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Mitochondrial Complex II: At the Crossroads

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Abstract

Mitochondrial complex II (CII), also called succinate dehydrogenase (SDH), is a central purveyor of the reprogramming of metabolic and respiratory adaptation in response to various intrinsic and extrinsic stimuli and abnormalities. In this review we discuss recent findings regarding SDH biogenesis, which requires four known assembly factors, and modulation of its enzymatic activity by acetylation, succinylation, phosphorylation, and proteolysis. We further focus on the emerging role of both genetic and epigenetic aberrations leading to SDH dysfunction associated with various clinical manifestations. This review also covers the recent discovery of the role of SDH in inflammation-linked pathologies. Conceivably, SDH is a potential target for several hard-to-treat conditions, including cancer, that remains to be fully exploited.

Introduction

Mitochondria are indispensable for eukaryotes, with a few notable exceptions [1], being the principal site of important cellular functions including ATP production via **oxidative phosphorylation (OXPHOS)** (see Glossary). Mitochondria also play a central role in cell physiology, from the synthesis of intermediary metabolites to modulation of cell death pathways. OXPHOS and the tricarboxylic acid (TCA) cycle are key metabolic pathways of mitochondria. OXPHOS comprises five **respiratory complexes (RCs)**: CI, CII, CIII, and CIV, and CV. While CI, CIII, CIV, and CV have subunits encoded by the nuclear and mitochondrial genomes, CII (SDH) comprises four subunits (SDHA, SDHB, SDHC, and SDHD) encoded by nuclear DNA (nDNA). In additional to its role in OXPHOS, SDH is a component of the TCA cycle, making a functional link between these two essential processes [2–6]. In the TCA cycle, SDH oxidizes succinate to fumarate [3,7]. As part of OXPHOS, SDH transfers electrons from succinate via its [Fe–S] clusters to ubiquinone (UbQ) [2,3,7]. Since SDH is at the crossroads of two essential pathways, there is growing

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evidence pointing to tight regulation of its biogenesis and function at the post-translational level in response to multiple (patho)physiological stimuli.

SDHx genes are mutated both germinally and somatically in several types of cancer, indicating their role as tumor suppressors. Epigenetic modulation of *SDHx* genes has been shown to contribute to various pathological states. A possible molecular mechanism underlying SDH dysfunction leading to tumor formation is linked to the failure of mitochondria to regulate apoptosis and the generation of **reactive oxygen species (ROS)** [8–10]. The phenomenon of pseudohypoxia based on stabilization of the transcriptional factors hypoxia-inducible factor 1 alpha (HIF-1α) and HIF-2α is also linked to SDH dysfunction [11–14]. In support, gain-of-function mutations of *HIF2A* (*EPAS1*) may be linked to a pathological condition similar to the clinical presentation of SDH dysfunction [15]. Additional, novel mechanisms have now been proposed to explain the frequent clinical presentations associated with SDH dysfunction, which are discussed in this review.

SDH Dysfunction and Disease

Paraganglioma (PGL) and Pheochromocytoma (PHEO)

These tumors are derived from paraganglia, which sense oxygen levels and play a major role in adaptation to hypoxia [16–18]. Paraganglia are located along the paravertebral axis from the base of the skull and the neck to the pelvis. PGLs and PHEOs are classified as clinically distinct, where tumors derived from the adrenal medulla are designated PHEOs and those formed from paraganglia extra-adrenally are referred to as PGLs [19]. Mutations in ~17 genes cause PGLs and PHEOs, including *RET, VHL, NF1, TMEM127, MAX, KIF1B, EGLN1*, and *SDHx* [20–22]. In rare cases PGL can be derived from organs where chromaffin cells are not normally present, perhaps due to developmental abnormalities leaving chromaffin cells in certain locations/organs [22].

While many PGLs and PHEOs with mutant *SDH* genes are considered benign tumors, they often become malignant and can metastasize [16,17,21]. About 40% of PGLs and PHEOs are associated with hereditary mutations in *SDHx* and other genes while about 60% of cases are linked to sporadic mutations [23]. Mutations in all subunits of SDH and in the **assembly factor** SDHAF2 are linked to PGL and PHEO (Box 1).

Gastrointestinal Stromal Tumor (GIST)

This disease is mainly located within the gastrointestinal tract [24]. *KIT, PDGFRA, PDGFRB, BRAF*, and *SDHx* are susceptible loci for GIST. Mutations in genes encoding subunits of SDH have been shown to cause GIST, which is mainly referred to as ‘wild-type GIST’ or dSDH-GIST [24,25]. Since the assembly factors SDHAF1, SDHAF2, SDHAF3, and SDHAF4 play roles in the maturation/assembly of functional SDH, future studies should reveal whether genes encoding these assembly factors contribute to the etiology of GIST. In support, negative staining for SDHB without detectable mutation of any of the *SDH* subunit genes has been demonstrated in pediatric patients with metastatic GIST [24].

Renal Cell Carcinoma (RCC)

This tumor arises from the urinary tract, mainly the kidney. In addition to germline mutations in the *VHL, c-Met*, and *FLCN* genes and translocations of chromosome 3, germline and somatic mutations of *SDHx* have been shown to be a cause of RCC [26–28]. Morphologically, SDH-deficient RCC shows similarities to GIST and PGL associated with *SDHx* mutations [27,28]. RCC linked to mutations in subunits of SDH other than SDHB is rare (unlike for PGL and GIST), those with mutant SDHB accounting for about 80% of SDH-dysfunctional RCCs. The molecular mechanism underlying the role of SDHx in RCC is unknown.

Leigh Syndrome

This is a progressive neurodegenerative disorder in infants, although it is also sporadically observed in teenagers and adults, with no current cure [29]. Leigh syndrome arises from a broad spectrum of genetic defects, with mutations in mitochondrial and nuclear genes [29] including mutations in *SDHA* [30]. Patients carrying the R451C, A524V, R554Y, or G555Q mutations in the *SDHA* gene have been diagnosed with Leigh syndrome [30–33]. However, detailed biochemical and structural analysis is required to understand which mutations in *SDHA* cause PGL and which give rise to Leigh syndrome.

Structural Insight into Complex II Function

The high-resolution X-ray crystal structure of SDH from porcine heart [2] revealed a head-to-tail arrangement of the hydrophobic subunits SDHC and SDHD, which are embedded in the inner mitochondrial membrane (IMM) where they anchor the flavoprotein SDHA and the iron–sulfur SDHB subunits, which protrude into the matrix.

