Mitochondrial complex II, a novel target for anti-cancer agents

Katarina Kluckova,[a] Ayanachew Bezawork-Geleta,[b] Jakub Rohlena,[a] Lanfeng Dong,[c] Jiri Neuzil[a,c,*]

[a]Institute of Biotechnology, Czech Academy of Sciences, Prague, Czech Republic; [b]La Trobe University, Melbourne, Vic, Australia; [c]School of Medical Science, Griffith University, Southport, Qld, Australia

Key words: Mitochondria, complex II, anti-cancer agents, cancer therapy, mitocans.

Abbreviations: BH3, Bcl-2 homology-3; 3BP, 3-bromopyruvate; CI, complex I; DCA, dichloroacetate; ETC, electron transport chain; HIF, hypoxia-inducible factor; MIM, mitochondrial inner membrane; MitoVES, mitochondrially targeted vitamin E succinate; MOM, mitochondrial outer membrane; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; 3NP, 3-nitropropionic acid; ODD, oxygen-dependent destruction; OXPHOS, oxidative phosphorylation; Q_p, proximal UbQ-binding site in CII; Q_d, distal UbQ-binding site in CII; PDK, pyruvate dehydrogenase kinase; PGL, paraganglioma; PHD, prolyl hydroxylase; PHEO, pheochromocytoma; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SDHA, succinate dehydrogenase subunit A; SDHAF1, succinate dehydrogenase assembly factor 1; SQR, succinate quinone reductase; TCA cycle, tricarboxylic acid cycle; α-TOS, α-tocopheryl succinate; α-TEA, α-tocopheryloxyacetic acid; TPP^+, triphenylphosphonium; TTFA, thenoyltrifluoroacetate; UbQ, ubiquinone; $UbQH_2$, ubiquinol; VE, vitamin E;

*Correspondence: Jiri Neuzil Apoptosis Research Group, School of Medical Science and the Griffith Institute of Health and Medical Research, Griffith University, Southport, 4222 Qld, Australia; phone: +61-2-55529109; fax: +61-2-555-28444; e-mail: j.neuzil@griffith.edu.au

ABSTRACT

With the arrival of the third millennium, in spite of unprecedented progress in molecular medicine, cancer remains as untamed as ever. The complexity of tumours, dictating the potential response of cancer cells to anti-cancer agents, has been recently highlighted in a landmark paper by Weinberg and Hanahan on hallmarks of cancer [1]. Together with the recently published papers on the complexity of tumours in patients and even within the same tumour (see below), the cure for this pathology seems to be an elusive goal. Indisputably, the strategy ought to be changed, searching for targets that are generally invariant across the landscape of neoplastic diseases. One such target appears to be the mitochondrial complex II (CII) of the electron transfer chain, a recent focus of research. We document and highlight this particularly intriguing target in this review paper and give examples of drugs that use CII as their molecular target.

1. Introduction

The improvement in sequencing and bioinformatic technology lead to a realisation that tumours are heterogeneous, as exemplified by recent reports revealing the extraordinary variability of mutations in tumours of the same type in different patients[2-4]. This grim notion has been further accentuated by a publication documenting the differences in mutational signatures in different regions of a single tumour and the derived metastases, as shown for renal carcinoma patients [5]. These findings underscore the improbable task of finding a good cancer cure that would target only a single gene or a single signalling pathway. This is most likely also a major reason why cancer incidence is either stagnating or on the rise, depending on the particular type of tumour [6-8]. Therefore, mitochondria came into the focus of contemporary research of cancer biologists.

The importance of mitochondria as potential targets in cancer cells stems from the fact that they are a reservoir of proteins that promote the apoptotic death when mobilised into the cytosol [9, 10]. This notion and the fact that mitochondria are functional, at least to some extent, in most if not all cancer cells, has fuelled considerable interest in these [10-16]. Due to the intriguing nature of mitochondria and accumulating evidence for these organelles comprising a variety of targets for anti-tumour agents, we decided to group these agents under the collective name of 'mitocans', an acronym epitomising the terms 'mitochondria' and 'cancer' [17, 18]. The classification of mitocans has been proposed: we defined 8 classes, according to the individual molecular targets at and inside mitochondria (Figure 1) [19]. Some of these agents have been trialled pre-clinically and are undergoing clinical trials, showing promise to be developed into clinically relevant drugs [20].

Our major interest has been focused on class 5 mitocans, *i.e*. agents acting on the mitochondrial electron transport chain (ETC) [20]. The reason for this has been that in our aspiration to understand the molecular mechanism of the induction of apoptosis by the redox-

silent vitamin E (VE) analogue α-tocopheryl succinate (α-TOS), which is also a selective anti-cancer drug as shown in pre-clinical experiments [21-25], we discovered that the molecular target for the agent is the mitochondrial complex II (CII) [26, 27]. Since CII is invariant in most types of cancer and since VE analogues are likely to enter clinical trials, we believe that it is of importance to document the role of CII as a site of the action of anti-cancer drugs, which is the thrust of this review paper.

2. Biology of complex II

Mitochondrial complex II (CII), also known as succinate dehydrogenase (SDH) or succinate:ubiquinone oxidoreductase (SQR), contains four nuclear encoded sub-units: SDHA, SDHB, SDHC and SDHD (called Sdh1-4 in yeast and SdhA-SdhD in bacteria) and, unlike other mitochondrial complexes, lacks subunits encoded by the mitochondrial genome. CII has a dual role, *i.e*. in the ETC and the tricarboxylic acid (TCA) cycle, linking the two essential energy-producing processes of the cell [28-30]. In TCA cycle, SDH oxidises the metabolite succinate to fumarate, and this reaction is inherent to the SDH activity of CII [30]. As a component of the respiratory complex, it transfers electrons from succinate to ubiquinone (UbQ), referred to as the SQR activity. Bacterial fumarate reductase (also known as succinate:quinone oxidoreductase), which has functional and structural homology to CII [31, 32], catalyses the reverse reaction of reducing fumarate to succinate during anaerobic respiration [31, 33]. The structure of CII purified from porcine heart resolved at 2.4 Å resolution [34] revealed the head-tail arrangement of the hydrophobic subunits (SDHC and SDHD) embedded within the mitochondrial inner membrane (MIM) with a short segment extended into the intermembrane space, while the catalytic subunit SDHA and the SDHB subunit are projected into the matrix (see Figure 2 for CII structure and function).

In contrast to the semi-sequential assembly manner of CI [35], the presence of all subunits of hydrophilic and hydrophobic cores is essential for the stability of functional CII as demonstrated in yeast. Thus, yeast lacking one of the subunits shows a low abundance of the other subunits [36]. However, the catalytic subunits of *E. coli* fumarate reductase are stable and partially active even in the absence of hydrophilic subunits [37]. These findings led to the question how mammalian mitochondria SDH evolved and whether it features unique assembly and regulatory mechanisms.

To carry out its dual activity of electron transfer to UbQ and of oxidising succinate to fumarate, prosthetic groups are required. Accordingly, CII comprises five prosthetic groups including FAD in the SDHA subunit, three iron-sulfur clusters ([2Fe-2S], [4Fe-4S] and [3Fe-4S]) bound in SDHB, and heme inserted within the hydrophobic pocket between SDHC and SDHD. Attachment of these prosthetic groups to the individual subunits, although considered to be autocatalytic, has been documented to require specific proteins collectively termed 'assembly factors' that assist the insertion of prosthetic groups into the holo-form of subunits. Studies on the biogenesis of CI indicated that it requires more than 9 assembly factors [35, 38]. Similarly, numerous factors are also required for the assembly of CIII, CIV and CV. On the other hand, the assembly of mammalian CII is not known in much detail and thus far only two assembly factor proteins have been identified. SDH assembly factor-1 (SDHAF1) is required for the insertion of iron-sulfur clusters in SDHB [39] and SDHAF2, also known as SDH5, for the flavination of SDHA [36].

SDHAF1 belongs to the LYR family of proteins that contain the conserved LYR (LYK) tripeptide motif in the N-terminal region, which is a typical signature of proteins involved in iron-sulfur cluster metabolism. Analogously, other LYR protein family members localise to mitochondria, such as NDUFA6 and NDUFB9 of CI. This is implicates a role for the iron-sulfur cluster formation in CI, while the yeast Mzm1 protein assists the assembly of the

Rieske iron-sulfur protein Rip1 in CIII [40]. Molecular details of the role of SDHAF1 in the insertion of iron-sulfur clusters in the SDHC subunit, which is associated with CII assembly, however, remain to be determined.

SDH5 has been discovered in an effort to characterise previously unannotated mitochondria proteins [36, 41]. Subsequent sequence analyses have suggested that SDH5 is not a *bona fide* member of any superfamily and is not homologous with any known domain or motif; it is now assigned its own specific group called 'SDH5 superfamily'. It has been shown that SDH5 physically associates with SDHA and that it is essential for incorporation of FAD to form the active SDHA flavoprotein. The molecular mechanism of SDH5-assisted insertion of FAD to SDHA is still to be completely resolved in eukaryotes, while recent studies using a homologous bacterial protein SdhE revealed that SdhE itself directly binds FAD via a covalent chemical interaction [42]. It is likely that SdhE acquires FAD from the aqueous environment and hands it over to SdhA. Similarly as for eukaryotes, the mechanism of FAD mobilisation from SdhE to SdhA is not precisely known, and neither is it known whether other macromolecules are involved in the flavination of SdhA. In yeast mitochondria, the flavin transporter Flx1 [43] and the 'chaperone-like' Tcm62 protein [44] have also been reported as assembly factors of CII, although it is not clear yet whether they play a broader role in mitochondria or are dedicated solely to the assembly process [45-47].

Additional modification of CII was proposed on the post-translational level that could modulate its activity. Proteome-wide screen showed that SDHA features 13 acetylated lysine residues [48], which lead to the proposition that deacetylation of these residues by SIRT3 might modulate the SDH activity of CII in a tissue-dependent manner [48, 49]. Also, *in vitro* experiments revealed that the Fgr tyrosine kinase phosphorylates two Tyr residues (Y535 and Y596) of SDHA [50], although the physiological significance of this post translational modification remains unclear. Additional experiments are needed to better understand the

precise molecular mechanism of the assembly and modifications of the subunits of CII in order to comprehend the importance of the role of CII at the 'crossroads' of the TCA cycle and the ETC. The importance of the assembly factors in the function of CII is accentuated by their recently discovered role as tumour suppressors [36, 39].

3. The role of complex II in mitochondrial bioenergetics and mutagenesis

With respect to CII as a target for anti-cancer drugs, it is useful to note that CII subunits function as tumour suppressors. Genes coding for complex II (CII) subunits have received considerable attention in the context of cancer susceptibility genes since 2000, when first germline mutations in SDHD and SDHC were reported in families with hereditary paraganglioma (PGL) [51, 52]. Very soon familial pheochromocytoma (PHEO) revealed SDHD germline mutations [53], and in the same year these authors found germline SDHB mutations in both familial diseases [54]. Finally, very recently a mutation in the SDHA subunit was identified in a PGL patient [55]. The role of CII in PGL was further strengthened by the finding that the CII assembly factor SDH5 was also marked as a tumour susceptibility gene [36]. In addition, mutations in several other genes have been found to be associated with this syndrome [56]. More on SDH mutations and associated cancers can be found in a recent review by Bardella, Pollard and Tomlinson [57] and elsewhere in this issue.

It is not yet entirely clear why CII deficiency gives rise to cancer. It has been reported that the activity of CII in tumors bearing SDHA-D mutations is compromised [58-61]. As CII converts succinate to fumarate in the tricarboxylic acid cycle (TCA), this results in succinate accumulation and substrate inhibition of prolyl hydroxylase enzymes (PHDs), which also form succinate as an end product. PHDs play an important regulatory role, as they hydroxylate hypoxia-inducible factors (HIF1 and HIF2) and in this way mark it for proteasomal degradation [62]. When HIFs are present, they activate transcription of various

genes implicated in cancer like glucose transporters, enzymes promoting glycolysis and pro-angiogenic vascular endothelial growth factor. Indeed, HIF protein stabilization has now been demonstrated in a number of CII deficient tumors [59, 60, 63]. In addition, it has been shown in a yeast model of SDHB deficiency that increased succinate levels may inhibit histone demethylases [64]. Similarly to isocitrate dehydrogenase mutations [65], it was subsequently demonstrated that CII mutations may result in altered epigenetics in mammalian cells [66], but this has as yet not been shown for human cancers.

