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# Respiratory chain complex II as general sensor for apoptosis ☆

Stefan Grimm *

*Department of Experimental Medicine and Toxicology, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, UK*

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ABSTRACT

I review here the evidence that complex II of the respiratory chain (RC) constitutes a general sensor for apoptosis induction. This concept emerged from work on neurodegenerative diseases and from recent data on metabolic alterations in cancer cells affecting the RC and in particular on mutations of complex II subunits. It is also supported by experiments with many anticancer compounds that compared the apoptosis sensitivities of complex II-deficient versus WT cells. These results are explained by the mechanistic understanding of how complex II mediates the diverse range of apoptosis signals. This protein aggregate is specifically activated for apoptosis by pH change as a common and early feature of dying cells. This leads to the dissociation of its SDHA and SDHB subunits from the remaining membrane-anchored subunits and the consequent block of it enzymatic SQR activity, while its SDH activity, which is contained in the SDHA/SDHB subcomplex, remains intact. The uncontrolled SDH activity then generates excessive amounts of reactive oxygen species for the demise of the cell. Future studies on these mitochondrial processes will help refine this model, unravel the contribution of mutations in complex II subunits as the cause of degenerative neurological diseases and tumorigenesis, and aid in discovering novel interference options. This article is part of a Special Issue entitled: Respiratory complex II: Role in cellular physiology and disease.



## 1. Introduction

### 1.1. Complex II in healthy cells: structure and function

In order to understand the role of complex II during apoptosis it is appropriate to first revisit the composition and function of this protein aggregate in normal cells. The mitochondrial respiratory chain (mRC) consists of four multimeric protein complexes, all of which are anchored to the inner mitochondrial membrane (IMM). Together they catalyze the oxidation of reducing equivalents, mainly NADH, using molecular oxygen ($O_2$) as the terminal electron acceptor, which finally yields water (Fig. 1) [1]. The electron transfer within the mRC is coupled at specific points to the extrusion of protons into the mitochondrial intermembrane space. This fuels the ATP synthase complex (also known as complex V) thereby generating ATP. The coupling between mRC and ATP synthesis is called oxidative phosphorylation (OXPHOS). All mRC complexes (mRCC) are multimeric protein aggregates that are composed of factors encoded by either nuclear or mitochondrial DNA. Complex II (also known as Succinate Dehydrogenase (SDH) or Succinate Coenzyme Q Reductase (SQR)) is made up of only four subunits (SDHA, SDHB, SDHC and SDHD) and as such is the smallest mRCC. It is the only complex to be fully encoded by nuclear DNA. Apart from mRCC I, complex II is the second entry point of reducing equivalents into the mRC via FADH, which is generated by the oxidation of succinate to fumarate as part of the tricarboxylic acid ((TCA), also known as citric acid or Krebs) cycle. The electrons provided by complex II to the mRC then reduce coenzyme Q (CoQ) to ubiquinol, which is further shuffled along the mRC [1] (Fig. 1). Another particularity of complex II, and the exception among mRCCs, is that it is the only complex that does not serve to pump protons across the IMM [2]. From the crystal structure of complex II it was deduced that two transmembrane proteins, SDHC and SDHD, anchor the complex to the IMM [3,4]. Their transmembrane domains hold a redox group, a heme *b*, bound at the interface between SDHC and SDHD whose role for the transfer of electrons within complex II is so far unknown for mammals [5,6]. In eukaryotic cells the SDHB subunit is associated with the membrane anchors SDHC and SDHD and together with SDHA forms the hydrophilic head of the protein aggregate that protrudes into the mitochondrial matrix. SDHA and SDHB constitute the catalytic core of the complex that on its own can oxidize succinate (which directly binds to SDHA) to fumarate in the TCA cycle. Within the SDHA/B subcomplex the electrons are transported to the FAD cofactor contained in SDHA and finally to the three [Fe–S] clusters in SDHB [2,3]. To monitor the enzymatic activity of this part of complex II the electrons can be captured *in vitro* by artificial, exogenously added electron acceptors. The corresponding enzymatic activity of complex II is called succinate dehydrogenase activity (SDH) [7]. At the SDHC/SDHD interface two CoQ-binding sites have been found: $Q_P$ ("p" for proximal to

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* Tel.: +44 7594 6898; fax: +44 7594 7393.
*E-mail address:* s.grimm@imperial.ac.uk.