The crystal structure of SDH confirmed previous biochemical data suggesting the presence of three prosthetic groups in the complex: FAD, [Fe–S] clusters, and heme (Figure 1). The SDHA subunit resembles the Rossmann-type fold in its crystal structure, with a large FAD-binding domain at its N terminus. The SDHB subunit contains a [2Fe–2S] cluster ligated to its N terminus, while the remaining [4Fe–4S] and [3Fe–4S] clusters are attached to the C terminus. The SDH crystal structure documents that the two integral membrane proteins contain one heme b and two UbQ-binding sites. They are referred to as the Q_P site, which is proximal to the matrix face of the IMM, and the Q_D site, which is distal from the matrix [2].

During oxidation of succinate by SDHA, the flavin cofactor accepts two electrons. One at a time, they transfer from the flavin via the [2Fe–2S], [4Fe–4S], and [3Fe–4S] clusters in SDHB to reduce UbQ at the Q_P site. Although it was anticipated that the electron flow from FAD to the [Fe–S] clusters and then to heme would result in UbQ reduction, the crystal structure and biochemical analyses do not support a direct role of heme in the electron transfer relay [2,34]. The crystal structure of SDH indicates direct transfer of electrons from the [3Fe–4S] cluster to the Q_P site. There are no unequivocal data clarifying the role of the heme or explaining the function of the less well-characterized Q_D site [2,34,35].

Assembly of CII and the Role of SDH Assembly Factors in Disease

Four assembly factors have been reported to play a role in the maturation of holo-SDH: SDH assembly factor 1 (SDHAF1), SDHAF2, and the chaperone-like SDHAF3 and SDHAF4 (Figure 2 and Box 2).

In the mitochondrial matrix, SDHA was proposed to be flavinated by the SDHAF2 (SDH5) protein (Sdh5 in yeast), and SDHAF2 is required for the covalent attachment of FAD to the catalytic SDHA (Sdh1 in yeast) subunit; the G78R mutation in SDHAF2 is associated with PGL [36]. Yeast cells lacking Sdh5 are respiration deficient due to the absence of the SDH activity. Studies in other model systems indicated that other, species-specific players are important for the covalent attachment of FAD to SDHA. Fully assembled and catalytically active SDH is formed in the absence of SDH5 in *Arabidopsis* [37]. Thermophilic bacteria lacking the SDH5/SDHAF2 homolog SdhE feature flavinated SdhA [38]. Recent findings also indicate that, at least in some human cells, SDHAF2 is redundant for SDHA flavination. We discovered this for triple-negative breast cancer cells that maintained flavinated SDHA and assembled SDH, as well as retaining SDH and SQR activities, despite SDHAF2 depletion by gene editing [39]. It needs to be investigated whether this redundancy for SDHAF2 extends to other mammalian systems and to uncover how SDHA is flavinated in these cells.

A study in yeast showed that the chaperon-like Sdh8 protein (SDHAF4 in mammals) binds to flavinated Sdh1 [40]. Binding of SDHA4 is required to reduce auto-oxidation, which would otherwise lead to the generation of excess ROS from flavinated SDHA, and interaction between Sdh1 and Sdh8 facilitates formation of the Sdh1–Sdh2 complex [40]. In agreement with a role in protection from ROS generation, SDHAF4-knockout *Drosophila* was found to be more sensitive to hypoxia than its parental counterpart, as was also found for yeast in which Sdh8 was deleted [40].

Assembly factors are needed for the insertion of [Fe–S] clusters into SDHB and for maturation of the subunit. SDHAF1 (Sdh6 in yeast) and SDHAF3 (Sdh7 in yeast) have been reported as assembly factors for SDHB biogenesis (Figure 2). A homozygous missense mutation in the *SDHAF1* gene (169G>C) corresponding to a G57R mutation in the SDHAF1 protein, gives rise to the clinical presentation of infantile leukoencephalopathy [41]. SDHAF1 contains a LYR tripeptide motif at its N terminus. Biochemical studies showed that the C terminus of SDHAF1 binds the SDHB protein while the TYR peptide at the N terminus binds the [Fe–S] of the cluster donors, containing the ISCU protein and the HSC20–HSPA9 chaperones [42]. SDHAF1 thus forms a ‘bridge’ and facilitates the transfer and incorporation of [Fe–S] clusters into SDHB. The G57R mutation of SDHAF1 impairs its interaction with SDHB, which in turn affects biogenesis of the SDH holoenzyme. Similarly, as for SDHAF2, the SDHAF1 G57R mutation makes this polypeptide unstable; it is rapidly degraded by Lon proteases (Figure 2), with pathological consequences [42,43]. This indicates that the biogenesis of SDH subunits is under the surveillance of mitochondrial proteases. The chaperone-like assembly factor SDHAF3 is also required for maturation of SDHB [44]. It is likely that SDHAF3 and SDHAF1 prevent the generation of superoxide

from SDHB. This is suggested by experiments in *Drosophila* and yeast SDHAF3-knockout strains sensitive to paraquat [40,44,45].

We know very little about the biogenesis and possible assembly factors for the membrane-buried SDHC and SDHD and our knowledge of the role of the heme group in the function of SDH is also largely unknown. There remains much more to be discovered about the assembly and function of SDH and its subunits, including its involvement in 'noncanonical' processes, such as the role of SDHC in the formation of the TIM complex [46].

Post-translational Modifications of SDH Subunits and Their Role in Metabolic Adaptation

Phosphorylation

An *in vitro* kinase assay experiment using purified mitochondria and mass spectrometry showed that the tyrosine kinase Fgr phosphorylates two tyrosine residues (Y535 and Y596 in the rat sequence) of SDHA [47]. Further experiments revealed that SDHA phosphorylation by the Fgr kinase is enhanced under certain conditions [48]. Generation of hydrogen peroxide triggers SDHA phosphorylation and increases CII-dependent respiration, suggesting that SDHA phosphorylation serves as a metabolic adaptation in response to ROS production [49]. Mitochondrially localized phosphatases are involved in the reverse process, dephosphorylation of SDHA. PTEN-like mitochondrial phosphatase-1 (PTPMT1), shown to play a role in cardiolipin biogenesis via dephosphorylation of phosphatidylglycerol phosphate [50,51], also dephosphorylates SDHA in relation to the glucose level as an adaptive mechanism [52]. Other possible signaling cascades or metabolic reprogramming that would modulate SDHA (de) phosphorylation and regulate CII activity (Figure 3) as adaptation mechanisms to pathophysiological conditions remain to be uncovered.

