Mitochondrial complex II and reactive oxygen species in disease and therapy

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ABSTRACT

Increasing evidence points to the respiratory Complex II (CII) as a source and modulator of reactive oxygen species (ROS). Both functional loss of CII as well as its pharmacological inhibition can lead to ROS generation in cells, with a relevant impact on the development of pathophysiological conditions, i.e., cancer and neurodegenerative diseases. While the basic framework of CII involvement in ROS production has been defined, the fine details still await clarification. It is important to resolve these aspects to fully understand the role of CII in pathology and to explore its therapeutic potential in cancer and other diseases.

Introduction

For decades, reactive oxygen species (ROS) have captivated many researchers because of their intractable nature and both beneficial and detrimental roles in cell physiology and pathology. Mitochondria, the site of cellular respiration, are considered the primary site of endogenous ROS production in most cell types. Depending on their concentration, ROS can initiate diverse cellular actions. At physiological levels, they support signaling pathways involved in cell growth and protection, while their high levels lead to cellular damage followed by cell death. The balance between ROS generation and ROS scavenging needs to be tightly regulated [1–3]. Indeed, mitochondrial ROS production has been connected to numerous pathological conditions including neurodegenerative diseases [4], aging [5], oxidative damage during ischemia/reperfusion injury [6], and cancer [7,8].

Initially, respiratory Complex I (NADH:ubiquinone oxidoreductase, CI) and Complex III (ubiquinol:cytochrome c oxidoreductase, CIII) were considered the main sources of mitochondrial ROS, while the contribution of Complex II (succinate dehydrogenase, CII) were considered the main sources of mitochondrial ROS production has been defined, the fine details still await clarification. It is important to resolve these aspects to fully understand the role of CII in pathology and to explore its therapeutic potential in cancer and other diseases.

Complex II structure and function

The research on mitochondrial CII as a source of redox cofactors dates back more than 6 decades [20]. CII is unique in linking the tricarboxylic acid (TCA) cycle and the respiratory chain. CII catalyzes the oxidation of succinate to fumarate, while the contribution of Complex II (succinate dehydrogenase, SDH, CII) was overlooked [9–11]. Identification of mutations in CII resulting in increased ROS production in cancer and neurodegenerative diseases [12–14] and realization that CII plays a crucial role in ROS production also during the reverse electron transfer (RET) through CI [15–18] changed the traditional view. Presently we know that CII contributes significantly to ROS both directly and indirectly (via RET), with important implications in physiology and disease. Mutations in CII are associated with familiar and sporadic forms of cancer, particularly with pheochromocytoma/paraganglioma (PHEO/PGL), gastrointestinal stromal tumors (GIST), and renal cancer, but also with the Leigh syndrome, a neurodegenerative disease. Moreover, CII inhibitors are cardioprotective in ischemia/reperfusion injury (I/R), and can be also applied to cancer therapy [19].

KEYWORDS

Respiratory complex II; succinate dehydrogenase; reactive oxygen species; OXPHOS; mitochondria; cancer; succinate; tricarboxylic acid cycle
Complex II and generation of ROS

In recent years, it has become apparent that CI has an important role in ROS production. This role is either direct, when ROS are generated at CI, or indirect, when ROS are produced at other sites from electrons supplied by CI (Figure 2). The indirect role was described first, because of the old observation that isolated mitochondria produce large amounts of ROS in the presence of high concentrations (≥5 mM) of succinate, the substrate of CI [15–17,33,34], implicating CI in the process. This is due to RET, when succinate-derived electrons from CI reduce the ubiquinone pool, and electrons are forced backwards from ubiquinone towards CI, where vast quantities of ROS are formed. It was suggested that CI can produce ROS under these conditions, but this is somewhat controversial and may be tissue-specific [35].

The direct role of CI in ROS production went long unrecognized. This is because the primary ROS producing site in CI, FAD in SDHA, cannot generate ROS when succinate concentration is high (≥5 mM). The mechanism of succinate-mediated inhibition of ROS production at FAD is not entirely clear, but succinate may block access of oxygen to FAD [36]. Respiratory measurements are traditionally performed at 5-10 mM succinate, which masks FAD contribution to ROS production. At 0.5 mM succinate, a concentration similar to normal intracellular succinate levels, the contribution of CI's electron transport through CI is blocked at the Q site or further downstream (at CIlow, for example), suggesting that ROS is produced when FAD is reduced, but the active site is not occupied [36,37]. Under specific conditions, ROS generation was also observed at the Q site [38], however, this is likely infrequent in mammalian CI.