The accumulation of succinate is undoubtedly an important feature of CII deficient cancers, but the 'succinate theory' cannot explain all of the clinical observations. For example, the deficiency in SDHA subunit should result in massive succinate accumulation as SDHA contains the catalytic site responsible for the succinate to fumarate conversion, yet the SDHA-deficient tumors are extremely rare, while mutations in SDHB-D are much more common. SDHA was in fact not considered a tumor suppressor until 2010 when heterozygous mutation was identified in a catecholamine-secreting abdominal paraganglioma [55]. An alternative theory has been proposed, suggesting reactive oxygen species (ROS) as culprits of increased tumorigenesis. CII has been shown to be a site of ROS generation (references [67, 68] and Moreno-Sanchez et al., submitted for publication), and in SDHA-deficiency there could be no ROS produced from CII due to the absence of electron flow from the non-existent active site, which is consistent with the limited occurrence of SDHA tumors. Increased ROS may result in HIF1 accumulation, but also in increased DNA mutational rate and tumorigenesis [69-71]. However, various groups using various models have reported either increased or normal levels of ROS in CII deficient cells, and this issue remains to be resolved [69, 70, 72, 73]. Increased ROS have not been detected in SDHB-deficient tumours, but the tumour tissue featured elevated superoxide dismutase expression, suggesting that the cells could have adapted to an increase in ROS production over time [61]. It is possible that the

individual factors (such as succinate accumulation and ROS production) may be of varying importance based on the tissue from which the tumour originates and the individual CII subunit that is absent or harbours the tumour-associated mutation(s).

Clinical manifestation of cancer differs, depending on which one of the CII subunits is defective. In PGL/PHEO, the most aggressive tumours are those deficient or malfunctional in SDHB due to their high metastatic potential; they are generally diagnosed at younger age [74, 75]. In contrast, SDHD-deficient tumours give rise to metastasis only very infrequently, and SDHC-deficient tumours even less so, while SDHA-derived tumours are extremely rare. SDHB tumours are relatively frequent in sporadic cases, while SDHC and SDHD tumours are mostly hereditary [75]. This appears an enigma, as 'SDHC/SDHD cases' often involve a complete loss of the subunit, which then results in the absence of SDHB as well (probably due to the failure to properly assemble CII), and one would therefore expect that the manifestation will be the same or not too dissimilar. It was even suggested that the absence of SDHB in PGL/PHEO biopsies could be a surrogate marker for the presence of mutations in SDHB, SDHC or SDHD [76]. To our knowledge, these differences remain unexplained, and no rigorous biochemical examination has been performed to compare SDHB and SDHC/SDHD tumours or *in vitro* models. However, a possibility exists that these differences derive from the chromosomal location of individual SDH genes. For the development of PGL/PHEO, the second allele of the particular SDH gene harbouring the germline mutation is usually inactivated, and this may result in the concomitant elimination of additional tumour suppressor genes in the vicinity of the loci during somatic loss of the second allele [75].

Based on the two mechanisms proposed to be responsible for tumour formation, α-ketoglutarate derivatives and antioxidants were proffered as a therapy, or as a prevention in individuals with familial history of CII mutations. Cell permeable α-ketoglutarate derivatives, substrates for PHD enzymes, were shown to restore the PHD activity in CII-deficient cells,

relieve pseudohypoxia [77] and reverse the hypoxia-mediated HIF1α stabilisation with decrease in glycolysis and ensuing cell death in a xenograft tumour model [78]. A lipid soluble antioxidant, vitamin E, protected cells with CII mutations from oxidative damage represented by lipid peroxidation and reversed their phenotype characterised by apoptosis resistance [79].

Since CII may serve as a target for anti-cancer agents, it is necessary to realise that CII deficiency is present only in the minority of cancers, and even though CII-deficient cells display lower response to CII-targeted agents such as α-TOS/MitoVES [27, 80], this should not present a substantial problem overall. In addition, despite the typical pro-glycolytic changes in many cancer cells, the oxidative phosphorylation (OXPHOS) in large majority of cancer cells is present, albeit often attenuated. This may stem from the fact that in most cancers the glycolytic pathway may be required to supply necessary building blocks for biosynthetic pathways, and not from the necessity to supply the cell with ATP, and cancer cells still mostly have functional OXPHOS [81-84]. Therefore, in most cancers, components of OXPHOS are a suitable target not only for CII-directed agents, but also for other compounds that kill cancer cells by targeting the other respiratory complexes [20]. In addition, it could be perhaps possible to pharmacologically manipulate a cancer cell in such a way that it would be 'primed' for CII-targeted therapy. For example, Pistollato and colleagues reported that the glucose analogue 2-deoxyglucose, which inhibits hexokinase, the first enzyme of the glycolytic pathway, caused an increase in the SDH activity of CII and decreased succinate levels in glioblastoma multiforme cells [85]. It is important to bear in mind, though, that acute inhibition of glycolysis would reduce OXPHOS as well, because the end product of glycolysis, pyruvate, would be less abundant and hence there would be less 'fuel' for the TCA cycle to produce the NADH and $FADH_2$ for respiration. A plausible approach is to re-direct the metabolite flow of cancer cells to mitochondria using

dichloroacetate (DCA), which inhibits the key enzyme regulating the fate of pyruvate, pyruvate dehydrogenase kinase (PDK) and is already used in the clinic to treat lactic acidosis [86]. PDK is overexpressed in cancer cells and phosphorylates and inhibits pyruvate dehydrogenase, so that the end product of glycolysis, pyruvate, cannot enter the mitochondrial TCA cycle and is instead converted to lactate in the cytoplasm. Using DCA, glycolysis could still supply pyruvate to maintain the electron flow through OXPHOS, and further with the inhibition of lactate production by lactate dehydrogenase [87] the redox balance would be impaired and the cell would be more vulnerable to the oxidative stress induced by CII targeting.

4. Complex II as a target for anti-cancer agents

4.1. General aspects

CII, the smallest of the respiratory complexes [34, 88], has recently attracted considerable attention, one reason being since it is at the branching point connecting the TCA cycle and the ETC. While other complexes of the respiratory chain contribute to the maintenance of the proton gradient across the MIM, CII lacks this activity. It can be postulated that the major role of CII is to drive the TCA cycle in the 'clockwise' direction by the conversion of succinate to fumarate, *i.e.* in the direction whereby the generation of NADH, feeding into CI. The electrons released form the conversion of succinate and fumarate are then mobilised to the UbQ molecule bound in the membrane 'portion' of CII, causing its reduction to $UbQH_2$. Further, UbQ in its reduced form relaxes its association of CII and donates the two electrons to CIII, where it causes reduction of CIII's UbQ, itself being re-oxidised. Due to its increased affinity for its CII site, it re-associates with its natural binding domain. Thus, the diversion of electrons form the CII's FAD keeps the SDH activity at the optimum rate.

The crystal structure of CII of both prokaryotes (*E. coli*) and eukaryotes (porcine complex) has been resolved, which gave us a detailed and reasonably precise understanding of its structure and its potential functional consequences [31, 34]. This also made it more feasible to design strategies of targeting CII for apoptosis induction in cancer cells. Apart from papers studying agents that bind to various sites of CII and are considered excellent molecular tools rather than potential anti-cancer agents, the first report, to the best of our knowledge, indicating that CII plays a role in apoptosis, was published by Albayrak and colleagues [89]. In this paper the authors reported on efficient apoptosis in parental cells and its suppression upon mutation in one of the CII subunits (SDHC). The triggers used were both small pharmacological agents as well as ligands activating the death receptors. This breadth of inducers suggested that CII is not an actual target for all these agents but, rather, a mediator of, probably, the earlier stages of the apoptotic process. This is in particular true for apoptosis induced via activation of the death receptors.

4.2. Vitamin E analogues

We have been studying for some time the molecular mechanism and the anti-oxidant properties of a redox-silent VE analogue α-TOS (Table I). This agent is of considerable interest, since it is derived from the nutritionally essential α-tocopherol but, unlike its redox-active counterpart, lacks the anti-oxidant activity and suppresses cancer in animal models [21, 90]. A particularly intriguing feature of α-TOS is its selectivity for cancer cells [91]. The potential importance of α-TOS stems from findings that it is efficient, without secondary toxicity, against a number of experimental cancers, including colon, breast, prostate and lung cancer, as well as mesotheliomas and melanomas, to name only a few [21, 22, 24, 92-97]. A variant of α-TOS, α-tocopheryloxyacetic acid (α-TEA), was shown to suppresses ovarian and breast cancer in experimental animals [98, 99]. We found that the ether α-TEA is more

efficient in suppressing spontaneous breast carcinomas in the transgenic FVB/N *c-neu* mice due to its longer half-life *in vivo* compared to the ester α-TOS [100].

α-TOS induces apoptosis in cancer cells by causing a rapid generation of reactive oxygen species (ROS), causing the formation of a pore in the mitochondrial outer membrane (MOM) [23, 101]. More detailed analysis identified that, at least in some types of cancer cells (as shown for Jurkat T lymphoma and non-small cell lung carcinoma cells), following the initial ROS generation, the Mst1 kinase is activated, phosphorylating and activating the transcription factor FoxO1. This results in transcriptional upregulation of the Bcl-2 homology-3 (BH3)-only protein Noxa [25]. Noxa then diverts the anti-apoptotic protein Mcl-1 from Bak, which is free to form a channel in the MOM [102].

Shiau et al [103] reported that α-TOS and its analogues have the propensity to interact with the BH3 domains of Bcl-2 and Bcl-x_L, whereby inhibiting their anti-apoptotic function. This may be a reason why α-TOS and its analogues have been shown to synergise with several anti-cancer agents, including TRAIL [27, 104], paclitaxel [105], cisplatin [106], or tamoxifen [107]. The notion that α-TOS interacts with BH3 domains of Bcl-2 family of proteins places the agent and its analogues to Class 2 of mitocans [19] (Figure 1). However, the fact that cancer cells respond to the agent by rapid ROS generation [23, 108-111] indicates that α-TOS targets a particular site in mitochondria that results in ROS formation. Such a target has been proposed for α-TOS to be the mitochondrial CI, although the precise mechanism for the interaction with this multicomponent respiratory complex has not been documented [111].

Our attempt to find the molecular target for α-TOS resulted in the identification of CII as the species via which the VE analogue interacts to induce apoptosis in cancer cells [26]. This was shown initially using biochemical methods, which allowed us to suggest the UbQ site(s) in CII as the target for α-TOS, rather than the FAD site in its SDHA subunit. Molecular

modelling indicated strong interaction of α-TOS with both the proximal (Q_p) and the distal UbQ site (Q_d) [34] of CII [26]. More specifically, α-TOS was found to have strong hydrogen bond with the UbQ-binding SDHC's Q_p in the SDHC subunit (Figure 3 A,B) and with SDHD's Lys128/135 [26] of the less well characterised Q_d [34]. The notion that CII is a target for α-TOS was corroborated by loss of apoptosis and ROS generation in cells with a mutation causing a loss of the SDHC subunit, which was overcome by reconstitution of the functional complex [26]. Importantly, too, tumours derived from CII-compromised H-Ras-transformed Chinese hamster lung fibroblasts were resistant, while the tumours derived from CII-competent (either parental or reconstituted) cells were susceptible to α-TOS treatment [27] (Figure 3C-E). These findings clearly document CII as a novel target for compounds with a clinical potential, epitomised by the promising α-TOS. Consistent with this, we also reported that α-TOS and α-TEA selectively suppress angiogenesis by interacting with CII of the proliferating endothelial cells [112].

Identification of the target for α-TOS within the MIM prompted us to develop a novel group of anti-cancer agents by tagging VE analogues with a cationic triphenylphosphonium (TPP^+) group. This reasoning was based on the pioneering work of Murphy and Smith, who attached the TPP^+ group to a range of compounds, in particular the redox-active UbQ to form mitochondrially targeted ubiquinone (MitoQ) as well as to radical scavengers accumulating in mitochondria, such as MitoB and MitoP used to evaluate the production of hydrogen peroxide inside mitochondria of live cells [113-115]. The principle of targeting a hydrophobic agent into mitochondria by tagging it with TPP^+ is based on the fact that the delocalised charge on the quarternary phosphorus will cause up to 1,000-fold accumulation of the modified compound in mitochondria, such that the TPP^+ group will be at the matrix face of the MIM and the bioactive, hydrophobic part (as in MitoQ) inside the MIM [114]. We applied this

principle to vitamin E succinate (VES) to generate mitochondrially targeted VES (MitoVES) (Table I) [80, 116].

Based on the above assumptions, we can reason that due to its chemico-physical properties, MitoVES will span the interface of the MIM and the mitochondrial matrix with the bioactive tocopheryl succinyl group in the proximity of Ser68 of the Q_p site (Figure 4A,B). For reasons of being anchored at the interface by the TPP^+ group, MitoVES will never reach the Q_d site. In other words, by tagging VES with TPP^+, we send the agent directly where it matters, *i.e*. to its site in the MIM, increasing its concentration within the mitochondria theoretically by the factor of 10^3 compared to that of the untagged parental compound. MitoVES was found to be very efficient in apoptosis induction in cancer cells, some 20-50 more than found for α-TOS [80, 116]. It triggered apoptosis by interaction with the CII's Q_p, causing rapid generation of ROS followed by activation of Mst1, nuclear translocation of FoxO1, increased expression of the Noxa protein and generation of the Bak pore in the MOM [80, 116], essentially as found earlier for α-TOS [25, 102]. Interestingly, too, unlike α-TOS that could induce apoptosis, albeit delayed, MitoVES was completely inefficient in apoptosis induction in Jurkat cells lacking both Bak and Bax proteins, a prerequisite for the formation of an MOM pore [80, 116]. This documents that while α-TOS can also destabilise other structures than mitochondria (such as lysosomes) [117, 118], MitoVES is mitochondria-specific due to its localisation (Figure 4 A,B).

