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Mitochondrial Dysfunction in Cancer Chemoprevention by Phytochemicals from Dietary and Medicinal Plants

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Abstract

Cancer chemoprevention, a scientific term coined by Dr. Sporn in the late seventies, implies use of natural or synthetic chemicals to block, delay or reverse carcinogenesis. Phytochemicals derived from edible and medicinal plants have been studied rather extensively for cancer chemoprevention using preclinical models in the past few decades. Nevertheless, some of these agents (e.g., isothiocyanates from cruciferous vegetables like broccoli and watercress) have already entered into clinical investigations. Examples of widely studied and highly promising phytochemicals from edible and medicinal plants include cruciferous vegetables constituents (phenethyl isothiocyanate, benzyl isothiocyanate, and sulforaphane), withaferin A (WA) derived from a medicinal plant (*Withania somnifera*) used heavily in Asia, and an oriental medicine plant component honokiol (HNK). An interesting feature of these structurally-diverse phytochemicals is that they target mitochondria to provoke cancer cell-selective death program. Mechanisms underlying cell death induction by commonly studied phytochemicals have been discussed rather extensively and thus are not covered in this review article. Instead, the primary focus of this perspective is to discuss experimental evidence pointing to mitochondrial dysfunction in cancer chemoprevention by promising phytochemicals.

Keywords

Phytochemicals; Mitochondrial Dysfunction; Mitochondrial Dynamics; Electron Transport Chain; Chemoprevention

2. Introduction

Practicality and promise of cancer chemoprevention is demonstrated by clinical integration of selective estrogen receptor modulators and aromatase inhibitors for breast cancer and

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human papilloma virus vaccines for cervical cancer (Fisher et al. 1998; Goss et al. 2011; McNamara et al. 2016). Small molecule phytochemicals present in dietary and medicinal plants appear promising for cancer chemoprevention (Surh 2003; Powolny et al. 2012; Singh and Singh 2012). Cancer chemoprevention research on phytochemicals derived from the dietary plants was originally inspired by cues from the population-based epidemiological studies (Verhoeven et al. 1996; Kolonel et al. 2000; Greenwald et al. 2001). More recently, the scientific community has witnessed a surge in research focused on identification of cancer chemopreventive phytochemicals from medicinal plants (Garodia et al. 2007; Teiten et al. 2013; Vyas and Singh 2014). Isothiocyanates (ITCs) from cruciferous vegetables (e.g., watercress, broccoli, mustard, and so forth), which are naturally stored as glucosinolates in these plants, have been widely studied for cancer chemoprevention using preclinical models (Hecht 1999; Singh and Singh 2012). Examples of well-characterized cancer chemopreventive ITCs include phenethyl isothiocyanate (PEITC) from watercress, benzyl isothiocyanate (BITC) from garden cress, and sulforaphane (SFN) from broccoli (Powolny et al. 2012; Singh and Singh 2012; Sehrawat and Singh 2013). All of these ITCs have shown *in vivo* cancer chemopreventive activity in rodent models (Powolny et al. 2012; Singh and Singh, 2012; Sehrawat and Singh 2013). Even though the evidence for cancer chemopreventive activity of ITCs in humans is still lacking, PEITC and SFN have entered clinical arena to determine their safety, bioavailability, and biological activity (Cipolla et al. 2015; Yuan et al. 2016). Similarly, the preclinical evidence for cancer chemoprevention by some phytochemicals isolated from medicinal plants is quite persuasive. Examples of promising cancer chemopreventative phytochemicals isolated from medicinal plants include withaferin A (WA) from *Withania somnifera* and honokiol (HNK) from *Magnolia officinalis* (Arora et al. 2012; Vyas and Singh 2014). Evidence continues to accumulate to suggest that mitochondrial dysfunction is a critical event in cancer chemoprevention by ITCs, WA, and HNK. The net result of mitochondrial dysfunction triggered by these phytochemicals in cancer cells is apoptotic death that is mediated by generation of reactive oxygen species (ROS) (Arora et al. 2012; Singh and Singh 2012; Sehrawat and Singh 2013; Vyas and Singh 2014). The mechanisms by which these chemicals cause generation of ROS and the underlying pathways in cell death induction have been reviewed extensively by us and others, and therefore, are not covered in this article (Antosiewicz et al. 2008; Singh and Singh 2012; Sehrawat and Singh 2013; Hahm et al. 2014; Vyas and Singh 2014). The primary focus of this perspective is to discuss experimental evidence pointing to mitochondrial dysfunction as a critical event leading to generation of reactive oxygen species (ROS) and eventual cancer cell death by promising phytochemicals. Gaps in our knowledge and unanswered questions pertaining to the mitochondrial dysfunction in cancer chemopreventive mechanisms for selected phytochemicals (ITCs, WA, and HNK) are also highlighted.

