Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Review:

Effects of sulforaphane on brain mitochondria: mechanistic view and future directions*

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Received Nov. 24, 2019; Revision accepted Feb. 3, 2020; Crosschecked Mar. 3, 2020

Abstract: The organosulfur compound sulforaphane (SFN; $C_6H_{11}NOS_2$) is a potent cytoprotective agent promoting antioxidant, anti-inflammatory, antiglycative, and antimicrobial effects in in vitro and in vivo experimental models. Mitochondria are the major site of adenosine triphosphate (ATP) production due to the work of the oxidative phosphorylation (OXPHOS) system. They are also the main site of reactive oxygen species (ROS) production in nucleated human cells. Mitochondrial impairment is central in several human diseases, including neurodegeneration and metabolic disorders. In this paper, we describe and discuss the effects and mechanisms of action by which SFN modulates mitochondrial function and dynamics in mammalian cells. Mitochondria-related pro-apoptotic effects promoted by SFN in tumor cells are also discussed. SFN may be considered a cytoprotective agent, at least in part, because of the effects this organosulfur agent induces in mitochondria. Nonetheless, there are certain points that should be addressed in further experiments, indicated here as future directions, which may help researchers in this field of research.

Key words: Sulforaphane; Brain; Mitochondria; Antioxidant; Nuclear factor erythroid 2-related factor 2 (Nrf2)
https://doi.org/10.1631/jzus.B1900614 **CLC number:** R285

1 Introduction

Sulforaphane (SFN; $C_6H_{11}NOS_2$), an organosulfur compound obtained from cruciferous vegetables, such as broccoli and cauliflower, exhibits several cytoprotective effects in human cells (Houghton et al., 2016). SFN is an antioxidant, antiglycative, anti-inflammatory, antitumor, and antimicrobial agent, depending on circumstances such as the concentration and duration of exposition (Leoncini et al., 2011; Bergantin et al., 2014; Angeloni et al., 2015a, 2015b; de Oliveira et al., 2018a, 2018b; Russo et al., 2018). SFN can also promote mitochondrial protection in brain cells. Nonetheless, the complete mechanisms by which SFN can benefit mitochondria remain to be demonstrated. There is evidence indicating that the antioxidant and anti-inflammatory effects caused by SFN treatment may be linked at the subcellular level by the modulation of mitochondrial function (Krysko

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et al., 2011; Jin et al., 2017; de Oliveira et al., 2018a, 2018b). In spite of this, the underlying mechanism linking the effects of SFN on the mitochondria and on the redox environment of mammalian cells remains to be fully understood.

Studies of SFN bioavailability in several experimental models have shown that SFN peak plasma concentration is observed between 1 and 3 h after SFN intake (Hanlon et al., 2008; Keum et al., 2009). Clarke et al. (2011) have demonstrated that SFN is metabolized in the kidney, lung, liver, colon, and brain, among other organs, of mice after oral ingestion of variable amounts of SFN. No gender differences in SFN metabolism or tissue distribution were observed. Importantly, it was demonstrated that the most abundant compounds found in the tissue of mice that ingested SFN were the SFN forms conjugated with glutathione, cysteine, and *N*-acetylcysteine (NAC), and not free SFN. Also, the kinetics of metabolism and the tissue distribution of SFN accompanied the plasma concentration of SFN, except in the small intestine, colon, and prostate. Vermeulen et al. (2008) studied SFN bioavailability in humans who consumed cooked or raw broccoli and found that the absorption of SFN was delayed when broccoli was cooked. Also, the peak plasma concentration of SFN was higher when raw broccoli was ingested by the volunteers. Fahey et al. (2015) tested the administration of glucoraphanin (the precursor of SFN found in broccoli) or the combination of glucoraphanin and the enzyme myrosinase (that converts glucoraphanin into SFN) in humans in relation to SFN bioavailability. They observed that the presence of myrosinase increased SFN bioavailability in humans. Importantly, SFN also induces some toxicity. For example, Socała et al. (2017) showed that SFN increased seizure vulnerability in mice (LD_{50}=212.67 mg/kg; LD_{50}, median lethal dose).

The use of SFN as an antioxidant is of particular interest in the case of brain cells, since the brain presents some particularities that increase its vulnerability during redox impairment, as reviewed by Cobley et al. (2018). Briefly, the brain consumes oxygen gas (O_2) at very high rate to maintain the bioenergetic status needed to sustain its high activity (Magistretti and Allaman, 2015). Also, brain cells contain lower levels of both enzymatic and non-enzymatic antioxidant defenses when compared to other cell types (Uttara et al., 2009; Baxter and Hardingham, 2016; Ren et al., 2017; Salim, 2017). Some neurotransmitters, such as dopamine and adrenaline, may undergo autoxidation, generating reactive species, such as the superoxide anion radical ($O_2^{-}\cdot$) and reactive quinones (Heikkila and Cohen, 1973; Cohen and Heikkila, 1974). The autoxidation reactions may be catalyzed by transition metals, whose concentrations are high in brain cells (Bandy and Davison, 1987; Miller et al., 1990). The mitochondria-located monoamine oxidase (MAO) enzymes also generate hydrogen peroxide (H_2O_2) during the degradation of neurotransmitters (mainly dopamine, tyramine, tryptamine, noradrenaline, 5-hydroxytryptamine, and 2-phenylethylamine) (Ramsay and Gravestock, 2003; Youdim et al., 2006). Neurotransmission involves drastic alterations in the intracellular concentration of calcium ions (Ca^{2+}). Increased Ca^{2+} levels are commonly associated with mitochondrial dysfunction and general damage in neurons and glial cells (Lipton et al., 1993; Brown, 1999, 2001). In addition to the autoxidation of neurotransmitters, brain cells consume glucose at very high rates, and this carbohydrate may also undergo autoxidation, giving rise to reactive species that also affect mitochondrial function and dynamics (Thornalley, 1985; Wolff and Dean, 1987; Hunt et al., 1988). The brain's structure and function are highly dependent on lipids, mainly the polyunsaturated fatty acids and cholesterol (Bazinet and Layé, 2014). Lipid peroxidation occurs at high rates in the brain and, depending on the extent of this type of redox impairment, the function of mitochondria in neurons and glial cells may be compromised (Niki et al., 2005; Reis and Spickett, 2012; Sultana et al., 2013; di Domenico et al., 2017). During neuroinflammation, the brain undergoes redox stress due to increased production of reactive species by the mitochondria in both microglial and neuronal cells (Bedard and Krause, 2007; Sumimoto, 2008; Schain and Kreisl, 2017). In summary, the brain is very sensitive to redox impairment due to several endogenous factors. Moreover, exogenous redox active agents, including xenobiotics present in the diet, may alter the redox balance in brain cells by affecting mitochondrial function and causing a pro-oxidant status that may favor cell loss and neurodegenerative processes (Aoyama and Nakaki, 2013; de Oliveira, 2015). In this review, we describe and discuss the effects of SFN on the mitochondria of brain cells.