Acetylation and Deacetylation

In addition to their presence in the nucleus and cytosol, acetyltransferases mediating acetylation of lysine residues have been found in mitochondria [53]. The enzyme referred to as 'general control of amino acid synthesis 5-like-1' (GCN5L1), a member of the acetyltransferase family, modifies certain respiratory chain proteins in mitochondria. Such acetylation will exert a negative effect on RCs, as GCN5L1-knockdown cells exhibit high oxygen consumption [53]. However, there are no data on direct involvement of GCN5L1 in acetylation of SDH proteins.

The deacetylation process is performed mainly by the NAD^+-dependent deacetylase SIRT3 [54]. Tandem mass spectrometry has revealed that four lysine residues (K179, K485, K498, and K538 in the mouse protein) are acetylated in SDHA [54,55]. Modeling of mouse SDHA using the CII crystal structure [2] showed that acetylation of these residues negatively regulates entry of the substrate into the SDHA active site [54]. SDH activity was increased in SIRT3-knockout mice, indicating that acetylation may inhibit SDH activity (Figure 3). As SIRT3 activity is modulated by the metabolic 'sensor' NAD^+/NADH ratio, reflected by SDH activity, the question arises of whether the TCA cycle, which involves SDH, also modulates the activity of SIRT3 by controlling the availability of NADH. Further studies are needed to

resolve whether unidirectional or bidirectional crosstalk between SDH and SIRT3 plays a role in the adaptation of the cell to alterations in metabolic state.

Succinylation

Lysine succinylation is another post-translational modification that regulates protein function. Unlike other Lys deacetylases of the sirtuin family, SIRT5 has desuccinylation activity [56,57]. A proteomics study identified succinylation sites in both SDHA and SDHB [58]. SIRT5-knockout mouse embryonic fibroblasts showed higher levels of SDH activity and cellular respiration, indicating the possibility of succinylation-mediated modulation of the SDH complex (Figure 3). The molecular events leading to SIRT5-dependent desuccinylation of SDH under (patho) physiological conditions are not fully understood. Inactivation of SDH leads to the accumulation of succinate as well as accumulation of the downstream metabolite succinyl-CoA, resulting in hypersuccinylation of other cellular proteins [59]. Although there is evidence for (de)succinylation and its physiological role [57,58,60], no proteins have been identified to perform the succinylation process. Several reports document that protein succinylation and acetylation may be non-enzymatic processes [61]. Recent proteomics data have shown partial overlap of succinylation and acetylation sites in mitochondrial proteins [62]. Future studies will dissect the role of acetylation and succinylation in the modulation of SDH activity.

Other Types of Post-translational Modification

Several other types of post-translational modification regulate the activity of mitochondrial proteins. *O*-GlcN-acylation, *S*-nitrosylation, lysine malonylation, lysine glutarylation, and ADP-ribosylation have been proposed to play roles in mitochondrial activity and function [63–65]. For instance, an affinity enrichment and label-free quantitative proteomics approach revealed that K179 of SDHA is malonylated in SIRT5-knockout mice [62]. The effect of these emerging post-translational modifications on SDH activity and their link to clinical presentations remain to be established.

ROS Production by SDH and its Relevance in Pathologies

It has long been assumed that OXPHOS-derived ROS can be produced only at CI and CIII. However, new data indicate that CII is also a significant source of ROS, relevant in several pathological conditions and in cell death [11,66,67]. Along with CI and CIII, CII/SDH is one of the top mitochondrial ROS producers in terms of maximal capacity [68]. Work in isolated mitochondria demonstrated that mammalian CII produces ROS at the FAD prosthetic group in SHDA under conditions that favor FAD reduction [69,70]; for example, when the Q_p site of CII is inhibited. Importantly, ROS generation from FAD is suppressed by high concentrations of the CII substrate succinate or by the intrinsic inhibitor 2-oxalacetate to FAD. In some specific situations, such as in the presence of manganese ions, the Q_p site can also produce ROS [71].

Conditions that favor reduction of FAD (such as Q^p site inhibition or mutations therein) will also reduce CII activity, favoring intracellular succinate accumulation. This complicates the situation in intact cells. Using site-directed mutagenesis, we have recently demonstrated that

CII-derived ROS are induced on direct Q_p site blockade in intact cells, but only when succinate levels do not rise quickly (e.g., with medium-affinity inhibitors) [72]. We have also shown that CII mutations that significantly reduce CII activity and therefore elevate intracellular succinate suppress CII-derived ROS [72]. This suggests that these mutations might induce substantial ROS production from CII only in a heterozygous state, where the remaining wild-type allele is able to keep the intracellular succinate concentration low. This is in agreement with findings that overexpression of a mutant CII SDHC subunit on the wild-type CII background resulted in chronic ROS elevation that could lead to cancer [73].

Besides its direct role, CII also plays a crucial indirect role in ROS generation. This occurs at high intracellular succinate concentrations that allow CII to efficiently reduce UbQ, leading to **reverse electron transfer (RET)** to CI and CI-derived ROS generation. The physiological relevance of this scenario in intact cells and tissues was first unequivocally demonstrated during ischemia–reperfusion (IR) injury [74]. In this case succinate accumulates during ischemia via reverse activity of SDH, represented by reduction of fumarate to succinate under anaerobic conditions. During reperfusion the accumulated succinate rapidly oxidizes and feeds the RET-generated ROS production. Recently, the RET-derived ROS have also been shown to promote a proinflammatory state in lipopolysaccharide-stimulated M1 **macrophages** [75] and this was dependent on high intracellular succinate and CII activity. In another study addition of **itaconate**, which is a newly discovered inhibitor of SDH, suppressed the expression of proinflammatory genes in macrophages while exerting anti-inflammatory effects [76], and macrophages could not control bacterial infection when their SDH activity was inhibited [49]. Based on emerging data, CII significantly contributes to ROS generation in cells and tissues both directly and indirectly. However, it remains unknown whether the post-translational modifications discussed earlier modulate the propensity of CII to induce ROS generation.

Emerging Role of SDH in Epigenomic Regulation

Previously described pathological conditions associated with SDH dysfunction are largely due to mutations in or deletions of genes encoding SDH subunits. Interestingly, **epigenetic regulation** by DNA methylation of the promoter regions of these genes has been shown to cause a pathological phenotype. A case report indicated that hypermethylation of CpG islands of the *SDHC* promoter caused decreased transcription of the gene resulting in GIST [77]. In this case, hypermethylation of the *SDHC* promoter resulted in a decrease in the protein levels of SDHB and SDHC [77], with lower SDH activity. *SDHC* promoter methylation, without mutation of the gene, has been demonstrated in several GIST and PGL patients [78–80]. Emerging data thus indicate a link between mutations and the epigenomic regulation of genes encoding SDHC subunits and neoplasias [78,80].