The molecular target of MitoVES in CII is particularly intriguing. We found that the length of the aliphatic chain linking the tocopheryl succinyl group and the TPP^+ group of 11 carbons is optimal for MitoVES, since its shortening by 2 carbons at a time causes gradual loss of the activity of the agent, such that MitoVE7S and MitoVE5S have a barely detectable ROS-and apoptosis-inducing activity [80, 116]. Further, we found that MitoVES suppressed the two activities of CII with different efficacy: the SDH activity with IC_{50} ~ 70 μM and the

SQR activity with IC_{50} ~ 1.7 μM. This is a very important finding, since it points to preferential inhibition by MitoVES of the SQR activity by way of interfering with UbQ binding in the Q_p site. At relatively low levels of MitoVES, the SDH activity will be relatively high, allowing for conversion of succinate to fumarate, giving rise to electron generation and their mobilisation towards Q_p. Due to displacement of UbQ with MitoVES, however, electrons will not be intercepted by their natural acceptor and will give recombine with molecular oxygen to form the apoptosis-inducing superoxide [80, 116] (Figure 4C).

The clinical relevance of MitoVES is documented by its highly efficient anti-cancer effect in two models of neoplastic disease: the transgenic FVB/N *c-neu* mice with spontaneous $HER2^{high}$ breast carcinomas and nude mice with colon cancer xenografts [80, 116] (Figure 5 A,B). The agent was more than 20-fold more efficient than the untargeted α-TOS with only marginal if any secondary toxicity, which is of considerable importance for its translational potential of MitoVES. Interestingly, we observed that the molecular mode of action of the agent uncovered in tissue culture experiments is similar to that exerted by the agent *in vivo*, as documented in Figure 5C. In these experiments, we used HCT116 (colorectal) cells stably transfected with the oxygen-dependent destruction (ODD) domain of HIF1α, that controls its stability, fused with GFP. Treatment of the cells with MitoVES for extended periods of time generated green fluorescence, presumably due to pseudohypoxia based on higher accumulation of succinate [62, 78]. The same cells were used to form a tumour in nude mice (*c.f.* Figure 5B), and the treatment of the mice with MitoVES induced development of green fluorescence, as detected in tumour sections (Figure 5C).

We have found yet another intriguing effect of MitoVES. In Figure 1, MitoVES is also placed in Class 8 of mitocans, *i.e.* compounds that affect mtDNA. The reason is that the agent at concentrations below those needed to induce apoptosis, considerably suppresses the level of mtDNA transcripts, in particular the *D-LOOP* transcript, affecting mitochondrial homeostasis

and cancer cell proliferation (Truksa et al., submitted for publication). This was not observed for the untargeted α-TOS. The fact that the effect on the *D-LOOP* transcript was gradually lost with shortening the aliphatic chain of MitoVES indicates that in this case, the effect of MitoVES on mtDNA may be, after all, mediated by its initial interaction with CII, although this has to be resolved. In any case, this is an interesting bioactivity of MitoVES that makes the agent to suppress cancer cell proliferation in an apoptosis-independent manner. Collectively, the above data endow mitochondrially targeted VE analogues, epitomised by MitoVES, with a great translational potential

4.3. 3-Bromopyruvate

3-Bromopyruvate (3BP) is an interesting compound acting via several modes (Table I, Figure 1). It is a Class 1 mitocan, interfering with hexokinase (HK) activity [119-121].]. Its effect on HK stems from its being an alkylating agent, binding to HK2 and causing its dissociation from VDAC [122]. It is apparent that 3BP causes the death of cancer cells by means of fast depletion of ATP, which translated into anti-tumour activity in animal models [119, 123]. 3BP is also an inhibitor of CII, interfering with the SDH activity [124], and we used 3BP to document that α-TOS does not inhibit CII by preferential interaction with the SDHA subunit [26]. Notwithstanding the effect of 3BP on CII, its main target is considered to be HK2. The clinical relevance of this compound is documented by a recent issue of *J. Bioenerg. Biomembr*. dedicated to 3BP [125]. In this issue, a case study was published in which 3BP was successful to extend the life of a fibrolamellar hepatocellular carcinoma patient, indicating a possible clinical application of the agent [126].

4.4. Malonate and 3-nitropropionic acid

Malonate and 3-nitropropionic acid (3NP) are compounds specifically inhibiting the SDH activity of CII; this results in the generation of ROS and induction of apoptosis [127, 128]. Malonate is an important tool when studying the contribution of individual complexes to respiration of cells and tissues. 3NP has also been used in the elucidation of the precise architecture of CII [34]. As anti-cancer agents, the two compounds are of limited value due to their secondary toxicity, in particular being neurotoxic as documented in experimental animals, where they cause severe neurological disorders [129, 130].

Hence, with regard to future anti-cancer drug development, the UbQ-binding sites in the SDHC and SDHD subunits may prove to be better suited targets than sites in the SDHA catalytic domain given the associated problem of neurotoxicity. Inhibiting SDH activity also blocks the TCA cycle as well as the transfer of electrons to the UbQ pool and hence, the metabolic TCA cycle arrest could have much more serious consequences for the cell than the selective inhibition of electron transfer involving the UbQ sites. In the latter case, the TCA cycle would not become fully inhibited, but the disruption of the electron flow at the UbQ sites would still allow for efficient superoxide formation.

4.5. Thenoyltrifluoroacetone

While malonate and 3NP act at the level of the SDHA subunit of CII, thenoyltrifluoroacetone (TTFA) interferes with the UbQ-binding site between the SDHC and SDHD subunits. It was discovered in the 1960s as a compound that interferes with the oxidation of succinate [131]. It was further found that TTFA affects the UbQ site in CII and SQR activity that causes reduction of UbQ to $UbQH_2$ [132, 133]. TTFA was shown to induce apoptosis, cause generation of superoxide/hydrogen peroxide and increase the Ca^{2+} levels [134]. The problem with clinical use of TTFA is that it is highly toxic to non-cancerous cells such as hepatocytes [135] in stark contrast to VE analogues [91]. Thus, TTFA is used as a

molecular tool to study the structure and function of CII, and was useful in precise defining the Q_p site in the porcine CII that was recently crystallised [34].

4.6. Troglitazone

Similarly as the anti-diabetic agent metformin that was found to also act on CII to induce ROS generation and apoptosis [136], the group of diabetes drugs from the group of thiazolidinedione compounds [137] represented by troglitazone were found to interfere with the activity of CII [138]. However, since troglitazone and some other thialidinediones have been found to be highly toxic, including hepatotoxicity and cardiotoxicity, they have been withdrawn [139, 140], which limits their use as potential anti-cancer agents.

4.7. Atpenins

An interesting group of agents acting via targeting CII are atpenins. They were discovered some 25 years ago as fungal metabolites [141]. Soon after, one of the members of the group, atpenin B, was found to considerably suppress ATP levels in the Raji B lymphoma cells [142]. Some 10 years later, it was found that atpenins, epitomised by atpenin A5, act by interfering with the UbQ site in CII and prevent reduction of UbQ [143, 144]. While a lot has been done in terms of understanding the molecular target for atpenins, not much is known about their anti-cancer activity. Thus far there is only one paper reporting on the inhibitory effect of synthetic atpenins on prostate cancer cells [145]. A recent report describes that natural compounds, including atpenins, can suppress the growth of prostate cancer cells by targeting the prostate stromal cells via reducing the expression of the insulin-like growth factor-I [146]. Of considerable interest are findings that by targeting CII, atpenins protect non-malignant tissues, such as the heart muscle, from the pathological effect of events like ischemic insult [147-149]. Very recently, atpenin A5 was used to better understand the

mechanism of ROS generation from CII [67]. This is an important report, since it clearly documents that CII can contribute to the formation of superoxide, which has biological and pathological implications. Since more analogues of atpenins are being synthesised, it is only a question of time when their effects on cancer cells and tumours will be tested and reported in the literature [150-152].

5. Conclusions, further perspectives and clinical relevance

CII is a pharmacologically interesting target, given the possibility to relatively independently interfere with its two enzymatic activities, the 'main' SDH activity which maintains the TCA cycle, and the SQR activity, which supports the SDH activity by electron transfer to UbQ. The inhibition of the former may be clinically problematic, due to high level of side effects such as neurotoxicity, possible related to high dependence of neurons on oxidative phosphorylation and TCA. From our experience and from the literature, it appears that the SQR activity may be a preferred target within CII that may be of clinical relevance. Differences in affinity (full blockade versus partial blockade of SQR activity at intracellular concentrations) between indiscriminately cytotoxic TTFA and cancer-specific VE analogues may perhaps explain the different effects in non-cancerous cells. TTFA may inhibit SQR too efficiently and thus compromise the SDH activity too much in all cells, leading to the loss of specificity. In addition, unknown side effects and indiscriminate uptake by various types of cells may also contribute to TTFA toxicity. The specificity of VE analogues for cancer cells may be also co-determined by the following issues. First, the succinyl moiety of the ester analogues is hydrolysed by esterases abundant in non-cancerous cells. Second, they are increased levels of anti-oxidant defense systems in normal cells. Finally, being weak acids, agents like α-TOS will be more readily taken up by cancer cells due to the acidic nature of the tumour interstitium. MitoVES then appears to derive its selectivity for cancer cells largely

from the fact that it crosses the plasma membrane on the basis of the potential, which is higher in cancer cells than in normal cells. It then specifically associates with mitochondria (spanning the MIM/matrix interface) due to high MIM potential of cancer cells. MitoVES has the optimum length of the aliphatic chain to span the interface and reach and interfere with the Q_p site of CII, yet allowing SDH activity to proceed to some degree. The electrons, however, cannot be all intercepted by UbQ and form the apoptosis-inducing superoxide [80, 116].

The eligibility of CII as a target for anti-cancer drugs is not compromised by the occurrence of mutations in its subunits in cancers. These mutations are associated with relatively infrequent and non-aggressive familiar neoplasias such as paragangliomas and pheochromocytomas, certain percentage of gastrointestinal stromal tumours and pre-cancerous harmatomas in the Cowden syndrome [58, 153-155]. Therefore, CII represents an invariant target in most cancers.

We have started with low scale clinical trials of α-TOS focusing on the currently fatal mesotheliomas. To this end, a single mesothelioma patient was receiving trans-dermal α-TOS, which increased her survival by several years. While we still not completely understand the reasons for possible development of resistance to α-TOS, we found that in cancer cells, their long-term exposure to the agent causes upregulation of a member of the ABC family protein that renders the cells resistant to the drug. We also found that this resistance is overcome by MitoVES (Prochazka et al., submitted for publication). These findings provide us with knowledge how to proceed in the planned clinical trials. If successful, this will place CII at the pedestal of clinically relevant targets, whose exploitation is at its very beginning but is already showing considerable promise.

Acknowledgements

J.N. was supported in part by the Australian Research Council, National Health and Medical Research Council of Australia, Cancer Council Queensland, the Clem Jones Foundation and the Czech Science foundation (P301/10/1937), L.F.D. by the Australian Research Council, K.K. and J.R. by the Czech Science Foundation (P301/12/1851).

References

[1] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell, 144 (2011) 646-674.

[2] S. Jones, X. Zhang, D.W. Parsons, J.C. Lin, R.J. Leary, P. Angenendt, P. Mankoo, H. Carter, H. Kamiyama, A. Jimeno, S.M. Hong, B. Fu, M.T. Lin, E.S. Calhoun, M. Kamiyama, K. Walter, T. Nikolskaya, Y. Nikolsky, J. Hartigan, D.R. Smith, M. Hidalgo, S.D. Leach, A.P. Klein, E.M. Jaffee, M. Goggins, A. Maitra, C. Iacobuzio-Donahue, J.R. Eshleman, S.E. Kern, R.H. Hruban, R. Karchin, N. Papadopoulos, G. Parmigiani, B. Vogelstein, V.E. Velculescu, K.W. Kinzler, Core signaling pathways in human pancreatic cancers revealed by global genomic analyses, Science, 321 (2008) 1801-1806.

