Published in final edited form as:

Mitochondrion. 2010 June ; 10(4): 393–401. doi:10.1016/j.mito.2010.03.001.

Succinate Dehydrogenase—Assembly, Regulation and Role in Human Disease

Jared Rutter¹, Dennis R. Winge^{1,2}, and Joshua D. Schiffman^{3,4}

¹Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT

²Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, UT

³Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT

⁴Department of Oncological Sciences, University of Utah School of Medicine, Salt Lake City, UT

Abstract

Succinate dehydrogenase (or Electron Transport Chain Complex II) has been the subject of a focused but significant renaissance. This complex, which has been the least studied of the mitochondrial respiratory complexes has seen renewed interest due to the discovery of its role in human disease. Under this heightened scrutiny, the succinate dehydrogenase complex has proven to be a fascinating machine, whose regulation and assembly requires additional factors that are beginning to be discovered. Mutations in these factors and in the structural subunits of the complex itself cause a variety of human diseases. The mechanisms underlying the pathogenesis of SDH mutations is beginning to be understood.

1. Mitochondrial Respiratory Chain

The generation of ATP in mitochondria is coupled to the oxidation of NADH and FADH₂ and reduction of oxygen to water within the respiratory chain. Energy from the oxidative respiratory chain is converted into a proton gradient across the mitochondrial inner membrane (IM) that drives ATP synthesis. The respiratory chain consists of four multisubunit protein complexes embedded within the IM in addition to mobile electron carriers, coenzyme Q (ubiquinone) and cytochrome *c*. Electrons from the oxidation of NADH are routed through Complex I to coenzyme Q, whereas electrons from the oxidation of carbon fuel substrates in the citric acid cycle that reduce FAD are funneled to ubiquinone through Complex II (succinate dehydrogenase). A third entry point to the electron transfer chain is the mammalian flavoprotein-ubiquinone oxidoreductase (ETF-QO) that directs electrons from the oxidation of fatty acids and some amino acids to the respiratory chain via reduction of ubiquinone. Reduced ubiquinol is oxidized by Complex III and subsequently electrons are transferred via cytochrome *c* to Complex IV where molecular oxygen is reduced to water. Proton pumping by Complexes I, III and IV generates the electrochemical gradient that is then utilized to drive ATP synthesis by Complex V (ATP synthase).

The electron transfer pathway in the oxidation of NADH by Complex I involves initial reduction of a FMN cofactor and subsequent transfer through 7 FeS clusters to the ubiquinone

binding site. The electron transfer pathway in the oxidation of succinate by Complex II involves initial reduction of a FAD cofactor followed by electron transfer through 3 FeS centers to ubiquinone (Hagerhall 1997; Sun, et al. 2005). In contrast, reduction of ubiquinone by the IM-associated ETF-QO links oxidation of 9 distinct matrix flavoprotein dehydrogenases with the respiratory chain. Electron transfer by ETF-QO occurs through a FeS center to a FAD moiety where ubiquinone is reduced (Zhang, et al. 2006).

2. Enzymology and Structure of SDH

Succinate dehydrogenase (SDH) is part of both the citric acid cycle and respiratory electron transfer chain. Within the citric acid cycle, SDH oxidizes succinate to fumarate. SDH is homologous in structure to an enzyme that catalyzes the reverse reaction during anaerobic respiration in bacteria, fumarate reductase (Hagerhall 1997). In fact, fumarate reductase in *E. coli* can functionally replace SDH in aerobic respiration and SDH can replace fumarate reductase in *E. coli* when expressed during anaerobic growth (Maklashina, et al. 1998).

Eukaryotic SDH consists of 4 subunits encoded by the nuclear genome. SDH is the only oxidative phosphorylation complex to lack subunits encoded by the mitochondrial genome and the only respiratory complex to not pump protons across the IM during its catalytic cycle. The structure of the porcine heart SDH consists of a hydrophilic head that protrudes into the matrix compartment and a hydrophobic tail that is embedded within the IM with a short segment projecting into the soluble intermembrane space (IMS) (Yankovskaya, et al. 2003; Sun, et al. 2005) (Fig. 1). The hydrophilic head consists of two subunits forming the catalytic core (Sdh1, Sdh2 in yeast and SdhA, SdhB in mammals). For simplicity and consistency, we will use the yeast nomenclature in this review.

The catalytic core Sdh1 and Sdh2 subunits contain the redox cofactors that participate in electron transfer to ubiquinone. Sdh1 contains the covalently bound FAD cofactor and the binding site for succinate. Sdh2 contains the 3 Fe/S centers that mediate electron transfer to ubiquinone (Hagerhall 1997; Sun, et al. 2005). The Fe/S centers in Sdh2 consist of a 2Fe-2S center proximal to the FAD site, an adjacent 4Fe-4S center followed by a 3Fe-4S center (Yankovskaya, et al. 2003; Sun, et al. 2005). Sdh2 also forms the interface between the catalytic domain and the membrane anchor domain of the complex. The packing interface of Sdh2 with Sdh1 and Sdh3 consists of a similar surface area for each interaction. This suggests that the catalytic core doesn't exist as a free dimeric entity in the absence of the membrane anchor. In fact, yeast lacking one of the membrane anchor subunits shows a marked decrease in abundance of both of the hydrophilic subunits, Sdh1 and Sdh2 (Hao, et al. 2009). In contrast, the *E. coli* SDH exists as an active soluble succinate dehydrogenase in the absence of the membrane domain subunits (Nakamura, et al. 1996). The soluble enzyme lacks ubiquinone reductase activity and shows activity only with artificial electron acceptors.

The membrane domain consists of two subunits (Sdh3, Sdh4 in yeast and SDHC, SDHD in mammals). The membrane domain contains a bound heme *b* moiety at the subunit interface with Sdh3 and Sdh4 each providing one of the two axial His ligands. Two ubiquinone binding sites have been identified in SDH complexes in mammals and *E. coli* (Yankovskaya, et al. 2003; Sun, et al. 2005). The high affinity ubiquinone site (Q_P -proximal) lies on the matrix side of the IM and is formed by residues in Sdh2, Sdh3 and Sdh4. The Q_P site lies within 7 Å (Angstrom sign) to the 3Fe-4S redox center and is the dominant ubiquinone site in yeast SDH (Oyedotun, et al. 2001; Silkin, et al. 2007). The second, low affinity ubiquinone site (Q_D – distal) resides closer to the IMS side of the IM. Ubiquinone reduction occurs in two stepwise single electron reactions, in contrast to the two electron reduction of FAD. The Q_P site markedly stabilizes the partially reduced semiquinone thereby permitting full reduction to

the ubiquinol (Yankovskaya, et al. 2003). Protonation of ubiquinol is likely attained by a conserved Tyr residue in the Q_p pocket (Silkin, et al. 2007).

The heme moiety associated with Sdh3 and Sdh4 is present in mammalian, yeast and *E. coli* SDHs, but diverse SDH species vary in the number of heme moieties and in their redox properties. This is consistent with the observation that membrane domain subunits show greater variability between SDHs and fumarate reductases than the highly conserved catalytic core domains (Hagerhall 1997). The membrane anchor heme can be reduced by succinate in certain SDH complexes, but not in others, including bovine SDH (Oyedotun, et al. 2007). Mutation of both axial heme His ligands results in a heme-free SDH complex that is competent to assemble and mediate succinate oxidation in yeast (Oyedotun, et al. 2007). The catalytic efficiency of the double mutant is only modestly impaired. Thus, the membrane domain heme lacks any critical role in catalysis. Similarly, the *E. coli* fumarate reductase lacks heme in its membrane domain, but is functional in succinate oxidation when expressed under aerobic conditions (Maklashina, et al. 1998).

The significance of the conserved heme moiety in eukaryotic SDHs and the distal Q_D site remain unclear. Whereas the heme is not essential for the reduction of ubiquinone at the Q_P site, it may mediate electron transfer to the distal Q_D site. SDH complexes that exhibit succinate reduction of heme may also form ubiquinol at the Q_D site, although proof of this is lacking. The presence of two Q sites in SDH does not result in any Q cycle as in the bc₁ Complex III since SDH does not pump protons.