Metabolites whose abnormal accumulation promotes malignant transformation are referred to as oncometabolites. The list of potential oncometabolites includes α-ketoglutarate, fumarate, and succinate [81], which are intermediates of the TCA cycle (Box 1). Recent findings show that succinate modulates the epigenomic landscape, translating into pathological states [82]. Succinate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases due to its structural similarity with α-ketoglutarate [82–85]. Among the large

family of α-ketoglutarate-dependent dioxygenases (>60 in mammals), succinate inhibits the jumonji domain-containing (JMJC) and 'ten–eleven translocation' (TET) proteins [86]. Succinate has also been shown to inhibit another dioxygenase family of proteins, the prolyl 4-hydroxylases-2 and -3, that post-translationally regulate Hif1α by its stabilization [12,23].

JMJC proteins, of which about 30 have been identified in humans, play an important role in demethylation of histones, utilizing α-ketoglutarate as a cofactor [87]. Inhibition of SDH activity with 2-thenoyl trifluoroacetic acid or depletion of SDHB and SDHD resulted in an increase in steady-state histone-3 methylation, the phenotype being rescued by overexpression of JMJD3, implicating inhibition of JMJD3 by succinate [88]. Addition of cell-permeable methyl succinate to HeLa and HEK293T cells lead to a two- to fourfold increase in histone methylation, mainly monomethylation of H3K4, dimethylation of H3K27 and H3K79, and trimethylation of H3K4 [85].

The TET proteins belong to the same family as JMJD proteins, also using α-ketoglutarate as a cofactor; their major role is the removal of methyl groups from cytosine, particularly within CpG sites [89]. The tumor suppressors TET1 and TET2 are responsible for the oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) (Box 3). It has been shown that succinate inhibits the enzymatic activity of TET proteins and leads to the loss of 5hmC [85,89]. This stems from results with knockdown of SDHx in HEK293T cells showing loss of 5hmC, implicating succinate-mediated inhibition of TET1 and TET2 [85].

The role of succinate in epigenetics was further documented by genome-wide methylation analysis in PGL [90] and GIST [91] patients. These studies showed that SDH-malfunctional GISTs feature a global increase in DNA methylation and deficiency of 5hmC [91], in support of the above *in vitro* studies. SDH-mutant GIST patients have over 400 hypermethylated sites, while only 19 hypermethylated sites were discovered in *KIT*-mutant tumors [91]. A genome-wide study of methylation in PGL and PHEO tumors with mutations in the *SDHx, VHL, NF1*, and *RET* genes showed that the hypermethylated phenotype was associated with mutations in *SDHx* [90]. This is accentuated by the survival of patients, which is negatively correlated with methylation, with the prognosis linked to *SDHx* mutation-associated hypermethylation being much worse than that for other PGL/PHEO-susceptible genes [90]. This points to the role of succinate as an underlying cause of DNA methylation alterations with pathological presentation. Taking these findings together, mitochondria-mediated regulation of apoptosis, ROS production, and accumulation of HIF-α are not the only molecular mechanisms underlying the pathogenesis of diseases associated with SDH dysfunction, since recent findings link chromatin remodeling and DNA methylation alterations that contribute to the pathologies as a result of malfunctional SDH. In support of this notion, mutations in epigenetic regulatory proteins are also linked to PGL tumorigenesis [92].

SDH as a Potential Therapeutic Target

CII has been proposed as a target for therapy in highly debilitating pathologies including cancer, IR injury, and inflammatory diseases, with potential clinical relevance. In the context of cancer, CII plays a role in apoptosis induced by intrinsic and extrinsic agents, a notion

from Grimm's group reporting on the involvement of SDHC [93]. We then identified SDH as a target for anticancer agents, epitomized by the malignant cell-selective vitamin E analog α-tocopheryl succinate (α-TOS) [94] and documented the UbQ-binding Q_P site as its molecular target [95], showing that-TOS binds to Ser68 of SDHC (which also binds UbQ [2]) with affinity similar to that for UbQ [95] (Figure 4). We then tagged α-TOS, a member of the mitocan group of anticancer agents [96], with the triphenylphosphonium (TPP^+) group targeting the IMM to enhance its anticancer efficacy via its juxtaposition to its molecular target. Mitochondria-targeted vitamin E succinate (MitoVES) was found to interact with SDH and to kill cancer cells and suppress experimental tumors with 20–50-fold increased efficacy compared with the parental compound by inducing CII-derived ROS without substantial succinate accumulation [10,72,97]. Several other potential anticancer agents targeting SDH have been reported (reviewed in [4]). The SQR activity of CII was recently shown to be inhibited by the anticancer agent lonidamine [98].

Emerging data also show a promising prospect for CII as a target for IR injury and inflammatory diseases. For instance, intravenous infusion of dimethyl malonate or dimethyl itaconate in an *in vivo* model of cardiac and brain IR injury alleviated the symptoms [74,76]. In addition, CII appears to protect cells by maintaining the integrity of OXPHOS, which can be enhanced by small molecules [97]. Whether these CII-targeting compounds, such as the highly efficient and selective MitoVES, will be translated into clinical use remains to be shown.

Concluding Remarks

Novel approaches in the fields of proteomic, metabolomic, genomic, and epigenomic regulation have allowed in-depth analysis of SDH/CII function. It is clear that SDH has emerged as a key regulator of both mitochondrial and cellular metabolism. Promising data are being reported on the involvement of accumulation of succinate as a secondary signaling molecule in human diseases with potential to be used in personalized medicine. However, further work is required to understand the link of SDH dysfunction and the various relevant signaling cascades (see Outstanding Questions). This research is considerably hindered by the paucity of appropriate models. For example, there is no cell line derived from SDH-deficient PGL patients despite the concerted effort of several prominent laboratories. The recent introduction of patient-derived xenografts may be a way to study PGL in a more realistic setting. We believe that the emerging novel findings will facilitate badly needed progress in translating our improving understanding of the molecular biology and biochemistry of CII into clinical practice.

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Glossary

Assembly factors

assessory proteins that help insert prosthetic groups into the subunits of mitochondrial RCs, including CII, resulting in their full assembly and acquisition of function; also help stabilize subunits of CII or intermediate complexes before complete assembly of CII.

Epigenetic regulation
altered expression of genes mediated by simple modification of bases in specific regions of genes (e.g., methylation of CpG islands).

