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Sulforaphane: Expected to Become a Novel Anti-tumor Compound

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Running Title: Sulforaphane as a novel anti-tumor compound

Abstract

Natural products are becoming increasingly popular in a variety of traditional, complementary and alternative systems due to their potency and slight side effects. Natural compounds have been shown to be effective against many human diseases, especially cancers. Sulforaphane (SFE) is a traditional Chinese herbal medicine. In recent years, an increasing number of studies have been conducted to evaluate the anti-tumor effect of SFE. The roles of SFE in cancers are mainly through the regulation of potential biomarkers to activate or inhibit related signaling pathways. SFE has exhibited promising inhibitory effects on breast cancer, lung cancer, liver cancer and other malignant tumors. In this review, we summarized the reports on the activity and functional mechanisms of SFE in cancer treatment and explored the efficacy and toxicity of SFE.

Key Words: Sulforaphane; malignant tumor; anti-tumor effects

Introduction

Cancers have become a major public health threat, representing one of the leading causes of death worldwide[1,2]. Radiation, surgery and drugs are currently effective treatments for malignant tumors. However, they all have different risks, especially with chemotherapy. Although chemical drugs are effective in treating cancer, their resistance and serious side effects, such as damage to liver function, bone marrow suppression, and neurotoxicity, often lead to treatment failure[3,4]. Therefore, we still need to find new drugs for treating cancer that are more effective and have fewer side effects than existing drugs. In this regard, plant-derived products, such as triptolide[5], have received considerable attention due to their lower levels of side effects and effective inhibition of various signaling-mediated pro-survival roles. Many antitumor natural compounds have been shown to be highly effective against a variety of solid tumors[6,7]. It has been reported that angelica blood-enriching decoction can induce autophagic death of colorectal cancer cells by upregulating autophagy-related protein Atg7[8]. Diosmetin, a flavone found in legumes and olive leaves, enhances the radiosensitivity of radioresistant nonsmall cell lung cancer (NSCLC) cells by attenuating phosphatidylinositol 3' phosphokinase/protein kinase B (PKB/Akt) activation[9]. Therefore, the extraction and identification of new compounds from Chinese herbal medicine has gained great potential for the development novel anti-cancer drugs.

Sulforaphane (4-methylsufinyl-3-butenyl isothiocyanate, SFE), a member of isothiocyanate family (ITCs), derived from Raphanus sativus seeds. Raphanus sativus is

a cultivated radish, which is a common cruciferous vegetable.[10] Given that the extracts derived from the roots of Raphanus sativus L. can significantly induce cell apoptosis and inhibit cell proliferation in a variety of human cancer cells by the induction of apoptosis-associated signaling pathways[11], these isolated compounds have long been used to treat a variety of human malignant diseases. Modern pharmacological studies have shown that ITCs have great potential as anticancer agents[12]. It has been reported that ITCs are capable of inhibiting cell proliferation in a dose-dependent manner and inducing apoptosis in HCT-116, LoVo, and HT-29 colon cancer cell lines[13]. ITCs can also reduce the cell proliferation of human erythroleukemic cells, T-lymphoid cells and cervical carcinoma cells[14]. Under the guidance of biological experiments, Kim et al. isolated and identified 7 ITCs derivatives by extraction and chemical methods from Raphanus sativus seeds, which included SFE[15]. The chemical structure of SFE is highly similar to that of Sulforaphane (SFN), another ITCs derivative which is mainly extracted from broccoli[16]. Compared to SFN, SFE has an additional double bond in its chemical structure. In order to better separate and extract SFE, Sangthong et al. for the first time used high performance liquid chromatography (HPLC) to simultaneously determine the content of SFN and SFE in Raphanus sativus extract, and separated them effectively[17]. The anticancer effect of SFN has been demonstrated: (a) blocking the initiation state by inhibiting Phase I enzymes to convert original carcinogen to proximate or final carcinogens; and (b) inducing Phase II enzymes that detoxify carcinogens and promote their excretion from the body[18]. Due to the similarity of chemical structures to SFN, SFE has the potential to be an effective

chemical preventive agent for cancer as well. In this review, we mainly discuss the anti-tumor activity of SFE and the related mechanisms in detail (Fig 1, Table 1). At the same time, as a promising natural anti-tumor product that could be widely used in the future, its toxic side effects and clinical application value are also be discussed.