2 Overview of mitochondrial structure, function, redox biology, and dynamics

Mitochondria are organelles whose ability to function according to cellular needs is highly dependent on their structure. They have a double-membrane structure formed by the outer and inner mitochondrial membranes (OMM and IMM, respectively) (van der Laan et al., 2016). The OMM and IMM surround the intermembrane space (IMS), which is fundamental to the synthesis of adenosine triphosphate (ATP) by the organelles (Porcelli et al., 2005; Herrmann and Riemer, 2010; Papa et al., 2012). Protein import machinery is present in both the OMM and IMM, and mitochondrial function is highly dependent on this process (Stojanovski et al., 2008; Prasai, 2017). In the mitochondrial matrix, a viscous space limited by the IMM, there are several enzymes that mediate the reactions of metabolic pathways, as will be summarized here. Moreover, the matrix contains the mitochondrial DNA (Gustafsson et al., 2016). The electron transfer chain (ETC, also known as the respiratory chain) comprises the complexes I (nicotinamide adenine dinucleotide (NADH) dehydrogenase), II (succinate dehydrogenase (SDH)), III (coenzyme Q-cytochrome *c* reductase), and IV (cytochrome *c* oxidase), and the mobile electron transfer components ubiquinone (also known as coenzyme Q10, which carries electrons from complexes I and II to complex III) and cytochrome *c* (a heme protein that transfers electrons from complex III to complex IV) (Chaban et al., 2014). Ubiquinone also transfers electrons from other sources, such as β-oxidation, to complex III (Zhang et al., 2006; Watmough and Frerman, 2010). The electrons that flow between the complexes I, III, and IV transfer energy that is used by those proteins to pump H^+ ions from the mitochondrial matrix into the IMS. This generates an electrochemical gradient, which is quantified as the mitochondrial membrane potential (MMP) (Signes and Fernandez-Vizarra, 2018). In complex IV, the electrons are transferred to O_2 (the final acceptor of electrons in the ETC), producing H_2O (Chaban et al., 2014). When the concentration of H^+ ions reaches a threshold, the H^+ ions return to the mitochondrial matrix through complex V (the so-called ATP synthase/ATPase), producing ATP in the process (Papa et al., 2012). Loss of MMP, as may occur for example during redox impairment of the mitochondrial membranes or in the case of inhibition of any part of the ETC, leads to decreased ATP production and increased production of reactive oxygen species (ROS) by the mitochondria (Murphy, 2009), as will be discussed below. Importantly, a reverse transport of electrons has been observed in certain situations, as reviewed by Scialò et al. (2017). There are several sources of electrons for the ETC, such as the tricarboxylic acid cycle (TCA, also known as Krebs' cycle), and the oxidation of fatty acids, α-ketoacids (derived from amino acids), and ketone bodies (Mailloux et al., 2007; Akram, 2014). The use of a given energetic substrate depends on the cell type and the expression of certain enzymes. For example, oxidation of ketone bodies does not occur in the liver due to the absence of the enzyme succinyl-CoA-acetoacetate-CoA transferase (the so-called thiophorase enzyme) that is responsible for the activation of acetoacetate (Puchalska and Crawford, 2017). The involvement of mitochondria in metabolic pathways also depends on the organ's particularities. In the liver, for example, the mitochondria take a central role by mediating some reactions of the gluconeogenesis and urea cycle (during fasting, some types of exercise, some types of diets, or stress) (Bigot et al., 2017; Petersen et al., 2017). Hepatic mitochondria are also involved in the synthesis of lipids, such as fatty acids and cholesterol (in these cases, mitochondria are a source of citrate that is needed in the cytosol as a source of acetyl-coenzyme A) (Nguyen et al., 2008). In the kidney, mitochondria also participate in some gluconeogenesis reactions during starvation (Gerich et al., 2001). However, brain mitochondria are not associated with the synthesis of ketone bodies or gluconeogenesis, for example, during fasting (Tracey et al., 2018). Thus, there are differences between cell types in their mitochondrial function and substrate consumption (Spinelli and Haigis, 2018). However, virtually all mitochondria are central to the homeostasis of Ca^{2+}, which is particularly important in neurons due to the role of Ca^{2+} ions in mediating neurotransmission (Pinton et al., 1998; Raffaello et al., 2016; Paupe and Prudent, 2018).