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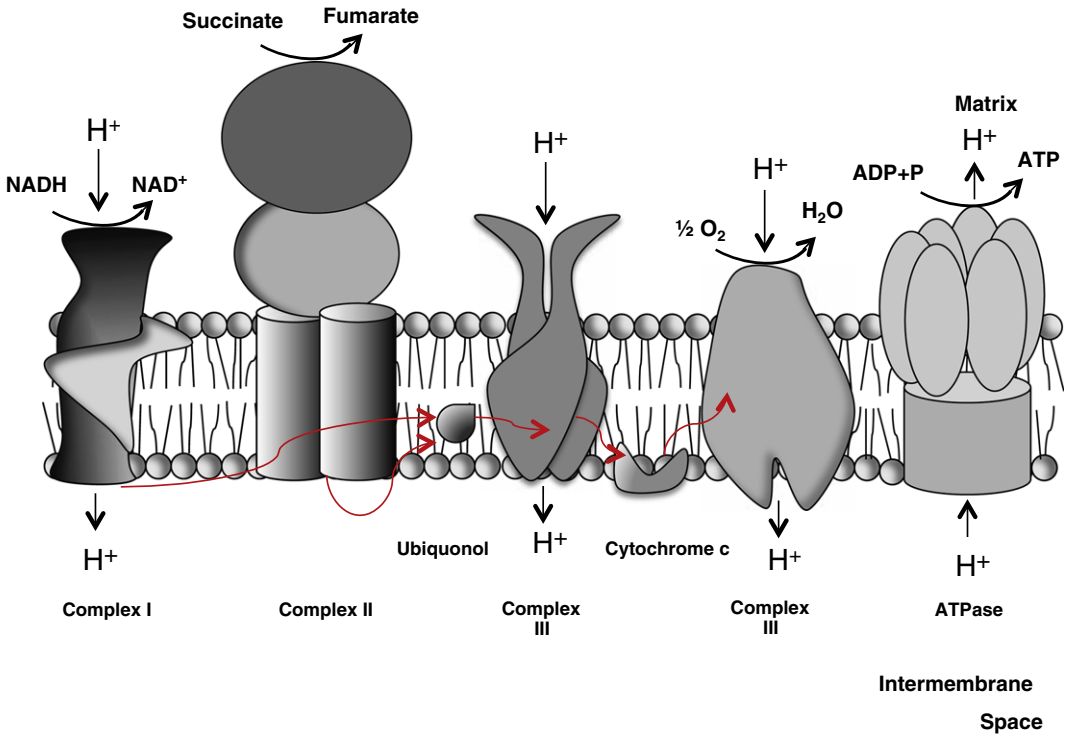


**Fig. 1.** Schematic representation of the mitochondrial respiratory chain complexes and the OXPHOS system. The four complexes of the respiratory chain and the ATP synthase and the electron/proton movements along these complexes are depicted. RCC I and II are the two main entries of reducing equivalents into the RC. RCC I receives electrons from NADH and transfers them via a flavin mononucleotide (FMN) cofactor to iron–sulfur (Fe–S) clusters to eventually reduce ubiquinone (coenzymeQ, CoQ, Q) to ubiquinol, which is coupled to the translocation of protons from the matrix into the intermembrane space (IMS). RCC II (succinate:ubiquinone oxidoreductase) also contributes to the ubiquinol pool via the transfer of electrons from succinate, a tricarboxylic acid (TCA) cycle intermediate, to the complex II-embedded cofactor FAD (flavin-adenin dinucleotide) and then to several Fe–S clusters. The ubiquinol pool delivers its electrons to RCC III (ubiquinol:cytochrome c oxidoreductase) through two heme centers (cytochromes b and c1) and one Fe–S cluster. This is likewise coupled to a translocation of protons across the IMM. Finally, RCC III delivers its electrons to cytochrome c (Cyt C), which transfers them to RCC IV (cytochrome c oxidase). This terminal RCC possesses uses the energy generated by the electron transfer to translocate protons. The final complex, ATP synthase (complex V), uses the protomotive force to generate ATP. This coupling between RC and ATP synthesis is called oxidative phosphorylation (OXPHOS).