Action of Sulforaphane in Breast Cancer

Breast cancer is one of the most common malignant tumors in the world, especially in women[19,20]. According to statistics, among women younger than 45, breast cancer is undoubtedly the leading cause of cancer-related death[21]. At present, the treatment of breast cancer mainly includes surgical resection, radiotherapy and chemotherapy. However, the therapy responses are often disappointing[22]. As to these questions, Pawlik et al. found that pretreatment with SFE, at as low concentration as 5 μM, inhibited cell clonogenicity by nearly 70% in breast cancer cells, when compared to untreated cells. However, SFN administration could inhibit the clonogenic potential of breast cancer cells only by about 30% at the similar dose. This indicates that SFE might be considered as a more effective anticancer drug than SFN [23,24]. Human epidermal growth factor receptor 2 (HER-2) is known to be involved in the proliferation and division of breast cancer cells[25], specifically through the Akt-mTOR-S6K kinase pathway[26,27]. The anti-HER2-targeted drug lapatinib is often used in breast cancer patients with HER2 overexpression[28]. Studies have found that the combination of SFE (2.5 μM) and lapatinib (100 nM) could effectively induce cell apoptosis and decrease cell viability mainly by

inhibiting the Akt-mTOR-S6K pathway in breast cancer cells, thus improving the therapeutic effect of lapatinib[29]. Triple-negative breast cancer (TNBC) is a common subtype of breast cancer lacking estrogen receptor, progesterone receptor and HER2 gene overexpression[30-32]. SFE also has the significant therapeutic potential against TNBC. In recent years, the Hedgehog (Hh) pathway has been identified as a key signaling pathway that drives tumorigenesis in TNBC[33]. Downregulation of the Hh signaling pathway by inhibitors can reduce cell migration and invasion[34,35]. SFE can significantly inhibit the Hh pathway, thereby reducing the activity of the downstream signal modulators matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) and inhibiting the invasion of human TNBC cells[36]. Early growth response 1 (EGR1) is an immediate early gene induced by estrogen, growth factor or stress signal that can exert both cancer-suppressive and promoter activities[37]. At the same time, EGR1 was successfully verified as a uniformly activated marker after SFE treatment in TNBC cell lines MDA-MB453 and MDA-MB-436. The data indicated that SFE could inhibit the expression of cyclinB1 and phosphorylated Cdc2 by mediating tumor suppressor EGR1, thus inducing G2/M phase arrest of TNBC cells[38].

Action of Sulforaphane in Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is one of the deadliest and most common cancers in humans. The treatment of liver cancer mostly involves surgical resection, transplantation and ablation, but the therapeutic effect is not good[39,40]. Some researchers

have found that SFE can promote the apoptosis of HCC cells, which is morphologically manifested as cell contraction, blistering, chromatin condensation and nuclear fragmentation. They also found that SFE was most toxic in HepG2 cells. SFE exhibited an IC_{50} value of 33.8 μM when incubated with HepG2 cells for 72 h. An Annexin V assay found that the same treatment increased caspase-3/7 and -9 activities, while caspase-8 activity decreased[41]. Oxidative reactive oxygen species (ROS), which are responsible for killing cancer cells, also affect secondary signaling networks[42]. SFE can induce the generation of intracellular ROS and inhibit the polymerization of microtubules, leading to the apoptosis and necrosis of HCC cells[43]. The transcription factor nuclear factor-κB (NF-κB) is a key transcriptional regulator in the inflammatory response. The NF-κB pathway is one of the important pathways activated during liver injury and inflammation and has been widely studied in the development of liver cancer[44]. SFE can inhibit NF-κB activity and downstream gene expression of the NF-κB pathway in HCC cells. Meanwhile, SFE can increase the radiation sensitivity of HCC by blocking the NF-κB pathway[45].