The ETC may fail in the transfer of electrons between the complexes and the electron transfer components, generating ROS, such as $O_2^{-}\cdot$, whose major sources are the complexes I and III (Grivennikova

and Vinogradov, 2006; Bleier and Dröse, 2013). Mitochondrial intoxication also promotes the production of ROS by the organelles (de Oliveira, 2015, 2016; de Oliveira and Jardim, 2016). Actually, mitochondria are the main source of ROS in human cells (Murphy, 2009). The dismutation of $O_2^{-}\cdot$ by the manganese-dependent mitochondrial enzyme superoxide dismutase (Mn-SOD), which is located in the mitochondria, generates H_2O_2 (Sies et al., 2017). There is evidence pointing to the presence of several enzymes that convert H_2O_2 into H_2O in the mitochondria. A major H_2O_2-detoxifying enzyme is glutathione peroxidase (GPx), which has a mitochondrial isoform (Esposito et al., 2000). Also, some authors have suggested that mitochondria contain catalase (CAT), another enzyme involved in the detoxification of H_2O_2 (Bai and Cederbaum, 2001; Salvi et al., 2007; Bakala et al., 2012). Furthermore, mitochondria contain a peroxiredoxin enzyme that also consumes H_2O_2, as reviewed by Cao et al. (2007). H_2O_2 metabolism in the mitochondria (and in the cytoplasm) depends on high levels of the major non-enzymatic antioxidant glutathione (GSH), which is consumed by GPx (Sies et al., 2017). The recycling of GSH depends on the availability of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), which is used by the enzyme glutathione reductase (GR) to reduce the glutathione disulphide (GS-SG) formed from the oxidation of GSH (Huang and Philbert, 1995). The increased generation of ROS by the mitochondria or their decreased ability to metabolize such pro-oxidant agents causes oxidative stress in the organelles, leading to bioenergetic collapse and cell death (Maes et al., 2011; Sies et al., 2017). Moreover, mitochondria may undergo nitrosative stress when there is an increase in the production of reactive nitrogen species (RNS) in the cells (Sies et al., 2017). The detection of increased levels of 3-nitrotyrosine, for example, indicates increased formation of peroxynitrite ($ONOO^{-}$), which is generated from nitric oxide ($NO\cdot$) and $O_2^{-}\cdot$ (Calcerrada et al., 2011; Radi, 2013). A growing body of evidence points to a role for mitochondria in the generation of $NO\cdot$, which would modulate several signaling pathways associated with the organelles (Poderoso et al., 1996, 2019). Recently, de Armas et al. (2019) have reported that peroxiredoxin 3 is responsible for the reduction of $ONOO^{-}$ in human mitochondria.

The control of the antioxidant enzymes (both enzymatic and non-enzymatic) located in the mitochondria is mediated by some signaling pathways commonly associated with cell survival. Activation of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is a major antioxidant route in human cells (Ma, 2013). Nrf2 modulates the expression of antioxidant enzymes, such as SOD, CAT, GPx, and GR (Nguyen et al., 2009; Ma, 2013). The expression of the rate-limiting enzyme in the synthesis of GSH, γ-glutamate-cysteine ligase (γ-GCL, which is formed by the catalytic and modifier subunits, GCLC and GCLM, respectively), is also controlled by Nrf2, among other transcription factors (e.g., nuclear factor-κB (NF-κB)) (Franklin et al., 2009; Lu, 2009, 2013). Nrf2 also coordinates the expression of enzymes involved in phase II detoxification reactions, such as glutathione-*S*-transferase (GST), responsible for the conjugation of GSH with xenobiotics and/or toxicants (Deponte, 2013). The glyoxalase system, which participates in the detoxification of reactive dialdehydes (such as methylglyoxal), is also dependent on GSH (Deponte, 2013). GSH is consumed in the glutathionylation of proteins in the post-translational control of protein function (Ghezzi, 2013). Thus, the metabolism of GSH needs intense regulation to attend at least three groups of reactions necessary to maintain cell homeostasis. The cellular needs of GSH are described in several excellent publications by other research groups (Deponte, 2013). The transport of GSH into mitochondria occurs in virtually any nucleated human cell and is determinant in modulating the vulnerability of neurons to redox impairment resulting from the exposure to ROS or RNS (Fernández-Checa et al., 1997, 1998; Lash, 2006; Marí et al., 2013; Wilkins et al., 2013). Cell fate is closely related to mitochondrial function and production of ROS by these organelles, as may be seen during the triggering of apoptosis through cytochrome *c* release (Green et al., 2014).

The number of mitochondria can be increased by the mitochondrial biogenesis (mitogenesis) process, which needs the expression of both nuclear and mitochondrial genes (Scarpulla, 2006, 2008). The peroxisome proliferator-activated receptor γ coactivator 1-α (*PGC-1α*) is a major modulator of mitochondrial biogenesis, as reviewed by Scarpulla (2008, 2011) and Jardim et al. (2018). Mitochondrial number, size,

and shape can also be changed by fusion (when two or more mitochondria combine, generating a single organelle) or fission (when one mitochondrion divides, leading to the formation of two or more organelles) (Scott and Youle, 2010; Westermann, 2012). In this context, mitophagy is a biological process by which the number of mitochondria is reduced (Wang et al., 2019). Mitophagy is important in the turnover and quality control of this organelle (Zhang, 2013; Campello et al., 2014). Modulation of mitochondrial number, size, and architecture has therapeutic relevance, and research in this area of mitochondrial medicine is important for the treatment or prevention of mitochondria-related diseases, such as cancer, neurodegeneration, and metabolic disorders (Campello and Scorrano, 2010; Corrado et al., 2012; Rodolfo et al., 2018).

3 Efects of SFN on brain mitochondria in in vitro experimental models

Several research groups have examined whether and how SFN would be able to modulate mitochondrial physiology in cultured cells (Table 1). Certain signaling pathways have been explored to understand how SFN affects mitochondrial parameters (Fig. 1). In spite of this, the complete mechanism of action elicited by SFN has not yet been elucidated. In this section, we discuss the effects induced by SFN on mitochondria and the ability this natural agent presents in modulating cell fate in different in vitro experimental models.