The SDH enzymatic reaction commences with the binding of succinate to an open state in Sdh1. Binding of succinate leads to domain closure bringing succinate into juxtaposition of the isoalloxazine ring of FAD, where it is oxidized. Succinate oxidation is dependent on the covalent attachment of FAD at an active site His residue (Hagerhall 1997). Replacement of the His residue in the *E. coli* SDH leads to retention of bound FAD, but the mutant enzyme fails to oxidize succinate. The covalent attachment increases the FAD redox potential by ~60 mV to permit succinate oxidation (Tomasiak, et al. 2008). SDH is the major covalent flavoprotein in yeast (Hao, et al. 2009). Since oxidation of succinate involves the two electron reduction of FAD and the subsequent Fe/S centers are one electron carriers, two successive electron transfer steps are required from the FADH₂ to the 2Fe-2S center. Calculations based on the midpoint potentials of the *E. coli* SDH redox cofactors indicate that electrons in FADH₂ are rapidly transferred to the 3Fe-4S center and heme moiety restoring oxidized FAD. The lack of partially reduced FAD may account for the low ROS generation from SDH (Yankovskaya, et al. 2003). ROS generation may arise from dissociation of semiquinone. However, the Q_p is effective in stabilizing the semiquinone, thus favoring release of fully reduced ubiquinol (Yankovskaya, et al. 2003).

3. Regulation of SDH

The catalytic activity of SDH is modulated by post-translational phosphorylation and acetylation as well as active site inhibition. Reversible acetylation at multiple Lys residues in mouse Sdh1 was shown to attenuate catalytic activity of Sdh1 (Cimen, et al. 2009). SIRT3 is the major deacetylase controlling the level of Sdh1 acetylation. The Sdh1 subunit of SDH is phosphorylated in mammalian cells and, like acetylation, this modification appears to attenuate activity (Tomitsuka, et al. 2009). The Fgr tyrosine kinase is capable of phosphorylation of Sdh1 at two tyrosine residues in vitro, although the physiological significance of Fgr-mediated modification is not known (Salvi, et al. 2007).

SDH catalytic activity is also modulated by Krebs cycle intermediates including oxaloacetate, which is a potent inhibitor. Succinate promotes the dissociation of oxaloacetate from SDH

thereby activating the enzyme. The oxaloacetate inhibition may contribute to the known modulation of SDH activity by the metabolic status of mitochondria (Gutman, et al. 1975).

4. Assembly of succinate dehydrogenase

The assembly of electron transport chain complexes poses a difficult problem for the eukaryotic cell as Complexes I,III,IV and V contain subunits encoded by both nuclear and mitochondrial genomes thereby requiring coordination of synthesis and assembly. As a result, the cell dedicates a large number of proteins specifically for the assembly of these complexes. An expanding list of assembly factors is known for Complex I, whereas Complex IV or cytochrome oxidase requires >20 factors for its assembly and activity. The assembly of Complex II, on the other hand, has been remained relatively enigmatic. Prior to 2009, only a couple of factors were known to be required for SDH assembly and their roles remain poorly understood. Further, these factors are either not evolutionarily conserved or only act on SDH assembly indirectly. During 2009, however, two new factors have been described with dedicated and evolutionarily conserved roles in SDH assembly. Each of these factors will be discussed in turn, followed by a comment on the future of SDH assembly research.

4.1. Tcm62

The gene encoding Tcm62 was originally identified in a screen for mutants specifically lacking SDH activity (Dibrov, et al. 1998). Lemire and colleagues showed that the *tcm62* mutant almost completely lacked SDH activity, but had only modest defects in the activity of other ETC complexes. Furthermore, the *tcm62* mutant had normal levels of components of Complexes III, IV and V, but undetectable Sdh2 (Dibrov, et al. 1998). Finally, Lemire and colleagues provided evidence that Tcm62 directly interacts with SDH structural subunits. Tcm62 migrated in blue-native gel electrophoresis in a wild-type strain as a roughly 200kDa complex. In an *sdh1* or *sdh2* mutant, however, Tcm 62 migrated in a much larger ~450kDa form. The content of each of these complexes hasn't been defined. Taken together, these results suggested an essential role for Tcm62 in the assembly of the SDH complex.

While the importance of Tcm62 in SDH assembly has not been called into question, the specificity of that role has been. Langer and colleagues subsequently showed that Tcm62 was required for thermostability of mitochondrial respiratory function (Klanner, et al. 2000). Specifically, a *tcm62* mutant exhibited severe respiratory defects at 24°C, but complete loss of respiratory growth at 37°C. The same temperature sensitivity profile was observed when measuring rates of mitochondrial protein synthesis in the *tcm62* mutant strain (Klanner, et al. 2000). To examine whether Tcm62 might exert its function on mitochondrial protein synthesis through supporting mitochondrial protein folding, Langer and colleagues examined the solubility of Var1. Var1 is the only soluble protein encoded by the mitochondrial genome. At 24°C, Var1 was found predominantly in the soluble fraction in both wild-type and *tcm62* strains. At 37°C, however, Var1 became insoluble in the *tcm62* mutant but not the wild-type strain (Klanner, et al. 2000). These authors suggest that Tcm62 acts more generally than just in the assembly of the SDH complex, by supporting mitochondrial protein stability under stress.

Both the Lemire and Langer groups recognized the sequence similarity of Tcm62 to the Hsp60 class of protein chaperones (Dibrov, et al. 1988; Klanner, et al. 2000). While the sequence identity is not overwhelming, it is clearly significant and is suggestive that Tcm62 functions in a manner related to the Hsp60 family, which includes GroEL. Both groups show that Tcm62 is in a high-molecular weight complex, although the exact size is quite different. The Langer group shows that endogenous, untagged Tcm62 migrates in a ~850kDa complex, which is quite similar to GroEL and other similar chaperone complexes (Bukau, et al. 1998). Interestingly, however, the sequence of the Tcm62 protein indicates that it is probably incapable of ATP

binding and hydrolysis. Therefore, any chaperone function would be mechanistically distinct from the ATP-driven process carried out by GroEL and other Hsp60 family members.

In 2002, (Vander Heiden, et al. 2002) described the recovery of Tcm62 in a screen for genes that are required for respiratory growth except when the mammalian apoptosis inhibitor Bcl-x(L) was overexpressed. The authors show that the impaired diauxic growth of the *tcm62* mutant strain is partially rescued by Bcl-x(L) overexpression and that Tcm62 overexpression in mammalian cells inhibits apoptosis caused by growth factor withdrawal. These authors again speculate that the effects of Tcm62 might be due to general protein folding capacity in the mitochondria (Vander Heiden, et al. 2002).

While it is clear that Tcm62 is required for SDH assembly, it is not clear that it is dedicated to this function or that it plays this role specifically and directly. The answer to this question awaits an understanding of the precise biochemical activity of Tcm62. If it is a chaperone, are SDH subunits direct substrates? Are they the only substrates, or, as is more likely, does Tcm62 catalyze the folding of a variety of mitochondrial matrix proteins?

4.2. Flx1

Alex Tzagaloff and colleagues originally described the *FLX1* gene as being required for maintenance of a normal FAD/FMN ratio in mitochondria (Tzagaloff, et al. 1996). In addition to altered flavin cofactor levels, they showed that the *flx1* mutant failed to respire and had decreased mitochondrial FAD transport in assays of purified mitochondria performed in vitro. A role in FAD transport into mitochondria is supported by the primary structure of Flx1, which places it in the Mitochondrial Carrier Family of membranous small molecule transporters.

