Title: Targeting Apoptosis Pathways in Cancer and Perspectives with Natural Compounds from Mother Nature

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Financial supports for this article

Ministry of Science and Technology (No. 2010DFA31430),
Ministry of Education of China (NCET-10–0316),
National Natural Science Foundation of China (No. 30871301, 30700827),
Jilin Provincial Science & Technology Department (20130521010JH , YYZX201241),
Changchun Science & Technology Department (No. 2011114-11GH29),
the Program for Introducing Talents to Universities (No. B07017),
and the Fundamental Research Funds for the Central Universities (12SSXM005).
Abstract

While the incidences are increasing day after day, scientists and researchers taken individually or by research group are trying to fight against cancer by several ways and also by different approaches and techniques. Sesquiterpenes, flavonoids, alkaloids, diterpenoids and polyphenolic represent a large and diverse group of naturally occurring compounds found in a variety of fruits, vegetables and medicinal plants with various anticancer properties. In this review, our aim is to give our perspective on the current status of the natural compounds belong to these groups and discuss their natural sources, their anticancer activity, their molecular targets, and their mechanism of actions with specific emphasis on apoptosis pathways, which may help the further design and conduct of preclinical and clinical trials.

Unlike pharmaceutical drugs, the selected natural compounds induce apoptosis by targeting multiple cellular signaling pathways including transcription factors, growth factors, tumor cell survival factors, inflammatory cytokines, protein kinases and angiogenesis that are frequently deregulated in cancers and suggest that their simultaneous targeting by these compounds could result in efficacious and selective killing of cancer cells. This review suggests they provide a novel opportunity for treatment of cancer, but clinical trials are still required to further validate them in cancer chemotherapy.

**Keywords:** Cancer, Apoptosis, Natural Compounds, Chemoprevention, Signaling pathways.
1. Introduction

Cancer is a major public health problem and the second leading cause of mortality around the world, mainly Europe and USA with an incident rate of about 2.6 million cases per year [1, 2]. It is characterized by unscheduled and uncontrolled cellular proliferation in the spectrum of cell. Cancer incidence in developing countries has been prevailed by tumor types that are related to viral, genetic mutations and bacterial contamination [3]. Cancer has a high incidence and a long period of latency on its development and in the progression of the sickness. There are numerous risk factors known concerning the development of cancer including age, geographic area and race [4]. However, cancer is mostly a preventable disease.

Regardless of whether a cancer specifically results from a genetic mutation and viral or bacterial contamination, the recent extensive research indicated that most cancers are caused by dysfunction of many genes coding for proteins such as, antiapoptotic proteins, growth factors, growth factor receptors, transcription factors and tumor suppressors; which constituted the target for cancer treatment. Prevailing treatment options have limited therapeutic success in cancer in the past decade. The concept of chemoprevention is gaining increasing attention because it is a cost-effective alternative for cancer treatment [5]. Cancer chemoprevention by natural compounds, especially phytochemicals, minerals and vitamins, in a number of studies under both in vitro and in vivo conditions has shown promising results against various malignancies [6].

In the development of bioactive chemical, natural products have a rich and long history. Herbal medicines, as an important novel source with a wide range of pharmaceutical potential, are being used to treat human ailments including almost all kinds of cancer [7].

The involvement of multiple factors underlying developmental stages of cancer at epigenetic,
genetic, cellular and molecular levels is opening up enormous opportunities to interrupt and reverse the initiation and progression of the disease and provide scientists and researchers with numerous targets to arrest by physiological and pharmacologic mechanisms to delay the development of cancer. The aim of this review is to summarize recent researches on twelve (12) natural compounds, such as flavonoids (Honokiol, Magnolol, Jaceosidin and Casticin), sesquiterpenes (Parthenolide, Costunolide, Isoalantolactone and Alantolactone), alkaloid (Evodiamine), diterpenoids (Oridonin and Pseudolaric Acid B) and polyphenolic (Wedelolactone) focusing on anticancer activity. The literature was screened from various sites including PubMed, Scopus, and Elsevier Science Direct Journal. Access to the Elsevier Science Direct Journal was made possible through library of Northeast Normal University, Changchun, China. We propose that the development of natural compounds into new anticancer agents has a bright future despite some difficulties.