3.1 Mitochondria-related antioxidant and anti-apoptotic effects induced by SFN

de Oliveira et al. (2018b) showed that a 30-min pretreatment with 5-μmol/L SFN effectively blocked mitochondria-related apoptosis triggered by a chemical challenge with the prooxidant H_2O_2 in the human neuroblastoma cell line SH-SY5Y. SFN prevented upregulation in the levels of *Bax*, reducing the release of cytochrome *c* from the mitochondria to the cytosol. Consequently, SFN blocked the H_2O_2-induced activation of the pro-apoptotic *caspases-9* and *-3*, thereby preventing the cleavage of poly(ADP-ribose) polymerase (PARP) and DNA fragmentation, the hallmarks of apoptosis (Green et al., 2014). SFN also

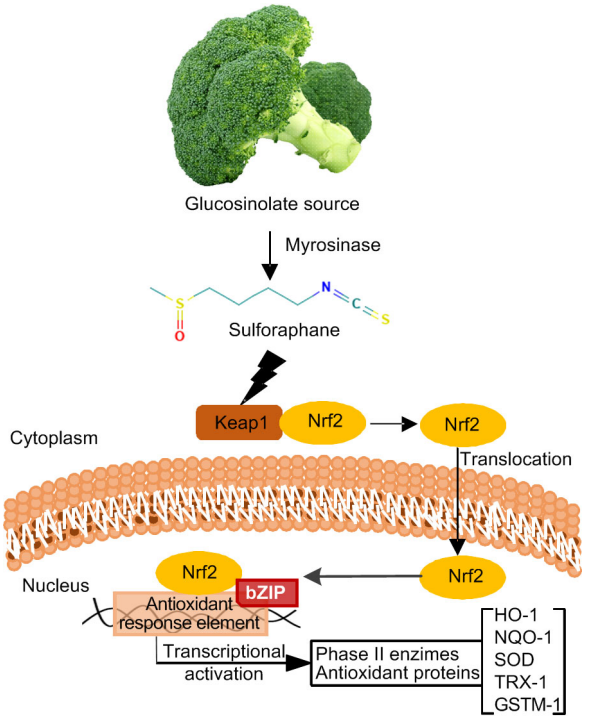

Fig. 1 Effect of SFN on the transcription factor Nrf2
After the reaction of the enzyme myrosinase, free sulforaphane (SFN) is obtained by the brain cells and activates nuclear factor erythroid 2-related factor 2 (Nrf2) by reacting with Kelch-like ECH-associated protein 1 (Keap1) in the cytosol. Free Nrf2 translocates to the nucleus of the cells, stimulating the expression of genes whose products are involved in antioxidant defenses and phase II detoxification reactions, as well as in the maintenance of mitochondrial function and dynamics. Those genes contain an antioxidant response element that interacts with the basic leucine zipper domain (bZIP) of Nrf2. HO-1: heme oxygenase-1; NQO-1: nicotinamide adenine dinucleotide phosphate (NADPH): quinone oxidoreductase-1; SOD: superoxide dismutase; TRX-1: thioredoxin 1; GSTM-1: glutathione-*S*-transferase M 1

modulated the mitochondria-related redox parameters by decreasing the impact of H_2O_2 on the levels of markers of lipid peroxidation, protein carbonylation, and nitration in the membranes of the organelles. SFN also promoted an increase in GSH levels and attenuated $O_2^{-}\cdot$ production by mitochondria isolated from the H_2O_2-challenged SH-SY5Y cells. In summary, SFN promoted mitochondrial protection by modulating the mitochondria-associated redox biology in H_2O_2-treated SH-SY5Y cells. Mitochondrial function was also examined by de Oliveira et al. (2018b). SFN prevented the H_2O_2-induced decline in the activity of the enzymes aconitase, α-ketoglutarate dehydrogenase (α-KGDH), and SDH, which are crucial to

Table 1 Effects of SFN on mitochondria in in vitro experimental models

Cell type	Experimental model	Main findings	Reference
Human neuroblastoma SH-SY5Y cells	SFN at 5 μmol/L for 30 min before a challenge with H_2O_2	Blocked the H_2O_2-induced mitochondria-dependent cell death Prevented the H_2O_2-induced lipid peroxidation and protein carbonylation and nitration in mitochondrial membranes Increased the mitochondrial GSH levels Reduced the production of $O_2^{-}\cdot$ by the mitochondria Prevented the H_2O_2-induced decrease in the activity of the TCA cycle enzymes and in components of the OXPHOS Silencing of Nrf2 abrogated the mitochondrial protection promoted by SFN	de Oliveira et al., 2018b
Human malignant GBM T98G and U87MG cells	SFN at 20–40 μmol/L for 24 h	Enhanced the Bax/Bcl-2 ratio, induced cytochrome *c* release from the mitochondria, and activated caspase-3 Induced AIF release from the mitochondria Upregulated calpain (by a Ca^{2+}-dependent manner), caspase-12, and caspase-9 levels	Karmakar et al., 2006
Human malignant glioma GBM 8401 cells	SFN at 12.5–50.0 μmol/L for 24 h	Activated the MEK and ERK1/2 signaling pathway Inhibited NF-κB Caused MMP collapse and activated caspases-9 and -3	Huang et al., 2012
Human GBM U251 and U87 cell lines	SFN at 20–40 μmol/L for 24 h	Increased the Bax/Bcl-2 ratio Activated caspase-3 NAC administration blocked the pro-apoptotic effects induced by SFN	Miao et al., 2017
Human GBM U87 and M-HBT32 cell lines	SFN at 10–30 μmol/L for 24 h	Activated caspases-9 and -3 Inhibition of the mitochondrial production of reactive species abrogated the SFN-induced apoptosis	Bijangi-Vishehsaraei et al., 2017
Human GBM U251MG cell line	SFN at 20–40 μmol/L for 24 h	Upregulated Bad and Bax levels Promoted cytochrome *c* release from the mitochondria Downregulated Bcl-2 and survivin	Zhang et al., 2016
Human GBM U251 glioma cell line	SFN at 25 μmol/L (combined or not with resveratrol at 25 μmol/L) for 24 h	Upregulated Bax level Caused cytochrome *c* release Activated caspase-3 Downregulated Akt	Jiang et al., 2010
Different human GBM cell lines	SFN at 40 μmol/L for 24 h	Increased the susceptibility of GBM cell lines to TMZ by a mitochondria-related manner Increased the Bax/Bcl-2 ratio Activated caspases-3 and -7	Lan et al., 2015
Human retinal pigment epithelial RPE-1 cells	SFN at 50 μmol/L for 4 h	Downregulation of Drp1 by a mechanism not associated with the regulation of Nrf2	O'Mealey et al., 2017