The straightforward model of Tzagaloff, which proposes Flx1 as a mitochondrial FAD importer, has been complicated, however, by the work of Barile and colleagues over the past six years. As would be expected, they found that two FAD-containing mitochondrial enzymes, Sdh1 and lipoamide dehydrogenase (Lpd1) had markedly impaired activity in an *flx1* mutant strain (Bafunno, et al. 2004). Unlike Tzagaloff, however, they suggest that Flx1 catalyzed FAD export and that mitochondrial FAD levels are unaffected by deletion of *FLX1*. Why then is the activity of SDH impaired? The authors suggest that this is due to a regulatory function of Flx1 on the post-transcriptional expression of Sdh1. To demonstrate this regulation, the authors constructed a reporter strain wherein the Sdh1 coding sequence was replaced by β -galactosidase. They showed that β -galactosidase activity was markedly reduced in the *flx1* mutant relative to a wild-type strain and this was independent of effects on *SDH1* transcription (Giancaspero, et al. 2008).

It is clear that Flx1 is a mitochondrial transporter and very likely is a flavin transporter. If the model of Barile is correct, it is hard to understand why the activity of FAD-dependent mitochondrial enzymes is impaired. Certainly, a direct role in Sdh1 regulation could account for a loss of SDH activity in the *flx1* mutant, but parsimony would suggest that the post-transcriptional regulation of Sdh1 by Flx1 is a secondary effect of altered mitochondrial flavins. It would not be at all surprising if Sdh1 synthesis were regulated to ensure that it was only made when adequate levels of its FAD cofactor were available.

Why would loss of mitochondrial FAD export lead to a loss of intramitochondrial SDH activity? Our experiments suggest that it is very unlikely to be due to impaired Sdh1 expression. As reviewed below, we observed a very modest decrease of Sdh1 protein levels in the *flx1* mutant, but a complete loss of covalent FAD incorporation (Hao, et al. 2009). Overexpression of *SDH5*, which is required for FAD incorporation, is able to partially restore the Sdh1-FAD covalent interaction that is lost in the *flx1* mutant. This is in the absence of any effects on Sdh1 protein levels. Interestingly, while *SDH5* overexpression rescues FAD incorporation into Sdh1,

it doesn't enable growth on non-fermentable carbon sources (Hao, et al. 2009). Therefore, we suggest that Flx1 is required for FAD incorporation into Sdh1 in a wild-type strain, but it is also necessary for additional functions required for respirative growth. The complexities of the data suggest that the *flx1* phenotype is probably not simply a manifestation of impaired FAD transport, although that seems to be clearly a component. The post-transcriptional regulation of Sdh1 protein levels is one additional layer and there are likely more that await discovery.

4.3. SDHAF1

The field of SDH assembly took two major steps forward in 2009. The first was the discovery of SDH Assembly Factor 1 (SDHAF1) by Zeviani and colleagues (Ghezzi, et al. 2009). This group had previously identified an Italian kindred with a highly progressive infantile leukoencephalopathy and showed that this pathology was accompanied by a significant decrease in SDH protein and activity (Bugiani, et al. 2006). Using genome-wide linkage analysis in this pedigree and a Turkish pedigree with nearly identical disease manifestations, the authors mapped the trait to a 1.2Mb region of chromosome 19. Utilizing mitochondrial protein prediction score, they sequenced candidate genes in this interval and found a homozygous mutation in a previously unannotated gene, which they named *SDHAF1* (Ghezzi, et al. 2009). This gene is highly conserved throughout eukaryotes and the two mutations found in the two pedigrees both disrupt highly conserved residues. The encoded protein is a small, 115 amino acid soluble protein of the mitochondrial matrix. Being a soluble protein, the authors concluded that SDHAF1 is not a stable component of the SDH complex and, therefore, must be an assembly factor.

Zeviani and colleagues took advantage of the evolutionary conservation of the *SDHAF1* gene family to study SDHAF1 in yeast. The yeast *S. cerevisiae* contains one *SDHAF1* homolog, named *YDR379C-A*. Deletion of *YDR379C-A* caused a complete failure to grow using acetate as the carbon source. This was accompanied by a 60-70% decrease in SDH activity, but no change in Complex IV activity. Mutations designed to mimic the disease-causing mutations found in the human gene failed to rescue these phenotypes, whereas a plasmid-borne copy of the wild-type yeast gene was fully functional (Ghezzi, et al. 2009).

It is clear the SDHAF1 is required for the stable assembly and full function of the SDH complex. A number of questions remain, however. First, is SDHAF1 indeed a dedicated SDH assembly factor (as hypothesized by Zeviani and colleagues) or does it play a broader role that has simply not yet been assayed? Why do mutations in *SDHAF1* present with infantile leukoencephalopathy, while other SDH mutations present with either tumor syndromes or Leigh syndrome? Perhaps the most intriguing question is the biochemical function of SDHAF1? Zeviani and colleagues provided no data pertaining to this question, but raised an interesting possibility based on the presence of an LYR motif in SDHAF1. A subset of other proteins bearing this motif have been implicated in functions related to the metabolism of Fe-S centers (Shi, et al. 2009). The authors, therefore, speculate that SDHAF1 might play a role in the insertion or retention of the Fe-S centers in the SDH complex. This intriguing possibility awaits biochemical analysis.

4.4. SDH5 (SDHAF2)

We recently initiated a project to determine the function of uncharacterized, but highly evolutionarily conserved mitochondrial proteins (Hao, et al. 2009). One of the proteins we chose to study was known by the systematic names Yol071 in yeast and C11orf79 in humans (Hao, et al. 2009). Initially using yeast as the primary model system, we showed the Yol071 was a soluble mitochondrial matrix protein that was required for growth on non-fermentable carbon sources and for normal respiration. The key observation that pointed us toward the SDH complex came from purifying the Yol071 protein and discovering that it specifically co-

purified with Sdh1. Following this observation, we went on to show that the *yo1071* mutant had undetectable SDH activity, while the activity of other TCA cycle enzymes and electron transport chain complexes were normal. The SDH complex seemed to partially assemble in the absence of Yoh1071 but was unstable. Based on its requirement for SDH function, we renamed *YOL071* as *SDH5*.

As with the other proposed SDH assembly factors, the major question for Sdh5 was its biochemical function. A dedicated role for Sdh5 in promoting the covalent FAD incorporation into Sdh1 is supported by the following pieces of evidence (Hao, et al. 2009). First, an *sdh5* mutant has undetectable FAD-Sdh1 conjugate, but only modestly reduced Sdh1 protein level. Second, overexpression of *SDH5* partially reduces the FAD incorporation defect of an *flx1* mutant, as described above. Finally and most directly, co-expression of Sdh5 but not Sdh2 with Sdh1 in *E. coli* increases FAD incorporation. We, therefore, propose that Sdh5 is a dedicated SDH assembly factor required for the covalent insertion of FAD into the catalytic Sdh1 subunit.

Nearly three decades earlier, Van Baars, et al. (van Baars, et al. 1982) had described a Dutch family with hereditary paraganglioma. In subsequent years, the gene was mapped to an interval on chromosome 11 (Mariman, et al. 1993; Mariman, et al. 1995), but the gene eluded identification. As we began to contemplate the potential disease relevance of our findings on the function of SDH5, we found that it lies in the exact interval implicated by Mariman and colleagues. In collaboration with Dr. Hannie Kremer and colleagues, we determined that the paraganglioma in this Dutch family is due to a G78R mutation in human *SDH5*. This mutation is found in all affected family members and leads to a severe decrease in SDHA FAD incorporation. When introduced into an *sdh5* mutant yeast strain, the wild-type but not the G78R mutant rescues both respiratory growth and Sdh1-FAD conjugation. The discovery and characterization of Sdh5 marks a new day in the study of the succinate dehydrogenase complex. We now know the identity and biochemical function of at least one SDH assembly factor. There are certainly more that await discovery.

