodegenerative disease [29]. This claim has, however, been challenged by the observation that among families of patients with paraganglioma (PGL), non-symptomatic

individuals may harbour a heterozygous deleterious mutation in one of the *SDH* genes.

Isolated SDH defects due to mutation of *SDH* genes

Paediatric cases

Inherited SDH deficiency is a rare cause of early onset encephalopathy, and only a few cases have been reported so far, all resulting from mutations in the gene encoding the SDHA subunit. The first deleterious mutation in the *SDHA* gene was identified in two sibblings presenting with Leigh syndrome and SDH deficiency [2]. The two patients were homozygous for an R554W substitution in a conserved domain of the protein, while the consanguineous parents were heterozygous for this mutation. The deleterious effect of the R554W substitution on the catalytic activity of the enzyme was established in an SDH-negative mutant yeast strain [2]. One other case with a similar clinical presentation – SDH deficiency and mutations in the *SDHA* gene – was subsequently reported [9]. Finally, a homozygous G555E substitution, inherited from consanguineous parents both carrying one mutated SDHA, resulted in reduced SDHA and SDHB subunits and lower SDH activity in a young girl who died in infancy from a respiratory infection and severe hypoglycemia before any sign of Leigh syndrome [30].

Later-onset diseases

During the last 5 years, linkage analysis – further strengthened by a candidate gene approach – has disclosed an unpredictable association between germ-line mutations in genes encoding SDH and PGLs, usually benign neuroendocrine tumours [5]. *SDH* gene mutations can also apparently cause sporadic forms of PGLs and pheochromocytomas (PHEOs) as well [5]. PGLs are neuroendocrine tumours which can secrete catecholamines. These highly vascularized tumours, found close to the major blood vessels and cranial nerves, are mainly localized in the head (glomus tympanicum and jugulare), the neck (carotid body and glomus vagale), the adrenal medulla and the extra-adrenal sympathetic ganglias. Like other elements of the autonomic nervous system, PGLs arise from neural crest cells, and are distributed from the skull base to the pelvic floor. The parasympathetic system (carotid body, glomus vagale, glomus jugulare and tympanicum) PGLs usually have no endocrine activity, while PGLs from the adrenomedulla and the sympathetic ganglia can produce catecholamines. PHEOs are functional PGLs evolving from the adrenal medulla as opposed to functional PGLs referring to extra-adrenal PGLs. Hereditary PGLs usually manifest earlier than isolated forms, with a more severe presentation. They are often bilateral and multiple, being recurrent or malignant. One or more secreting tumours are frequent, adding to the severity of the disease. PGLs are dominantly inherited, and four different loci have been identified, PGL1 (11q23), PGL2 (11q13), PGL3 (1q21) and PGL4 (1p36), with genomic imprinting of the maternal allele for the PGL1 locus [31].

Baysal et al. first reported deleterious mutations in the *SDHD* gene, corresponding to the PGL1 locus [32]. Thereafter, mutations were found in *SDHC* (PGL3 locus; [33]) and *SDHB* (PGL4 locus; [34]) genes. Five years later, a number of mutations in these three genes have been reported in both hereditary and apparently sporadic PGLs [5].

Beside the PGL formation, a late-onset neurodegenerative disease with progressive optic atrophy, ataxia and myopathy was tentatively ascribed to an inherited SDH deficiency [29]. The affected members of this family had a 50% residual SDH activity and harboured a heterozygous Arg408Cys substitution in a highly conserved region of the SDHA protein. The corresponding mutation in *Escherichia coli* resulted in an enzyme unable to bind the flavin adenine dinucleotide moiety. The absence of mutation in the other allele was claimed to be compatible with the 50% residual activity found in these patients. However, the absence of such phenotypes in a number of individuals (members of the kindred of patients with PGL) with presumably 50% residual SDH activity casts doubt on this observation, and it remains to be demonstrated that the 50% decrease in activity is indeed causative of the disease.

Why are tumours associated with SDH defects?

A dysfunction of the respiratory chain, whatever the RC component affected, has long been known to result in cell death, either through a necrotic process due to ATP deprivation or to cell apoptosis, possibly through superoxide overproduction and/or changes in mitochondrial membrane permeability favouring release of proapoptotic signals [35]. How then could blockade of SDH result in an increase in the cell proliferation associated with tumour formation?