3. Mitochondrial dysfunction and cancer

Mitochondrial function is not limited to ATP generation from oxidative phosphorylation (OXPHOS) but this organelle is implicated in numerous biochemical reactions (Nunnari and Suomalainen 2012). Mitochondrial involvement in carcinogenesis has also been reviewed extensively (Carew and Huang 2002; Gogvadze et al. 2008; Scatena 2012). Furthermore, this

organelle is a target of cancer therapy because of its vital role in pro-death and pro-survival pathways (Fulda et al. 2010; Wenner 2012). Mitochondrial dysfunction is also implicated in cancer initiation and progression (Carew and Huang 2002). Defects in the mitochondrial genome in cancer cells lead to deficient respiration and ATP generation and overproduction of ROS causing damage to the mitochondria and other macromolecules (Modica-Napolitano and Singh 2004; Galluzzi et al. 2010). Germline mutations in mitochondrial DNA have been suggested to predispose to cancer development (Canter et al. 2005; Petros et al. 2005). Canter et al. demonstrated the association of the mitochondrial DNA G10398A polymorphism in susceptibility to breast cancer in African-Americans women (Canter et al. 2005). The mitochondrial DNA mutations were suggested to increase prostate cancer (Petros et al. 2005). It is interesting to note that four of the conserved mutations were detectable in multiple patients (Petros et al. 2005). Experimentally, introduction of a mitochondrial DNA mutant through cybrid transfer increased tumorigenic potential of PC-3 cells (Petros et al. 2005). Mitochondrial dysfunctions are also linked to impaired cell death (Carew and Huang 2002; Kroemer and Pouyssegur 2008). In summary, it is clear that mitochondrial dysfunction is intimately linked to cancer development.

4. Inhibition of electron transport chain (ETC) by cancer chemopreventive phytochemicals

In this section, we review scientific evidence implicating inhibition of ETC by cancer chemopreventive phytochemicals shown in Figure 1. PEITC and BITC are aromatic ITCs with a minor difference, whereas SFN is a thioalkyl type ITC compound. Several other naturally-occurring thioalkyl type ITCs have also been identified in plants but SFN is the best studied member of this subclass (Fahey et al. 2001). It is important to mention that SFN occurs naturally as an L-isomer but most studies have used synthetic racemic D,L-SFN for mechanistic and efficacy studies in cancer prevention research. WA is a steroidal lactone and HNK is a phenolic compound. Despite structural difference, they all have been shown to inhibit ETC. Inhibition of ETC causes generation of ROS to trigger Bax and/or Bak-mediated apoptosis as summarized for PEITC in Figure 2 (Hahm et al., 2008; Xiao et al. 2008; Xiao et al. 2009; Xiao et al. 2010; Hahm et al. 2011; Hahm et al. 2014; Pan et al. 2014).