Action of Sulforaphane in Lung cancer

As we all know, lung cancer is the leading cause of cancer death in the world[46]. NSCLC, the most frequent subtype of lung cancer, has increased in both incidence and mortality[47]. At present, research advancement in the field has revealed the tumor promotion roles of PI3K-Akt overactivation in NSCLC[48]. The PI3K-Akt pathway promotes proliferation, migration, invasion, and resistance to treatment by activating a

variety of mechanisms, including the loss of the negative regulator PTEN and/or Akt1 itself[49,50]. SFE-treated NSCLC cells have significant inhibitory effects on the PI3K-Akt signaling pathway, including inhibition of PTEN expression and inhibition of Akt phosphorylation[51]. SFE (7.5 μM) combined with the chemotherapy drug carboplatin (20 μM) can significantly induce mitochondrial membrane potential and intracellular ROS depolarization. By activating caspases, destroying MMPs and arresting the cell cycle, combination treatment with SFE and carboplatin synergistically promotes the apoptosis and anti-proliferative effects of human NSCLC cells A549d and enhances the tumor toxicity effect of conventional therapy alone[52].

Action of Sulforaphane in cervical cancer

Cervical cancer remains the third most common cancer in developing countries, despite a wide range of screening procedures[53]. The therapeutic effects of photodynamic therapy in cervical intraepithelial neoplasia (CIN) and cervical cancer have been extensively studied[54,55]. Effects of photodynamic therapy with a very low dose of SFE (2.0 μg/ml) and radachlorin (0.5 μg/ml) at a fluence of 27 J/cm2 (30 milliwatts/cm2, λmax ~ 670 ± 3 nm) on human cervical cancer cells HeLa shown a synergistic effect in inducing cell apoptosis. This combination therapy activates the mitochondrial apoptotic pathway primarily through upregulating the levels of caspase-3 and caspase-9. This therapeutic strategy also activates the caspase-8-dependent death receptor pathway and inhibits cell proliferation by downregulating EGFR[56].

Action of Sulforaphane in other tumors

SFE also has obviously cytotoxic effects on other human malignant tumor models. For example, cisplatin is a first-line chemotherapy drug for a variety of cancers, including ovarian cancer[57,58]. SFE can sensitize cisplatin by enhancing ROS and mitochondrial membrane depolarization and can activate multiple apoptotic pathways to synergistically inhibit the proliferation of ovarian cancer SKOV3 and SNU8 cells and induce apoptosis. Therefore, SFE could be used as a promising chemotherapy sensitizer to improve the efficacy of cisplatin in ovarian cancer[59]. SFE can also reduce the viability of gastric cancer cells and induce apoptosis[60]. In addition, SFE can induce cell cycle arrest and apoptosis in the G2/M phase of colon cancer cells, accompanied by the phosphorylation of CDK1 and CDC25B inhibitory sites and the upregulation of the p38 and JNK pathways[61]. Surprisingly, SFE can selectively clear lymphoma cells via CRM1-mediated SQSTM1/p62 overexpression and AMPK activation. At the same time, SFE protects normal lymphocytes by inducing cophage and apoptosis[62]. SFE and photosensitive fiber-mediated photodynamic therapy can induce the apoptosis of thyroid cancer cells via significantly upregulating Ras, MEK, ERK and B-Raf protein expression levels. After combined treatment, their pro-apoptosis and anti-proliferative effects were both significantly enhanced to a much higher level than the single dose[63,64].