It has been shown that, under some conditions, increasing the superoxides necessary for ensuring normal cell multiplication results in cell proliferation [36]. As SDH-deficient cells have previously been shown to overproduce superoxides, either due to increased production [37, 38] or possibly to decreased elimination [15], it is tempting to suggest that superoxides are responsible for this cell proliferation [36]. This view was reinforced by observation of significant oxidative stress in an SDH-deficient worm, *Caenorhabditis elegans (*mutant *mev-1)* [39]. The role of oxygen and its activated derivatives in SDH deficiency is also supported by the correlation that exists between PGL frequency and atmospheric oxygen

tension [40, 41]. However, because increased superoxide production is quite commonly observed in the case of RC dysfunction [42], this proposal is challenged by the rather specific link between SDH defects and formation of PGL. A specific link between the type of metabolic blockade and tumour formation was suggested by the observation that mutations of the fumarase gene, functionally associated with SDH in the Krebs cycle, also cause tumours (skin and uterus and/or renal cancer) [43] .

The observation that SDH deficiency results in abnormal activation of the hypoxia-inducible factors HIF1α and EPAS1/HIF2α identified a potential culpable mechanism: unwanted induction of a pseudo-hypoxia pathway [44]. Additional support came from the fact that adrenomedulla tumours or pheochromocytomas could also be associated with mutations in the tumour suppressor gene encoding the so-called von Hippel-Lindau protein, known to be an active partner in the proteasomal destruction of HIF1α [45]. As for SDH deficiency, impaired activity of the VHL protein would thus favour unwanted induction of a pseudo-hypoxia pathway. HIF1α metabolism thus became a perfect candidate to account for the tumor formation associated with SDH mutations. Interestingly, the first step of HIF1α degradation involves prolyl hydroxylation of HIF1α catalysed by a prolyl hydroxylase (PH) which uses α-ketoglutarate as a substrate and leads to succinate as a product. The highly plausible idea arose that a succinate accumulation simply triggers PH inhibition. Although product inhibition of PH by succinate was not reported in initial studies [46], recent work provides experimental evidences for in vitro PH inhibition by succinate [47].

There are, however, pending questions raised by this seductive scheme. First, activation of the hypoxia-responsive pathway is observed in other types of tumours where it does not appear to be causative in tumorigenesis but rather a consequence [48]. Tumour growth obviously favours intra-tumoral hypoxia and thus tends to trigger the hypoxia-responsive pathway. As a result, the causative link between the hypoxia-responsive pathway and tumorigenesis is still a debatable issue. Second, if accumulated succinate can inhibit PH and trigger a pseudo-hypoxia-responsive pathway, superoxides can trigger tumour formation as well [49]. As noted above, if SDH-deficient cells accumulate succinate, they also overproduce superoxides [37, 50]. Third, a complex I deficiency resulting from a *GRIM-19* gene mutation also appears to favour cell proliferation [51]. To our knowledge, specific succinate accumulation has never been reported as resulting from a complex I deficiency, while a complex I blockade could lead to overproduction of superoxides [52]. However, other organic acids might also accumulate in these cells, resulting in PH inhibition, but this remains to be shown. The specificity of the link between the SDH deficiency (as compared with other RC deficiencies) and tumour formation therefore has to considered cautiously. Alternatively, totally unsuspected mechanisms may also be at work. Neurofibromin, the product of the *NF1* gene (also known to be associated with PGL when mutated) co-localizes with mitochondria in cultured cells [53], and the significance of this location is thus far unknown. The fact that VHL protein has been claimed to be one of the factors responsible for controlling the biogenesis of OXPHOS complexes, at least in clear cell renal carcinoma [54], also deserves attention. Obviously, as shown by the increasing number of papers now dealing with the relationship between tumours and mitochondria, this has become a very active field of research. But more should be learned before making any firm conclusions on the mechanisms of mitochondrial dysfunction and tumorigenesis.

SDH defects: several causes, several consequences

Beside the cases/diseases whose molecular bases have been identified, a number of SDH-deficient patients can be found in registers and/or databases in screening centers. In infancy, although isolated SDH deficiency is a rare cause of respiratory chain dysfunction associated with encephalopathy and/or cardiomyopathy, it exhibits wide variety of clinical phenotypes [7, 55]. A similarly large spectrum of clinical presentations is observed when considering a combined deficiency, which often frequently associates a complex II with either a complex V (ATPase) or a fumarase deficiency, again frequently with encephalopathy. This may indicate that the pathological cell processes that cause these diseases share some similarities, SDH deficiency being among the most important.