4.1 ETC inhibition by ITCs

Chemopreventive effect of PEITC has been shown in chemically-induced as well as transgenic rodent models of various cancers (Powolny et al. 2012; Singh and Singh 2012). Several mechanisms have been implicated in cancer chemoprevention by PEITC, including inhibition of carcinogen activation through inhibition of phase I metabolism and induction of phase II systems to boost detoxification of carcinogenic intermediates (Hecht 1999; Powolny et al. 2012; Singh and Singh 2012). Furthermore, PEITC triggers death of cancer cells by causing apoptosis and autophagy (Xiao et al. 2006a; Bommareddy et al. 2009; Cheung and Kong 2010). Various reports suggest involvement of ROS in PEITC-induced apoptosis (Trachootham et al. 2006; Zhang et al. 2008; Xiao et al. 2010; Powolny and Singh 2010). We have used prostate cancer cells to study the mechanism underlying ROS generation by PEITC (Xiao et al. 2010). Prostate cancer cells exhibit a decrease in OXPHOS after PEITC

treatment that is associated with complex III inhibition (Xiao et al. 2010). This effect of PEITC seems cancer cell-selective because OXPHOS inhibition is not seen in PrEC, a normal human prostate epithelial cell line (Xiao et al. 2010). However, the mechanism for cancer cell-selective inhibition of OXPHOS, and potentially complex III is yet to be determined. We believe that the differential sensitivity is likely due to differences in uptake of PEITC by cancer cells *versus* normal cells. The sequential mechanism downstream of ROS in PEITC-mediated apoptosis involves activation of Bax that is observed in wild-type LNCaP (androgen-responsive) and PC-3 (androgen-independent) human prostate cancer cells but not in their Rho-0 variants (Xiao et al. 2010). The Rho-0 cells lack OXPHOS. Inhibition of complex III and oxygen consumption as well as Bax activation were also shown in a hepatoma HepG2 cell line (Rose et al. 2005). Condensed mitochondria and mitochondrial cristae structure perturbations (rounded and dilated shape) were observed in ultrastructure studies within 4 hours of PEITC treatment in prostate cancer cells (Xue et al. 2014). However, the *in vivo* evidence for inhibition of OXPHOS or ROS production upon PEITC treatment is still lacking. Another unanswered question in this context is precise mechanism by which PEITC inhibits complex III. Nevertheless, breast cancer chemoprevention by PEITC in a transgenic mouse model representative of human epidermal growth factor receptor 2 subtype is associated with alterations of metabolism-related proteins, including pyruvate kinase isozymes M1/M2, mitochondrial ATP synthase H^+ transporting F1 complex beta subunit, hexokinase-1, isoform CRA_f, and L-lactate dehydrogenase A chain isoform 1 (Singh et al. 2012).

BITC is a close structural analog of PEITC and exhibits *in vivo* preventive efficacy in rodent models of cancer (Hecht 1999; Warin et al. 2009; Sehrawat and Singh 2013). The proapoptotic effect of BITC is also cancer cell-selective and linked to ROS production as exemplified by our own data in breast cancer cells (Xiao et al. 2006b; Xiao et al. 2008). BITC treatment in breast cancer cell lines MCF-7 (estrogen-responsive) and MDA-MB-231 (estrogen-independent) resulted in inhibition of complex III, ROS production, c-Jun NH_2-terminal kinase-dependent activation of Bax leading to apoptosis (Xiao et al. 2008). Overexpression of antioxidative enzymes attenuated ROS production and apoptosis by BITC in these breast cancer cells (Xiao et al. 2008). Mitochondrial damage and loss of the mitochondrial membrane potential with BITC treatment were shown in rat liver epithelial cells (Nakamura et al. 2002). Similar to PEITC, the mechanism for complex III inhibition by BITC is yet to be elucidated but likely involves a common mechanism for both these agents.

Cancer chemoprevention by SFN was first shown for 9,10-dimethyl-1,2-benzanthracene-induced breast cancer in rats (Zhang et al. 1994). A cancer chemopreventive role for SFN has also been established in other types of cancers (Clarke et al. 2008). Our own data implicated ROS in SFN-induced apoptosis in prostate cancer cells (Singh et al. 2005). In contrast to PEITC or BITC (Xiao et al. 2008; Xiao et al. 2010), the ROS production by SFN was accompanied by inhibition of complex I, II, and III of the ETC (Xiao et al. 2009). However, the relative contribution of different complexes in ROS generation by SFN is yet to be established. Nevertheless, our unpublished results show inhibition of OXPHOS in LNCaP human prostate cancer cell line after 9 h treatment with SFN (Figure 3).