Safety and efficacy

SFE is often used as an anti-cancer and/or anti-inflammatory drug in traditional medicine[15]. SFE is unstable in aqueous medium and at high temperature; thus, the stability of SFE during storage is the focus of its biological activity research. Studies have shown that -20 ℃ and 4 ℃ and are the best storage temperatures for SFE[12]. As a potential anti-tumor drug, SFE exhibits a wide range of activities in vivo and in vitro against most tumors[10]. Because of its certain cytotoxicity, it is of great significance to evaluate the clinical safety of SFE[65]. Some researchers have tested SFE in acute toxicity analyses. After fasting overnight, 48 mice were given 5 different doses of SFE at 400, 300, 225, 168.8, and 126.6 mg/kg (8 in each group), and any serious effects or mortality were carefully observed after administration. After 14 days, all eight mice treated with 126.6 mg/kg SFE survived during treatment. However, 8, 7, 4, or 2 animals treated with 400, 300, 225, or 168.8 mg/kg SFE died within 24 hours of dosing. In addition, one mouse treated with 225 or 168.8 mg/kg SFE died within 48 hours. For the 126.6 mg/kg SFE group, no physical or abnormal changes were observed in sleep patterns, behavior patterns, fur, skin, eyes, mucous membranes, tremors or salivation[51]. In another study, scientists implanted lymphoma cells in nude mouse xenografts and administered SFE to them twice a week, 100 mg/kg each time. After 10 days, there was no significant change in body weight compared with the control group, indicating that SFE is less toxic[62]. Thus, the dose-associated superiority of SFE in reducing adverse reactions is obvious in current preclinic research. In addition, the findings from Li M et al. [66] have shown that SFE could be able to evidently restrain the pathological process of diseases in C57BL/6J mice

associated with increased intestinal inflammatory factors. Meanwhile, they demonstrated that no apparent toxicity to animals induced by SFE administration.

Currently, it is well known that evaluation of the bioavailability of natural compounds is one challenge in the design of clinical trials for studying their biological activity. Recently, Fahey JW et al. identified that changes of inflammatory-related genes in peripheral blood mononuclear cells have significantly influence on the SFE bioavailability in twenty healthy participants [67]. Similarly, another research has been carried out to evaluate the bioavailability of SFE in fourteen women, and found that repeated dosing of SFE could not result in the accumulation of toxic metabolites in urine over time [68]. Moreover, SFN-loaded nanostructured lipid carriers (NLC) were developed and optimized to effectively improve its bioavailability and cytotoxicity efficacy against cancers [69]. These findings provide valuable recommendation to better design the clinical trials to study the SFE functionality in future. To data, a preliminary randomized controlled trial was performed to demonstrate that pre-treat with broccoli sprout extract could improve the bioavailability and chemopreventive activity of SFE, together with downregulation of several prostate cancer development-associated genes in the biopsy form 98 men [70]. However, unfortunately, there are no clinical trials for direct evaluation of SFE on its anti-tumor effect. Therefore, further additional investigation, mainly well-designed clinical trials, are required to establish correlations and allow for further verify the efficacy, safety and possible adverse reactions of SFE products.

Discussion

SFE extracted from Raphanus sativus is unstable in aqueous solutions and at high temperatures[11]. This instability undermines many useful applications of SFE. Generally, the degradation rate of SFE increases with increasing of temperature. Some researchers have found that the optimal storage temperature of SFE is -20 °C and 4 °C by ESI/MS, NMR and other research methods. After 5 weeks of storage, the residual rates remained around 96.56 ± 0.15% and 95.18 ± 0.20% respectively[12]. To overcome the instability of SFE at high temperatures, some researchers have developed hydroxypropyl-β-cyclodextrin (HP-β-CD) and maltodextrin (MD) microcapsules loaded with SFE[71]. As ROS-induced oxidative stress has been shown to be involved in the pathogenesis of many diseases, a recent study showed that MD microcapsules can increase the antioxidant capacity of natural compound anthocyanins and reduce ROS levels[72]. This suggests that HP-β-CD and MD microcapsules containing SFE might also have similar potential and need to be further clarified in other clinical applications.