Inhibitors of CI show ambivalent effect on mitochondrial ROS production depending on substrate supply, membrane potential and overall metabolic activity of the cell as well as intracellular succinate concentration [39,40]. Specific inhibitors of CI bind either to the succinate-binding site, i.e. oxaloacetate and malonate (reviewed in [14]), or to the Q site, i.e. thenoyltrifluoroacetone (TFFA) [22], atpenin [41], α-tocopheryl succinate [42], or mitochondrially targeted vitamin E succinate (MitoVES) [43–45]. Generally, succinate-binding site inhibitors suppress ROS production from CI as they block FAD, while Q site inhibitors stimulate ROS generation as they reduce FAD by blocking electron transfer to ubiquinone. However, in intact cells only intermediate affinity (TFFA, MitoVES) Q site inhibitors induce ROS (and cell death), while high-affinity Q site inhibitors such as atpenin do not induce ROS [45]. The explanation is that the plasma membrane is impermeable for succinate, and succinate rapidly accumulates when high-affinity Q site inhibitors are employed, canceling ROS production from CI. This is perhaps the case for atpenin A5, which immediately blocks all CI molecules in a cell, so that succinate cannot be consumed. Lower affinity Q site inhibitors do not occupy all CI molecules at the same time, which keeps succinate down, allowing ROS production at FAD of those CI molecules that have the Q site blocked. Indeed, atpenin treatment [46], unlike TFFA and MitoVES, do not induce ROS-mediated cell death [45], and atpenin is quite well tolerated by cultured cells. Finally, both the succinate-binding site and the Q site inhibitors suppress ROS production under high succinate concentrations during RET [47] as they all prevent the transfer of electrons from succinate via CI to the ubiquinone pool.

The paradox of ROS production from CI

It has been shown that functional loss of CI can lead to succinate accumulation and ROS generation in cells [19]. Guzy et al.
found that pharmacological inhibition of CII or silencing of SDHB can lead to ROS production and ROS-dependent stabilization of hypoxia-inducible factor-α [48], while others ascribed this effect to the accumulation of succinate [49]. Similarly, CII dysfunction, increased ROS formation, and mtDNA mutability were observed in a yeast model with mutated SDHB [50]. Mutations in the SDHC subunit of CII in fibroblasts from a transgenic mouse enhance ROS generation due to dysfunction of mitochondrial respiration [51]. Similarly, downregulation of the expression of the SDHC subunit in hepatocellular carcinoma was linked to increased cancer cell growth and metastasis due to elevated ROS production with subsequent nuclear factor-κB signaling [52]. A study using hamster fibroblasts revealed that mutation in SDHD resulted in elevated ROS production [53]. A similar effect on the production of ROS and instability of DNA was observed in yeast mutant of SDH [54].

These observations are puzzling given recent strong evidence for FAD in SDHA being the principal site of ROS production in the mature mammalian CII, coming both from isolated mitochondria and from intact cells [36,37,45]. We face the following paradox. Mutations and/or manipulations that interfere with CII and therefore favor reduced FAD will also increase intracellular succinate to concentration over 5 mM which is incompatible with ROS production from FAD in mammalian CII. Indeed, PHEO/PGL-associated mutations in the SDHC subunit that stimulate ROS at low (0.5 mM) succinate levels in isolated mitochondria often do not stimulate ROS in intact cells [45]. There are several relevant aspects that should be considered when thinking about CII-derived ROS in pathology. When wild-type CII alleles are present (heterozygous mutations, incomplete knockdown), these will control succinate levels to some degree to allow ROS production at FAD by mutated CII. Indeed, inherited PHEO/PGL-associated germline mutations are heterozygous during tumor development. Yeast results could perhaps be explained by a different behavior of mammalian/Escherichia coli CII compared to Saccharomyces cerevisiae CII with respect to ROS production. While the amount of ROS produced at different succinate concentrations follows the typical bell-shaped curve for human and E. coli CII (with a maximum at about 0.5 mM succinate, corresponding to a typical concentration in normal cells) [36,47,55], this is not the case for S. cerevisiae CII. In yeast, ROS production at CII is succinate-insensitive and the likely source is the Q site [56,57]. For this reason, yeast CII may not be the optimal model to study ROS-related aspects of CII-dependent tumorigenesis.