4.2 ETC inhibition by WA

WA is a steroidal lactone that is abundant in the leaf and root of *Withania somnifera* plant (also known as Ashwagandha or Indian winter cherry), which is a key ingredient in Ayurvedic medicine practiced in India. Cancer chemopreventive effect of WA has been demonstrated for oral, breast, and skin cancers (Manoharan et al. 2009; Hahm et al. 2013; Li et al. 2015). ROS-dependent apoptosis seems to be an important mechanism in anticancer effects of WA in leukemia (Malik et al. 2007), melanoma (Mayola et al. 2011), and breast cancer (Hahm et al. 2011). However, the mechanism for ROS generation following WA treatment has been characterized in breast cancer cells (Hahm et al. 2011). WA treatment inhibited both basal and reserve OXPHOS in MCF-7 and MDA-MB-231 human breast cancer cells (Hahm et al. 2011). Inhibition of OXPHOS after WA treatment was associated with suppression of complex III in MDA-MB-231 cells (Hahm et al. 2011). Interestingly, a modest but significant increase in complex IV activity was also discernible in WA-treated MDA-MB-231 cells, but implications of this finding are unclear (Hahm et al. 2011). Similar to the PEITC effect observed in prostate cancer cells, a normal human mammary epithelial cell line was resistant to WA-mediated ROS production and apoptosis (Hahm et al. 2011). A role for ROS in apoptosis following WA treatment was further established using mitochondrial DNA-deficient Rho-0 variants of MDAMB-231 and MCF-7 cells (Hahm et al. 2011). Because breast cancer is a heterogeneous disease broadly grouped into different subtypes (luminal, basal, HER2 amplified, and normal like breast cancer), an obvious unanswered question is whether OXPHOS and/or complex III inhibition by WA occurs in other subtypes of breast cancer. Inhibitory effect of WA on OXPHOS has been shown in luminal (MCF-7) and basal-like MDA-MB-231 cells but the experimental evidence for complex III inhibition only exists for MDA-MB-231 cells (Hahm et al. 2011). Unlike ITCs, however, we were able to demonstrate that breast cancer prevention by WA in a transgenic mouse model was associated with *in vivo* inhibition of complex III in the mammary tumor (Hahm et al. 2013). While involvement of ROS in anticancer effect of WA is indisputable, the mechanism for complex III inhibition by WA is still unresolved similar to ITCs. It is also important to establish if inhibition of OXPHOS and complex III by WA treatment is not specific for breast cancer cells. In this regard, a recent study using skin epidermal JB6 P+ cells showed reversal of 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced changes by WA (Li and Zhao 2013). TPA treatment inhibited mitochondrial membrane potential, complex I activity and mitochondrial respiration, which were reversed by WA (Li and Zhao 2013).

4.3 ETC inhibition by HNK

HNK is present in *Magnolia* tree whose bark extract is widely used in the oriental traditional medicine (Lee et al. 2011). Anticancer properties of HNK were first demonstrated in leukemia cell lines (Hirano et al. 1994). However, Bai et al (2003) were the first to show *in vivo* anticancer activity of HNK. Chemoprevention of cancer by HNK has also been documented (Chilampalli et al. 2010; Pan et al. 2014). For example, *N*-nitroso-trischloroethylurea-induced lung squamous cell carcinoma development in mice was inhibited significantly by HNK treatment (Pan et al. 2014). A role for ROS in anticancer effects of HNK has also been suggested previously (Han et al. 2009; Hahm et al. 2014; Lin et al. 2016). One group of investigators showed that HNK can induce rat liver mitochondrial

swelling, decrease membrane potential and modulate respiration (Dong et al. 2013). Another study also reported inhibition of basal OXPHOS by HNK treatment in lung cancer cells that was accompanied by stimulation of extracellular acidification rate, a measure of glycolysis, in H226 cells but not in H520 cells (Pan et al. 2014). HNK is not electrophilic and thus post-translational modification of ETC complex subunits is not a likely explanation for inhibition of OXPHOS.