the [3Fe-4S] cluster) and $Q_D$ ("D" for distal to the [3Fe-4S] cluster) [2,3]. CoQ reduction at the $Q_P$ site, which has a high affinity for CoQ [8], is assumed to be a two-step process. A partially reduced semiquinone is formed by the first electron transferred. This semiquinone radical appears to be stabilized for complete reduction by the second electron that then produces ubiquinol [2,9]. This mechanism is thought to safeguard complex II against excessive electron leakage under normal physiological conditions [2,9]. The entire electron transfer within RCCII, from succinate at the catalytic site of SDHA to CoQ at the $Q_P$ site at the SDHC/D interface, constitutes the succinate CoQ oxidoreductase (SQR) activity of RCC II, which can be measured, similar to the SDH activity, with an appropriate enzymatic assay *in vitro* [7].

## 2. A general role of complex II in apoptosis

The concept that complex II contributes to apoptosis evolved only relatively recently based on its role in Leigh syndrome, also known as Subacute Necrotizing Encephalomyelopathy (SNEM), a neurodegenerative disease that affects the central nervous system and is associated with neuronal cell death, which eventually leads to impaired motor functions [10]. The connection was discovered through SDHA mutations that were linked to this disease [11], an observation that was later corroborated by various studies in neuronal cells showing the pro-apoptotic effects of specific complex II inhibitors, in particular the irreversible complex II inhibitor 3-nitropropionic acid (3-NP) and the competitive inhibitor methylmalonate [12,13]. Both reagents target the succinate-binding site in SDHA and both, as a consequence, inhibit the SDH as well as the downstream SQR activity [7,14]. The most recent, and most prominent, indication that complex II is involved in apoptosis regulation was made when the tumor-suppressor gene function of the SDHD, SDHC and SDHB subunits were discovered [15–17] (see below). Three mechanisms were proposed to account for these results: Firstly, the accumulation of succinate in mitochondria as a consequence of complex II inhibition and its subsequent transport to the cytosol where it inhibits HIF1α prolyl hydroxylase (PHD), leads to HIF1 stabilization and the establishes a pseudo-hypoxic state. This favours glycolysis and promotes tumor formation possibly through the Warburg effect and its growth-promoting consequences, among them, notably, apoptosis inhibition [18–20].

A second possible scenario of complex II inhibition, whose connection to apoptosis might be indirect, is that sublethal levels of superoxides are formed, which can contribute to either genomic instability or tumorigenesis. In fact, it has been acknowledged for some time that modest oxidative stress is furthering the proliferation of cells [21–25]. The third explanation of the defects of complex II subunits in cancer cells assumes a direct role of this protein aggregate in apoptosis, *i.e.* that it acts as a sensor for cell death. If this sensor is not properly functioning anymore, resistance to apoptosis signals builds up that otherwise curtail tumor formation. Indeed, various studies brought to light the role of complex II as a pro-apoptotic sensor. The first study suggesting than complex II is a transmitter of apoptosis signals observed that after mitochondrial outer membrane (MOM) permeabilization, a crucial step in apoptosis signalling, complex II (and complex I) are inhibited in a caspase-dependent manner and contribute to apoptotic cell death via reactive oxygen species (ROS) production and $\Delta\Psi\mu$ collapse [26]. ROS formation is a common theme when complexes of the RC are integrated into the apoptosis signalling process. This is achieved through the production of high,