[3] D.W. Parsons, S. Jones, X. Zhang, J.C. Lin, R.J. Leary, P. Angenendt, P. Mankoo, H. Carter, I.M. Siu, G.L. Gallia, A. Olivi, R. McLendon, B.A. Rasheed, S. Keir, T. Nikolskaya, Y. Nikolsky, D.A. Busam, H. Tekleab, L.A. Diaz, Jr., J. Hartigan, D.R. Smith, R.L. Strausberg, S.K. Marie, S.M. Shinjo, H. Yan, G.J. Riggins, D.D. Bigner, R. Karchin, N. Papadopoulos, G. Parmigiani, B. Vogelstein, V.E. Velculescu, K.W. Kinzler, An integrated genomic analysis of human glioblastoma multiforme, Science, 321 (2008) 1807-1812.

[4] E. Check Hayden, Cancer complexity slows quest for cure, Nature, 455 (2008) 148.

[5] M. Gerlinger, A.J. Rowan, S. Horswell, J. Larkin, D. Endesfelder, E. Gronroos, P. Martinez, N. Matthews, A. Stewart, P. Tarpey, I. Varela, B. Phillimore, S. Begum, N.Q. McDonald, A. Butler, D. Jones, K. Raine, C. Latimer, C.R. Santos, M. Nohadani, A.C. Eklund, B. Spencer-Dene, G. Clark, L. Pickering, G. Stamp, M. Gore, Z. Szallasi, J. Downward, P.A. Futreal, C. Swanton, Intratumor heterogeneity and branched evolution revealed by multiregion sequencing, N. Engl. J. Med., 366 (2012) 883-892.

[6] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, 2012, CA. Cancer J. Clin., 62 (2012) 10-29.

[7] R. Siegel, C. DeSantis, K. Virgo, K. Stein, A. Mariotto, T. Smith, D. Cooper, T. Gansler, C. Lerro, S. Fedewa, C. Lin, C. Leach, R.S. Cannady, H. Cho, S. Scoppa, M. Hachey, R. Kirch, A. Jemal, E. Ward, Cancer treatment and survivorship statistics, 2012, CA. Cancer J. Clin., 62 (2012) 220-241.

[8] E.P. Simard, E.M. Ward, R. Siegel, A. Jemal, Cancers with increasing incidence trends in the United States: 1999 through 2008, CA. Cancer J. Clin., (2012).

[9] D.R. Green, G. Kroemer, The pathophysiology of mitochondrial cell death, Science, 305 (2004) 626-629.

[10] S.J. Ralph, S. Rodriguez-Enriquez, J. Neuzil, E. Saavedra, R. Moreno-Sanchez, The causes of cancer revisited: "mitochondrial malignancy" and ROS-induced oncogenic transformation - why mitochondria are targets for cancer therapy, Mol. Aspects Med., 31 (2010) 145-170.

[11] V. Gogvadze, S. Orrenius, B. Zhivotovsky, Mitochondria in cancer cells: what is so special about them?, Trends Cell Biol., 18 (2008) 165-173.

[12] S. Fulda, L. Galluzzi, G. Kroemer, Targeting mitochondria for cancer therapy, Nat Rev Drug Discov, 9 (2010) 447-464.

[13] D.C. Wallace, W. Fan, V. Procaccio, Mitochondrial energetics and therapeutics, Annu Rev Pathol, 5 (2010) 297-348.

[14] F. Wang, M.A. Ogasawara, P. Huang, Small mitochondria-targeting molecules as anti-cancer agents, Mol. Aspects Med., 31 (2010) 75-92.

[15] S. Fulda, G. Kroemer, Mitochondria as therapeutic targets for the treatment of malignant disease, Antioxid Redox Signal, 15 (2011) 2937-2949.

[16] O. Kepp, L. Galluzzi, M. Lipinski, J. Yuan, G. Kroemer, Cell death assays for drug discovery, Nat Rev Drug Discov, 10 (2011) 221-237.

[17] J. Neuzil, X.F. Wang, L.F. Dong, P. Low, S.J. Ralph, Molecular mechanism of 'mitocan'-induced apoptosis in cancer cells epitomizes the multiple roles of reactive oxygen species and Bcl-2 family proteins, FEBS Lett., 580 (2006) 5125-5129.

[18] J. Neuzil, J.C. Dyason, R. Freeman, L.F. Dong, L. Prochazka, X.F. Wang, I. Scheffler, S.J. Ralph, Mitocans as anti-cancer agents targeting mitochondria: lessons from studies with vitamin E analogues, inhibitors of complex II, J. Bioenerg. Biomembr., 39 (2007) 65-72.

[19] J. Neuzil, L.F. Dong, J. Rohlena, J. Truksa, S.J. Ralph, Classification of mitocans, anti-cancer drugs acting on mitochondria, Mitochondrion, (2012).

[20] J. Rohlena, L.F. Dong, S.J. Ralph, J. Neuzil, Anticancer drugs targeting the mitochondrial electron transport chain, Antioxid Redox Signal, 15 (2011) 2951-2974.

[21] J. Neuzil, T. Weber, A. Schroder, M. Lu, G. Ostermann, N. Gellert, G.C. Mayne, B. Olejnicka, A. Negre-Salvayre, M. Sticha, R.J. Coffey, C. Weber, Induction of cancer cell apoptosis by alpha-tocopheryl succinate: molecular pathways and structural requirements, FASEB J., 15 (2001) 403-415.

[22] T. Weber, M. Lu, L. Andera, H. Lahm, N. Gellert, M.W. Fariss, V. Korinek, W. Sattler, D.S. Ucker, A. Terman, A. Schroder, W. Erl, U.T. Brunk, R.J. Coffey, C. Weber, J. Neuzil, Vitamin E succinate is a potent novel antineoplastic agent with high selectivity and cooperativity with tumor necrosis factor-related apoptosis-inducing ligand (Apo2 ligand) in vivo, Clin. Cancer Res., 8 (2002) 863-869.

[23] T. Weber, H. Dalen, L. Andera, A. Negre-Salvayre, N. Auge, M. Sticha, A. Lloret, A. Terman, P.K. Witting, M. Higuchi, M. Plasilova, J. Zivny, N. Gellert, C. Weber, J. Neuzil, Mitochondria play a central role in apoptosis induced by alpha-tocopheryl succinate, an agent with antineoplastic activity: comparison with receptor-mediated pro-apoptotic signaling, Biochemistry (Mosc). 42 (2003) 4277-4291.

[24] M. Stapelberg, N. Gellert, E. Swettenham, M. Tomasetti, P.K. Witting, A. Procopio, J. Neuzil, Alpha-tocopheryl succinate inhibits malignant mesothelioma by disrupting the fibroblast growth factor autocrine loop: mechanism and the role of oxidative stress, J. Biol. Chem., 280 (2005) 25369-25376.

[25] K. Valis, L. Prochazka, E. Boura, J. Chladova, T. Obsil, J. Rohlena, J. Truksa, L.F. Dong, S.J. Ralph, J. Neuzil, Hippo/Mst1 stimulates transcription of the proapoptotic mediator NOXA in a FoxO1-dependent manner, Cancer Res., 71 (2011) 946-954.

[26] L.F. Dong, P. Low, J.C. Dyason, X.F. Wang, L. Prochazka, P.K. Witting, R. Freeman, E. Swettenham, K. Valis, J. Liu, R. Zobalova, J. Turanek, D.R. Spitz, F.E. Domann, I.E. Scheffler, S.J. Ralph, J. Neuzil, Alpha-tocopheryl succinate induces apoptosis by targeting ubiquinone-binding sites in mitochondrial respiratory complex II, Oncogene, 27 (2008) 4324-4335.

[27] L.F. Dong, R. Freeman, J. Liu, R. Zobalova, A. Marin-Hernandez, M. Stantic, J. Rohlena, K. Valis, S. Rodriguez-Enriquez, B. Butcher, J. Goodwin, U.T. Brunk, P.K. Witting, R. Moreno-Sanchez, I.E. Scheffler, S.J. Ralph, J. Neuzil, Suppression of tumor growth in vivo by the mitocan alpha-tocopheryl succinate requires respiratory complex II, Clin. Cancer Res., 15 (2009) 1593-1600.

[28] B.A. Ackrell, Progress in understanding structure-function relationships in respiratory chain complex II, FEBS Lett., 466 (2000) 1-5.

[29] M. Saraste, Oxidative phosphorylation at the fin de siecle, Science, 283 (1999) 1488-1493.

[30] G. Cecchini, Function and structure of complex II of the respiratory chain, Annu. Rev. Biochem., 72 (2003) 77-109.

[31] V. Yankovskaya, R. Horsefield, S. Tornroth, C. Luna-Chavez, H. Miyoshi, C. Leger, B. Byrne, G. Cecchini, S. Iwata, Architecture of succinate dehydrogenase and reactive oxygen species generation, Science, 299 (2003) 700-704.

[32] E. Maklashina, D.A. Berthold, G. Cecchini, Anaerobic expression of Escherichia coli succinate dehydrogenase: functional replacement of fumarate reductase in the respiratory chain during anaerobic growth, J. Bacteriol., 180 (1998) 5989-5996.

[33] C. Hagerhall, Succinate: quinone oxidoreductases. Variations on a conserved theme, Biochim. Biophys. Acta, 1320 (1997) 107-141.

[34] F. Sun, X. Huo, Y. Zhai, A. Wang, J. Xu, D. Su, M. Bartlam, Z. Rao, Crystal structure of mitochondrial respiratory membrane protein complex II, Cell, 121 (2005) 1043-1057.

[35] M. McKenzie, M.T. Ryan, Assembly factors of human mitochondrial complex I and their defects in disease, IUBMB Life, 62 (2010) 497-502.

[36] H.X. Hao, O. Khalimonchuk, M. Schraders, N. Dephoure, J.P. Bayley, H. Kunst, P. Devilee, C.W. Cremers, J.D. Schiffman, B.G. Bentz, S.P. Gygi, D.R. Winge, H. Kremer, J. Rutter, SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma, Science, 325 (2009) 1139-1142.

[37] K. Nakamura, M. Yamaki, M. Sarada, S. Nakayama, C.R. Vibat, R.B. Gennis, T. Nakayashiki, H. Inokuchi, S. Kojima, K. Kita, 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., 271 (1996) 521-527.

[38] J. Nouws, L.G. Nijtmans, J.A. Smeitink, R.O. Vogel, Assembly factors as a new class of disease genes for mitochondrial complex I deficiency: cause, pathology and treatment options, Brain, 135 (2012) 12-22.

[39] D. Ghezzi, P. Goffrini, G. Uziel, R. Horvath, T. Klopstock, H. Lochmuller, P. D'Adamo, P. Gasparini, T.M. Strom, H. Prokisch, F. Invernizzi, I. Ferrero, M. Zeviani, SDHAF1, encoding a LYR complex-II specific assembly factor, is mutated in SDH-defective infantile leukoencephalopathy, Nat. Genet., 41 (2009) 654-656.

[40] A. Atkinson, O. Khalimonchuk, P. Smith, H. Sabic, D. Eide, D.R. Winge, Mzm1 influences a labile pool of mitochondrial zinc important for respiratory function, J. Biol. Chem., 285 (2010) 19450-19459.

[41] H.X. Hao, J. Rutter, Revealing human disease genes through analysis of the yeast mitochondrial proteome, Cell Cycle, 8 (2009) 4007-4008.

[42] M.B. McNeil, J.S. Clulow, N.M. Wilf, G.P. Salmond, P.C. Fineran, SdhE is a conserved protein required for flavinylation of succinate dehydrogenase in bacteria, J. Biol. Chem., 287 (2012) 18418-18428.

[43] T.A. Giancaspero, R. Wait, E. Boles, M. Barile, Succinate dehydrogenase flavoprotein subunit expression in Saccharomyces cerevisiae--involvement of the mitochondrial FAD transporter, Flx1p, FEBS J, 275 (2008) 1103-1117.

[44] E. Dibrov, S. Fu, B.D. Lemire, The Saccharomyces cerevisiae TCM62 gene encodes a chaperone necessary for the assembly of the mitochondrial succinate dehydrogenase (complex II), J. Biol. Chem., 273 (1998) 32042-32048.

[45] J. Rutter, D.R. Winge, J.D. Schiffman, Succinate dehydrogenase - Assembly, regulation and role in human disease, Mitochondrion, 10 (2010) 393-401.

[46] A. Tzagoloff, J. Jang, D.M. Glerum, M. Wu, FLX1 codes for a carrier protein involved in maintaining a proper balance of flavin nucleotides in yeast mitochondria, J. Biol. Chem., 271 (1996) 7392-7397.

[47] C. Klanner, W. Neupert, T. Langer, The chaperonin-related protein Tcm62p ensures mitochondrial gene expression under heat stress, FEBS Lett., 470 (2000) 365-369.

[48] L.W. Finley, W. Haas, V. Desquiret-Dumas, D.C. Wallace, V. Procaccio, S.P. Gygi, M.C. Haigis, Succinate dehydrogenase is a direct target of sirtuin 3 deacetylase activity, PLoS One, 6 (2011) e23295.

[49] H. Cimen, M.J. Han, Y. Yang, Q. Tong, H. Koc, E.C. Koc, Regulation of succinate dehydrogenase activity by SIRT3 in mammalian mitochondria, Biochemistry (Mosc). 49 (2010) 304-311.