SFN: sulforaphane; GBM: glioblastoma; RPE-1: retinal pigment epithelial; GSH: glutathione; TCA: tricarboxylic acid; OXPHOS: oxidative phosphorylation; Nrf2: nuclear factor erythroid 2-related factor 2; Bcl-2: B-cell lymphoma-2; Bax: Bcl-2-associated X protein; AIF: apoptosis-inducing factor; MEK: mitogen-activated protein kinase; ERK1/2: extracellular signal-regulated kinase 1/2; NF-κB: nuclear factor-κB; MMP: mitochondrial membrane potential; NAC: *N*-acetylcysteine; TMZ: temozolomide; Drp1: dynamin-1-like protein

maintaining TCA function in virtually any nucleated cell. Mitochondrial function-related effects induced by SFN were also observed on the activity of complex I (the initial part of the ETC) and complex V. In H_2O_2-treated cells, SFN also prevented the loss of MMP and the decline in the levels of ATP. Silencing of the transcription factor Nrf2 inhibited the protective effect of SFN on mitochondrial function (complexes I, V, and MMP) in the H_2O_2-treated SH-SY5Y cells.

3.2 Mitochondria-related anti-tumor effects induced by SFN

SFN is also able to induce mitochondria-related cell death in tumor cells. Depending on the concentration, SFN activates certain signaling pathways that trigger cell death in a mitochondria-dependent manner. This is particularly important in the context of gliomas, whose mortality and morbidity rates are considered high (Ostrom et al., 2014).

Karmakar et al. (2006) observed that SFN at 20–40 μmol/L induced cell death in human malignant glioblastoma (GBM) T98G and U87MG cells by increasing the Bax (B-cell lymphoma-2 (Bcl-2)-associated X protein)/Bcl-2 ratio, triggering cytochrome *c* release from the mitochondria, and activating caspase-3. SFN also caused the release of apoptosis-inducing factor (AIF) from the mitochondria by a mechanism associated with increased mitochondrial membrane permeability. Furthermore, SFN at 40 μmol/L (the highest concentration tested by the authors) upregulated calpain, caspase-12, and caspase-9 levels in both cell lines. The calpain upregulation was associated with an increase in the levels of Ca^{2+} ions in the cytosol, which may have been related to endoplasmic reticulum stress triggered by exposure to SFN in the GBM cells. Thus, SFN activated multiple pro-apoptotic pathways in both cell lines by mitochondria-dependent and -independent routes. This effect is very likely to amplify the pro-apoptotic effect induced by SFN in tumor brain cells.

Huang et al. (2012) tested SFN at different concentrations (12.5–50.0 μmol/L) on human malignant glioma GBM 8401 cells and found decreased rates of cell viability in all the experimental groups. SFN at any concentration tested also caused MMP collapse and activation of caspases-9 and -3. SFN activated the mitogen-activated protein kinase (MEK) and extracellular signal-regulated kinase 1/2 (ERK1/2)-dependent signaling pathways, causing a mitochondria-related increase in the rates of apoptosis. SFN also inhibited the transcription factor NF-κB, which has been associated with tumor resistance, in the GBM 8401 cells. The authors also demonstrated that SFN (200–400 μmol/L) decreased the growth of tumors in an experimental model of cancer xenografts. Their experimental design did not allow them to test whether SFN induced any type of interplay between the MEK/ERK and NF-κB signaling pathways. Such an effect may occur because of the role the MEK/ERK axis has in the regulation of NF-κB in different cell types (Carter and Hunninghake, 2000; Jiang et al., 2004; Kloster et al., 2011).

Miao et al. (2017) showed that SFN-induced apoptosis in GBM cell lines was dependent on a pro-oxidant effect of the natural compound. SFN at 20–40 μmol/L increased the level of Bax and reduced the level of Bcl-2, causing activation of caspase-3 and triggering mitochondria-related apoptosis in U251 and U87 cell lines. Those effects were blocked by NAC (3 mmol/L), a well-known antioxidant, indicating that SFN is able to induce an increase in the production of reactive species, depending on its concentration (i.e., SFN is a redox-active agent that acts as an anti- or pro-oxidant according to its concentration and the cellular conditions). The type of reactive species produced by SFN, and the source of the pro-oxidant agents in the cell lines were not addressed. Therefore, studies aiming to reveal the pro-oxidant agents involved in SFN-induced mitochondria-related cell death are welcome.