lethal doses of ROS – in contrast to the sublethal concentrations that contribute to tumor formation – and is caused by the abrupt bock of the electron flow within the mRC. How exactly ROS are produced at complex II has not fully been resolved, mostly due to the fact that complexes I and III were so far predominantly investigated as principal sources of mitochondrial ROS [1,27,28]. In the few studies that looked at ROS formation by complex II, different sites for superoxide production were identified, depending on the respective chemical inhibitors used or the specific sequence of the mutations analyzed. The CoQ-binding site formed by SDHB/C/D [29] was proposed, possibly through the stabilization of the semiquinone radical [30]; alternatively the FAD in SDHA was identified [4]; and finally a so far undefined site between the SDHA-embedded FAD and a downstream blockade was singled out [7,22]. After the initial implication of complex II in apoptosis signalling [26], a later study showed that apoptosis induced by nerve growth factor (NGF) withdrawal depends on a functional complex II in neurons [31]. This observation was subsequently extended by demonstrating that NGF deprivation activates the transcription factor c-Jun and apoptosis via PHD3 (EglN3), a prolyl hydroxylase involved in HIF1α degradation. In this case, however, the apoptosis induction appeared to be independent of HIF1α and instead relied on KIF1Bβ, a motor protein implicated in anterograde transport of synaptic vesicles whose connection to apoptosis induction remains unknown [32]. Taken together, these data strongly suggested that the protein aggregate of complex II acts as a sensor for apoptosis induction.

## 3. Complex II as a pH sensor for apoptosis

A recent series of experiments not only broadened the scope of apoptosis signals mediated through complex II but also led to a mechanistic understanding how this complex is activated for apoptosis induction. A genetic high-throughput screen led to the isolation of various apoptosis genes, among them SDHC and SDHD (but not SDHA nor SDHB) [33–39]. Overexpression of SDHC caused robust apoptosis and cells deficient of its protein and therefore lacking an intact complex II were significantly more resistant to numerous structurally diverse anticancer drugs, ranging from cisplatin, etoposide, doxorubicin, to paclitaxel and also to proapoptotic cytokines [5,40,33]. Based on the efficiency and swiftness of the pro-effect of SDHC, a direct effect, possibly on the assembly of complex II is likely, rather than an indirect such as compromising the mitochondrial import [41]. Importantly, all agents, including SDHC expression, inhibited the activity of complex II even before overt signs of cell death were observable implying that it is an early cellular response [33]. While this cell death was shown to be dependent on ROS formation, the exact mechanism of their formation remained unresolved [33]. This was addressed in a follow-up study, which revealed that all those divers apoptosis agents listed above specifically reduced the SQR of complex II without impairing its SDH activity [7]. In agreement with this, the complex II inhibitors thenoyltrifluoroacetate (TTFA), α-tocopheryl succinate (α-TOS), and Aptenins, which target the SQR activity through binding the proximal $Q_P$ site [3], likewise caused apoptosis [7,42,43]. The specific SDH inhibitor 3-nitroproprionic acid (3-NP) and succinate competitors (malonate derivatives), on the other hand, did not only not lead to the demise of the cells but actually rescued them. This is also supported by mutants of the complex II subunits such as the mev-1 mutant of SDHC in the CoQ-binding site, which features mitochondrial superoxide production and apoptosis through the specific inhibition of the SQR activity without SDH function impairment [44]. Other mutations in SDHB/C/D at the CoQ-binding site have been shown to replicate these effects [24,29,45,46]. This suggested that it is crucial exactly how complex II is inhibited for apoptosis induction: when SQR activity is blocked while the enzymatic SDH activity is still intact. The ensuing massive leakage of electrons from complex II to molecular oxygen can then generate enough superoxides for apoptosis induction [7]. When the integrity of complex II during apoptosis was investigated with blue native gels, it was observed that apoptosis induction is correlated with the specific disintegration of complex II by the release of SDHA and SDHB from the membrane-anchoring subunits into the matrix. As the SDHA/SDHB sub-complex is still enzymatically active [47,48], it can efficiently remove electrons from its substrate succinate. However, since the physiological downstream acceptors for the electrons (the SDHC/D subunits) are missing, the electron flow is blocked and the electrons are transferred to molecular oxygen instead. This uncontrolled enzymatic activity thereby generates excessive ROS and causes apoptosis induction [7,33]. This, however, is not the only way in which complex II can, even though much less efficiently, generate ROS. We have observed that over a long incubation period 3-NP also generates oxidative stress and leads to cell death, albeit inefficiently (unpublished). This is probably the scenario that is relevant for the neurodegenerative Leigh syndrome with mutations in SDHA (above). The findings on complex II disintegration seamlessly integrate the data on the differential inhibition of the two enzymatic activities of complex II (SDH and SQR) observed with chemicals (above) and led to a mechanistic understanding of how complex II is activated for apoptosis induction.