[50] M. Salvi, N.A. Morrice, A.M. Brunati, A. Toninello, Identification of the flavoprotein of succinate dehydrogenase and aconitase as in vitro mitochondrial substrates of Fgr tyrosine kinase, FEBS Lett., 581 (2007) 5579-5585.

[51] B.E. Baysal, R.E. Ferrell, J.E. Willett-Brozick, E.C. Lawrence, D. Myssiorek, A. Bosch, A. van der Mey, P.E. Taschner, W.S. Rubinstein, E.N. Myers, C.W. Richard, 3rd, C.J. Cornelisse, P. Devilee, B. Devlin, Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma, Science, 287 (2000) 848-851.

[52] S. Niemann, U. Muller, Mutations in SDHC cause autosomal dominant paraganglioma, type 3, Nat. Genet., 26 (2000) 268-270.

[53] D. Astuti, F. Douglas, T.W. Lennard, I.A. Aligianis, E.R. Woodward, D.G. Evans, C. Eng, F. Latif, E.R. Maher, Germline SDHD mutation in familial phaeochromocytoma, Lancet, 357 (2001) 1181-1182.

[54] D. Astuti, F. Latif, A. Dallol, P.L. Dahia, F. Douglas, E. George, F. Skoldberg, E.S. Husebye, C. Eng, E.R. Maher, Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma, Am J Hum Genet, 69 (2001) 49-54.

[55] N. Burnichon, J.J. Briere, R. Libe, L. Vescovo, J. Riviere, F. Tissier, E. Jouanno, X. Jeunemaitre, P. Benit, A. Tzagoloff, P. Rustin, J. Bertherat, J. Favier, A.P. Gimenez-Roqueplo, SDHA is a tumor suppressor gene causing paraganglioma, Hum. Mol. Genet., 19 (2010) 3011-3020.

[56] L. Fishbein, K.L. Nathanson, Pheochromocytoma and paraganglioma: understanding the complexities of the genetic background, Cancer Genet, 205 (2012) 1-11.

[57] C. Bardella, P.J. Pollard, I. Tomlinson, SDH mutations in cancer, Biochim. Biophys. Acta, 1807 (2011) 1432-1443.

[58] K.A. Janeway, S.Y. Kim, M. Lodish, V. Nose, P. Rustin, J. Gaal, P.L. Dahia, B. Liegl, E.R. Ball, M. Raygada, A.H. Lai, L. Kelly, J.L. Hornick, M. O'Sullivan, R.R. de Krijger, W.N. Dinjens, G.D. Demetri, C.R. Antonescu, J.A. Fletcher, L. Helman, C.A. Stratakis, Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations, Proc. Natl. Acad. Sci. U. S. A., 108 (2011) 314-318.

[59] P.J. Pollard, J.J. Briere, N.A. Alam, J. Barwell, E. Barclay, N.C. Wortham, T. Hunt, M. Mitchell, S. Olpin, S.J. Moat, I.P. Hargreaves, S.J. Heales, Y.L. Chung, J.R. Griffiths, A. Dalgleish, J.A. McGrath, M.J. Gleeson, S.V. Hodgson, R. Poulsom, P. Rustin, I.P. Tomlinson, Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations, Hum. Mol. Genet., 14 (2005) 2231-2239.

[60] J. Favier, J.J. Briere, N. Burnichon, J. Riviere, L. Vescovo, P. Benit, I. Giscos-Douriez, A. De Reynies, J. Bertherat, C. Badoual, F. Tissier, L. Amar, R. Libe, P.F. Plouin, X.

Jeunemaitre, P. Rustin, A.P. Gimenez-Roqueplo, The Warburg effect is genetically determined in inherited pheochromocytomas, PLoS One, 4 (2009) e7094.

[61] S.M. Fliedner, N. Kaludercic, X.S. Jiang, H. Hansikova, Z. Hajkova, J. Sladkova, A. Limpuangthip, P.S. Backlund, R. Wesley, L. Martiniova, I. Jochmanova, N.K. Lendvai, J. Breza, A.L. Yergey, N. Paolocci, A.S. Tischler, J. Zeman, F.D. Porter, H. Lehnert, K. Pacak, Warburg Effect's Manifestation in Aggressive Pheochromocytomas and Paragangliomas: Insights from a Mouse Cell Model Applied to Human Tumor Tissue, PLoS One, 7 (2012) e40949.

[62] M.A. Selak, S.M. Armour, E.D. MacKenzie, H. Boulahbel, D.G. Watson, K.D. Mansfield, Y. Pan, M.C. Simon, C.B. Thompson, E. Gottlieb, Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase, Cancer Cell, 7 (2005) 77-85.

[63] Y. Ni, X. He, J. Chen, J. Moline, J. Mester, M.S. Orloff, M.D. Ringel, C. Eng, Germline SDHx variants modify breast and thyroid cancer risks in Cowden and Cowden-like syndrome via FAD/NAD-dependant destabilization of p53, Hum. Mol. Genet., 21 (2012) 300-310.

[64] E.H. Smith, R. Janknecht, L.J. Maher, 3rd, Succinate inhibition of alpha-ketoglutarate-dependent enzymes in a yeast model of paraganglioma, Hum. Mol. Genet., 16 (2007) 3136-3148.

[65] C. Lu, P.S. Ward, G.S. Kapoor, D. Rohle, S. Turcan, O. Abdel-Wahab, C.R. Edwards, R. Khanin, M.E. Figueroa, A. Melnick, K.E. Wellen, D.M. O'Rourke, S.L. Berger, T.A. Chan, R.L. Levine, I.K. Mellinghoff, C.B. Thompson, IDH mutation impairs histone demethylation and results in a block to cell differentiation, Nature, 483 (2012) 474-478.

[66] M. Xiao, H. Yang, W. Xu, S. Ma, H. Lin, H. Zhu, L. Liu, Y. Liu, C. Yang, Y. Xu, S. Zhao, D. Ye, Y. Xiong, K.L. Guan, Inhibition of alpha-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors, Genes Dev., 26 (2012) 1326-1338.

[67] C.L. Quinlan, A.L. Orr, I.V. Perevoshchikova, J.R. Treberg, B.A. Ackrell, M.D. Brand, Mitochondrial Complex II Can Generate Reactive Oxygen Species at High Rates in Both the Forward and Reverse Reactions, J. Biol. Chem., 287 (2012) 27255-27264.

[68] C. Gleason, S. Huang, L.F. Thatcher, R.C. Foley, C.R. Anderson, A.J. Carroll, A.H. Millar, K.B. Singh, Mitochondrial complex II has a key role in mitochondrial-derived reactive oxygen species influence on plant stress gene regulation and defense, Proc. Natl. Acad. Sci. U. S. A., 108 (2011) 10768-10773.

[69] R.D. Guzy, B. Sharma, E. Bell, N.S. Chandel, P.T. Schumacker, Loss of the SdhB, but Not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis, Mol. Cell. Biol., 28 (2008) 718-731.

[70] K.M. Owens, N. Aykin-Burns, D. Dayal, M.C. Coleman, F.E. Domann, D.R. Spitz, Genomic instability induced by mutant succinate dehydrogenase subunit D (SDHD) is mediated by O2(-*) and H2O2, Free Radic. Biol. Med., 52 (2012) 160-166.

[71] T. Ishii, K. Yasuda, A. Akatsuka, O. Hino, P.S. Hartman, N. Ishii, A mutation in the SDHC gene of complex II increases oxidative stress, resulting in apoptosis and tumorigenesis, Cancer Res., 65 (2005) 203-209.

[72] A.M. Cervera, N. Apostolova, F.L. Crespo, M. Mata, K.J. McCreath, Cells silenced for SDHB expression display characteristic features of the tumor phenotype, Cancer Res., 68 (2008) 4058-4067.

[73] M.A. Selak, R.V. Duran, E. Gottlieb, Redox stress is not essential for the pseudo-hypoxic phenotype of succinate dehydrogenase deficient cells, Biochim. Biophys. Acta, 1757 (2006) 567-572.

[74] V. Kantorovich, K. Pacak, Pheochromocytoma and paraganglioma, Prog. Brain Res., 182 (2010) 343-373.

[75] B. Pasini, C.A. Stratakis, SDH mutations in tumorigenesis and inherited endocrine tumours: lesson from the phaeochromocytoma-paraganglioma syndromes, J. Intern. Med., 266 (2009) 19-42.

[76] F.H. van Nederveen, J. Gaal, J. Favier, E. Korpershoek, R.A. Oldenburg, E.M. de Bruyn, H.F. Sleddens, P. Derkx, J. Riviere, H. Dannenberg, B.J. Petri, P. Komminoth, K. Pacak, W.C. Hop, P.J. Pollard, M. Mannelli, J.P. Bayley, A. Perren, S. Niemann, A.A. Verhofstad, A.P. de Bruine, E.R. Maher, F. Tissier, T. Meatchi, C. Badoual, J. Bertherat, L. Amar, D. Alataki, E. Van Marck, F. Ferrau, J. Francois, W.W. de Herder, M.P. Peeters, A. van Linge, J.W. Lenders, A.P. Gimenez-Roqueplo, R.R. de Krijger, W.N. Dinjens, An immunohistochemical procedure to detect patients with paraganglioma and phaeochromocytoma with germline SDHB, SDHC, or SDHD gene mutations: a retrospective and prospective analysis, Lancet Oncol, 10 (2009) 764-771.

[77] E.D. MacKenzie, M.A. Selak, D.A. Tennant, L.J. Payne, S. Crosby, C.M. Frederiksen, D.G. Watson, E. Gottlieb, Cell-permeating alpha-ketoglutarate derivatives alleviate pseudohypoxia in succinate dehydrogenase-deficient cells, Mol. Cell. Biol., 27 (2007) 3282-3289.

[78] D.A. Tennant, C. Frezza, E.D. MacKenzie, Q.D. Nguyen, L. Zheng, M.A. Selak, D.L. Roberts, C. Dive, D.G. Watson, E.O. Aboagye, E. Gottlieb, Reactivating HIF prolyl hydroxylases under hypoxia results in metabolic catastrophe and cell death, Oncogene, 28 (2009) 4009-4021.

[79] Y. Ni, C. Eng, Vitamin E Protects against Lipid Peroxidation and Rescues Tumorigenic Phenotypes in Cowden/Cowden-like Patient-derived Lymphoblast Cells with Germline SDHx Variants, Clin. Cancer Res., (2012).

[80] L.F. Dong, V.J. Jameson, D. Tilly, J. Cerny, E. Mahdavian, A. Marin-Hernandez, L. Hernandez-Esquivel, S. Rodriguez-Enriquez, J. Stursa, P.K. Witting, B. Stantic, J. Rohlena, J. Truksa, K. Kluckova, J.C. Dyason, M. Ledvina, B.A. Salvatore, R. Moreno-Sanchez, M.J. Coster, S.J. Ralph, R.A. Smith, J. Neuzil, Mitochondrial targeting of vitamin E succinate enhances its pro-apoptotic and anti-cancer activity via mitochondrial complex II, J. Biol. Chem., 286 (2011) 3717-3728.

[81] M.G. Vander Heiden, L.C. Cantley, C.B. Thompson, Understanding the Warburg effect: the metabolic requirements of cell proliferation, Science, 324 (2009) 1029-1033.

[82] X.L. Zu, M. Guppy, Cancer metabolism: facts, fantasy, and fiction, Biochem. Biophys. Res. Commun., 313 (2004) 459-465.

[83] R. Moreno-Sanchez, S. Rodriguez-Enriquez, A. Marin-Hernandez, E. Saavedra, Energy metabolism in tumor cells, FEBS J, 274 (2007) 1393-1418.

[84] M. Busk, M.R. Horsman, P.E. Kristjansen, A.J. van der Kogel, J. Bussink, J. Overgaard, Aerobic glycolysis in cancers: implications for the usability of oxygen-responsive genes and fluorodeoxyglucose-PET as markers of tissue hypoxia, Int. J. Cancer, 122 (2008) 2726-2734.

[85] F. Pistollato, S. Abbadi, E. Rampazzo, G. Viola, A. Della Puppa, L. Cavallini, C. Frasson, L. Persano, D.M. Panchision, G. Basso, Hypoxia and succinate antagonize 2-deoxyglucose effects on glioblastoma, Biochem. Pharmacol., 80 (2010) 1517-1527.

[86] S. Bonnet, S.L. Archer, J. Allalunis-Turner, A. Haromy, C. Beaulieu, R. Thompson, C.T. Lee, G.D. Lopaschuk, L. Puttagunta, G. Harry, K. Hashimoto, C.J. Porter, M.A. Andrade, B. Thebaud, E.D. Michelakis, A mitochondria-K+ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth, Cancer Cell, 11 (2007) 37-51.