2. Natural Sources and Biological Activities of Anticancer Chemopreventive Agents

Natural products are important and valuable resources for drug development. Extensive researches have been carried out on the phytochemicals, for their health-promoting potential. They have been found in fruits, vegetables, nuts, seeds, herbs, spices, stems, flowers and tea. The phyto-constituent from these plants was extracted by several techniques, mainly HPLC, micellar electrokinetic chromatography (MEKC), microemulsion electrokinetic chromatography (MEEKC), and their structures were elucidated on the basis of NMR analysis (Figure 1).

The selected natural compounds among diterpenoids, sesquiterpenes, flavonoids, alkaloids and polyphenolic have been reported for their wide spectrum of biological effects, including
antifungal, anti-helmintic, antimicrobial, anti-inflammatory, anti-trypanosomal, and antiproliferative effects on various cancer types as described in Table 1 and Table 2.

3. Role of Natural compounds in Cancer Prevention

Plants provide an extensive reservoir of natural products, demonstrating important structural diversity, and offer a wide variety of novel and exciting chemical entities and have a long history of use in the treatment of several illnesses. The significance of natural products in health care is supported by a report that 80% of the global population still relies on plant derived medicines to address their health care needs [8]. It is also reported that 50% of all drugs in clinical use are natural products, or their derivatives, or their analogs [9], and 74% of the most important drugs consist of plant-derived active ingredients [10]. There are more than 3000 plant species that have reported to be used in the treatment of cancer in modern medicine [11-14].

There is a continued interest in the investigation of extracts of microorganisms, terrestrial plants and marine life forms to search for anticancer compounds [12]. Indeed, since 1920s with Berrenblum, chemopreventive have began [11], after a period of relative dormancy, re-entered the cancer research mainstream in the 1970s through the work of Sporn [15]. Till now, molecules derived from Mother Nature have played and continue to impart a dominant role in the discovery of compounds for the development of conventional drug for the treatment of most human diseases [16].

Medical indications of natural compounds and related drugs, including anticancer, antibacterial, anti-parasitic, anticoagulant, and immune suppressant agents, are being used to treat 87% of all categorized human diseases [12]. Since 1970s, drug discovery was based on screening of a large
number of natural and synthetic compounds; until with the advent of computer and other molecular biology techniques, resulting in the modern and rational drug discovery [17]. The selected compounds and many other natural products have traditionally provided a rich source of drugs for cancer treatment [11].

Although different approaches are available for the discovery of novel and potential therapeutic agents, natural products from medicinal plants are still one of the best reservoirs for novel agents with new medicinal activities. Thus, identification of natural compound that selectively has ability to not only block or inhibit initiation of carcinogenesis but also to reverse the promotional stages by inducing apoptosis and growth arrest in cancer cells without cytotoxic effects in normal cells [18]. The chemopreventive properties and molecular targets of selected promising natural compounds are detailed in Table 1, Figure 2 and Figure 3.

4. Apoptosis Signaling Pathways

Programmed cell death also called apoptosis play crucial roles for embryonic development and tissue homeostasis of multicellular organisms. It’s carried out in a regulated way, which is associated with typical morphological features like cell shrinkage, chromatin condensation and cytoplasmic membrane blabbing. Dysregulated apoptosis has been implicated in a variety of diseases, including tumor formation or even development of cancer cell drug resistance [19].

Apoptosis is triggered through two well-characterized pathways in mammalian cells. The first one is extrinsic pathway, depending on triggering of death receptors (e.g. TNF), transmembrane proteins expressed on the cell surface, and the second is intrinsic pathway, mediated by molecules released from the mitochondria (e.g. Bcl-2 protein family) [20].
The extrinsic apoptosis pathway is initiated through the binding of ligand (Fas-associated death domain (FADD)) to death receptors that contain an intracellular death domain (death-inducing signaling complexes (DISC)) [21, 22]. The intrinsic pathway is activated by physical or chemical stimulations, such as hypoxia, growth factor deprivation, cell detachment, or stress signals.

A set of cysteine proteases, both pathways cause the activation of the initiator caspases, which then activate effector caspases. Caspases are cysteine-dependent aspartate-specific proteases and are regulated at a post-translational level which ensures that they can be rapidly activated. They are first synthesized or expressed in cells as inactive proenzyme which consists of a prodomain, a small subunit and a large subunit forms that require oligomerization and/or cleavage for activation. However, caspase-independent apoptosis is also reported [23].