Bijangi-Vishehsaraei et al. (2017) reported that SFN (10.0–30.0 μmol/L for 24 h) induced apoptosis in U87 and M-HBT32 GBM cells by activating caspase-3 and caspase-9. They also found that inhibition of ROS production by the mitochondria suppressed SFN-induced apoptosis in GBM cells. Therefore, ROS originating from the mitochondria are very likely to play a role during the triggering of the intrinsic apoptotic pathway stimulated by SFN. A co-treatment with NAC blocked the SFN-induced cell death, reinforcing the conclusion that SFN induced a pro-oxidant pulse that activated mitochondria-dependent cell death in that experimental model. Similarly, Zhang et al. (2016) observed that SFN (20.0–40.0 μmol/L for 24 h) stimulated apoptosis in

U251MG GBM cells by upregulating Bad and Bax, consequently promoting cytochrome *c* release into the cytosol. The authors found evidence of downregulation of Bcl-2 and survivin in SFN-treated U251MG cells. Thus, SFN is able to trigger apoptosis by a mechanism associated with mitochondria in GBM cells.

Some research groups have demonstrated an effect of the combination of SFN with other bioactive molecules in the triggering of cell death in brain tumor cells. Jiang et al. (2010) reported that SFN (25.0 μmol/L) alone or in combination with resveratrol (RESV, 25.0 μmol/L) upregulated Bax levels, leading to cytochrome *c* release from the mitochondria to the cytosol, consequently activating caspase-3 in human U251 glioma cells. Moreover, SFN induced Akt downregulation, favoring apoptosis in the glioma cells. Other mitochondria-related apoptotic signaling pathways were not investigated by the authors in that experimental model.

SFN has also been tested in an attempt to reduce the resistance of GBM cells to temozolomide (TMZ). Lan et al. (2015) found that SFN (40.0 μmol/L for 24 h) enhanced the mitochondria-related apoptotic effects promoted by TMZ, including upregulation of Bax and caspase-3/7 activity and downregulation of Bcl-2. Other aspects of the mitochondria-mediated apoptotic cell death signaling pathway were not examined by the authors, but would be a useful subject for further studies of GBM cells exposed to SFN and TMZ.

SFN has been used at concentrations ranging from 10.0 to 50.0 μmol/L as an antitumor agent in in vitro experimental models. Nonetheless, this concentration range is considered high compared with the concentrations SFN reaches in in vivo experimental models. Also, the passage of SFN across the blood-brain barrier (BBB) is limited, which may reduce the concentration of SFN reaching brain cells. Thus, the development of strategies that would amplify the availability of SFN to brain cells in a secure form is very important. In this regard, toxicological studies are necessary to investigate whether SFN may induce toxicity in normal cells as well as tumor cells in in vivo experimental models and clinical trials.

3.3 Effects of SFN on aspects related to mitochondrial dynamics

In human retinal pigment epithelial-1 (RPE-1) cells, O'Mealey et al. (2017) reported that SFN blocked mitochondrial fission by a mechanism that was not associated with Nrf2. Using an elegant experimental design, they demonstrated that SFN (50 μmol/L for 4 h) downregulated dynamin-1-like protein (DNM1L, also called Drp1), a major regulator of mitochondrial fission events (Smirnova et al., 2001). Their data indicated that SFN may modulate mitochondrial quality control, among other parameters, such as mitochondrial function and dynamics, by a more complex mechanism not solely dependent on the activation of the classical antioxidant axis associated with Nrf2. The authors suggested that this is a promising new route to be examined when considering the treatment of neurodegenerative and other diseases in which mitochondrial dynamics is impaired.

4 Effects of SFN on brain mitochondria in in vivo experimental models

There has been a lack of studies aiming to reveal the effects of SFN on brain mitochondria in in vivo experimental models. Some research groups have demonstrated that SFN administration in vivo promoted mitochondrial protection for which there was evidence from ex vivo experimental models (Table 2). Greco and Fiskum (2010) reported that treatment of rats with SFN at 10 mg/kg (intraperitoneal (i.p.) administration) 40 h before extraction of non-synaptic brain mitochondria decreased the impact of a challenge with $CaCl_2$ at 50 μmol/L followed by exposure to *tert*-butyl hydroperoxide (*t*BOOH) at 250 μmol/L on mitochondria in relation to the release of Ca^{2+} ions from the organelles. The $CaCl_2$ concentration used was insufficient to induce the opening of the mitochondrial permeability transition pore (MPTP) in that study. However, the addition of *t*BOOH triggered Ca^{2+} release from the organelles in a pro-oxidative manner by opening the MPTP. SFN did not inhibit MPTP opening induced by phenylarsine oxide (PhAsO, 30 μmol/L), which is able to directly oxidize sulfhydryl groups in the mitochondria, leading to MPTP opening and Ca^{2+} release from the organelles. Also, in vivo administration of SFN did not affect the oxidation of mitochondrial pyridine nucleotide in mitochondria exposed to *t*BOOH. Furthermore, SFN did not alter mitochondrial respiration rates, as assessed by the authors using different oxidizable substrates. In this regard, SFN did not change the immunocontents

Table 2 Effects of SFN on the mitochondria in ex vivo and in vivo experimental models

Experimental model	Main findings	Reference
SFN at 10 mg/kg (i.p. administration) 40 h before extraction of non-synaptic brain mitochondria and an ex vivo challenge with $CaCl_2$ (50 μmol/L) and *t*BOOH (250 μmol/L)	Partial mitochondrial protection evidenced by Ca^{2+} ion release from the organelle	Greco and Fiskum, 2010
SFN at 5 mg/kg (i.p. administration) 48 h before isolation of brain mitochondria and ex vivo challenge with 4-HNE (30 μmol/L)	Prevented mitochondrial impaired respiration Decreased the 4-HNE-induced redox impairment in the mitochondrial proteins	Miller et al., 2013
SFN at 5 mg/(kg·d) (i.p. administration) for 5 d before the induction of seizure in mice	Alleviated the seizure-induced decline in state 2 respiration Increased state 3u and state 3 ADP-state 4o respiration Upregulated both complex I- and complex II-driven OCR	Carrasco-Pozo et al., 2015
SFN at 5 mg/kg for 24 h (two doses of SFN through i.p. administration) before induction of excitotoxicity by quinolinic acid in rats	Prevented the decline in ATP production, loss of MMP, and decreased activity of the ETC components in the brain mitochondria of quinolinic acid-treated rats	Luis-García et al., 2017
SFN at 10–40 mg/kg once a day by i.p. injection after CO intoxication in rats	Improved mitochondrial structure in the hippocampus of CO-treated rats Restored MMP Upregulated Nrf2, Trx-1, NQO-1	Bi et al., 2017
SFN at 5 mg/kg administrated to rats that were exposed to iron during the nenonatal period	Upregulated DNM1L/Drp1 in the hippocampus	Lavich et al., 2015