The second most pressing question emerging from the studies on complex II inhibition was how so many structurally diverse signals can converge on one protein complex. What, in other words, is the common denominator that combines all those agents that signal through complex II? We found evidence that the specific disintegration of complex II was accomplished through intracellular acidification, which occurs both in the cytosol and the mitochondria as an early and universal change during the apoptotic process [49]. This seems – perhaps through the protonation of amino acid side chains in complex II subunits – to impact on the interactions within complex II and drives the release of the catalytic SDHA/SDHB subunits from the membrane-anchoring subunits of complex II [7]. When isolated mitochondria were incubated in buffers with different pH, a reduction from pH 7.3 to 6.7 caused a release of SDHA/B, which was also observed in mitochondria isolated from cells undergoing apoptosis. Conversely, the transfection of the sodium/hydrogen exchanger NHE1, which maintains the physiological pH, also inhibited the disintegration of complex II. However, how direct or indirect the effect of the pH drop during apoptosis on the release of complex II subunits is, remains unknown. Also, the pH sensor function of RCC II is certainly not the only mediator of apoptosis activated by acidification. Caspases, endonucleases and proapoptotic Bcl2 family members have also been shown to be activated by the apoptotic pH drop, though it could be argued that they are part of the execution machinery of cell death and hence act at a later point in apoptosis signaling [49]. In other scenarios of physiological pH decline, *e.g.* metabolic acidosis or lactic acidosis, which are not associated with cell death, the oxidative stress caused by complex II inhibition must be overcome but how this is accomplished remains to be determined.

ROS are formed as a consequence of the disintegration of complex II. These molecules then act in a pleiotropic way in the cell for apoptosis induction; they crosslink proteins, produce ceramide, oxidize GSH and proteins in the mitochondrial permeability transition (PT) pore, activate JNK, and damage DNA [50]. As various RCCs are directly impacted by ROS as revealed in isolated mitochondria and in intact cells ROS can also act on the RCCs by establishing a feed-forward loop of RC inhibition, ROS formation and further inhibition of the RCCs [51–53]. This can be accomplished by posttranslational modifications of the subunits by lipid peroxidation adducts (4-hydroxynonenal, HNE, or malondialdehyde MDA), carbonylation or nitration of residues and finally a block of their activities [54–57]. Superoxides generated through ischemia/reperfusion prevent the correct glutathionylation of SDHA in complex II, which facilitates its nitration by peroxynitrite on tyrosine residues and diminishes its activity [58,59]. Which one of those processes is the downstream apoptosis executioner of complex II disintegration remains to be determined. Hence, while the finding about the dissociation of complex II subunits and the establishment of oxidative stress answer a number of questions,

it also raises others. Nevertheless, taken together our results led us to propose the model depicted in Fig. 2 in which apoptosis leads to an early cellular pH drop causing the dissociation of complex II subunits SDHA/B so that its SDH activity remains intact and its SQR activity is curtailed. This produces the catalytic formation of ROS and finally cell death induction though oxidative stress. Hence, complex II is a mitochondrial sensor for pH change as a widespread initiating event in apoptotic cells [49]. It is therefore, apart from complex I of the RC whose subunit NDUFS1 is cleaved by caspases [60], the second complex of the RC of which we have a mechanistic understanding of the changes leading to apoptosis.