Apoptosis is characterized by chromatin condensation and DNA fragmentation, and it is mediated by caspases [24]. Many apoptotic signals are mediated to cell death machinery through p53 with other proteins such as TNF, Fas and TRAIL receptors are highly specific physiological mediators of the extrinsic signaling pathway of apoptosis. Mitochondria are involved in a variety of key events, such as release of caspases activators, changes in electron transport, loss of mitochondrial membrane potential, and participation of both pro-and anti-apoptotic Bcl-2 family proteins [25, 26]. This breakthrough finding may have important implication for targeted cancer therapy and modern application of natural compounds.

5. Molecular Targets of Natural Chemopreventive Agents

Natural compounds are being actively investigated for their chemopreventive potential, among flavonoids, sesquiterpenes lactones, alkaloid, diterpenoid and polyphenolic have been extensively
studied and founded to exhibit chemopreventive properties a broad spectrum of activity against multiple cancer types in both cell culture and animal models and currently several preventive trials are ongoing to treat cancers. For insistence, the cell signaling pathways activated by anticancer natural compounds agents are numerous and different for different targets. Moreover, the same compound activates different signaling pathways depending on the cell types. The main signaling pathways activated by anti-cancer chemopreventive agents are illustrated in Figure 2.

6. Targeting Cancer Cells by Regulating Apoptosis Related Proteins Pathway

In normal cells, certain cellular signals control and regulate their growth and all other mechanisms. When these signals and mechanisms are altered due to various factors, including mutations that prevent cells to undergo apoptosis, normal cells are transformed into cancerous cells. Studies thus so far suggest that inhibition of any one of these altered signals or mechanisms together are helpful in alleviation of cancer.

6.1 p53 and its Family Members Pathway

The tumor suppressor p53 considered as guardian of the genome plays a pivotal role in controlling the cell cycle, apoptosis, genomic integrity, and DNA repair in response to various genotoxic stresses [25, 27, 28]. Once active, p53 can bind to regulatory DNA sequences and activate the expression of target genes, which is important for the suppression of tumor formation as well as for mediating the cellular responses to many standard DNA damage inducing cancer therapies by cycle inhibition (p21, reprimo, cyclin G1, GADD45, 14-3-3) and angiogenesis (TSP1, Maspin, BAI1, GD-AIF), induction of apoptosis (PERP, NOXA, PUMA, p53AIP1, ASPP1/2, Fas, BAX, PIDD) and genetic stability (p21, DDB2, MSH2, XPC) [29-32].
Recently, it has also been documented that many natural chemopreventive agents induce cell cycle arrest and apoptosis by activating p53 and its target genes. Oridonin induced up-regulation of the functional p53 protein in A2780 [33]. Oridonin increased p53 and its target Bax and p21waf1 in prostate cancer LNCaP and NCI-H520 cells with wild-type p53 gene [33, 34]. Oridonin also stabilize p53 protein and sensitize TRAIL (TNF receptor apoptosis-inducing ligand) induced apoptosis, and prevent or delay chemotherapy-resistance in A2780 cells [35]. In human prostate cancer, honokiol activated p21 (PC-3 and LNCaP) and p53 protein expression (LNCaP) [36].

Honokiol increased phosphorylated p53 in both HCT116H and CT116-CH3 cell lines [37]. In skin cancer, p53 activation is lead to the induction of DNA fragmentation and apoptosis [38]. Honokiol is particularly effective in several tumor xenograft systems with deficits in p53 signaling, including PC3, MDA-MD-231, and SVR cells [39]. Furthermore, honokiol in a concentration-and time-dependent manner independent of their androgen responsiveness or p53 status induced Bax, Bak, and Bad in PC-3, LNCaP, and C4-2 cells [40]. p53 expression had no remarkable changes in honokiol-induced in human colorectal RKO cell line [41].

Casticin also induced p53-mediated apoptosis by activating its pro-apoptotic protein Bax in U251, U87 and U373 glioma cell [42]. Casticin induce a p53-independent apoptosis in a human non-small-cell lung carcinoma cell lines H460, A549 and H157 [43]. Mechanism of casticin for malignant tumors is suppressed through c-Myc in p53 mutated Hs578T cells [44].