4.5. The future of SDH assembly

This past year witnessed the discovery of the two first (apparently) dedicated SDH assembly factors, SDHAF1 and SDH5. The question remains whether there are others? Based on the precedent from other electron transport chain complexes, we would have to expect the answer to be yes. While Sdh5 might be responsible for insertion of the FAD cofactor, what about the four Fe-S centers and the heme? Even if SDHAF1 promotes insertion or stability of the Fe-S centers, it is unlikely to act alone. Unlike the *sdh5* mutant, the *ydr379c-a* mutant (lacking SDHAF1) has 30-40% residual SDH activity. Perhaps this is indicative of another factor, possibly bearing an LYR motif, which assists in Fe-S center insertion in SDH. As witnessed by the disease manifestations of mutations in SDHAF1 and SDH5, the SDH complex and its assembly is critical for human health. As we discover additional SDH assembly factors, we are very likely to uncover the molecular bases for currently enigmatic human diseases.

5. Disease associated with impaired SDH activity

5.1. Leigh syndrome—mutations in SDHA

Leigh syndrome, also known as Subacute Necrotizing Encephalomyelopathy (SNEM), is an early-onset progressive neurodegenerative disorder (Finsterer 2008). Patients with Leigh syndrome present with a characteristic neuropathology consisting of developmental delay or psychomotor regression, weakness, external ophthalmoplegia, lactic acidosis, ataxia, dystonia, vomiting, and seizures. The progressive neuropathy and accompanying symptoms are often recognized in early infancy and are due to either a sporadic or inherited metabolic dysfunction of the mitochondria. Patients will often have bilateral lesions consisting of foci of necrosis along

the spinal cord, brain stem, or brain. Specific symptoms will depend on the location of these progressively necrotic lesions. There is no known cure for Leigh syndrome, and patients often die from their disease within several months of being diagnosed.

Leigh syndrome is a genetically heterogeneous disease with multiple causes for alteration in mitochondrial function including defects or deficiencies in: electron transport chain Complexes I-V, the pyruvate dehydrogenase complex (PDHC), mitochondrial DNA (mtDNA), and mutations in the SURF1 gene. Complex II deficiency is very rare and thought to account for only 2-4% of the respiratory chain deficiencies (Rustin, et al. 1997; Parfait, et al. 2000).

Bourgeron et al. (1995) first described a mutation of the nuclear-encoded flavoprotein (Fp) subunit gene, or *SDHA*, to contribute to the clinical presentation of two siblings with Complex II deficient Leigh's syndrome (Bourgeron, et al. 1995). The parents of these children were first cousins and were heterozygous for the *SDHA* mutation, which was absent in 120 controls. This case report was important because it was the first time in humans that a nuclear gene mutation (*SDHA*) was found to cause a mitochondrial respiratory chain deficiency (Bourgeron, et al. 1995). This study was followed several years later by Parfait et al. (2000) who reported another patient with Complex II deficient Leigh syndrome and compound heterozygous mutations in *SDHA* (Parfait, et al. 2000). Since then, two other case reports also have described mutations in *SDHA* contributing to Leigh syndrome, including homozygous Gly555Glu mutation (Van Coster, et al. 2003) and another patient with atcompound heterozygous mutations (Horvath, et al. 2006). Horvath et al. (2006) also investigated six other patients with neurodegenerative symptoms of Leigh syndrome with isolated Complex II deficiency but could not identify any *SDHA* mutations, further supporting the genetic heterogeneity of this disorder. Finally, Birch-Machin et al. (2000) described single gene *SDHA* mutations in a family with symptoms of late-onset optic atrophy, ataxia, and myopathy similar to Leigh syndrome, but tended to occur in the fourth decade of life (Birch-Machin, et al. 2000). Interestingly, these patients all demonstrated only partial deficiency of both complex II and SDH in muscle mitochondria, perhaps explaining their later onset of disease symptoms.

Of note, *SDHB*, -C, -D, or -5 mutations have never been described in any of these progressive neurodegenerative syndromes related to mitochondrial Complex II deficiencies.

5.2. Familial paraganglioma syndrome – mutations in *SDHB*, -C, -D, -5

Paragangliomas (PGLs) are neuro-endocrine tumors that can occur in cells of the neural crest anywhere from the skull base to pelvic floor. PGLs are most commonly located adjacent to oxygen-sensing tissues such as the carotid body, although they also can be found in the adrenal glands where they are called pheochromocytomas (PCCs). When they occur as head and neck paragangliomas (HNPGL), these tumors often arise from the parasympathetic chain and only rarely secrete catecholamines. HNPGLs are often found at the bifurcation of the carotid artery where they are referred to as carotid body tumors. HNPGLs also occur in areas surrounding the carotid artery and alternatively have been referred to as glomus tumors including glomus jugulare, glomus tympanicum, and nonchromaffin PGLs (which often arise in the ear) (Ruben 2007). When PGLs (or PCCs) occur in the abdomen – either adrenal or extra-adrenal – they most often arise along the sympathetic chain and can secrete catecholamines such as epinephrine, norepinephrine, or dopamine. HNPGLs are usually benign, whereas the extra-adrenal PGLs like PCCs can be malignant. HNPGLs are estimated to occur with an incidence of 1:30,000-100,000 in the general public (Pasini, et al. 2009) and are slow-growing tumors, which although benign, can have significant morbidity due to their compression of vital organs as well as complications upon surgical removal. PGLs that secrete catecholamines can also lead to health issues related to uncontrolled hypertension. Known risk factors for PGLs include hypoxic conditions, including living at high altitude or cardiorespiratory diseases. In fact, HNPGLs have been reported to occur at 10-fold higher frequencies among high-altitude

inhabitants. Rodriguez-Cuevas et al. (Rodriguez-Cuevas, et al. 1998) described the differences in high-altitude PGLs in Mexico (>670 feet above sea level) compared to low-altitude PGLs in patients in the United States and Europe (<450 feet above sea level), and found high-altitude PGLs had extreme female predominance (8:1), low bilaterality, and low family history. Data from this and others studies (Rodriguez-Cuevas, et al. 1998; Astrom, et al. 2003; Jech, et al. 2006) suggest that high-altitude PGLs result from increased response to chronic hypoxic stimulation rather than an underlying SDH mutation (which are associated with bilateral tumors and family history).

In contrast to the majority of spontaneous or *de novo* paragangliomas, some families have inherited a predisposition to develop both HNPGLs and PCCs (adrenal and extra-adrenal). These patients often develop multiple, bilateral, and occasionally malignant PGLs. These tumors will occur at a young age (sometimes as young as 10 years old) and these patients are said to have Familial Paraganglioma Syndrome (FPS). Patients with FPS can be classified genetically into four clinical entities, PGL1, PGL2, PGL3, and PGL4, each of which will be discussed below and are summarized in Table 1. These four FPS clinical entities each now have been associated with germline mutations in succinate dehydrogenase (SDH) genes: *SDHD* (PGL1), *SDH5* (PGL2), *SDHC* (PGL3), and *SDHB* (PGL4). As described above, each of these SDH genes is required for activity of Complex II and contributes to the Krebs cycle, respiratory chain, and oxygen sensing. Interestingly, although it is clearly required for SDH assembly and function, *SDHA* mutations only have been associated with Leigh syndrome as discussed above, but never with an inherited tumor syndrome. While the incidence of PGLs in the healthy public is relatively low, 30% of individuals with underlying *SDHB*, *SDHC*, or *SDHD* mutations will develop PGLs by 30 years old, and 70% will develop PGLs by 80 years old (Ricketts, C. J., et al. 2009; Timmers, et al. 2009). Approximately 10% of PGLs are estimated to occur due to FPS and underlying SDH mutations (Martin, et al. 2007). Interestingly, PGLs in FPS – including HNPGLs – are more aggressive with increased metastasis, morbidity, and mortality (Neumann, et al. 2004; Boedeker, et al. 2007; Boedeker, et al. 2009). Other tumors associated with FPS include thyroid cancer, gastrointestinal stromal tumors (GIST), pulmonary chondromas, renal cell carcinoma, and even neuroblastoma, and these are discussed in the following section.