Increasing numbers of studies have shown that SFE has potential as an effective cancer chemopreventive agent. For example, SFE can reduce cell proliferation in human and murine erythroleukemia cells, human T lymphocytes, human cervical cancer cells, and H3-T1-1 cells[12]. Studies have identified SFE, and its analogue, SFN, as ITC derivatives extracted from dextran[73]. SFN and SFE belong to the same family and exhibit similar effects through various mechanisms. Studies have found that low concentrations of ITCs can induce apoptosis in human malignant melanoma (A375) cells[74]. It is well

known that pSTAT3 is a key carcinogen in head and neck squamous cell carcinoma (HNSCC)[75]. SFN promotes non-NRF2-dependent dephosphorylation/inactivation of pSTAT3[76,77]. A high level of aldehyde dehydrogenase (ALDH) enzyme activity in breast cancer cells results in breast cancer stem cell (BCSC) properties by upregulating Notch-1 and epithelial mesenchymal markers[78]. Studies have shown that SFN can reduce the number of ALDH-positive cells in human breast cancer cells by 65% to 80%. At the same time, SFN downregulated the Wnt/β-catenin signaling pathway [79], an important regulator for the stem cell self-renewal. In addition, miR-616-5p was identified as a carcinogenic marker associated with the risk of recurrence and metastasis in patients with NSCLC[80]. Epithelial-mesenchymal transition (EMT) is an important mechanism leading to cancer metastasis[81-83]. SFN inhibits miR-616-5p expression and abrogates EMT processes in NSCLC cells, thereby inhibiting lung cancer metastasis[80]. These results further suggest the indirect anti-tumor effect of SFE. At the same time, the abovementioned findings can provide clues to finding more active substances to enrich our clinical drug classes.

Conclusion

In recent years, identifying active ingredients in plants that can be used to treat diseases has been the research approach for creating new drugs both at home and abroad. As shown in previous studies, SFE has significant anti-tumor effects and exhibits enormous clinical potential due to its undiscovered activity. However, studies on the mechanism of SFE anti-tumor activity have not been comprehensive, and there is a lack

of available information for evidence-based medicine. In addition, the safety and toxic side effects of SFE have yet to be further studied. In conclusion, with continuous research and increasing understanding of the cancer prevention and anti-cancer mechanisms, SFE has emerged as a very promising new drug in anti-tumor clinical treatment.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Conflict of interest statement

The authors declare no conflicts of interest.

Authors' contributions

GW, YY, JZ and ZX were the main authors of the manuscript; GW, YZ, XC, SZ, XW, JW, WL and CO contributed to the design and format of figures and tables; YY and ZX

296 revised the manuscript; GW, YY, JZ and ZX were responsible for the manuscript writing.

297 All authors read and approved the final manuscript.

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536 Table1: Mechanism of action of Sulforaphene(SFE) in human tumors.

Tumors	Action	Outcome	Model Used	Refs
Breast cancer	Akt-mTOR-S6K kinase pathway↓	Reversal multidrug resistance, Apoptosis↑	SKBR-3, BT-474	26
Triple-negative breast cancer	Hedgehog↓, MMP-2↓, MMP-9↓,	migration and invasion↓, Apoptosis↑, proliferation↓,	MCF7, T47D, MCF10A, MCF10AT1, MCF10CA1a, SUM159,	33
Triple-negative breast cancer	EGR1↑, cyclinB1↓, Cdc2↓	Apoptosis↑, Cell cycle G2/M phase arrest	MDA-MB-231, MDA-MB-453, MDA-MB-436, MDA-MB-468	35
Hepatocellular Carcinoma	caspases -3/7 and -9↑, caspase-8↓,	Apoptosis↑, Cell cycle G0/G1 phase arrest	MFC-7, HT-29	38
Hepatocellular Carcinoma	ROS↑, microtubule polymerization↑,	Apoptosis↑, radiation-induced cell death↑	HB-8065	40

Hepatocellular Carcinoma	NF-kB↓	Apoptosis↑, proliferation↓	HepG2, Hep3B	42
Lung cancer	PI3K-Akt↓, PTEN↓	Apoptosis↑, migration and invasion↓,proliferation↓	A549, H460, H446, HCC827, H1975, H1299	48
Non-Small Cell Lung Carcinoma	ROS↑, Bcl-2↓, Bax↑, cytochrome C↑, caspase-9/-3↑	Apoptosis↑,,proliferation↓	A549	49
Cervical cancer	caspase 3↑, caspase 9↑, EGFR↑	Apoptosis↑, proliferation↓	HeLa	53
Ovarian cancer	ROS↑, mitochondrial membrane depolarization	Apoptosis↑, proliferation↓	SKOV 3, SNU 8	56