The signaling pathways that depend on p53 are essential components of cellular responses to stress. Parthenolide in four cell lines, HCT116, RKO colon carcinoma, NCI-H1299 lung
carcinoma and HL60 myeloblastoma, induced a significant reduction in the frequency of apoptotic cells in UV-irradiated p53-proficient lines [45, 46]. Parthenolide activated p53 and other MDM2-regulated tumor-suppressor proteins [47]. Synergistic apoptotic effects of parthenolide and okadaic acid treatment increased p53 accompanied by lowering in p-Akt and pS166-Mdm2 levels under PTEN action [48].

It has also been documented that alantolactone significantly increased the expression of p53 in HepG2 cells [49, 50] with concomitant increase of its downstream target genes, mainly cyclin-dependent kinase inhibitor p21 in adriamycin (ADR)-resistant human erythroleukemia cell line K562/ADR [51]. Alantolactone induce p53-independent apoptosis in prostate cancer PC-3 cells [52].

6.2 Nuclear Factor-kappa B (NF-κB) and its Family Members Pathway

The pro-oncogenic nuclear factor-kappa B (NF-κB) is a master transcription factor consisting of closely related proteins that generally exist as dimers and bind to a common DNA sequence within the promoters of target genes, called the κB B site, which promote transcription of target genes through the recruitment of coactivators and corepressors [53]. NF-κB pathway plays an important role in tumorigenesis through transactivation of genes involved in cell proliferation, apoptosis, tumor cell invasion, metastasis, and angiogenesis [54]. The NF-κB1 family of transcription factors consists of five members, NF-κB1 (p50), NF-κB2 (p52), c-Rel, RelB, and RelA (p65), which share an N-terminal Rel homology domain responsible for DNA binding and homodimerization and heterodimerization through ankyrin repeats, covering the nuclear localization sequence of NF-κB [53, 55]. In this momentum, NF-κB is normally sequestered in the cytoplasm via association with its endogenous inhibitor IκB. Furthermore, IκB-α is rapidly phosphorylated by kinase IKK (IκB kinase) in two catalytic subunits, IKK-α and IKK-β, and one regulatory subunit IKK-γ [56].

Nuclear factor-kappa B and other signaling pathways that are involved in its activation by free radicals,
inflammatory stimuli, cytokines, carcinogens, tumor promoters, endotoxins, γ-radiation, ultraviolet (UV) light, and x-rays are highly significant in cellular growth and transformation, suppression of apoptosis, invasion, metastasis, chemotherapy resistance, radio resistance, and inflammation [57]. Furthermore, other agents including TNF-α, IL-1, IL-6, and COX-2,5 in an inflammatory microenvironment are also highly involved in tumor progression, incursion of adjoining tissues, angiogenesis, and metastasis [58].

Activation of NF-κB inhibits apoptosis by inducing the expression of Bcl-2 family members and caspases inhibitor [59]. The major activity of NF-κB and its family members is to help proteolytic matrix metalloproteinase’s enzyme that promote tumor invasion. Hence, IKKa promotes metastasis in prostate cancer via inhibition of mammary serine protease inhibitor (maspin) [60, 61] and also stimulates angiogenesis, by activating IL-8 and vascular endothelial growth factor (VEGF) [58]. However, accumulation of the IκB-α protein through proteasome inhibition prevents the activation of anti-apoptotic NF-κB, resulting in tumor cell apoptosis [62].

The detail of these studies validated NF-κB as a potent and novel target for cancer therapy. They demonstrated that NF-κB signaling pathways played critical role in a wide variety of biological, physiological and pathological processes, mainly in promoting cell survival through induction of its target genes. Each study individually taken, stimulate the motivation and dedicated insight for developing natural compounds NF-κB inhibitors.

Many studies have been carried out on whether natural compounds-related cancer inhibits expression of NF-κB or not. All the selected natural compounds chemopreventive agents act as potent inhibitors of NF-κB pathways. Wedelolactone, an inhibitor of I-κB kinase, suppressed both TNF-α-induced I-κB phosphorylation and NF-κB phosphorylation at Ser 536 and Ser 468 [63], parthenolide [64-66], honokiol [67, 68]. Costunolide inhibited the activation of Akt and NF-κB and the expression of anti-apoptotic factors B-cell lymphoma-extra large (Bcl-xL) and X-linked inhibitor of apoptosis protein (XIAP) in 11Z
cells [69-71], magnolol inhibits ERK1/2 phosphorylation and NF-κB translocation [72, 73], PI3K/Akt/caspase and Fas-L/NF-κB signaling pathways might account for the responses of A375-S2 cell death induced by evodiamine [74, 75]. Oridonin [76], alantolactone [77, 78], isoalantolactone [79], casticin [80], pseudolaric acid B [81], jaceosidin [82], each of them has an inhibitory effect on NF-κB and its associated proteins. These compounds may inhibit one or more steps in NF-κB signaling pathway and its upstream growth factor receptors that activate the signaling cascade, translocation of NF-κB to the nucleus, DNA binding of the dimers, or interactions with the basal transcriptional machinery. Thereupon, they can induce apoptosis in cancer cells, offering a promising strategy for the treatment of different malignancies including cancer (Table 1 and Figure 2) [83].