SFN: sulforaphane; i.p.: intraperitoneal; *t*BOOH: tert-butyl hydroperoxide; 4-HNE: 4-hydroxynonenal; CO: carbon monoxide; OCR: oxygen consumption rate; ATP: adenosine triphosphate; MMP: mitochondrial membrane potential; ETC: electron transfer chain; Nrf2: nuclear factor erythroid 2-related factor 2; Trx-1: thioredoxin-1; NQO-1: nicotinamide adenine dinucleotide phosphate (NADPH):quinone oxidoreductase-1; DNM1L: dynamin-1-like protein, also called Drp1

of cyclophilin D, a protein directly involved in the sensitivity of mitochondria to inducers of MPTP opening. Therefore, the effects of SFN on brain mitochondria may not be generalized, as commented by the authors, since some parameters were affected and others not, showing some limitations in the ability of SFN to promote mitochondrial protection in that experimental model. On the other hand, the in vitro challenge of mitochondria by different stressors following in vivo administration of SFN occurs in a context in which the organelles would not be able to produce additional antioxidant agents, such as Mn-SOD and GSH, capable of increasing the redox defenses when facing such challenges. The content of GSH in the organelles, for example, would be maintained only by some recycling that would occur in the mitochondrial matrix. Also, the synthesis of new GSH, which is a target of SFN in virtually any nucleated cell in humans, would not occur due to the absence of the transcriptions factors involved in the modulation of the production of this non-enzymatic antioxidant (Liu et al., 2014). The consumption of GSH by the administration of direct or indirect oxidant agents favors the opening of the MPTP in the case of adding a second stressor, as demonstrated by the authors. Even though this is an ex vivo experimental model, the data obtained by the researchers are very useful for interpreting what occurs in cells previously exposed to SFN when facing redox impairment. Future research is needed using different stressors and organelles isolated from other organs in order to evaluate whether there are differences in the mitochondrial response to induction of dysfunction based on the type of chemical or its source.

In a similar experimental model, Miller et al. (2013) demonstrated that the in vivo administration of SFN at 5 mg/kg (i.p., 48 h before chemical challenge) to male CF-1 mice attenuated the effects of 4-hydroxynonenal (4-HNE; 30 μmol/L) on the cortical mitochondria in an ex vivo experimental model. SFN prevented the mitochondrial respiration dysfunction (as assessed through quantification of the consumption of O_2 due to complex I) and the redox impairment caused by 4-HNE in the protein obtained from the organelles. SFN was unable to prevent the 4-HNE-induced decline in complex II-driven respiration in the isolated mitochondria. The authors also demonstrated that carnosic acid at a lower dose (1 mg/kg, i.p.

administration) caused a similar protection (or even better results, as in the case of complex II-driven respiration) in the 4-HNE-challenged mitochondria. The limitations of SFN in relation to mitochondrial protection need further investigation, since they may involve different modulation of signaling pathways associated with cell survival and antioxidant defense.

Carrasco-Pozo et al. (2015) found that SFN at 5 mg/(kg·d) (i.p. administration) for 5 d attenuated mitochondrial impairment resulting from the induction of seizure in male CD1 mice. SFN caused anticonvulsant and antioxidant effects, and prevented the mitochondria-related bioenergetic state in the mice hippocampus. SFN alleviated the decline in state 2 respiration following pilocarpine-induced status epilepticus. Moreover, SFN increased the state 3u respiration (the so-called state 3 uncoupled, which may be induced by adding an uncoupler to mitochondria in state 4) in the status epilepticus group. Moreover, the state 3 ADP-state 4o (mitochondrial respiration associated with ATP synthesis) was increased by SFN in the hippocampal mitochondria of both control and status epilepticus experimental groups. The authors also analyzed the ETC activity in separated parts and found that SFN upregulated both complexes I and II enzyme activities in the hippocampus of the pilocarpine-induced status epilepticus experimental group. SFN also upregulated both the complex I- and complex II-driven oxygen consumption rates (OCRs) of mitochondria obtained from that group. In spite of the mitochondrial function-related benefits promoted by SFN, this molecule was unable to prevent the cell loss observed in the hippocampus of the status epilepticus group. The authors did not determine whether SFN activated cellular signaling pathways associated with mitochondrial function and dynamics in that study. However, they examined in detail whether SFN would be able to modulate the function of mitochondria in the experimentally induced status epilepticus.

Luis-García et al. (2017) found that SFN suppressed the quinolinic acid-induced excitotoxic cascade in striatal neurons in rats. Quinolinic acid is used in experimental models of Huntington's disease and causes hyperactivation of the *N*-methyl-D-aspartate (NMDA) receptors, which are closely related to excitotoxicity associated with mitochondrial dysfunction (Beal et al., 1991; Bordelon et al., 1997; Mishra and Kumar, 2014). The authors tested SFN at 5 mg/kg for 24 h (two doses of SFN through i.p. administration) before the induction of excitotoxicity by quinolinic acid treatment. SFN prevented the quinolinic acid-induced decline in ATP production, loss of MMP, and decreased activity of the ETC components (complexes I–IV, with the exception of complex III). Why SFN did not induce any action in complex III in the animals exposed to quinolinic acid was not investigated. Moreover, the authors did not investigate the mechanism of action involved in the partial protection induced by SFN in the rat striatal mitochondria. Additional studies would be useful to understand exactly how SFN promotes mitochondrial protection in an experimental model of striatal excitotoxicity.