[87] A. Le, C.R. Cooper, A.M. Gouw, R. Dinavahi, A. Maitra, L.M. Deck, R.E. Royer, D.L. Vander Jagt, G.L. Semenza, C.V. Dang, Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression, Proc. Natl. Acad. Sci. U. S. A., 107 (2010) 2037-2042.

[88] I.E.Scheffler, Mitochondria, Second Edition, John Wiley & Sons, Inc., Hoboken, New Jersey, USA, 2008.

[89] T. Albayrak, V. Scherhammer, N. Schoenfeld, E. Braziulis, T. Mund, M.K. Bauer, I.E. Scheffler, S. Grimm, The tumor suppressor cybL, a component of the respiratory chain, mediates apoptosis induction, Mol. Biol. Cell, 14 (2003) 3082-3096.

[90] J. Neuzil, T. Weber, A. Terman, C. Weber, U.T. Brunk, Vitamin E analogues as inducers of apoptosis: implications for their potential antineoplastic role, Redox Rep, 6 (2001) 143-151.

[91] J. Neuzil, T. Weber, N. Gellert, C. Weber, Selective cancer cell killing by alpha-tocopheryl succinate, Br. J. Cancer, 84 (2001) 87-89.

[92] X.F. Wang, M. Birringer, L.F. Dong, P. Veprek, P. Low, E. Swettenham, M. Stantic, L.H. Yuan, R. Zobalova, K. Wu, M. Ledvina, S.J. Ralph, J. Neuzil, A peptide conjugate of vitamin E succinate targets breast cancer cells with high ErbB2 expression, Cancer Res., 67 (2007) 3337-3344.

[93] K. Kline, W. Yu, B.G. Sanders, Vitamin E and breast cancer, J. Nutr., 134 (2004) 3458S-3462S.

[94] M.P. Malafa, F.D. Fokum, J. Andoh, L.T. Neitzel, S. Bandyopadhyay, R. Zhan, M. Iiizumi, E. Furuta, E. Horvath, K. Watabe, Vitamin E succinate suppresses prostate tumor growth by inducing apoptosis, Int. J. Cancer, 118 (2006) 2441-2447.

[95] J. Quin, D. Engle, A. Litwiller, E. Peralta, A. Grasch, T. Boley, S. Hazelrigg, Vitamin E succinate decreases lung cancer tumor growth in mice, J. Surg. Res., 127 (2005) 139-143.

[96] M. Tomasetti, N. Gellert, A. Procopio, J. Neuzil, A vitamin E analogue suppresses malignant mesothelioma in a preclinical model: a future drug against a fatal neoplastic disease?, Int. J. Cancer, 109 (2004) 641-642.

[97] M.P. Malafa, F.D. Fokum, A. Mowlavi, M. Abusief, M. King, Vitamin E inhibits melanoma growth in mice, Surgery, 131 (2002) 85-91.

[98] K. Anderson, M. Simmons-Menchaca, K.A. Lawson, J. Atkinson, B.G. Sanders, K. Kline, Differential response of human ovarian cancer cells to induction of apoptosis by vitamin E Succinate and vitamin E analogue, alpha-TEA, Cancer Res., 64 (2004) 4263-4269.

[99] T. Hahn, L. Szabo, M. Gold, L. Ramanathapuram, L.H. Hurley, E.T. Akporiaye, Dietary administration of the proapoptotic vitamin E analogue alpha-tocopheryloxyacetic acid inhibits metastatic murine breast cancer, Cancer Res., 66 (2006) 9374-9378.

[100] L.F. Dong, G. Grant, H. Massa, R. Zobalova, E. Akporiaye, J. Neuzil, alpha-Tocopheryloxyacetic acid is superior to alpha-tocopheryl succinate in suppressing HER2-high breast carcinomas due to its higher stability, Int. J. Cancer, 131 (2012) 1052-1058.

[101] W. Yu, B.G. Sanders, K. Kline, RRR-alpha-tocopheryl succinate-induced apoptosis of human breast cancer cells involves Bax translocation to mitochondria, Cancer Res., 63 (2003) 2483-2491.

[102] L. Prochazka, L.F. Dong, K. Valis, R. Freeman, S.J. Ralph, J. Turanek, J. Neuzil, alpha-Tocopheryl succinate causes mitochondrial permeabilization by preferential formation of Bak channels, Apoptosis, 15 (2010) 782-794.

[103] C.W. Shiau, J.W. Huang, D.S. Wang, J.R. Weng, C.C. Yang, C.H. Lin, C. Li, C.S. Chen, alpha-Tocopheryl succinate induces apoptosis in prostate cancer cells in part through inhibition of Bcl-xL/Bcl-2 function, J. Biol. Chem., 281 (2006) 11819-11825.

[104] M. Tomasetti, M.R. Rippo, R. Alleva, S. Moretti, L. Andera, J. Neuzil, A. Procopio, Alpha-tocopheryl succinate and TRAIL selectively synergise in induction of apoptosis in human malignant mesothelioma cells, Br. J. Cancer, 90 (2004) 1644-1653.

[105] K. Kanai, E. Kikuchi, S. Mikami, E. Suzuki, Y. Uchida, K. Kodaira, A. Miyajima, T. Ohigashi, J. Nakashima, M. Oya, Vitamin E succinate induced apoptosis and enhanced chemosensitivity to paclitaxel in human bladder cancer cells in vitro and in vivo, Cancer Sci, 101 (2010) 216-223.

[106] K. Anderson, K.A. Lawson, M. Simmons-Menchaca, L. Sun, B.G. Sanders, K. Kline, Alpha-TEA plus cisplatin reduces human cisplatin-resistant ovarian cancer cell tumor burden and metastasis, Exp Biol Med (Maywood), 229 (2004) 1169-1176.

[107] R. Tiwary, W. Yu, L.A. deGraffenried, B.G. Sanders, K. Kline, Targeting cholesterol-rich microdomains to circumvent tamoxifen-resistant breast cancer, Breast Cancer Res, 13 (2011) R120.

[108] P. Ottino, J.R. Duncan, Effect of alpha-tocopherol succinate on free radical and lipid peroxidation levels in BL6 melanoma cells, Free Radic. Biol. Med., 22 (1997) 1145-1151.

[109] Y.H. Kang, E. Lee, M.K. Choi, J.L. Ku, S.H. Kim, Y.G. Park, S.J. Lim, Role of reactive oxygen species in the induction of apoptosis by alpha-tocopheryl succinate, Int. J. Cancer, 112 (2004) 385-392.

[110] V. Gogvadze, E. Norberg, S. Orrenius, B. Zhivotovsky, Involvement of Ca2+ and ROS in alpha-tocopheryl succinate-induced mitochondrial permeabilization, Int. J. Cancer, 127 (2010) 1823-1832.

[111] G.A. dos Santos, R.S. Abreu e Lima, C.R. Pestana, A.S. Lima, P.S. Scheucher, C.H. Thome, H.L. Gimenes-Teixeira, B.A. Santana-Lemos, A.R. Lucena-Araujo, F.P. Rodrigues, R. Nasr, S.A. Uyemura, R.P. Falcao, H. de The, P.P. Pandolfi, C. Curti, E.M. Rego, (+)alpha-Tocopheryl succinate inhibits the mitochondrial respiratory chain complex I and is as effective as arsenic trioxide or ATRA against acute promyelocytic leukemia in vivo, Leukemia, 26 (2012) 451-460.

[112] L.F. Dong, E. Swettenham, J. Eliasson, X.F. Wang, M. Gold, Y. Medunic, M. Stantic, P. Low, L. Prochazka, P.K. Witting, J. Turanek, E.T. Akporiaye, S.J. Ralph, J. Neuzil, Vitamin E analogues inhibit angiogenesis by selective induction of apoptosis in proliferating endothelial cells: the role of oxidative stress, Cancer Res., 67 (2007) 11906-11913.

[113] G.F. Kelso, C.M. Porteous, C.V. Coulter, G. Hughes, W.K. Porteous, E.C. Ledgerwood, R.A. Smith, M.P. Murphy, Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties, J. Biol. Chem., 276 (2001) 4588-4596.

[114] M.P. Murphy, R.A. Smith, Targeting antioxidants to mitochondria by conjugation to lipophilic cations, Annu. Rev. Pharmacol. Toxicol., 47 (2007) 629-656.

[115] H.M. Cocheme, C. Quin, S.J. McQuaker, F. Cabreiro, A. Logan, T.A. Prime, I. Abakumova, J.V. Patel, I.M. Fearnley, A.M. James, C.M. Porteous, R.A. Smith, S. Saeed, J.E. Carre, M. Singer, D. Gems, R.C. Hartley, L. Partridge, M.P. Murphy, Measurement of H2O2 within living Drosophila during aging using a ratiometric mass spectrometry probe targeted to the mitochondrial matrix, Cell Metab, 13 (2011) 340-350.

[116] L.F. Dong, V.J. Jameson, D. Tilly, L. Prochazka, J. Rohlena, K. Valis, J. Truksa, R. Zobalova, E. Mahdavian, K. Kluckova, M. Stantic, J. Stursa, R. Freeman, P.K. Witting, E. Norberg, J. Goodwin, B.A. Salvatore, J. Novotna, J. Turanek, M. Ledvina, P. Hozak, B. Zhivotovsky, M.J. Coster, S.J. Ralph, R.A. Smith, J. Neuzil, Mitochondrial targeting of alpha-tocopheryl succinate enhances its pro-apoptotic efficacy: a new paradigm for effective cancer therapy, Free Radic. Biol. Med., 50 (2011) 1546-1555.

[117] J. Neuzil, I. Svensson, T. Weber, C. Weber, U.T. Brunk, alpha-tocopheryl succinate-induced apoptosis in Jurkat T cells involves caspase-3 activation, and both lysosomal and mitochondrial destabilisation, FEBS Lett., 445 (1999) 295-300.

[118] J. Neuzil, M. Zhao, G. Ostermann, M. Sticha, N. Gellert, C. Weber, J.W. Eaton, U.T. Brunk, Alpha-tocopheryl succinate, an agent with in vivo anti-tumour activity, induces apoptosis by causing lysosomal instability, Biochem. J., 362 (2002) 709-715.

[119] S.P. Mathupala, Y.H. Ko, P.L. Pedersen, Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria, Oncogene, 25 (2006) 4777-4786.

[120] A. Nakano, H. Miki, S. Nakamura, T. Harada, A. Oda, H. Amou, S. Fujii, K. Kagawa, K. Takeuchi, S. Ozaki, T. Matsumoto, M. Abe, Up-regulation of hexokinaseII in myeloma cells: targeting myeloma cells with 3-bromopyruvate, J. Bioenerg. Biomembr., 44 (2012) 31-38.

[121] C. Rodrigues-Ferreira, A.P. da Silva, A. Galina, Effect of the antitumoral alkylating agent 3-bromopyruvate on mitochondrial respiration: role of mitochondrially bound hexokinase, J. Bioenerg. Biomembr., 44 (2012) 39-49.

[122] Z. Chen, H. Zhang, W. Lu, P. Huang, Role of mitochondria-associated hexokinase II in cancer cell death induced by 3-bromopyruvate, Biochim. Biophys. Acta, 1787 (2009) 553-560.

[123] Y.H. Ko, B.L. Smith, Y. Wang, M.G. Pomper, D.A. Rini, M.S. Torbenson, J. Hullihen, P.L. Pedersen, Advanced cancers: eradication in all cases using 3-bromopyruvate therapy to deplete ATP, Biochem. Biophys. Res. Commun., 324 (2004) 269-275.

[124] A.P. Pereira da Silva, T. El-Bacha, N. Kyaw, R.S. dos Santos, W.S. da-Silva, F.C. Almeida, A.T. Da Poian, A. Galina, Inhibition of energy-producing pathways of HepG2 cells by 3-bromopyruvate, Biochem. J., 417 (2009) 717-726.

[125] P.L. Pedersen, 3-Bromopyruvate (3BP) a fast acting, promising, powerful, specific, and effective "small molecule" anti-cancer agent taken from labside to bedside: introduction to a special issue, J. Bioenerg. Biomembr., 44 (2012) 1-6.

[126] Y.H. Ko, H.A. Verhoeven, M.J. Lee, D.J. Corbin, T.J. Vogl, P.L. Pedersen, A translational study "case report" on the small molecule "energy blocker" 3-bromopyruvate (3BP) as a potent anticancer agent: from bench side to bedside, J. Bioenerg. Biomembr., 44 (2012) 163-170.

[127] M. Gomez-Lazaro, M.F. Galindo, R.M. Melero-Fernandez de Mera, F.J. Fernandez-Gomez, C.G. Concannon, M.F. Segura, J.X. Comella, J.H. Prehn, J. Jordan, Reactive oxygen species and p38 mitogen-activated protein kinase activate Bax to induce mitochondrial cytochrome c release and apoptosis in response to malonate, Mol. Pharmacol., 71 (2007) 736-743.