PGL1—Baysal et al. (Baysal, et al. 2000) identified the *SDHD* gene (11q23) to be mutated in this clinical entity, which very frequently includes HNPGLs and occasionally adrenal PCCs, but less commonly extra-adrenal PCCs. The PGLs found in these *SDHD*-mutated patients are very rarely malignant and only occasionally secrete catecholamines. The HNPGLs in patients with PGL1 are often multifocal. In a recent review, Pasini and Stratakis reported that 68 different *SDHD* germline mutations have been identified in 218 index cases (Pasini, et al. 2009). The majority of these mutations were frameshift mutations (40%), followed by nonsense mutations (25%), and then splicing mutations (9%). Although PCCs are relatively rare in patients with *SDHD* germline mutations and occur only occasionally, Ricketts et al. (2010) recently described that mutations predicted to result in loss of expression or truncated or unstable proteins were associated with significantly increased risk of PCCs compared to missense mutations that do not affect protein stability (Ricketts, C. J., et al. 2009). The mean age of PGL diagnosis in PGL1 patients ranges from 20.7 (PCCs) to 40.1 (HNPGLs) years old (Neumann, et al. 2004; Ricketts, C. J., et al. 2009). Very interestingly, inherited PGLs associated with *SDHD* germline mutations seem to occur in offspring of male carriers but not the offspring of female carriers, suggestive of maternal imprinting (Baysal, et al. 2002; Baysal 2004; Neumann, et al. 2004).

PGL2—This FPS clinical entity was first described in a previously identified large Dutch kindred with multiple HNPGLs (van Baars, et al. 1982; Mariman, et al. 1995). The position of the involved gene in these affected families was localized by linkage analysis to 11q11.3, but

for almost two decades the specific gene remained unknown. Recently, we discovered that *SDH5* was the responsible gene for FPS in PGL2 (Hao, et al. 2009). The connection between PGL2 and *SDH5* mutations is very new, and the associated clinical features and tumors associated with this mutation are now being investigated - although thus far, the tumors seem to be isolated to the head and neck. Very recently, another FPS lineage in Spain has been shown to be due to the same Gly78Arg mutation in *SDH5* (Bayley, et al. 2010); based on haplotype analysis, the authors conclude that the mutation in the Dutch and Spanish kindreds is most likely recurrent, rather than the result of a founder effect. As with the *SDHD* mutant (PGL1) patients, these patients seem to also be affected in a manner consistent with maternal imprinting. As more patients with familial or bilateral HNGPLs are tested, we may learn that *SDH5* mutations could account for a subset of the nearly 30% of the inherited FPS patients without a previously identified *SDHB*, *-C*, or *-D* mutation.

SDH5 mutations were not found in the germline of 315 patients with sporadic PGLs or PCCs, and *SDH5* gross gene deletions were not found in a subset of 200 of these same patients (Bayley, et al. 2010). Moreover, 128 of PGLs and PCCs were screened and found to be negative for somatic *SDH5* mutations (Bayley, et al. 2010). Most recently, another cohort of 104 PGLs and PCCs (including 8 tumors with familial status but unknown genetic cause) were also found to be negative for somatic *SDH5* mutations (Yao, et al. 2010). Based on these reports, it seems unlikely at this point in time that *SDH5* mutations will contribute greatly to sporadically occurring PGLs or PCCs.

Interestingly, both PGL1 and PGL2 seem to be inherited with a parent of origin effect caused by maternal imprinting. Both *SDHD* (PGL1) and *SDH5* (PGL2) are encoded on chromosome 11, at 11q23 and 11q11.3, respectively. It is possible to speculate that this chromosome may be prone to a specific form of imprinting, leading to the unique inheritance patterns observed and limited to both of these inherited PGL syndromes.

PGL3—Shortly after the discovery of *SDHD* and PGL1, Niemann and Muller (2000) described the association of *SDHC* (1q21) mutations with PGL3 (Niemann, et al. 2000). Like patients with *SDHD* mutations, those with *SDHC* mutations very frequently will develop HNPGLs. However, adrenal and extra-adrenal PCCs are far less common with *SDHC* germline mutations. The HNGPLs that do occur are often localized and rarely malignant. *SDHC*-associated PGLs have been described to secrete catecholamines (Niemann, et al. 2003), but relatively few patients with such mutations have been described in the literature. Fifteen different *SDHC* germline mutations have been identified in 19 index cases, and the majority of these were nonsense mutations (47%), followed by splicing mutations (33%), and then large deletions (7%). Unlike *SDHD* or *SDHB* mutations, there have been no frameshift mutations described in *SDHC*. Because of its rarity, *SDHC* germline mutations are often clinically tested only after *SDHB* and *SDHD* mutations.

PGL4—Astuti et al. (2001) recognized that mutations in the *SDHB* gene (1p36) were associated with FPS in PGL4 patients (Astuti, et al. 2001). Unlike the other clinical entities, these patients very frequently develop malignant, extra-adrenal PCCs. These sympathetic PCCs can also be multi-focal, including adrenal, and very frequently secrete norepinephrine. They also have been described to secrete epinephrine and dopamine. In addition to the abdominal tumors, HNPGLs are frequently found in these patients (although these tend to be less malignant). *SDHB* mutations are some of the most common germline mutations in FPS, and 98 different alterations have been identified in 216 index cases (Ricketts, C. J., et al. 2009). The majority of these *SDHB* mutations were missense mutations (46%), followed by frameshift mutations (23%), and then splicing mutations (13%). The mean age of PGL diagnosis has been reported from 27.4 (PCCs) to 42.3 (HNPGLs) years old by one study (Ricketts, C. J., et al. 2009), and ~ 30 years old by another study (Timmers, et al. 2007). In

fact, the youngest patients with PGLs are seen in *SDHB* mutation carriers and include PCCs seen at 3 year old and HNPGLs seen at 9 years old (Ricketts, C. J., et al. 2009). A recent report described 3 unrelated pediatric patients with PGLs and PCCs found, each patient possessing a germline *SDHB* mutation (Mora, et al. 2006). Unlike *SDHD* germline mutations, no clear genotype-phenotype have been identified for *SDHB* mutations (Ricketts, C. J., et al. 2009). In summary, the biggest clinical concern with FPS caused by *SDHB* mutations is the multi-focal and highly aggressive nature of the PGL tumors that can occur at a young age.

The clinical testing for SDH-mutation in patients with inherited PGLs (HNPGL or PCC) is often based on the tumor location and whether the tumor secretes catecholamines. If one SDH gene is negative, then the genetic testing often proceeds to the next most likely candidate gene until all of the known SDH-genes related to PGLs have been sequenced for mutations or deletions. Of note, *SDH5* is not yet clinically available for mutation testing but as indicated above, may explain up third of the previously negative SDH-mutation results in patients with a striking clinical history for FPS. Some clinicians have argued that even patients who present apparently sporadic or PCCs should be screened for underlying SDH mutations (Amar, et al. 2005; Jimenez, et al. 2006; Cascon, et al. (2009).

5.3. *SDHAF1*-infantile leukoencephalopathy

Ghezzi et al. (2009) recently described the identification of *SDHAF1* mutations to be associated with two families with highly penetrant infantile leukoencephalopathy (Ghezzi, et al. 2009). One family was from a multiconsanguineous kindred of Turkish descent and the other family was from a small alpine village in Italy. Similar to Leigh syndrome, affected individuals in both families presented with infantile progressive psychomotor regression accompanied by lack of speech development, progressive quadriparesis, and dystonia. Brain imaging revealed severe leukodystrophy and blood lactate and pyruvate levels were elevated in all of these patients. Mitochondrial respiratory chain analyses from muscle and fibroblast biopsies revealed only up to 30% SDH and SCOQR activity with other respiratory chain activities reported to be normal. Two homozygous missense mutations were identified in *SDHAF1*, as described above.

5.4. Other tumors

In addition to the HNPGLs and PCCs commonly found in patients with FPS and underlying SDH mutations, several other types of neuroendocrine and non-neuroendocrine tumors have been associated with mutations in SDH. Specifically, the clinical triad of PGLs, gastrointestinal stromal tumors (GISTs), and pulmonary chondromas (Carney triad) and the clinical dyad of PGLs and GISTs (Carney-Stratakis syndrome) have been described in the literature (Perry, et al. 2006; Stratakis, et al. 2009). Interestingly, patients with the Carney triad have not been found to possess *SDHA*, *-B*, *-C*, or *-D* mutations. However, patients with Carney-Stratakis syndrome have been found to have germline mutations in *SDHB*, *-C*, and *-D* genes (McWhinney, et al. 2007; Stratakis, et al. 2009). Investigation is now underway to explore if isolated or familial GISTs (without associated PGLs) may be due to underlying SDH mutations.