Itaconate (methylene succinic acid)
a decarboxylation product of the TCA intermediate metabolite cis-aconitate in a reaction catalyzed by immune-responsive gene 1 (IRG1); has antimicrobial activity and inhibits SDH.

Macrophages
innate immune cells involved in the host defense system that are divided into two major subtypes: M1 and M2 cells. M1 cells are activated by classical stimuli such as lipopolysaccharide (LPS), interferon gamma (IFNγ), and bacteria. Activated M1 macrophages are marked by an increase in glycolysis, a decrease in mitochondrial respiratory activity, and a change in TCA cycle activity (mainly accumulation of succinate and citrate). M2 macrophages are activated by interleukin-4 (IL-4) and IL-13, maintaining active OXPHOS and the TCA cycle.

Oxidative phosphorylation (OXPHOS)
process of generation of ATP at the expense of oxygen.

Reactive oxygen species (ROS)
a collective name for various forms of oxygen-containing small molecules (such as superoxide) that have an important function in biological systems, including regulation of various signaling pathways as well as causing damage to a variety of biologically active molecules.

Respiratory complexes (RCs)
mitochondrial complexes located in the inner mitochondrial membrane that are involved in the movement of electrons between CI/CII and CIV via CIII and in generation of the proton motive force.

Reverse electron transfer (RET)
from CII via UbQ to CI; a major pathway of mitochondrial ROS production.

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Trends

Recent research points to a major role of complex II (CII) in various aspects of cell biology, being at the crossroads of oxidative phosphorylation and the tricarboxylic acid cycle. CII therefore represents a branching point of two essential mitochondrial pathways.

Dysfunction of succinate dehydrogenase (SDH) leads to accumulation of succinate, which is categorized as an oncometabolite and signaling molecule.

CII is a major player in mitochondrial reactive oxygen species (ROS) generation and contributes ROS either directly or indirectly via reverse electron transfer (RET).

Genetic mutations as well as epigenetic regulation of the *SDHx* genes are associated with several pathological conditions. Similarly, both metabolic and epigenetic malfunctions have emerged as the underlying molecular mechanisms of the pathologies.

Several novel accessory proteins have been identified as crucial for the biogenesis of the SDH complex. They are referred to as assembly factors and emerging data show mutations in their genes to be linked to dysfunction of CII with clinical presentation.

Modulation of SDH function under various metabolic conditions could be utilized as a promising therapeutic target.

Outstanding Questions

New players in the assembly of CII have been recently discovered but the assembly process is not yet fully understood. The association of CII assembly factors with pathological conditions is also unclear. Are there additional, unknown assembly factors and what is the extent of the evolutionary conservation of the CII assembly process?

What are the signaling cascades leading to post-translational regulation of SDH in response to various pathophysiological conditions? Only a few relevant proteins modulating these processes have been identified to date. Furthermore, it is unclear whether these post-translational modifications impact the propensity of CII to produce ROS and affect succinate accumulation.

Dysfunction of SDH leads to accumulation of succinate. Recent data show that this results in inhibition of the activity of the JMC, TET, and PHD enzymes, all of which belong to the large dioxygenase protein family. The effect of elevated succinate on the activity of other dioxygenases remains unknown.

What is the driving force for the competition of SDH and CI for ubiquinone and what are the molecular events leading to direct or RET-mediated ROS production? Do these events play a major role in pathologies such as IR or inflammatory diseases and can this be exploited for cancer therapy?

Are the roles of CII in the TCA cycle and OXPHOS equally important or is the role in the TCA cycle dominant?

Box 1.

Neplastic Pathologies Linked to SDH Mutation

Problem with CII	Pathology	Manifestation	Refs
SDHA-D	PHEO/PGL	Hypertention, palpitation, sweating	[99]
SDHA-D	GIST	Abdominal pain, gastrointestinal bleeding	[91, 100]
SDHA-D	Renal cell carcinoma	Hematuria, abdominal pain	[101–103]
SDHA-D	Carney triad	Similar to PHEO/PGL and GIST	[104]
SDHA-D	Carney dyad (Carney–Stratakis syndrome)	Similar to PHEO/PGL and GIST	[105,106]
SDHAF2	PHEO/PLG, preferentially head and neck PGL mainly in young adults	Similar to PHEO/PGL	[107,108]
SDHB	Pancreatic NET	Abdominal pain	[109]
SDHB	Ganglioneuroma	Abdominal pain	[109]
SDHA-D	PHEO/PGL and pituitary adenoma (3PAs)	Similar to PHEO/PGL; headache, visual problems, clinical characteristics related to hypersecretion of GH and PRL	[110–112]

Carney triad: PHEO/PGL together with pulmonary chondroma and GIST; very rarely ganglioneuroma or neuroblastoma is present.

Carney dyad (Carney–Stratakis syndrome): PHEO/PGL together with GIST.

NET, neuroendocrine tumor; GH, growth hormone; PRL, prolactin.

Box 2.

Composition of CII and the Accessory Proteins

Component in human (yeast/bacteria)	Function
SDHA (Sdh1/SdhA)	Component of CII; part of the matrix portion of CII, contains the SDH catalytic site containing FAD;~70 kDa
SDHB (Sdh2/SdhB)	Component of CII; part of the matrix portion of CII, contains three [Fe–S] clusters, carries electrons from the catalytic site in SDHA to the UbQ site;~30 kDa
SDHC (Sdh3/SdhC)	Component of CII, buried in the IMM, contributes to the UbQ site;~18 kDa; proposed to play a noncanonical role in the formation of the translocator of the inner membrane complex
SDHD (Sdh4/SdhD)	Component of CII; buried in the IMM, contributes to the UbQ site;~12 kDa
SDHAF1 (Sdh6/ns[a])	Assembly factor 1; involved in [Fe–S] insertion into SDHB
SDHAF2 or SDH5 (Sdh5/SdhE)	Assembly factor 2; also known as SDH5; shown to insert FAD into SDHA; recent data indicate its redundancy, at least in some types of mammalian cells
SDHAF3 (Sdh7 or Acn9/ns)	Assembly factor 3; thought to be involved in [Fe–S] insertion into SDHB
SDHAF4 (Sdh8/ns)	Assembly factor 5; proposed to facilitate interaction of SDHA and SDHB (demonstrated for their Sdha and Sdh2 *Drosophila* homologs)

[a] ns, not studied/uncharacterized

Box 3.