5. Alteration of mitochondrial dynamics by BITC in breast cancer cells

Persistent fission and fusion of mitochondria is essential for their integrity and normal physiology (Detmer and Chan 2007). A role for mitochondrial dynamics in regulation of apoptosis has been suggested (Brooks and Dong 2007; Suen et al. 2008). Generally speaking, mitochondrial fusion inhibits apoptosis and mitochondrial fission promotes release of apoptogenic factors to trigger apoptotic cell death (Brooks et al. 2007; Boland et al. 2013). In cells committed to apoptosis, the normal filamentous network of mitochondria is fragmented (punctate and spherical) due to increased fission or inhibition of fusion. We recently explored the possibility of whether mitochondrial dynamics was affected by BITC using human breast cancer cells as a model (Sehrawat et al. 2016). We showed that BITC treatment disrupted mitochondrial filamentous network and inhibited mitochondrial fusion (Sehrawat et al. 2016). On the other hand, mitochondrial integrity was preserved after BITC treatment in MCF-10A cells, which is a non-tumorigenic mammary epithelial cell line that was spontaneously immortalized (Sehrawat et al. 2016). Effect of BITC on mitochondrial dynamics was associated with a transient or sustained decrease in mitochondrial fission and fusion regulating protein levels, including Dynamin-related protein 1 (Drp1) and mitofusins. Immortalized mouse embryonic fibroblasts Drp1 knockout mice were resistant to BITC-induced apoptosis when compared with those from wild-type mice (Sehrawat et al. 2016). This protein regulates mitochondrial fission (Lennon and Salgia 2014). Besides apoptosis regulation, Drp1 may either directly or indirectly affect other anticancer functions of BITC. We found a decrease in S616 phosphorylation of Drp1 upon BITC treatment (Sehrawat et al. 2016) and this phosphorylation of Drp1 is mediated by cyclinB/cyclin-dependent kinase complex (Lennon and Salgia 2014). We have also shown previously that BITC-mediated G_2/M phase cell cycle arrest in MDA-MB-231 and MCF-7 cells is associated with suppression of cyclinB/cyclin-dependent complex (Xiao et al., 2006b). Our prior work also shows inhibition of breast cancer cell migration after BITC treatment (Kim et al., 2012). Interestingly, Drp1 silencing reduces breast cancer cell migration *in vitro* (Zhao et al., 2013). It would be interesting to determine if Drp1 overexpression attenuates breast cancer cell migration inhibition by BITC. It is equally important to determine if the BITC-mediated inhibition of mitochondrial fusion is specific for breast cancer cells or this ITC only. Because other ITCs and WA are electrophilic and exhibit mechanistic similarities (e.g., inhibition of complex III), it is highly likely that mitochondrial fusion is also inhibited by these agents. However, it is uncertain if the mitochondrial dynamic is also affected by HNK. Future research will likely address these questions.

6. Conclusions and gaps in knowledge

Even though the experimental evidence for ETC inhibition by the highlighted cancer chemopreventive phytochemicals is compelling, the underlying mechanism is still not fully resolved. One possibility for ETC inhibition by ITCs and WA may entail covalent modification of sulfhydryl groups in critical cysteine in complex III subunits owing to their electrophilic nature. It is also plausible that the agents covered in this review and possibly other cancer chemopreventive phytochemicals cause downregulation of ETC complex subunits. Further research is needed to probe these possibilities. An equally intriguing and unresolved question relates to differences in ETC inhibition by aromatic ITCs (complex III) *versus* SFN (complex I, II, and III) (Xiao et al. 2008; Xiao et al. 2009; Xiao et al. 2010). Another obvious gap in our knowledge is the *in vivo* evidence for inhibition of ETC and mitochondrial dynamics, although we were able to demonstrate complex III inhibition *in vivo* in mammary tumors from WA-treated mice (Hahm et al. 2013). We have shown recently that inhibition of mitochondrial fusion is an early and critical event in cell death induction by BITC (Sehrawat et al. 2016). However, the evidence for mitochondrial dynamics alterations by PEITC, SFN, WA, and HNK is still lacking. Finally, it is imperative that these agents are tried clinically for their cancer chemopreventive activity to move the field further. Nevertheless, this article clearly establishes that mitochondrion is a critical target of structurally-diverse cancer chemopreventive phytochemicals.