Renal tumors have been described in patients with underlying *SDHB* mutations, including renal cell carcinoma (Neumann, et al. 2004; Vanharanta, et al. 2004; Ricketts, C., et al. 2008) and oncocytoma (benign renal tumor)(Henderson, et al. 2009). In addition, both papillary and medullary thyroid cancer have been described in patients who are *SDHB* or *SDHD* mutation carriers (Neumann, et al. 2004; Zantour, et al. 2004). Previously, it was thought that SDH mutations did not play a role in the development of neuroblastoma (a pediatric neuro-endocrine tumor)(Astuti, et al. 2004; Grau, et al. 2005). More recently, however, isolated cases of neuroblastoma have been described in 2 patients with *SDHB* germline deletions; one patient had an underlying family history of familial PGLs (Cascon, et al. 2008) and the other patient

did not (Armstrong, et al. 2009). The tumor in the patient without any evidence of FPS was described to be a “composite PGL/neuroblastoma.”

As more patients are tested for underlying SDH mutations, including *SDH5*, we believe that more tumors will be found to be associated with germline defects in the SDH subunits assembly factors.

6. Mechanisms underlying SDH-associated disease

6.1. Leigh syndrome

The reported mutations in the nuclear-encoded flavoprotein (Fp) subunit gene (*SDHA*) of the SDH complex were all associated with Leigh syndrome and/or some form of progressive neurodegenerative disease (Bourgeron, et al. 1995; Birch-Machin, et al. 2000; Parfait, et al. 2000; Van Coster, et al. 2003; Horvath, et al. 2006). Interestingly, even though *SDHA* is part of the same mitochondrial Complex II comprised of *SDHB*, *-C*, and *-D* and assembled by *SDH5*, there are no published reports of these other SDH genes leading to neurological disorders. The molecular mechanism causing these respiratory chain enzyme deficiencies is not well understood. Bourgeron et al. reported an increased sensitivity of *SDHA* to the inhibitory effect of oxaloacetate (OAA), and speculated that the mutation could alter *SDHA*'s conformation or redox state which might alter enzyme catalysis and response to OAA (Bourgeron, et al. 1995). In Complex II deficiencies, both oxidative phosphorylation and the Krebs cycle do not function properly which may contribute to the hypoglycemia seen in these disorders. Van Coster et al. described a significant decrease of cross-reacting material (CRM) for the *SDHA* and *SDHB* subunits compared to normal controls, which the authors believe could contribute to the inadequate assembly or instability of Complex II (Van Coster, et al. 2003).

It remains unclear why *SDHA* mutations have not been found to cause tumors in the heterozygous state, unlike *SDHB*, *SDHC*, *SDHD* and *SDH5*. It could be related to an unappreciated aspect of chromosomal biology as *SDHB* and *SDHC* are located on chromosome 1 and *SDHD* and *SDH5* are located on chromosome 11. While it is quite clear that chromosome 11 is subject to imprinting and this is important for PGL formation, it is also possible that chromosome 1 dynamics could be important in the case of heterozygosity for *SDHB* and *SDHC* mutations.

6.2. Mechanisms of tumor formation

Mutations in *SDHB*, *-C*, *-D*, and now *SDH5* have been associated with tumor formation, specifically PGLs, including HNPGLs and PCCs. In addition, other tumors have been reported to occur in the context of these mutated SDH genes including GIST, thyroid cancer, renal tumors, and even neuroblastoma. It is still unknown why these specific tumors would arise from these mutations, but there is a growing literature that describes the connection between inadequate functioning of the mitochondrial II complex and tumor formation (Pollard, et al. 2003; Gottlieb, et al. 2005; Pollard, et al. 2005). SDH and fumarate hydratase (FH) help mediate metabolic signaling, and their dysfunction (through SDH mutations) leads to the accumulation of succinate or fumarate, respectively, in the mitochondria (King, et al. 2006); these metabolites accumulate and transit into the cytosol where they inhibit prolyl hydroxylase (PHD). Inhibition of PHD leads to the accumulation of hypoxia-inducible factor (HIF) by preventing its degradation, which is dependent on the von Hippel-Lindau (VHL) tumor suppressor protein (Esteban, et al. 2005). The inhibition of PHD enzymes (and HIF induction) can enhance tumorigenesis through the resistance to apoptotic signals (Lee, et al. 2005) and/or a pseudohypoxic signaling that enhances glycolysis for the tumor (King, et al. 2006). The increase in HIF – through lack of degradation - also can lead to the stimulation of angiogenic

signaling and the formation of new blood vessels that will feed and supply the tumor (Esteban, et al. 2005).

Interestingly, the SDH mutations associated with FPS often lead to PGLs of the carotid body (carotid body tumors). The carotid body sits at the bifurcation of the carotid vessels and functions as a peripheral chemoreceptor to provide afferent signaling to the respiratory center to control breathing. These receptors in the carotid body respond mostly to changes in the partial pressure of oxygen, but also to increases in carbon dioxide. If the mitochondrial Complex II does not function due to mutations in the SDH genes, then a state of pseudohypoxia develops due to increased HIF abundance— which usually only increases in the low oxygen states. It may be that the carotid body and other neuroendocrine tissues prone to PGLs are more sensitive to HIF induction due to their normal physiologic functions involving oxygen sensing. This could explain the predilection to form tumors in these specific sites when the SDH complex does not function or is not assembled properly due to SDH mutations. However, it still remains to be discovered exactly why *SDHA* mutations do not lead to these same types of tumors.

7. Conclusion

The SDH complex has been the subject of renewed interest over the past few years. This has been driven primarily by the discovery of its role in human disease and also by the discovery of new assembly factors. Like most important discoveries, these new discoveries supply more questions than answers. The coming years will likely witness new insights into SDH assembly, including new factors and greater clarity on mechanisms. We also anticipate a greater understanding of how SDH and SDH-related mutations cause human disease. As this happens, the concept of targeted therapies will enter the realm of possibility. Surely, this is an exciting time to be a student of the SDH complex.

Bibliography

- Amar L, Bertherat J, et al. Genetic testing in pheochromocytoma or functional paraganglioma. *J Clin Oncol* 2005;23(34):8812–8818. [PubMed: 16314641]
- Armstrong R, Greenhalgh KL, et al. Succinate dehydrogenase subunit B (SDHB) gene deletion associated with a composite paraganglioma/neuroblastoma. *J Med Genet* 2009;46(3):215–216. [PubMed: 19251979]
- Astrom K, Cohen JE, et al. Altitude is a phenotypic modifier in hereditary paraganglioma type 1: evidence for an oxygen-sensing defect. *Hum Genet* 2003;113(3):228–237. [PubMed: 12811540]
- Astuti D, Latif F, et al. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *Am J Hum Genet* 2001;69(1):49–54. [PubMed: 11404820]
- Astuti D, Morris M, et al. Investigation of the role of SDHB inactivation in sporadic pheochromocytoma and neuroblastoma. *Br J Cancer* 2004;91(10):1835–1841. [PubMed: 15505628]
- Bafunno V, Giancaspero TA, et al. Riboflavin uptake and FAD synthesis in *Saccharomyces cerevisiae* mitochondria: involvement of the Flx1p carrier in FAD export. *J Biol Chem* 2004;279(1):95–102. [PubMed: 14555654]
- Bayley JP, Kunst HP, et al. SDHAF2 mutations in familial and sporadic paraganglioma and pheochromocytoma. *Lancet Oncol*. 2010
- Baysal BE. Genomic imprinting and environment in hereditary paraganglioma. *Am J Med Genet C Semin Med Genet* 2004;129C(1):85–90. [PubMed: 15264276]
- Baysal BE, Ferrell RE, et al. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 2000;287(5454):848–851. [PubMed: 10657297]
- Baysal BE, Willett-Brozick JE, et al. Prevalence of SDHB, SDHC, and SDHD germline mutations in clinic patients with head and neck paragangliomas. *J Med Genet* 2002;39(3):178–183. [PubMed: 11897817]