Epigenetic Regulation and Function

Epigenetics regulate gene expression in response to various intrinsic and extrinsic stimuli, creating an adaptive mechanism of cellular physiology to environmental changes. It passes environmental stimuli to the genetic system by means of three mechanisms: DNA methylation, post-translational histone modification (chromatic remodeling), and the use of noncoding RNA [113]. DNA methylation is the addition of a methyl or hyroxymethyl group to the 5′ position of cytosine resulting in the generation of 5mC 5hmC. The cytosine residue preceding guanine repeats, referred to as the CpG island, is a preferred site of methylation that results in suppression of gene expression either by inhibition of the binding of a transcription factor or by recruitment of proteins involved in gene repression. Methylation is reversible (counteracted by demethylation), being a three-step process; interestingly, 5mC and 5hmC follow distinct demethylation processes. Demethylation of 5mC, achieved by deamination of 5mC to thymine, is followed by thymine deletion and then by repairvia base excision (replacement of cytosine). Demethylation of 5hmC involves either its oxidation initially to 5-formylcytosine (5fC) and then to 5-carboxycytosine (5caC) or its deamination into 5-hydroxymethyluracil (5hmU) [114]. Next, oxy-mC intermediates are excised and non-methylated cytosine is replaced by means of the DNA repair enzymes. There are data indicating that 5hmC is also an intermediate product of 5mC during the demthylation process [115,116]. In addition, demethylation could be a non-enzymatic process where DNA with methylcytosine is eventually diluted through mitosis, leading to the accumulation of non-methylated, newly synthesized DNA strands [114,116].

Histone modification modulates chromatin opening/condensation, playing a key role in gene replication and expression. Chromatin contains DNA, RNA, and proteins that wrap around histone octamers [117]. Each histone octamer contains two copies of the core histones (H2A, H2B, H3, and H4) [82,113,117]. This structure creates a highly compact chromatin that hinders its accessablity by factors that use DNA as a template, including the transcription machinery. It has been shown that post-translational modifications of histones lead to remodeling of the chromatin structure that alters its accessibility by the transcriptional machinery, resulting in either in transcriptional activation or repression [82,117]. In addition to succinate, other mitochondrial TCA cycle products such as fumarate, a-ketoglutarate, and acetyl-CoA contribute to post-translational modification of histones [82,113].

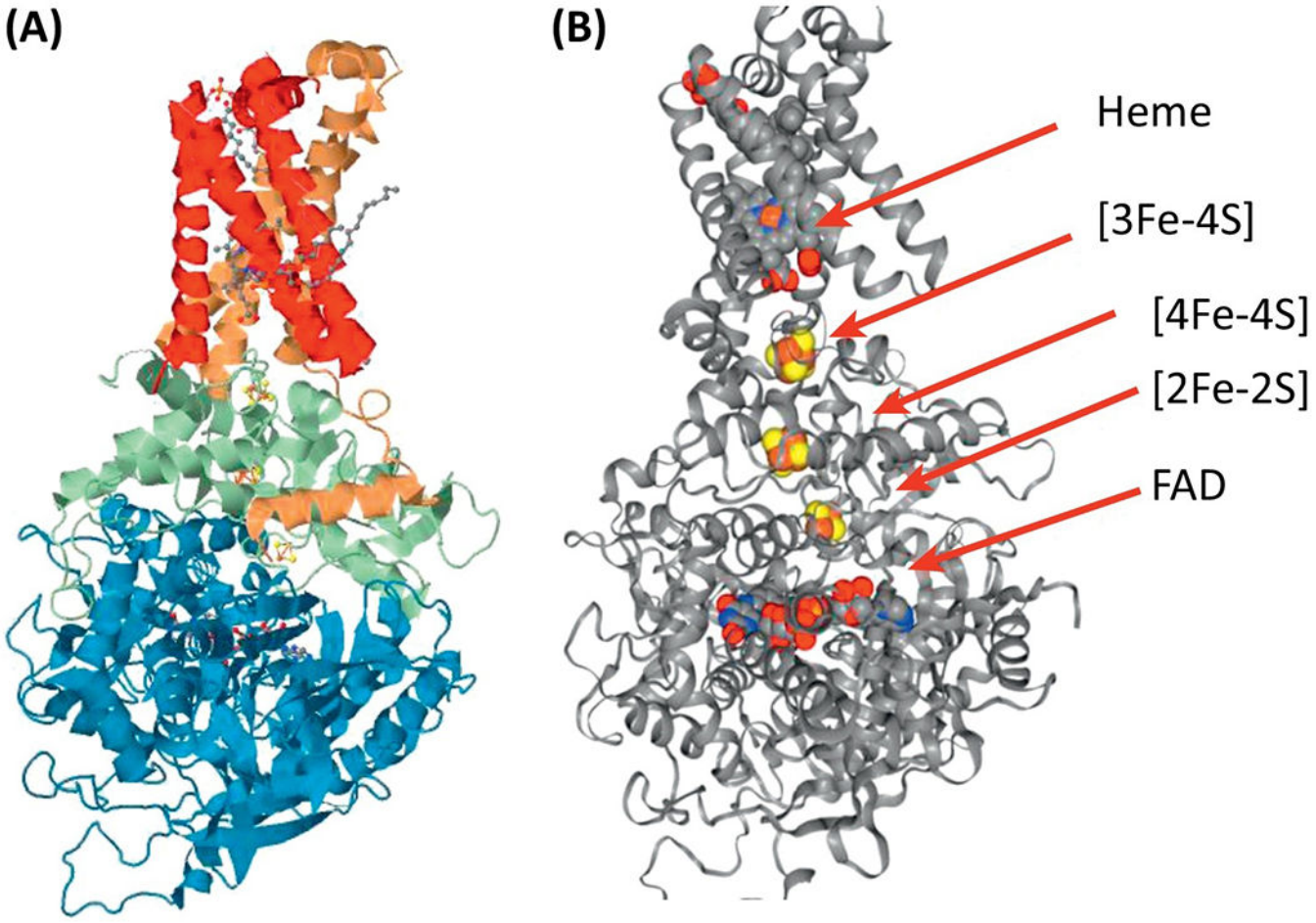

Figure 1. Crystal Structure of Succinate Dehydrogenase (SDH) Isolated from Porcine Heart Mitochondria and its Three Prosthetic Groups.

(A) shows subunits of SDH: SDHA (blue), SDHB (green), SDHC (brown), and SDHD (red). (B) highlights three prosthetic groups of SDH. For details see [2] (PDB:1ZOY).