Colon cancer	p38, CDK1, CDC25B	Apoptosis↑, Cell cycle G2/M phase arrest	HCT116, HT-29, DLD1, KM12	58
gastric cancer	ROS↑, cytochrome c↑, Casp-3↑, Casp-8 ↑, PARP-1↑	Apoptosis↑, migration and invasion↓	AGS	57
Lymphoma	CRM1, p62↑, AMPK↑	Apoptosis↑	U937, HUT78, Raji, JeKo-1, U2932	59
Thyroid cancer	Ras↑, MEK↑, ERK↑, B-Raf↑	Apoptosis↑, proliferation↓	FRO	60,61

Note. MMPs: Matrix Metalloproteinases; EGR1: early growth response 1; Cdc2: cell division cycle gene 2; ROS: reactive oxygen species; PTEN: phosphatase and tensin homolog; Bcl-2: B-Cell lymphoma 2; CDK1: cyclin dependent kinase 1; CDC25B: Cell division cycle 25 B; CRM1:chromosome-region-maintenance-1; AMPK: AMP-activated protein kinase; MEK: Mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase. ↑:activation/ upregulation; ↓:suppression/downregulation.

541 Figure Legends

542 Figure 1. Overview of the natural compound Sulforaphane (SFE) and the aberrant

543 signaling pathways for human malignant cancer research and therapy.

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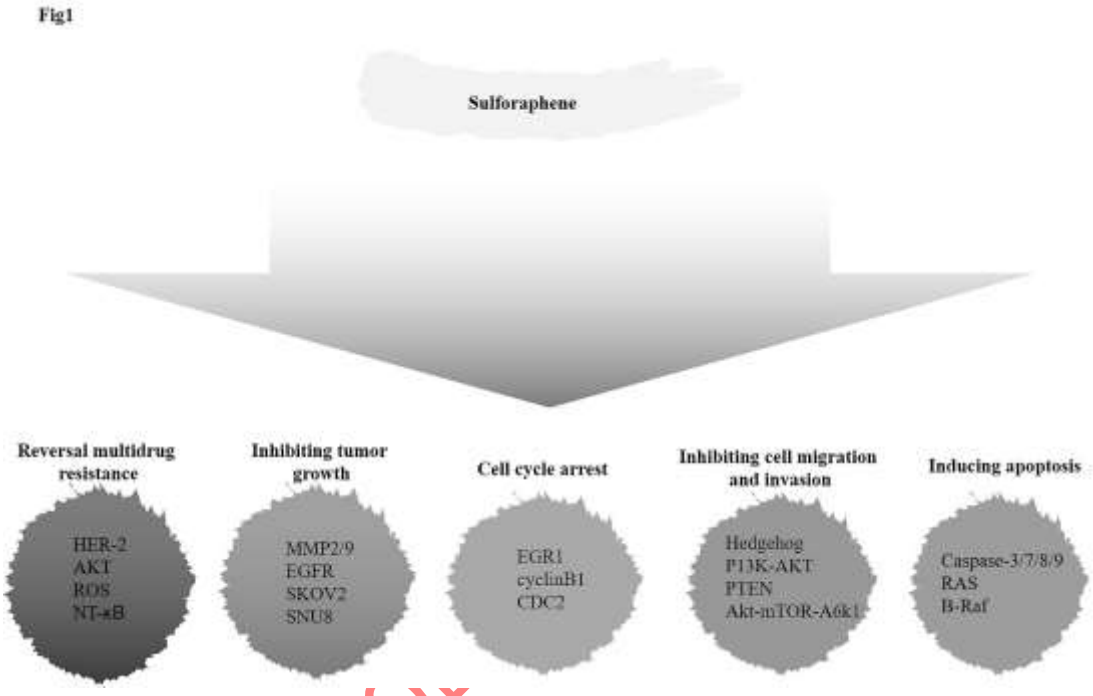

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