- Birth-Machin MA, Taylor RW, et al. Late-onset optic atrophy, ataxia, and myopathy associated with a mutation of a complex II gene. *Ann Neurol* 2000;48(3):330–335. [PubMed: 10976639]
- Boedeker CC, Neumann HP, et al. Malignant head and neck paragangliomas in SDHB mutation carriers. *Otolaryngol Head Neck Surg* 2007;137(1):126–129. [PubMed: 17599579]
- Boedeker CC, Neumann HP, et al. Clinical features of paraganglioma syndromes. *Skull Base* 2009;19(1):17–25. [PubMed: 19568339]
- Bourgeron T, Rustan P, et al. Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. *Nat Genet* 1995;11(2):144–149. [PubMed: 7550341]
- Bugiani M, Lamantea E, et al. Effects of riboflavin in children with complex II deficiency. *Brain Dev* 2006;28(9):576–581. [PubMed: 16737791]
- Bukau B, Horwich AL. The Hsp70 and Hsp60 Chaperone Machines. *Cell* 1998;92(3):351–366. [PubMed: 9476895]
- Cascon A, Landa I, et al. Molecular characterisation of a common SDHB deletion in paraganglioma patients. *J Med Genet* 2008;45(4):233–238. [PubMed: 18057081]
- Cascon A, Lopei-Jimenez E, et al. Rationalization of genetic testing in patients with apparently sporadic pheochromocytoma/paraganglioma. *Horm Metab Res* 2009;41(9):672–675. [PubMed: 19343621]
- Cimen H, Han MJ, et al. Regulation of Succinate Dehydrogenase Activity by SIRT3 in Mammalian Mitochondria. *Biochemistry*. 2009
- Dibrov E, Fu S, et al. The *Saccharomyces cerevisiae* TCM62 gene encodes a chaperone necessary for the assembly of the mitochondrial succinate dehydrogenase (complex II). *J Biol Chem* 1998;273(48):32042–32048. [PubMed: 9822678]
- Esteban MA, Maxwell PH. HIF, a missing link between metabolism and cancer. *Nat Med* 2005;11(10):1047–1048. [PubMed: 16211034]
- Finsterer J. Leigh and Leigh-like syndrome in children and adults. *Pediatr Neurol* 2008;39(4):223–235. [PubMed: 18805359]
- Ghezzi D, Goffrini P, et al. SDHAF1, encoding a LYR complex-II specific assembly factor, is mutated in SDH-defective infantile leukoencephalopathy. *Net Genet*. 2009
- Giancaspero TA, Wait R, et al. Succinate dehydrogenase flavoprotein subunit expression in *Saccharomyces cerevisiae*--involvement of the mitochondrial FAD transporter, Flx1p. *Febs J* 2008;275(6):1103–1117. [PubMed: 18279395]
- Gottlieb E, Tomlinson IP. Mitochondrial tumour suppressors: a genetic and biochemical update. *Nat Rev Cancer* 2005;5(11):857–866. [PubMed: 16327764]
- Grau E, Oltra S, et al. There is no evidence that the SDHB gene is involved in neuroblastoma development. *Oncol Res* 2005;15(7-8):393–398. [PubMed: 16491957]
- Gutman M, Silman N. The steady state activity of succinate dehydrogenase in the presence of opposing effectors.II. Reductive activation of succinate dehydrogenase in presence of oxaloacetate. *Mol Cell Biochem* 1975;7(3):177–185. [PubMed: 239334]
- Hagerhall C. Succinate: quinone oxidoreductases. Variations on a conserved theme. *Biochim Biophys Acta* 1997;1320(2):107–141. [PubMed: 9210286]
- Hao HX, Khalimonchuk O, et al. SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. *Science* 2009;325(5944):1139–1142. [PubMed: 19628817]
- Hao HX, Rutter J. Revealing human disease genes through analysis of the yeast mitochondrial proteome. *Cell Cycle* 2009;8(24):4007–4008. [PubMed: 19949294]
- Henderson A, Douglas F, et al. SDHB-associated renal oncocytoma suggests a broadening of the renal phenotype in hereditary paragangliomatosis. *Fam Cancer* 2009;8(3):257–260. [PubMed: 19184535]
- Horvath R, Abicht A, et al. Leigh syndrome caused by mutations in the flavoprotein (Fp) subunit of succinate dehydrogenase (SDHA). *J Neurol Neurosurg Psychiatry* 2006;77(1):74–76. [PubMed: 16361598]
- Jech M, Alvarado-Cabrero I, et al. Genetic analysis of high altitude paragangliomas. *Endocr Pathol* 2006;17(2):201–202. [PubMed: 17159253]
- Jimenez C, Cote G, et al. Review: Should patients with apparently sporadic pheochromocytomas or paragangliomas be screened for hereditary syndromes? *J Clin Endocrinol Metab* 2006;91(8):2851–2858. [PubMed: 16735498]

- King A, Selak MA, et al. Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. *Oncogene* 2006;25(34):4675–4682. [PubMed: 16892081]
- Klanner C, Neupert W, et al. The chaperonin-related protein Tcm62p ensures mitochondrial gene expression under heat stress. *FEBS Lett* 2000;470(3):365–369. [PubMed: 10745098]
- Lee S, Nakamura E, et al. Neuronal apoptosis linked to EglN3 prolyl hydroxylase and familial pheochromocytoma genes: developmental culling and cancer. *Cancer Cell* 2005;8(2):155–167. [PubMed: 16098468]
- Maklashina E, Berthold DA, et al. Anaerobic expression of *Escherichia coli* succinate dehydrogenase: functional replacement of fumarate reductase in the respiratory chain during anaerobic growth. *J Bacteriol* 1998;180(22):5989–5996. [PubMed: 9811659]
- Mariman EC, van Beersum SE, et al. Fine mapping of a putatively imprinted gene for familial non-chromaffin paragangliomas to chromosome 11q13.1: evidence for genetic heterogeneity. *Hum Genet* 1995;95(1):56–62. [PubMed: 7814027]
- Mariman EC, van Beersum SE, et al. Analysis of a second family with hereditary non-chromaffin paragangliomas locates the underlying gene at the proximal region of chromosome 11q. *Hum Genet* 1993;91(4):357–361. [PubMed: 8388849]
- Martin TP, Irving RM, et al. The genetics of paragangliomas: a review. *Clin Otolaryngol* 2007;32(1):7–11. [PubMed: 17298303]
- McWhinney SR, Pasini B, et al. Familial gastrointestinal stromal tumors and germ-line mutations. *N Engl J Med* 2007;357(10):1054–1056. [PubMed: 17804857]
- Mora J, Cascon A, et al. Pediatric paraganglioma: an early manifestation of an adult disease secondary to germline mutations. *Pediatr Blood Cancer* 2006;47(6):785–789. [PubMed: 16304664]
- Nakamura K, Yamaki M, et al. Two hydrophobic subunits are essential for the heme b ligation and functional assembly of complex II (succinate-ubiquinone oxidoreductase) from *Escherichia coli*. *J Biol Chem* 1996;271(1):521–527. [PubMed: 8550613]
- Neumann HP, Pawlu C, et al. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. *JAMA* 2004;292(8):943–951. [PubMed: 15328326]
- Niemann S, Muller U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. *Nat Genet* 2000;26(3):268–270. [PubMed: 11062460]
- Niemann S, Muller U, et al. Autosomal dominant malignant and catecholamine-producing paraganglioma caused by a splice donor site mutation in SDHC. *Hum Genet* 2003;113(1):92–94. [PubMed: 12658451]
- Oyedotun KS, Lemire BD. The Quinine-binding sites of the *Saccharomyces cerevisiae* succinate-ubiquinone oxidoreductase. *J Biol Chem* 2001;276(20):16936–16943. [PubMed: 11279023]
- Oyedotun KS, Sit CS, et al. The *Saccharomyces cerevisiae* succinate dehydrogenase does not require Heme for ubiquinone reduction. *Biochem Biophys Acta* 2007;1767(12):1436–1445. [PubMed: 18028869]
- Parfait B, Chretien D, et al. Compound heterozygous mutations in the falvoprotein gene of the respiratory chain complex II in a patient with Leigh syndrome. *Hum Genet* 2000;106(2):236–243. [PubMed: 10746566]
- Pasini B, Stratakis CA. SDH mutations in tumorigenesis and inherited endocrine tumours: lesson from the pheochromocytoma-paraganglioma syndromes. *J Intern Med* 2009;266(1):19–42. [PubMed: 19522823]
- Perry CG, Young WF Jr. et al. Functioning paraganglioma and gastrointestinal stromal tumor of the jejunum in three women: syndrome or coincidence. *Am J Surg Pathol* 2006;30(1):42–49. [PubMed: 16330941]
- Pollard PJ, Briere JJ, et al. Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. *Hum Mol Genet* 2005;14(15):2231–2239. [PubMed: 15987702]
- Pollard PJ, Wortham NC, et al. The TCA cycle and tumorigenesis: the examples of fumarate hydratase and succinate dehydrogenase. *Ann Med* 2003;35(8):632–639. [PubMed: 14708972]
- Ricketts C, Woodward ER, et al. Germline SDHB mutations and familial renal cell carcinoma. *J Natl Cancer Inst* 2008;100(17):1260–1262. [PubMed: 18728283]