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Abbreviations

BITC	benzyl isothiocyanate
Drp1	dynamin-related protein 1 (also known as DNML1 and dynamin 1 like
ETC	electron transport chain
HNK	honokiol
ITCs	isothiocyanates
OXPHOS	oxidative phosphorylation
PEITC	phenethyl isothiocyanate
ROS	reactive oxygen species
SFN	sulforaphane
WA	withaferin A

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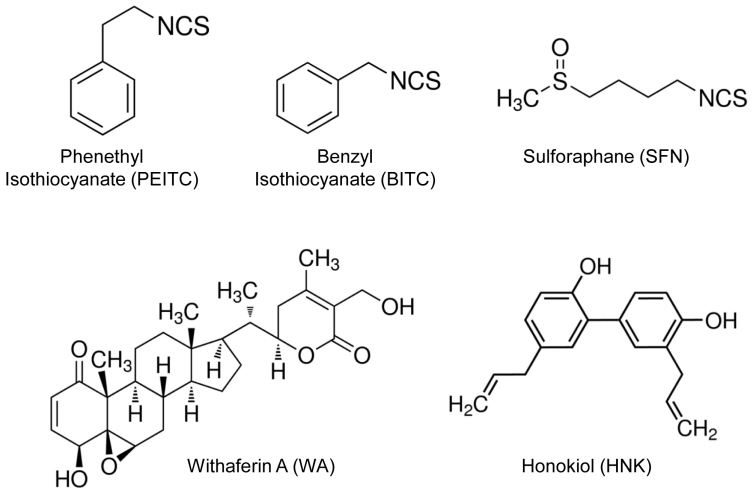

Figure 1.

Molecular structures of the cancer chemopreventive phytochemicals highlighted in this article.

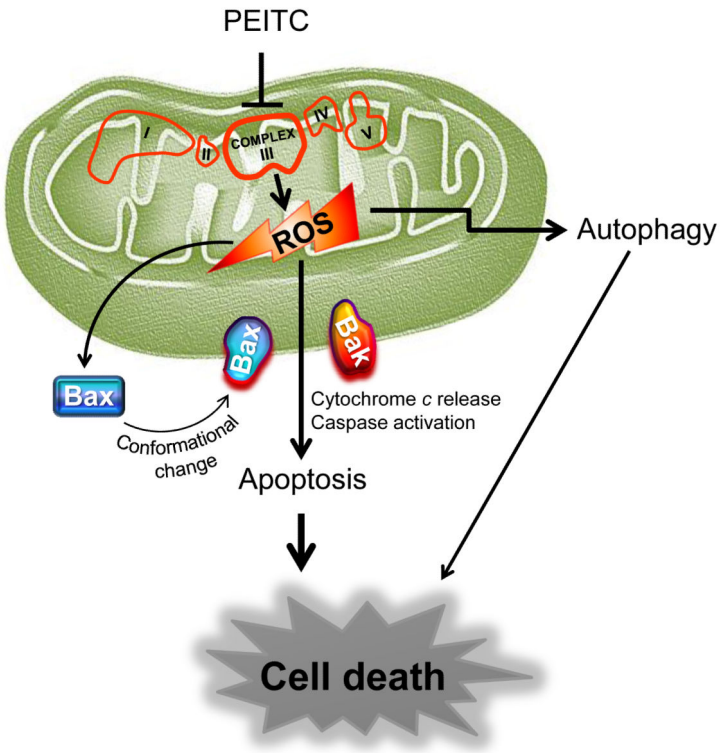

Figure 2.
Mechanistic summary of mitochondria-mediated apoptosis induction by PEITC based on our own findings in prostate cancer cells (Xiao et al. 2010).