[128] L.S. Huang, G. Sun, D. Cobessi, A.C. Wang, J.T. Shen, E.Y. Tung, V.E. Anderson, E.A. Berry, 3-nitropropionic acid is a suicide inhibitor of mitochondrial respiration that, upon oxidation by complex II, forms a covalent adduct with a catalytic base arginine in the active site of the enzyme, J. Biol. Chem., 281 (2006) 5965-5972.

[129] P. Bonsi, D. Cuomo, G. Martella, G. Sciamanna, M. Tolu, P. Calabresi, G. Bernardi, A. Pisani, Mitochondrial toxins in Basal Ganglia disorders: from animal models to therapeutic strategies, Curr Neuropharmacol, 4 (2006) 69-75.

[130] G. Liot, B. Bossy, S. Lubitz, Y. Kushnareva, N. Sejbuk, E. Bossy-Wetzel, Complex II inhibition by 3-NP causes mitochondrial fragmentation and neuronal cell death via an NMDA- and ROS-dependent pathway, Cell Death Differ., 16 (2009) 899-909.

[131] S. Takemori, T.E. King, Coenzyme Q: Reversal of Inhibition of Succinate Cytochrome C Reductase by Lipophilic Compounds, Science, 144 (1964) 852-853.

[132] R.R. Ramsay, B.A. Ackrell, C.J. Coles, T.P. Singer, G.A. White, G.D. Thorn, Reaction site of carboxanilides and of thenoyltrifluoroacetone in complex II, Proc. Natl. Acad. Sci. U. S. A., 78 (1981) 825-828.

[133] V.G. Grivennikova, A.D. Vinogradov, Kinetics of ubiquinone reduction by the resolved succinate: ubiquinone reductase, Biochim. Biophys. Acta, 682 (1982) 491-495.

[134] A. Mehta, C. Shaha, Apoptotic death in Leishmania donovani promastigotes in response to respiratory chain inhibition: complex II inhibition results in increased pentamidine cytotoxicity, J. Biol. Chem., 279 (2004) 11798-11813.

[135] J.G. Zhang, M.A. Tirmenstein, F.A. Nicholls-Grzemski, M.W. Fariss, Mitochondrial electron transport inhibitors cause lipid peroxidation-dependent and -independent cell death: protective role of antioxidants, Arch. Biochem. Biophys., 393 (2001) 87-96.

[136] M.R. Owen, E. Doran, A.P. Halestrap, Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain, Biochem. J., 348 Pt 3 (2000) 607-614.

[137] S. Mudaliar, R.R. Henry, New oral therapies for type 2 diabetes mellitus: The glitazones or insulin sensitizers, Annu. Rev. Med., 52 (2001) 239-257.

[138] M. Soller, S. Drose, U. Brandt, B. Brune, A. von Knethen, Mechanism of thiazolidinedione-dependent cell death in Jurkat T cells, Mol. Pharmacol., 71 (2007) 1535-1544.

[139] J.C. Parker, Troglitazone: the discovery and development of a novel therapy for the treatment of Type 2 diabetes mellitus, Adv Drug Deliv Rev, 54 (2002) 1173-1197.

[140] D.J. Graham, L. Green, J.R. Senior, P. Nourjah, Troglitazone-induced liver failure: a case study, Am. J. Med., 114 (2003) 299-306.

[141] S. Omura, H. Tomoda, K. Kimura, D.Z. Zhen, H. Kumagai, K. Igarashi, N. Imamura, Y. Takahashi, Y. Tanaka, Y. Iwai, Atpenins, new antifungal antibiotics produced by Penicillium sp. Production, isolation, physico-chemical and biological properties, J. Antibiot. (Tokyo). 41 (1988) 1769-1773.

[142] K. Oshino, H. Kumagai, H. Tomoda, S. Omura, Mechanism of action of atpenin B on Raji cells, J. Antibiot. (Tokyo). 43 (1990) 1064-1068.

[143] H. Miyadera, K. Shiomi, H. Ui, Y. Yamaguchi, R. Masuma, H. Tomoda, H. Miyoshi, A. Osanai, K. Kita, S. Omura, Atpenins, potent and specific inhibitors of mitochondrial complex II (succinate-ubiquinone oxidoreductase), Proc. Natl. Acad. Sci. U. S. A., 100 (2003) 473-477.

[144] R. Horsefield, V. Yankovskaya, G. Sexton, W. Whittingham, K. Shiomi, S. Omura, B. Byrne, G. Cecchini, S. Iwata, Structural and computational analysis of the quinone-binding site of complex II (succinate-ubiquinone oxidoreductase): a mechanism of

electron transfer and proton conduction during ubiquinone reduction, J. Biol. Chem., 281 (2006) 7309-7316.

[145] M. Kawada, I. Momose, T. Someno, G. Tsujiuchi, D. Ikeda, New atpenins, NBRI23477 A and B, inhibit the growth of human prostate cancer cells, J. Antibiot. (Tokyo). 62 (2009) 243-246.

[146] M. Kawada, H. Inoue, S. Ohba, T. Masuda, I. Momose, D. Ikeda, Leucinostatin A inhibits prostate cancer growth through reduction of insulin-like growth factor-I expression in prostate stromal cells, Int. J. Cancer, 126 (2010) 810-818.

[147] A.P. Wojtovich, P.S. Brookes, The complex II inhibitor atpenin A5 protects against cardiac ischemia-reperfusion injury via activation of mitochondrial KATP channels, Basic Res. Cardiol., 104 (2009) 121-129.

[148] S. Drose, L. Bleier, U. Brandt, A common mechanism links differently acting complex II inhibitors to cardioprotection: modulation of mitochondrial reactive oxygen species production, Mol. Pharmacol., 79 (2011) 814-822.

[149] S.J. Ralph, R. Moreno-Sanchez, J. Neuzil, S. Rodriguez-Enriquez, Inhibitors of succinate: quinone reductase/Complex II regulate production of mitochondrial reactive oxygen species and protect normal cells from ischemic damage but induce specific cancer cell death, Pharm. Res., 28 (2011) 2695-2730.

[150] T.P. Selby, K.A. Hughes, J.J. Rauh, W.S. Hanna, Synthetic atpenin analogs: Potent mitochondrial inhibitors of mammalian and fungal succinate-ubiquinone oxidoreductase, Bioorg. Med. Chem. Lett., 20 (2010) 1665-1668.

[151] M. Ohtawa, K. Sugiyama, T. Hiura, S. Izawa, K. Shiomi, S. Omura, T. Nagamitsu, Stereoselective total synthesis of atpenins a4 and b, harzianopyridone, and NBRI23477 B, Chem. Pharm. Bull. (Tokyo). 60 (2012) 898-906.

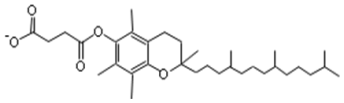
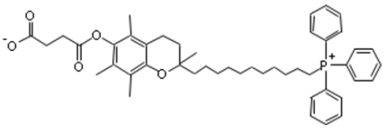
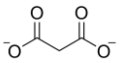
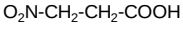
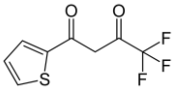
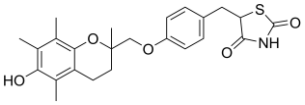
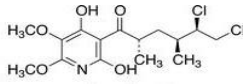
[152] C. Sakai, E. Tomitsuka, H. Esumi, S. Harada, K. Kita, Mitochondrial fumarate reductase as a target of chemotherapy: From parasites to cancer cells, Biochim. Biophys. Acta, 1820 (2012) 643-651.

[153] F. Schiavi, C.C. Boedeker, B. Bausch, M. Peczkowska, C.F. Gomez, T. Strassburg, C. Pawlu, M. Buchta, M. Salzmann, M.M. Hoffmann, A. Berlis, I. Brink, M. Cybulla, M. Muresan, M.A. Walter, F. Forrer, M. Valimaki, A. Kawecki, Z. Szutkowski, J. Schipper, M.K. Walz, P. Pigny, C. Bauters, J.E. Willet-Brozick, B.E. Baysal, A. Januszewicz, C. Eng, G. Opocher, H.P. Neumann, Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene, JAMA, 294 (2005) 2057-2063.

[154] M. Peczkowska, A. Cascon, A. Prejbisz, A. Kubaszek, B.J. Cwikla, M. Furmanek, Z. Erlic, C. Eng, A. Januszewicz, H.P. Neumann, Extra-adrenal and adrenal pheochromocytomas associated with a germline SDHC mutation, Nat Clin Pract Endocrinol Metab, 4 (2008) 111-115.

[155] Y. Ni, K.M. Zbuk, T. Sadler, A. Patocs, G. Lobo, E. Edelman, P. Platzer, M.S. Orloff, K.A. Waite, C. Eng, Germline mutations and variants in the succinate dehydrogenase genes in Cowden and Cowden-like syndromes, Am J Hum Genet, 83 (2008) 261-268.

Table 1. Compounds targeting complex II.

Compound	Structure
α-Tocopheryl succinate (α-TOS)	
Mitochondrially targeted vitamin E succinate (MitoVES)	
3-Bromopyruvate (3BP)	
Malonate	
Nitropropionic acid (3NP)	
Thenoyltrifluoroacetone (TTFA)	
Troglitazone	
Atpenin A5	

Legend to figures

Fig. 1. The classification of mitocans. Mitocans, small molecules with anti-cancer activity that act upon mitochondria, are classified into several classes. Class 1 mitocans comprises agents targeting HK, Class 2 compounds acting on Bcl-2 family proteins (BH3 mimetics and similar compounds), Class 3 and 4 compouds with redox-inhibitory function and acting on the VDAC and ANT channel proteins, Class 5 agents targeting the ETC, Class 7 lipophilic compounds targeting the inner membrane, Class 7 agents targeting the TCA cycle, and Class 8 compounds acting on mtDNA. Highlighted are the Class 5 mitocans and CII, which as a target for anti-cancer drugs is the subject of this paper. Adapted from [19].

Fig. 2. The structure of complex II. Complex II consists of four subunits, the 70 kD SDHA (F_p), the 30 kDa SDHB (I_p), the 15 kDa SDHC (Cyb_L) and the 13 kDa SDHD (Cyb_S) subunit. The SDHA subunit contains the prosthetic group FAD, which participate in the TCA cycle, converting succinate to fumarate (SDH activity of CII), resulting in the generation of $FADH_2$ that feeds electrons to CII. The two electrons generated from conversion of succinate to fumarate are mobilised to the SDHB subunit that comprises three [Fe-S] clusters arranged to force the movement of electrons to the membrane 'portion' of CII composed of the SDHC and SDHD subunits with the heme and UbQ prosthetic groups. Heme helps stabilise electrons adjacent to UbQ to help with the two-electron reduction of UbQ to $UbQH_2$ (SQR activity of CII). $UbQH_2$ then leaves its site in CII to tranverses to CII, where it is re-oxidised, returning to CII. Shown are also two assembly factors, SDHAF2 (SDH5) helping insert FAD into SDHA, and SDHAF1 with a role in the insertion of the [Fe-S] clusters into the SDHB subunit. Adapted from [19].

Fig. 3. Model of the interaction of α-TOS with CII and its effect on CII-functional and dysfunctional tumours. Molecular modeling indicates the position of α-TOS in relation to UbQ and heme between the SDHC and SDHD subunits of CII (A), and its interaction with the Ser68 (shown here as Ser42 as used in the study by Sun et al [34], which binds UbQ in the Q_p site. Strong hydrogen bonds of the oxo groups of the succinyl moiety of α-TOS with the SDHC's Ser68 are indicated (B). Parental B1, SDHC-deficient B9 and SDHC-reconstituted $B9_{SDHC}$ cells were transformed with H-Ras and grafted into nude mice to form tumours. The carcinomas were treated with α-TOS, indicating a good response of the CII-functional and very little response of the CII-compromised tumours (C-E). Adapted from [26] and [116].