## 4. Mutations in tumors affecting apoptosis though complex II

The inhibition of apoptosis is regarded as one of the so-called hallmarks of cancer cells [61]. It allows the malignant cells to continuously grow despite the many adverse conditions they encounter. Conversely, apoptosis induction is also the basis for the therapeutic effect of

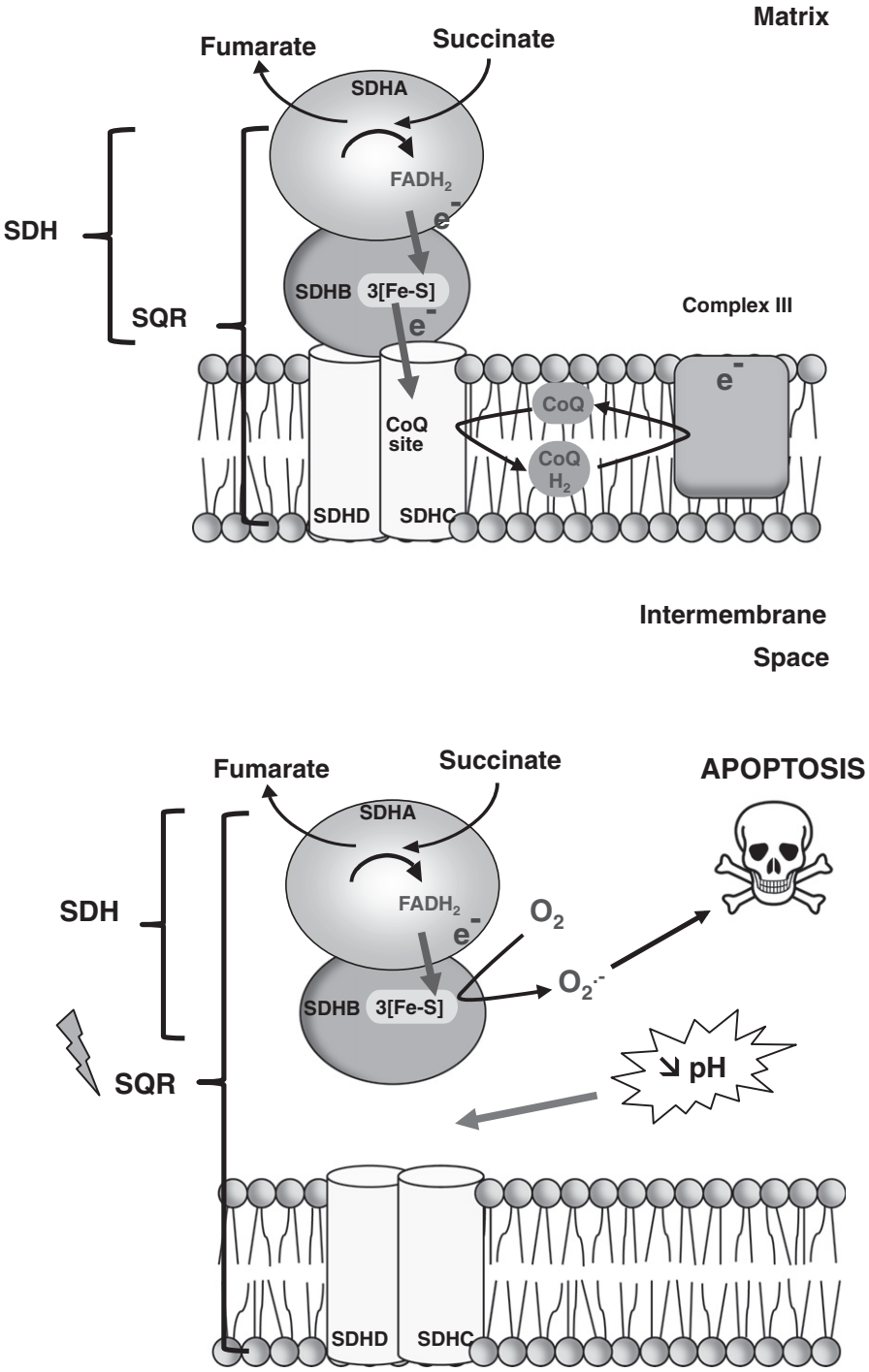


**Fig. 2.** Complex II disintegration for apoptosis induction: the specific disintegration of complex II is indicated by the schematic representation of its state in normal cells (top) and during apoptosis, which leads to the specific release of the SDHA/SDHB subcomplex into the matrix in response to a pH drop (bottom). This dissociation uncouples its two enzymatic activities (succinate dehydrogenase (SDH) and succinate coenzyme Q oxidoreductase (SQR) activities) for superoxide ($O_2^{\cdot-}$) production and subsequent apoptosis.