Improperly assembled CII, for example incorrect insertion of FeS clusters into SDHB, can result in increased ROS [26]. Yet, Maklashina et al. showed that free E. coli SDHA flavoproteins have only minor catalytic activity and generate little or no ROS. Their results suggest that the iron–sulfur protein SDHB in CII is necessary for robust catalytic activity and ROS generation by incomplete CII [58]. This could explain how CII could produce ROS to amplify the apoptotic response. In this scenario, SDHA/SDHB subcomplex disengages from the

Figure 2. Complex II contributes to ROS production in both physiological and pathophysiological conditions. (A) In the presence of high concentrations of succinate, CII does not produce ROS directly but can contribute to indirect ROS generation via reverse electron transfer (RET) by forcing the electrons onto CI. (B) At lower, physiological succinate concentration, succinate molecule passes the electrons to FAD forming FADH2 which is then able to react with oxygen within the unoccupied succinate binding site, therefore directly forming ROS. (C) The ROS generating ability of reduced FAD is significantly increased when the Q site is blocked by an inhibitor. In contrast, succinate binding site inhibitors block ROS production. (D) Incorrect assembly or damage to CII subunits can induce ROS formation either via reduced FAD or possibly via exposed FeS clusters.
membrane-bound SDHC/SDHD, and superoxide is formed [59]. The precise site of superoxide generation was not identified, but it could possibly originate from the exposed FeS clusters of SDHB that would be insensitive to succinate inhibition. This raises the possibility that CII mutations, which can alter CII conformation (particularly in SDHB), could allow ROS production even in the presence of accumulated intracellular succinate, circumventing the FAD paradox.

**CII in disease**

Isolated defects of CII are relatively rare, accounting for approximately 2% of all respiratory chain deficiency diagnoses [60]. Still, accumulating evidence links SDHx mutations to pathology of the nervous system and the brain. Deficiency of CII is recognized to cause encephalomyopathy in childhood and optic atrophy in adulthood [61]. Jain-Ghai et al. reviewed 37 clinical cases of CII deficiency, concluding that neurological findings, abnormal brain imaging, and developmental delay were the most common manifestation of CII defects, regardless of the large variation in the phenotype [62]. Chronic administration of 3-nitropropionic acid (3-NPA), an irreversible inhibitor of succinate dehydrogenase, replicates the neuropathologic and clinical features of Huntington disease (HD) in nonhuman primates [63]. Later it was shown that patients with HD have severe defects of CII in caudate nucleus [64], which can mediate striatal cell death and neurodegeneration mimicking the development of HD [65]. On the other hand, Naseri et al. recently measured an elevated SDH activity in HD patient lymphoblasts [66], pointing to a possible compartment-specific CII regulation.

One of the rare cases of documented autosomal inheritance of SDHA subunit defect was linked to bilateral optic atrophy, ocular movement disorder, progressive polyneuropathy, psychiatric involvement, and cardiomyopathy [60]. Mutations in SDHA, SDHB, and SDHAF1 were reported in leukodystrophy [67], Leigh syndrome and cardiomyopathy [23,68–70], and infantile leukoencephalopathy [25]. Recently, a case of encephalomyopathy has been connected to a recessive germline mutation in SDHD in nonhuman primates [63]. Later it was shown that patients with HD have severe defects of CII in caudate nucleus [64], which can mediate striatal cell death and neurodegeneration mimicking the development of HD [65]. On the other hand, Naseri et al. recently measured an elevated SDH activity in HD patient lymphoblasts [66], pointing to a possible compartment-specific CII regulation.

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SDHx defects show a strong association with tumorigenesis, and SDHx genes are considered tumor suppressors. Germline mutations in subunits SDHA-D, as well as assembly factor SDHAF2, were recognized to cause familial PHEO/PGL [13,23,72]. Further, SDH dysregulation is linked to GIST oncogenesis [23,73] and renal carcinoma [74,75], but less frequently. In addition, the familiar SDHx defects are connected to PTEN mutation-negative Cowden syndrome, associated with breast, thyroid, and endometrial neoplasias [76].

Unlike in neurological disorders and cancer, in other pathologies, the direct genetic link to CII has not been established. However, evidence is emerging for the role of mitochondrial ROS in obesity [77–79], insulin resistance/diabetes [79,80], cardiovascular diseases [81], and non-alcoholic fatty liver disease [79,82,83]. With regard to CII/Ros, skeletal muscle biopsy from patients with obesity and diabetes showed changes in CII activity [78,84,85]. Also visceral adipose tissue in obese patients exhibits decreased CII activity compared to subcutaneous adipose tissue which can be restored in vitro by addition of the mitochondria-specific oxidant scavenger mito-TEMPO [77]. Chemical inhibition of CII and CII by amiodarone followed by increased ROS production may result in steatohepatitis [86]. Moreover, Fazakerley et al. have suggested that loss of mitochondrial CoQ can drive adipocyte insulin resistance most likely via CII-dependent mitochondrial ROS production [87]. Altogether, CII should be considered when searching for novel therapeutic approaches in metabolic disorders.