- Ricketts CJ, Forman JR, et al. Tumour Risks and Genotype-Phenotype-Proteotype Analysis in 358 Patients with Germline Mutations in SDHB and SDHD. *Hum Mutat.* 2009
- Rodriguez-Cuevas S, Lopez-Garza J, et al. Carotid body tumors in inhabitants of altitudes higher than 2000 meters above sea level. *Head Neck* 1998;20(5):374–378. [PubMed: 9663663]
- Ruben RJ. The history of the glomus tumors - nonchromaffin chemodectoma: a glimpse of biomedical Camelot. *Acta Otolaryngol* 2007;127(4):411–416. [PubMed: 17453463]
- Rustin P, Bourgeron T, et al. Inborn errors of the Krebs cycle: a group of unusual mitochondrial diseases in human. *Biochim Biophys Acta* 1997;1361(2):185–197. [PubMed: 9300800]
- Salvi M, Morrice NA, et al. Identification of the flavoprotein of succinate dehydrogenase and aconitase as in vitro mitochondrial substrates of Fgr tyrosine kinase. *FEBS Lett* 2007;581(29):5579–5585. [PubMed: 17997986]
- Shi Y, Ghosh MC, et al. Human ISD11 is essential for both iron-sulfur cluster assembly and maintenance of normal cellular iron homeostasis. *Hum Mol Genet* 2009;18(16):3014–3025. [PubMed: 19454487]
- Silkin Y, Oyedotun KS, et al. The role of Sdh4p Tyr-89 in ubiquinone reduction by the *Saccharomyces cerevisiae* succinate dehydrogenase. *Biochim Biophys Acta* 2007;1767(2):143–150. [PubMed: 17208193]
- Stratakis CA, Carney JA. The triad of paragangliomas, gastric stromal tumours and pulmonary chondromas (Carney triad), and the dyad of paragangliomas and gastric stromal sarcomas (Carney-Stratakis syndrome): molecular genetics and clinical implications. *J Intern Med* 2009;266(1):43–52. [PubMed: 19522824]
- Sun F, Huo X, et al. Crystal structure of mitochondrial respiratory membrane protein complex II. *Cell* 2005;121(7):1043–1057. [PubMed: 15989954]
- Timmers HJ, Gimenez-Roqueplo AP, et al. Clinical aspects of SDHx-related pheochromocytoma and paraganglioma. *Endocr Relat Cancer* 2009;16(2):391–400. [PubMed: 19190077]
- Timmers HJ, Kozupa A, et al. Clinical presentations, biochemical phenotypes, and genotype-phenotype correlations in patients with succinate dehydrogenase subunit B-associated pheochromocytomas and paragangliomas. *J Clin Endocrinol Metab* 2007;92(3):779–786. [PubMed: 17200167]
- Tomasiaik TM, Maklashina E, et al. A threonine on the active site loop controls transition state formation in *Escherichia coli* respiratory complex II. *J Biol Chem* 2008;283(22):15460–15468. [PubMed: 18385138]
- Tomitsuka E, Kita K, et al. Regulation of succinate-ubiquinone reductase and fumarate reductase activities in human complex II by phosphorylation of its flavoprotein subunit. *Proc Jpn Acad Ser B Phys Biol Sci* 2009;85(7):258–265.
- Tzagoloff A, Jang J, et al. FLX1 codes for a carrier protein involved in maintaining a proper balance of flavin nucleotides in yeast mitochondria. *J Biol Chem* 1996;271(13):7392–7397. [PubMed: 8631763]
- van Baars F, Cremers C, et al. Genetic aspects of nonchromaffin paraganglioma. *Hum Genet* 1982;60(4):305–309. [PubMed: 6286462]
- Van Coster R, Seneca S, et al. Homozygous Gly555Glu mutation in the nuclear-encoded 70 kDa flavoprotein gene causes instability of the respiratory chain complex II. *Am J Med Genet A* 2003;120A(1):13–18. [PubMed: 12794685]
- Vander Heiden MG, Choy JS, et al. Bcl-x(L) complements *saccharomyces cerevisiae* genes that facilitate the switch from glycolytic to oxidative metabolism. *J Biol Chem* 2002;277(47):44870–44876. [PubMed: 12244097]
- Vanharanta S, Buchta M, et al. Early-onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. *Am J Hum Genet* 2004;74(1):153–159. [PubMed: 14685938]
- Yankovskaya V, Horsefield R, et al. Architecture of succinate dehydrogenase and reactive oxygen species generation. *Science* 2003;299(5607):700–704. [PubMed: 12560550]
- Yao L, Barontini M, et al. Mutations of the Metabolic Genes IDH1, IDH2, and SDHAF2 Are Not Major Determinants of the Pseudohypoxic Phenotype of Sporadic Pheochromocytomas and Paragangliomas. *J Clin Endocrinol Metab.* 2010
- Zantour B, Guilhaume B, et al. A thyroid nodule revealing a paraganglioma in a patient with a new germline mutation in the succinate dehydrogenase B gene. *Eur J Endocrinol* 2004;151(4):433–438. [PubMed: 15476441]

Zhang J, Frerman FE, et al. Structure of electron transfer flavoprotein-ubiquinone oxidoreductase and electron transfer to the mitochondrial ubiquinone pool. *Proc Natl Acad Sci U S A* 2006;103(44):16212–16217. [PubMed: 17050691]

Table 1

Summary of clinical features of the four described familial paraganglioma syndromes.

	PGL1	PGL2	PGL3	PGL4
SDH Gene	<i>SDHD</i>	<i>SDH5 (SDHAF2)</i>	<i>SDHC</i>	<i>SDHB</i>
Chromosomal Location	11q23	11q11.3	1q21	1p35-36.1
First Described	Baysal et al. (2000)	Hao et al. (2009)	Niemann and Muller (2000)	Astuti et al. (2001)
Most common mutation	frameshift	point	nonsense	missense
HNPGL	++	++	++	+
PCC (any abdominal)	+	–	+/-	++
Catecholamine secreting	+/-	–	+/-	++
Malignant	–	–	Unknown	++
Biochemistry	Structural SDH subunit	SDH assembly factor-FAD insertion	Structural SDH subunit	Structural SDH subunit

“–” = Rare

“+” = Occasionally

“++” = Frequently

HNPGL = Head & Neck Paraganglioma

PCC = Pheochromocytoma