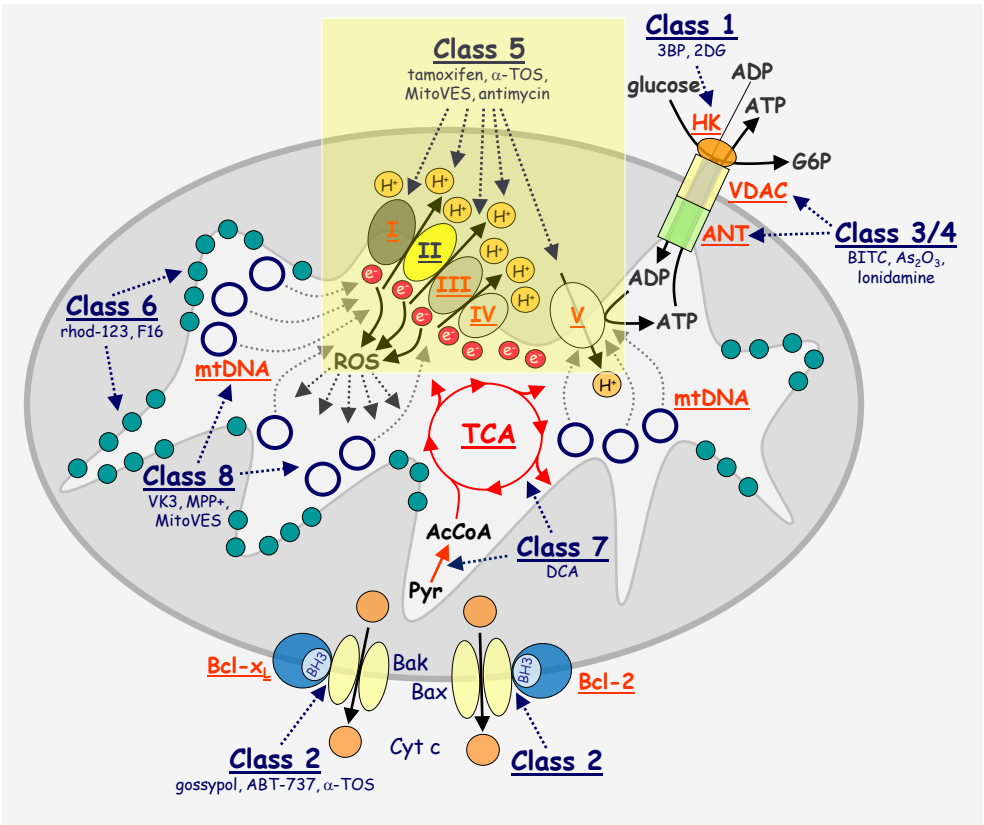
Fig. 4. Mitochondrial localisation of MitoVES, its interaction with CII and the molecular mechanism of its effect on CII. A. Mouse breast cancer NeuTL cells were incubated with MitoTracker Red and fluorescently labelled MitoVES or α-TOS and inspected by confocal microscopy. Hoechst 33342 was used to visualise the nuclei. B. Molecular modelling indicated the position of MitoVES at the interface of the MIM and matrix components of CII. C. MitoVES inhibits both SDH and SQR activity of CII, the former with IC_{50} ~ 70 μM, the latter with IC_{50} ~ 2 μM. Due to this scenario, the conversion of succinate to fumarate occurs in the presence of MitoVES, albeit at a lower rate, generating electrons that are forced to transverse to the membrane components of CII. In the situation when UbQ is displaced from the Q_p site in CII, electrons lack their natural acceptor and recombine with molecular oxygen to give rise to superoxide. This then triggers a series of reactions that result in the induction of the apoptotic cascade, resulting in the demise of the cell. Adapted from [19, 80, 116].

Fig. 5. MitoVES efficiently suppresses tumours and induces pseudohypoxia in cancer cells and in tumours. FVB/N *c-neu* transgenic mice with spontaneous breast carcinomas (A) and

nude mice with xenografts derived from colorectal HCT116 cells (B) were treated with α-TOS and with MitoVES at 10-fold lower doses than that of the former. Ultrasound imaging documents a superior activity of MitoVES over its untargeted counterpart. C. HCT116 colon cancer cells were stably transfected with the *ODD-GFP* gene-containing plasmid. The cells were exposed to 5 μM MitoVES for 12 h and green colour, indicative of the accumulation of the chimeric ODD-GFP protein observed using confocal microscopy (upper images). Sections from $HCT116_{ODD\text{-}GFP}$-derived tumours treated with MitoVES were inspected by confocal microscopy, revealing green fluorescence. The *in vitro* and *in vivo* experiments document that the mechanism by which MitoVES induces apoptosis is similar in both settings. Adapted from [80] and [116].

Figure

Figure 1



Figure

Figure 2

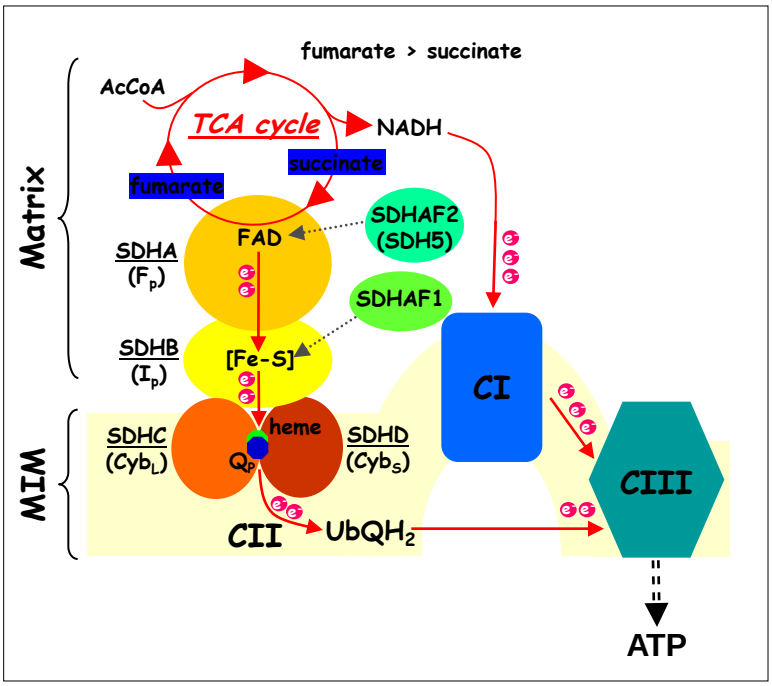
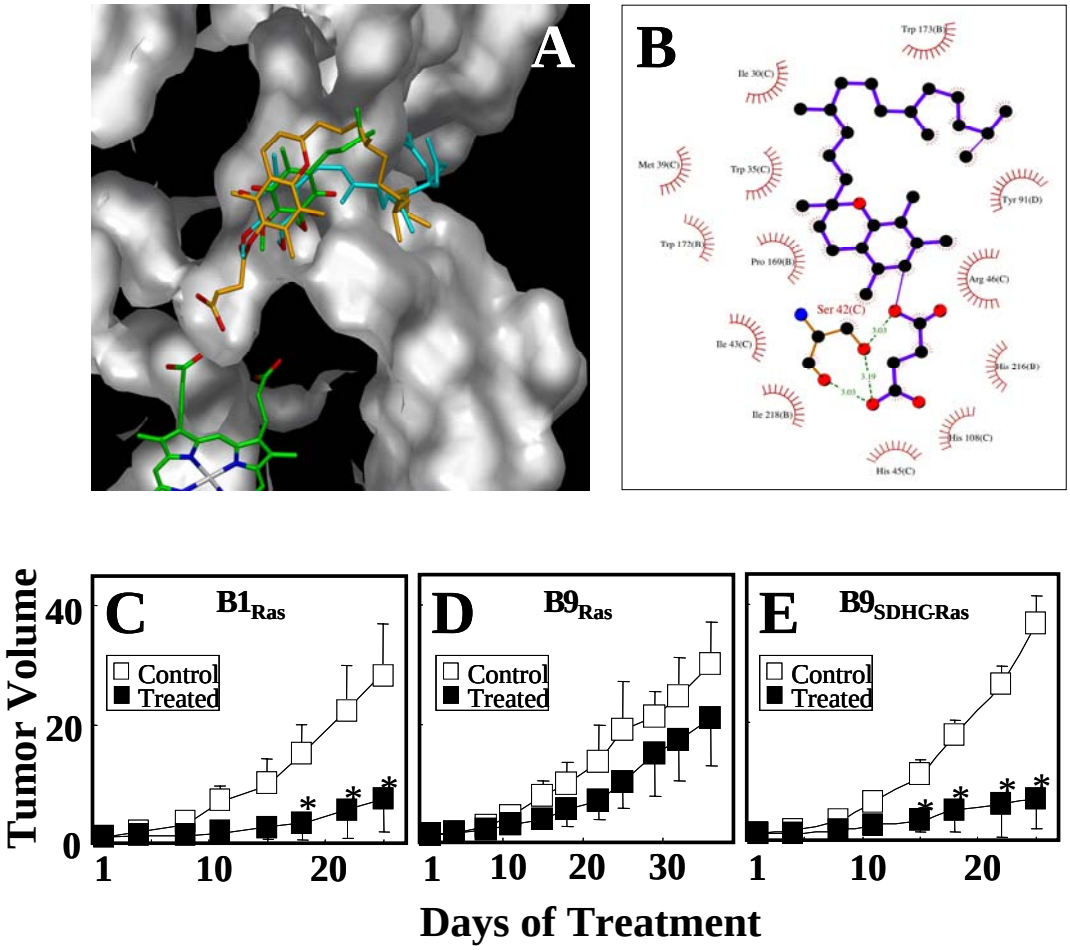
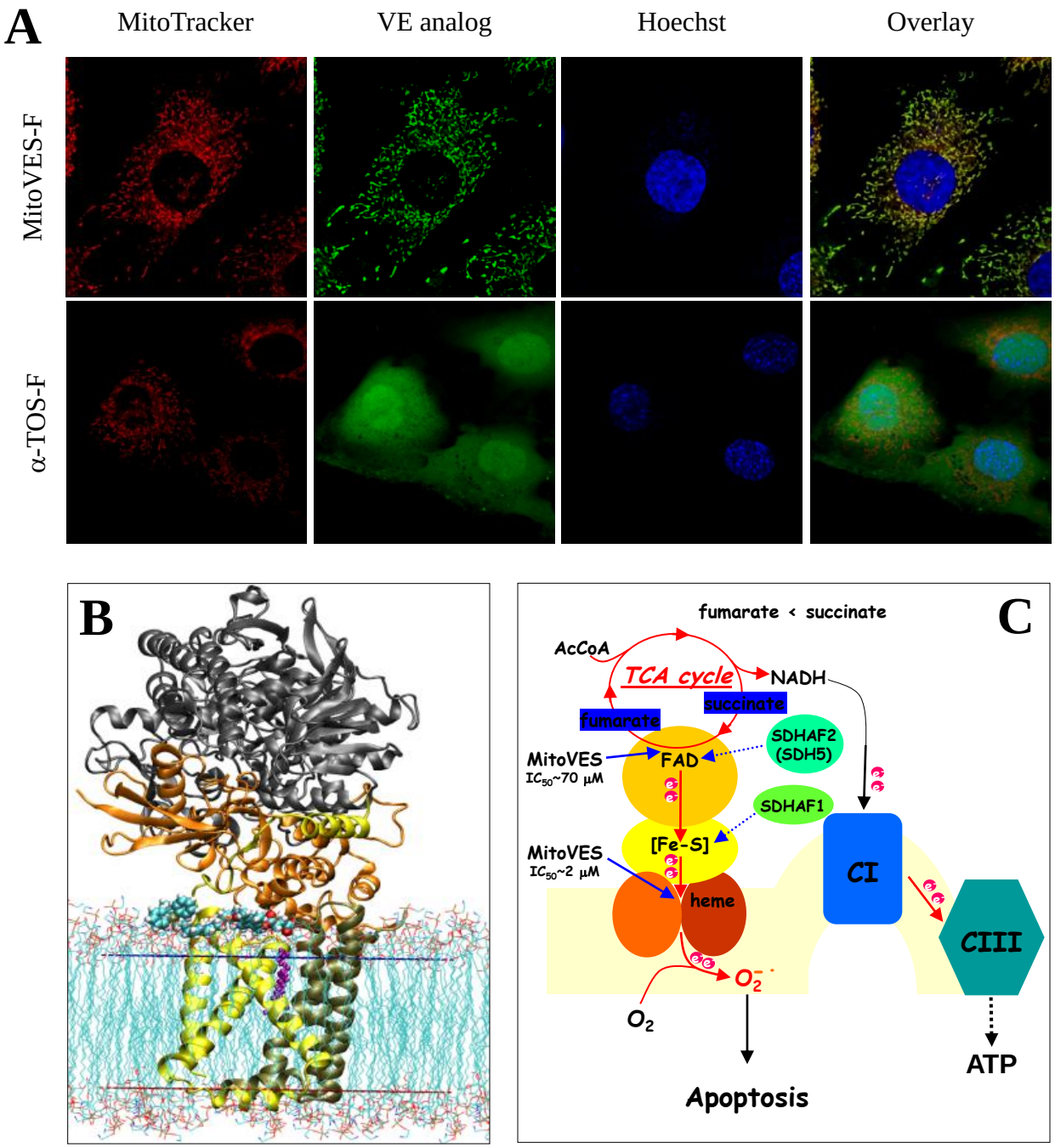


Figure 3


A
B
Trp 173(B)
Ile 30(C)
Met 39(C)
Trp 35(C)
Tyr 91(D)
Trp 172(B)
Pro 169(B)
Arg 46(C)
Ser 42(C)
Ile 43(C)
His 216(B)
Ile 218(B)
His 108(C)
His 45(C)
C
B1Ras
D
B9Ras
E
B9SDHCRas
Control
Treated
Tumor Volume
0
20
40
1
10
20
30
Days of Treatment

Figure

Figure 4



Figure

Figure 5