Succinate dehydrogenase and ATPase deficiencies may be associated

Despite their own scarcity, SDH and ATPase deficiencies are often associated. These two multi-subunit complexes are not known to form a super-complex, a structure that might be assumed to account for a combined respiratory chain deficiency [14]. As a result, another mechanism associated with one of the two deficiencies has to be tought that could subsequently hamper the functioning of the other complex. Notably, the genetically defined deficiencies of SDH resulting from mutations in the *SDHA* gene are not associated with ATPase deficiency, suggesting that this latter should probably not be considered to result from SDH deficiency. Conversely, we noticed an SDH deficiency in the muscle biopsy of a patient harbouring the NARP (neurogenic ataxia with retinitis pigmentosa) mutation in the mitochondrial gene encoding one of the ATPase subunits [56]. Although this is far from being systematically observed in cells harbouring a

variable load of the NARP mutation, it strongly suggests that SDH deficiency can indeed occur secondary to an ATPase deficiency. Hence, because this latter activity is not frequently measured in the context of routine analysis of the respiratory chain in patients at risk, the potential association with SDH deficiency has so far largely been ignored.

Fumarase deficiency may be associated with decreased complex II activity

A fumarase deficiency has been identified in a few cases of encephalomyopathy, and the molecular defect in the corresponding gene was reported as early as 1994 [25]. Interestingly, a handful of patients with low fumarase activity (but no identified mutation in the *FH* gene) have low succinate dehydrogenase activity as well, especially in the liver. This frequent association suggests that at some point the two enzymes share a common regulatory factor/mechanism controlling their co-expression that is directly affected in these patients or responsive to an as yet unknown additional event.

Friedreich ataxia

In 1997, we showed that a generalized deficiency of iron-sulfur proteins, including SDH, causes Friedreich ataxia [57], the most frequent recessive cardiomyopathy-associated ataxia resulting from a GAA triplet expansion in the first intron of the frataxin gene [58]. Under this condition as well, but in a more general context, SDH deficiency and superoxide overproduction appear to give rise to complex interference. Subsequent results from a number of groups have established the role of frataxin in the biogenesis of the iron-sulfur cluster [59–61]. The partial restoration of SDH activity in the heart of one patient after 3 years of antioxidant (idebenone) therapy suggested a double mechanism for SDH deficiency, i.e. decreased synthesis of the iron-sulfur clusters and an additional oxidant-induced insult to the residual iron-sulfur clusters [62].

SDH is a sensitive target for a number of toxic or unusual metabolites

Over the years, decreased SDH activity has been observed in relation to impaired iron metabolism, with riboflavin or vitamin E deficiency, pointing to the frequent secondary involvement of the enzyme under different conditions [3]. Inhibition of SDH by toxic intermediates that accumulate during propionic and methylmalonic aciduria has also been occasionally reported, but the toxic compound accumulating in these diseases has not yet been clearly identified [63]. Such a secondary involvement in a series of conditions underscores SDH sensitivity towards various insults, indeed often associated with oxidative stress. Conversely, secondary SDH impairment may predictably contribute to numerous phenotypes, especially in view of the central and irreplaceable role of SDH in a number of tissues and cells. Hence, assay of SDH activity has been recommended as a marker for living cells, and the enzyme plays a prominent role in the widely used tetrazolium salt reduction tests used to quantify living cells [64].

Accordingly, under various disease conditions, decreased SDH activity has been claimed to contribute to the phenotype. Such a decrease was reported in particular in Huntington disease [65], and this observation was extensively used to model this disease, and more generally neuronal diseases, by treating animals, mostly rodents, with SDH inhibitors [66]. This research shows the particular effect of SDH inhibition on neuronal cell death, possibly through Ca2+ overload and subsequent uncontrolled activation of the NMDA glutamate receptor [67]. Given this susceptibility of specific brain cell lines to SDH inhibition, the protection of the enzyme against a series of external insults would constitute a major achievement.

Conclusion

Although dealing with the smallest complex of the RC – four subunits, all encoded by nuclear genes – studies focused on complex II have resulted in surprising observations in the field of mitochondrial research. Indeed, studying patients with SDH deficiency has revealed a number of different mechanisms, primary and secondary, that result in SDH impairment, while SDH impairment has been shown to have unexpected consequences. Although great progress has been made in understanding these diseases, we still do not know much about the mechanisms linking cell proliferation/death to SDH dysfunction, or the complex interaction between SDH activity and oxygen sensing/response in the mitochondria. To answer these questions will be of tremendous importance in understanding these essential cellular processes, and possibly for better counteracting SDH-associated diseases.

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