chemotherapeutic drugs [62]. Hence, the sensitivity of the cancer cell to apoptosis signals determines both tumorigenesis and the clinical outcome of cancer treatment. Based to the wide range of cell death signals acting on malignant cells, many cellular mediators of apoptosis are impaired in cancers. In this section of the review I will give an overview of the mRC alterations found in tumor cells, both general ones and those specifically affecting complex II subunits and discuss how their occurrence can be explained by the new role of complex II as a general sensor for apoptosis signals and by the mechanistic understanding of how it causes apoptosis. I will first turn cover general changes in tumor cells that incapacitate complex II as an apoptosis sensor.

It is well known that cancer cells remodel their metabolism. As a result, the activity of the respiratory chain is reduced while glycolysis is increased (the so-called Warburg effect [63–65]). This is achieved through a range of biochemical changes that also confer a growth advantage to the malignant cells. Lactate, the end product of glycolysis, for example, sustains tumor growth by changing the microenvironment of the tumor cells and also diminish OXPHOS. While this might have a consequence for many other mediators of apoptosis, including Bax and Bak [18], this should also affect complex II as a general mediator of apoptosis signals. The electron flow through RCC II is disrupted during its activation for apoptosis and hence a reduced flow *a priori* should likewise reduce its apoptosis effect. Even though this seems to be a plausible view, it should be stressed that this has not formally been shown. Nevertheless, when the activity of the respiratory chain is reduced the cells' ability to form reactive oxygen species is likewise decreased [66]. More definitive data exists on the biochemical regulators of the Warburg effect and how they affect complex II: the HIF1α transcription factor is activated during hypoxia and enhances glycolysis by tilting the balance between pyruvate dehydrogenase (PDH) and lactate dehydrogenase (LDH) in favour of the latter [67–69], HIF1α targets the Bcl-2 family member BNIP3 [70] to establish a global RC inhibition [71], HIF1α's microRNA-210 target reduces RCC IV activity [72], and finally HIF1α even initiates the direct downregulation of the expression of SDHB [73,74].

Apart from these global RC curtailments specific mutations are also affecting complex II as it contains tumor suppressor proteins [15–17]. Germ-line loss-of-function mutations in SDHB, SDHD (also known as cytochrome bS or cybS) and, albeit more rarely, SDHC (also known as cytochrome bL or cybL) predispose carriers to paragangliomas. Paragangliomas originate from neuronal cells in paraganglia from the skull base to the pelvic floor of chromaffin-negative glomus cells derived from the embryonic neural crest. These cells are part of the sympathetic nervous system (a branch of the autonomic nervous system) and normally act as special chemoreceptors located along blood vessels, particularly in the carotid bodies and in aortic bodies. Complex II mutations cause head and neck paragangliomas (PGL), extra-adrenal PGL, and paragangliomas of the medulla of the adrenal gland known as pheochromocytomas (PH). The neuro-endocrine tumors PGL and PH are considered as relatively rare with a yearly incidence of 1–2 per 1,000,000, respectively [75]. These SDHB/C/D mutations account for 15% of all hereditary cases of those tumors [2] and, since they show loss of heterozygosity by somatic mutation of the normal allele, they constitute classical tumor suppressor genes [75]. Mutations of the same subunits have also been found in 11-16% of patients with apparently sporadic PGL [76,77] but appear to be absent in sporadic PH [78,79]. Mutated SDHB in those tumors is regarded as a high-risk factor for malignancy, poor prognosis and metastasis [80–82]. Complex II mutations have also been found in other types of cancers. SDHB mutations, for example, have been associated with renal cell carcinomas [83,84] and neuroblastoma [85], and SDHB or SDHD mutations were detected in papillary thyroid cancer [83]. Mutations in SDHB, SDHC, and SDHD have also been detected in hereditary PGL-associated gastrointestinal stromal tumors (GIST) [86] and sporadic GIST [87]. While numerous germ-line mutations were found in the iron–sulfur subunit of complex II (Ip, SDHB) and in its two small membrane-anchoring subunits SDHC and SDHD [88], only one mutation in SDHA leading to PGL has been reported so far [89]. These mutations have in common that the abundance of complex II in the IMM of carrier patients is reduced: Mutations in SDHA [89], SDHB [90,91], SDHC [91], SDHD [91–93] and SDHAF2 [94] all lead to the complete absence or at least a dramatic decrease of complex II and its enzymatic activity, both in PGL/PH tumors [91] and in GIST [87]. This is even found in the absence of mutations in the coding sequence of complex II-subunits; individual SDH genes can also be markedly downregulated in PGL/PH [78,95] and in other types of cancers [96,97] suggesting that downregulation of RCC proteins in tumors can have similar effects as mutations in the protein sequence.