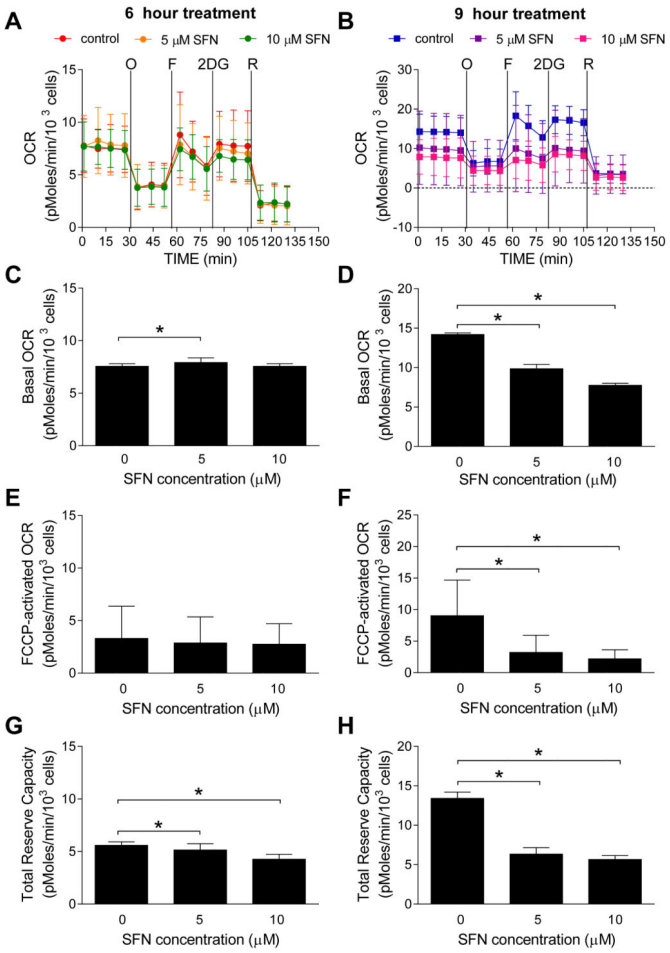

Figure 3.

Inhibition of OXPHOS by SFN in LNCaP human prostate cancer cell line. (**A**-**B**) Representative pharmacologic profiling of oxygen consumption rate (OCR), indicative of OXPHOS, in LNCaP cells treated with DMSO (control) or the indicated doses of SFN for 6 hours (**A**) or 9 hours (**B**) through real-time measurements using the Seahorse Bioscience XF24 Extracellular Flux Analyzer. After measurement of basal oxygen consumption, the cells were treated with oligomycin (“**O**”, 1 μM), FCCP (“**F**”, 0.3 μM), 2-deoxyglucose (“**2DG**”, 100 mM), and rotenone (“**R**”, 1 μM) as indicated. Results shown are mean ± SD (*n* = 3). (**C**-**D**) Basal OCR level in LNCaP cells treated with DMSO (control) or indicated doses of SFN for 6 hours (**C**) or 9 hours (**D**). (**E**-**F**) FCCP-activated OCR level in LNCaP cells treated with DMSO (control) or the indicated doses of SFN for 6 hours (**E**) or 9 hours (**F**). (**G**-**H**) Total reserve capacity in LNCaP cells treated with DMSO (control) or the indicated doses of SFN for 6 hours (**G**) or 9 hours (**H**). Combined results (**C**-**H**) from three independent experiments are shown as mean ± SD (*n* = 13~14). *Statistically significant compared to DMSO-treated control by one-way ANOVA followed by Dunnett’s adjustment.