**Targeting CII/ROS as a therapeutic approach**

Mitochondrial malfunction and increased ROS production are relevant in aging, neurodegenerative diseases, obesity, diabetes, and cancer [88,89]. ROS can be countered by antioxidants, but the therapeutic application of antioxidants has yielded disappointing results, possibly because only a small fraction of these compounds are taken up by mitochondria [88]. Hence, mitochondrial targeting was employed to accumulate antioxidants within mitochondria [89]. One of the best characterized mitochondria-targeted antioxidants is mitochondrially targeted coenzyme Q (MitoQ) containing the triphenylphosphonium (TPP+) moiety [reviewed in [89,90]]. In mitochondria, the reduced form of MitoQ is oxidized, followed by its rapid re-reduction at CII, which was documented to act as a protective mechanism in different cell models of mitochondrial oxidative stress [91] and neuroprotection [92]. Furthermore, MitoQ was studied in metabolic syndrome and proved to be effective against hypercholesterolemia, hypertriglyceridemia, mtDNA oxidative damage, hyperglycemia, and hepatic steatosis [reviewed in [83]].

Mitochondrial ROS production is involved in I/R injury, and CII inhibitors exert protective effects in different I/R models by suppressing RET [93–96]. Mitochondria-targeted tanshinone IIa, a new CII inhibitor, was developed and showed to be protective in I/R oxidative injury [97]. A similar effect was shown for the ferulic acid derivative hmy-paa (3-(4-hydroxy-3-methoxyphenyl)-N-(1H-pyrazol-3-yl)acylamide) [98]. This is because during the ischemic phase of I/R accumulated succinate is quickly oxidized upon oxygen availability, resulting in massive RET and ROS generation at CII. CII inhibitors, such as malonate, that prevent electron transfer through CII to the ubiquinone pool, therefore, prevent RET and ROS production, are protective during I/R or cold ischemia [99,100]. However, it has also been proposed that CII-dependent reserved respiratory capacity affords cardioprotection during cardiomyocyte recovery from hypoxia [101].

Mills et al. showed that CI-induced ROS production by RET is involved in LPS-stimulated macrophage activity. Succinate-dependent ROS generation was observed, resulting in pro-inflammatory responses, while inhibition of CII by malonate promoted an anti-inflammatory outcome [102]. Interventions at CII can thus regulate inflammation which is associated with numerous metabolic and cardiovascular disorders [103]. Cardioprotective effects of diazoxide was linked directly to inhibition of SDH [104]. In addition, inhibition of CII with 3-NPA reduced glucose-stimulated insulin secretion and ROS production, thereby offering new directions in treatment of cell damage in diabetes [105]. Conversely, while most therapies are focused on inhibiting CII and ROS production, stimulation of SDH activity by succinate administration drives production
of ROS and thermogenic respiration in brown adipose tissue, which may stimulate protection against diet-induced obesity and improve glucose tolerance [106]. These findings suggest that targeting CI and CI-driven ROS production may broaden the potential treatment of metabolic disorders.

It has been proposed that CI may function as a general sensor for apoptosis [59,107], making CI a regulator of cell death [108]. Indeed, blockade of the Q site of CI can induce apoptosis by stimulating ROS production from FAD. The amplitude of cell death is directly proportional to the amount of CI-produced ROS [45]. Thus, CI can be targeted for cancer therapy, and efficient experimental anti-cancer agents directed at the Q site have been developed [42–44,109]. The list of potential chemicals to manipulate CI and CI-dependent ROS has been recently updated, including α-TOS, mitoVES, 3-bromopyruvate, malonate, 3-NPA, TFFA, apernins, lindonamide, and DT-010 as possible candidates for cancer therapy [110]. In addition to the direct effect on cancer cells, some agents also reduce tumor angiogenesis [111,112]. Furthermore, non-toxic doses of the Q site inhibitor TFFA sensitize cancer cells to cell death regulated by other drugs [113], suggesting that CI has potential in combinational cancer therapy. This is in line with CI being an important player in cell death induction. Additionally, it has recently been shown that tumors carrying SDHB mutations produce more ROS and accumulate iron, and disruption of redox hemostasis by ascorbic acid to induce cell death seems to be a promising tool for the treatment of SDHB-mutated PGL/PHEO [114].

Conclusions

Accumulating evidence suggests that CI is an important and underestimated source and modulator of ROS in physiological and pathophysiological conditions that can be manipulated to both induce and suppress cell death, depending on the scenario. Since literature on the therapeutic application of CI modulation in cancer, neurodegeneration, and other pathologies is still fractional, a better understanding of the basic mechanisms of ROS regulation by CI in disease may lead to new therapeutic approaches.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Czech Science Foundation [grant numbers 19-20553S and 20-18513S], the institutional support of the Institute of Biotechnology RVO:66620306, and by the BIOCEN European Regional Development Fund CZ.1.05/1.1.00/02.0109.

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