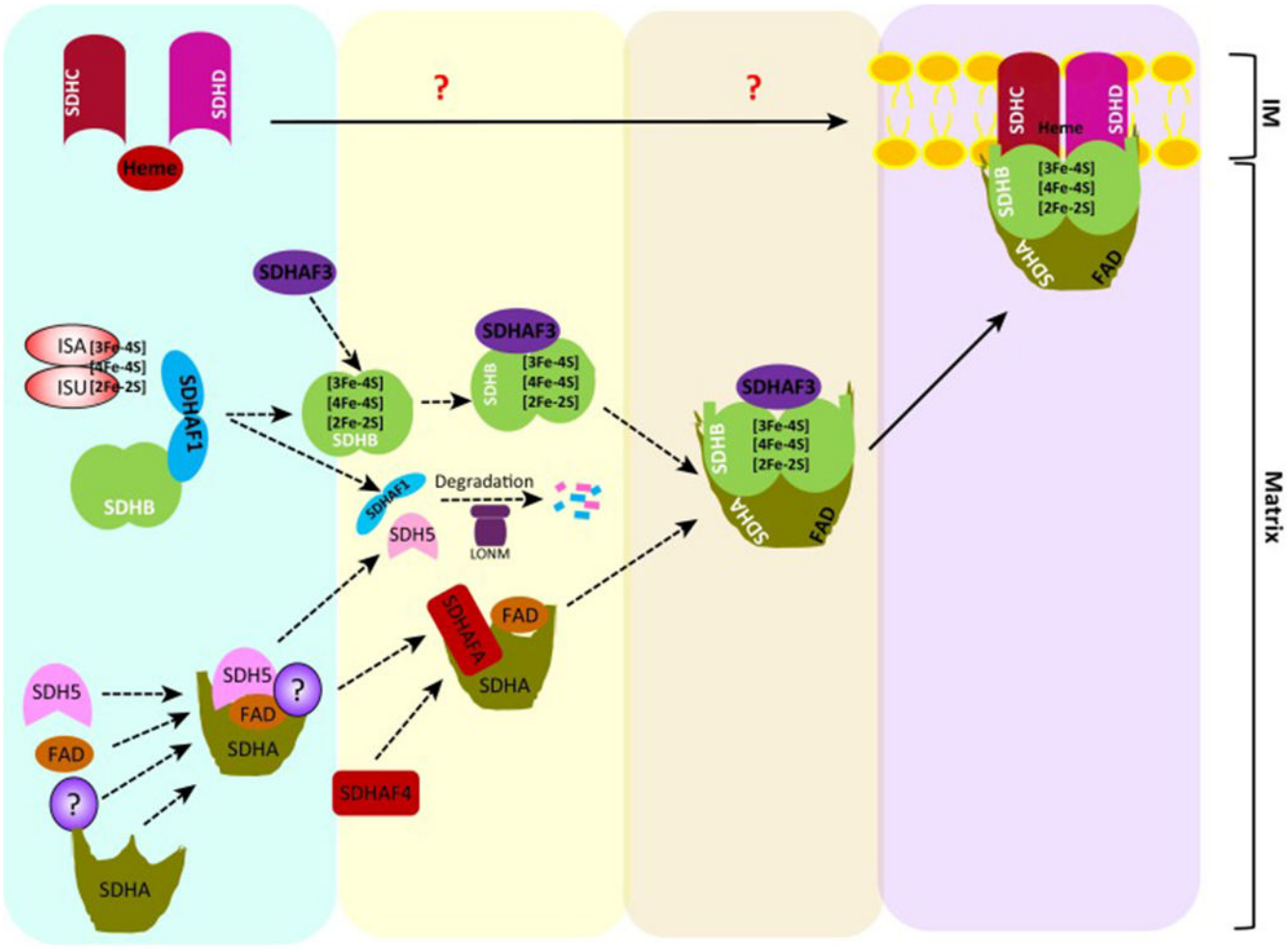

Figure 2. Step-Wise Assembly of Succinate Dehydrogenase (SDH) and the Putative Role of the Protease LON in the Degradation of SDHAF1 and SDHAF2/SDH5.

Flavination of SDHA requires SDH5/SDHAF2 and other putative factors. Following flavination, the chaperone-like assembly factor SDHAF4 binds to SDHA to reduce auto-oxidation. The insertion of [Fe–S] clusters into SDHB requires the assembly factor SDHAF1 and later SDHAF3 to protect the system from oxidative damage. SDHAF3 also facilitates the formation of a transitional SDHA–SDHB subcomplex. The assembly of the SDHC and SDHD subunits and heme to form the holoenyme is depicted. The detailed mechanism of this process remains to be discovered.