Hence, the inhibition of the RC is widespread in cancer and intimately connected to apoptosis resistance. In both scenarios, when complex II is downregulated as a consequence of the general shift from OXPHOS to glycolysis and in the tumors with specific complex II subunit mutations, the end result is a reduction of its activity and consequently its ability to contribute to ROS production and apoptosis induction upon receiving pro-apoptotic signals, through which the tumor cells become desensitized.

The model through which complex II induces apoptosis emphasises its specific disintegration. Recently evidence emerged that the assembly of complex II is regulated. This suggests, but does not prove, that its disassembly is reversible. Two assembly factors for complex II were discovered, one acting on SDHA flavination (SDH5 or SDHAF2, [94]) and the other putatively on SDHB (SDHAF1, [98]). A relatively rare mutation of SDH5 was identified in PGL [94,99]. In line with a role in apoptosis, Tcm62, another complex II assembly factor discovered in yeast [100] and prohibitin (PHB), its putative mammalian homologue, have been implicated in cell death inhibition following growth factor withdrawal [101]. Both proteins act as mitochondrial chaperones and are required for mitochondrial respiratory function [100,102–104]. This could indicate that members of this chaperone family can re-assemble complex II during apoptosis and thereby decrease ROS formation and cell death. In interesting recent study implicated cardiolipin, a mitochondrial lipid that is predominantly contained in the IMM, as being required for the assembly of complex II [105]. With the known role of cardiolipin for apoptosis regulation [106] this finding might represent an important connection to diseases of cardiolipin deficiency such as the X-linked cardioskeletal myopathy and neutropenia (Barth syndrome). This disease is caused by mutations in the tafazzin gene whose gene product is believed to function as an acyltransferase in lipid metabolism and cells from such patients displays reduced apoptosis [107].

It is interesting to note that attempts have been made, with promising-looking results, to pharmaceutically target complex II for apoptosis induction in tumor cells [43,108,109].

## 5. Known unknowns of complex II

While we have undoubtedly made progress in deciphering how complex II contributes to the demise of the cell as a general apoptosis sensor, a number of questions remain or even emerged from the new insights. Is, for example, complex II mediating only accidental cell death, *i.e.* when cells are stressed or is this complex also involved in developmental cell death, *i.e.* intentional cell death? Another important question concerns the exact molecular details of how complex II can sense the pH change and disintegrate during apoptosis. The assumption was made here that amino acid side chains are protonated but it could also be dependent on a more indirect effect. Also, the model about the disintegration of complex II for apoptosis puts into focus a long-ignored aspect of this protein aggregate: its assembly and potentially its re-assembly. The chaperones mentioned here are prime candidate for the regulation of the integrity of the protein complex and, as a consequence, of apoptosis induction. Moreover, the various mutations that have been observed within complex II, both in tumors and neurodegenerative diseases, have been explained in the context of the

model of how complex II is activated for apoptosis. However, this has to be rigorously tested in each case. In particular the consequences of a reduced electron flow on the disintegration of and ROS formation by complex II have to be investigated. Last but not least the tissue specificity of the complex II subunit mutations for certain tumors has to be explained.

In conclusion, future studies on the role of complex II in apoptosis will contribute to a better understanding of the degenerative and proliferation diseases complex II is involved in and hopefully to novel rational therapeutic interference.

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