Bi et al. (2017) studied the effects of SFN in male Sprague Dawley rats submitted to carbon monoxide (CO) poisoning. The animals were treated with SFN at 10–40 mg/kg once a day by i.p. injection after CO intoxication. SFN at 20 or 40 mg/kg ameliorated rat behavior in an open-field test and improved mitochondrial structure in the hippocampus of intoxicated rats. SFN also restored MMP values in the CO-treated animals, and induced the upregulation of Nrf2, thioredoxin-1 (Trx-1), and NADPH:quinone oxidoreductase-1 (NQO-1) in the hippocampus. Nonetheless, whether SFN would be able to alter the levels of mitochondria-located antioxidant defenses or the levels of markers of redox stress in the mitochondria of CO-treated rats was not determined. Alterations in the production of reactive species by the mitochondria of SFN- and/or CO-treated rats were not investigated in that experimental model. It would be useful to examine the redox effects of SFN and/or CO in mitochondria closely, since MMP, the single functional parameter associated with mitochondria analyzed by the authors, is a general parameter whose levels may be affected by several factors. MMP homeostasis depends on mitochondrial integrity (the structure of the membranes and cristae) and on the function of the oxidative phosphorylation (OXPHOS) system, as well as on the local production of reactive species, among other factors. Therefore, more detailed analyses directly related to mitochondria would lead to a better understanding of the effects of SFN in experimental models of CO poisoning. Importantly, CO is a mitochondrial toxicant able to inhibit complex IV of the ETC, leading to decreased ability of this protein to pump H^+ ions into the IMS (Alonso et al.,

2003). Consequently, the electrochemical gradient across the IMM is decreased and the MMP values are affected, leading to decreased ATP production by the organelles and release of cytochrome *c* into the cytosol (Green et al., 2014). Therefore, the investigation of parameters directly associated with the function of the mitochondria in the case of exposure to SFN and/or CO is welcome and would be very useful in the development of therapies against CO intoxication.

Lavich et al. (2015) showed that SFN at 5 mg/kg upregulated Drp1 in the hippocampus of adult rats exposed to iron during the neonatal period. DNM1L, a guanosine triphosphatase (GTPase) protein, modulates mitochondrial fission. Reductions in the levels of this protein have been seen in the brain of patients suffering from Alzheimer's disease (Wang et al., 2009). Therefore, SFN may be able to restore mitochondrial dynamics, leading to an increased number of the organelles by fission events (Smirnova et al., 2001). However, the mechanism underlying SFN-induced DNM1L upregulation was not addressed in that work.

5 Conclusions and future directions

There is evidence from both in vitro and in vivo experimental models indicating a role for SFN as a promising mitochondrial protectant agent. However, the mechanism of action is not yet completely understood. Future directions include:

(1) Investigating the effects of SFN on signaling pathways associated with mitochondrial biogenesis in both in vitro and in vivo experimental models focusing on brain cells. Some research groups have demonstrated that SFN modulated proteins associated with mitochondrial biogenesis, but the evidence was not sufficient to elucidate and/or confirm that SFN is an inducer of the synthesis of new mitochondria. The same applies to the effects of SFN on fusion and fission mitochondrial events;

(2) Examining whether there is a link between modulation of mitochondrial function and/or dynamics in microglial cells and the control of neuroinflammation in in vitro and in vivo experimental cells;

(3) Analyzing whether and how SFN would be able to suppress mitochondrial dysfunction induced by endoplasmic reticulum stress (and vice-versa) in in vitro and in vivo experimental cells;

(4) Evaluating the distribution of SFN and its derivatives among organelles, with a focus on the mitochondria;

(5) Testing nanotechnology-associated strategies to increase the availability of SFN to the mitochondria of cells undergoing redox and/or bioenergetic impairment;

(6) Comparing the effects of SFN-containing foods on mitochondrial parameters in experimental animals and humans.

Contributors

Marcos Roberto de OLIVEIRA wrote the manuscript. Fernanda Rafaela JARDIM participated in the writing of the manuscript. Fhelipe Jolner Souza de ALMEIDA created the figure. Marcos Roberto de OLIVEIRA, Fernanda Rafaela JARDIM, Matheus Dargesso LUCKACHAKI, and Fhelipe Jolner Souza de ALMEIDA analyzed the data and discussed about it. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Fernanda Rafaela JARDIM, Fhelipe Jolner Souza de ALMEIDA, Matheus Dargesso LUCKACHAKI, and Marcos Roberto de OLIVEIRA declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：萝卜硫烷对脑线粒体的影响：机理观点和未来发展方向

概　要：有机硫化物萝卜硫烷（SFN）是一种有效的细胞保护剂，在体外和体内实验模型中均可促进抗氧化、抗炎、抗糖化和抗菌作用。因为氧化磷酸化系统的存在，所以线粒体是三磷酸腺苷（ATP）的主要产生部位。同时，线粒体也是具核人体细胞中产生活性氧的主要场所。线粒体损伤在多种人类疾病中起着重要作用，包括神经退行性病变和代谢异常。本文描述和讨论了 SFN 调节哺乳动物细胞中线粒体功能和动力学的作用和机理，以及其对肿瘤细胞中线粒体促凋亡途径的促进作用。SFN 对线粒体的调节作用使得它在一定程度上被认为是一种细胞保护剂。本文还指出了几个需要通过进一步实验解决的问题，即未来的可能研究方向，这可能会对该领域的研究人员具有指导作用。

关键词：萝卜硫素；脑；线粒体；抗氧化剂；Nrf2