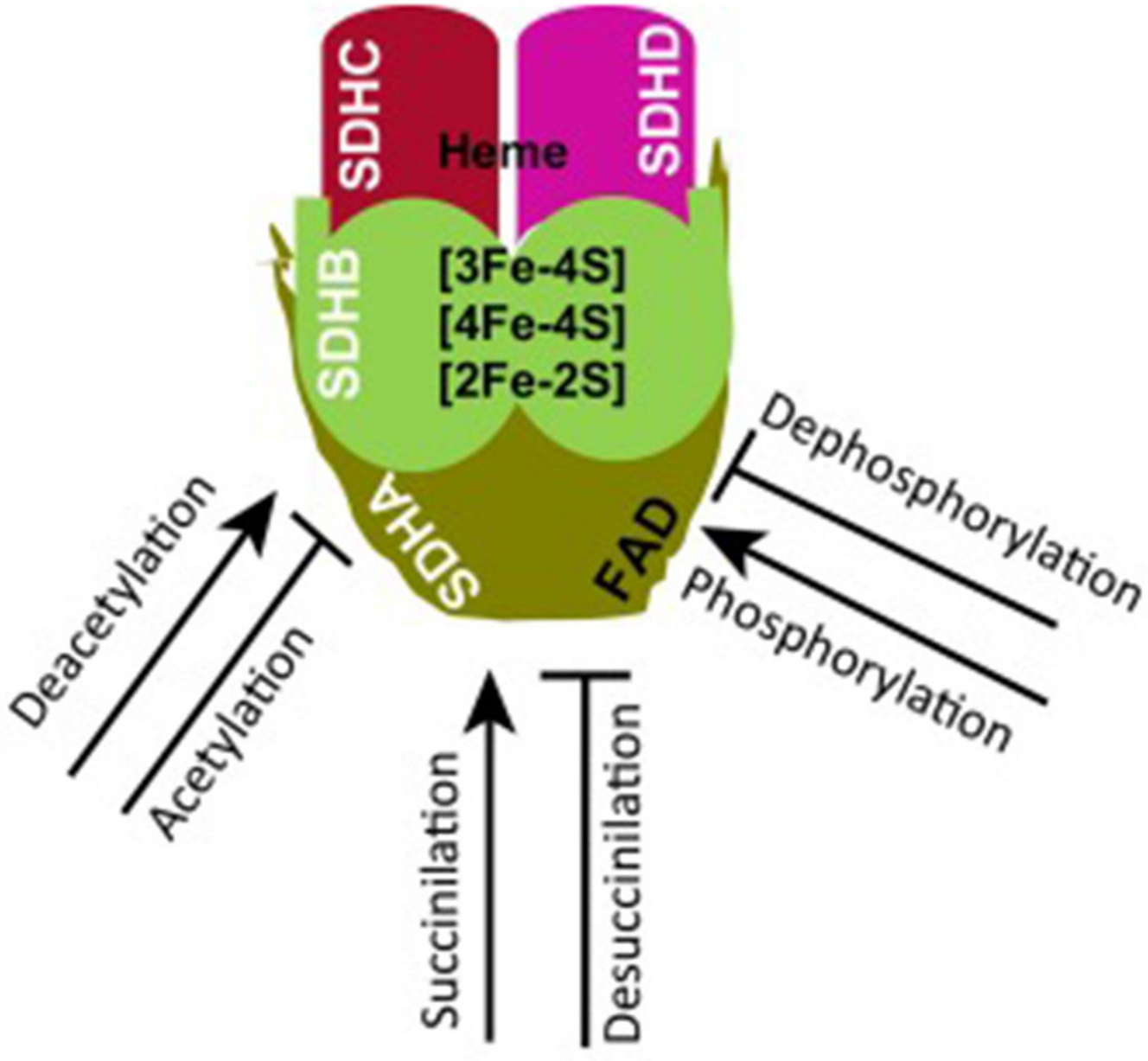

Figure 3. Post-translational Modifications of Succinate Dehydrogenase (SDH) Subunits that Affect the Activity of the Complex.

Phosphorylation, deacetylation, and succinylation are shown to increase the activity of SDH while the reverse modifications reduce its enzymatic activity.

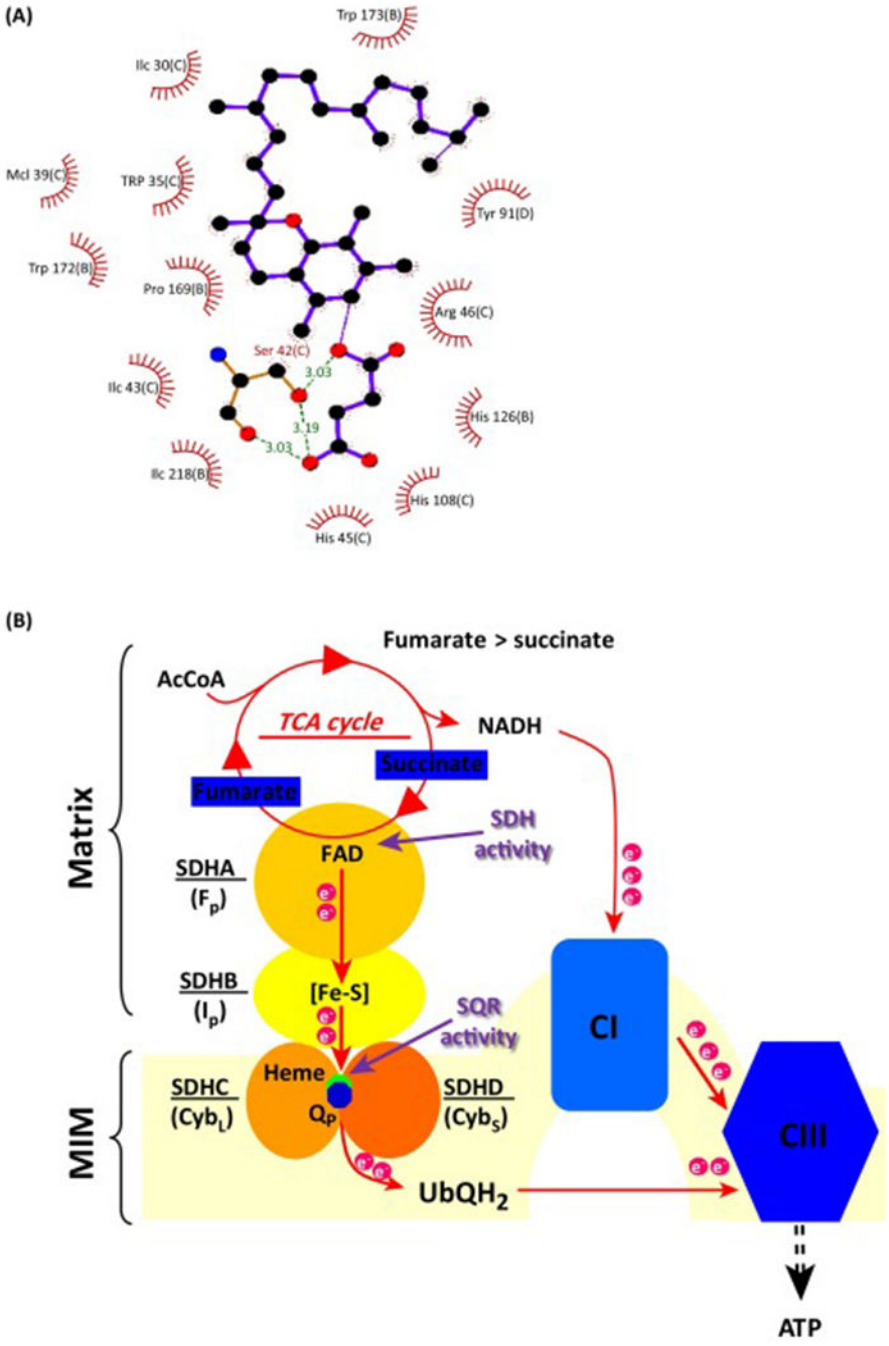

Figure 4. Succinate Dehydrogenase (SDH) as a Target for Anticancer Agents.

(**A**) The scheme documents the position of α-tocpheryl succinate (α-TOS) within the SDHC subunit, including its hydrogen bonding to the ubiquinone (UbQ)-binding Ser42 residue of the SDHC subunit. Adapted from [92]. (**B**) The scheme indicates the subunits of complex II (CII) and its SDH and SQR activities lined to the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS). α-TOS acts by interfering with the UbQ-binding site, resulting in ROS generation and, in turn, induction of apoptosis.