### 6.3 Nuclear factor-related factor 2 (Nrf2) signaling pathway

In cancer chemoprevention, Nrf2 is a potential molecular target for natural compounds. Several selected natural compounds are reported as a potential candidate for chemoprevention, by stimulating the accumulation of Nrf2 in the nucleus and plays a major role in transcriptional activation of phase 2 detoxification enzymes. Low concentrations of parthenolide led to Nrf2-dependent HO-1 induction accompanied by the attenuation of its apoptogenic effect in Choi-CK and SCK cells. Furthermore, with the protein kinase C-alpha inhibitor Ro317549 (Ro), parthenolide -mediated apoptosis inhibits expression and nuclear translocation of Nrf2, resulting in blockage of HO-1 expression. Parthenolide also stimulated oxidation of KEAP1 in normal prostate epithelial cells, leading to increased Nrf2 (NFE2L2) levels and subsequent Nrf2-dependent expression of antioxidant enzymes [84, 85]. Costunolide and CH2-BL induced HO-1 expression and Nrf2 nuclear accumulation in RAW264.7 macrophages [86]. Oridonin activates Nrf2 signaling pathway, leading to accumulation of the Nrf2 protein and activation of the Nrf2-dependent cytoprotective response [87]. Isoalantolactone stimulates the accumulation of Nrf2 in the nucleus of both Hepa1c1c7 cells and its mutant BPRc1 cells [88]. Alantolactone also stimulated the nuclear accumulation of Nrf2 in HepG2-C8 cells [89].
6.4 Transducers and Activators of Transcription and its Family Members Pathway

Signal transducer and activator of transcription is a novel signal transduction pathway to the nucleus has been uncovered through the study of transcriptional activation in response to interferon. It has been implicated in many processes including development, differentiation, immune function, proliferation, survival, and epithelial to mesenchymal transition (EMT) [90, 91].

Activation of various tyrosine kinases leads to phosphorylation, dimerization, and nuclear localization of the signal transducers and activators of transcription (STAT) proteins, binding to specific DNA elements and direct transcription. Constitutive activation of STAT3 and STAT5 has been reported to be implicated in many cancers such as myeloma, lymphoma, leukemia, and several solid tumors [90-92]. Furthermore, seven mammalian STAT family members known such as STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6 have been cloned and share common structural elements.

During the last decade, the natural compounds have been implicated to modulate STAT activation in tumor cells. Some selected agents are part, such as honokiol increases expression and activity of SPH-1 that further deactivates STAT3 pathway [93], wedelolactone inhibits STAT1 dephosphorylation through specific inhibition of T-cell protein tyrosine phosphatase (TCPTP), which is important tyrosine phosphatase for STAT1 [94]. Parthenolide shows strong STAT-inhibition-mediated transcriptional suppression of pro-apoptotic genes [64-66], alantolactone inhibits STAT3 activation in HepG2 cells [49]. Therefore, these cumulative observations from both in vitro and/or in vivo studies have not only validated STAT as a novel target for cancer chemotherapy, and also hence provided the rationale for developing natural
compounds STAT inhibitors.

6.5 Growth Factors and Their Receptors Family Pathway

Growth factors are proteins that bind to receptors on the cell surface and reported to regulate a number of cellular processes, with the primary result of activating cellular proliferation and differentiation [95], apoptosis, and rearrangement of cytoskeleton [96]. Several growth factor signaling molecules are implicated in carcinogenesis. Among of them include endothelial growth factor, platelet-derived growth factor, fibroblast growth factor, transforming growth factor, insulin-like growth factor, and colony-stimulating factor [97].

As an important intracellular pathway consequence of growth factor receptor activation, several downstream signaling, such as PI3K-Akt and Ras-MAPK also become active. These signaling pathways have significant impacts on the fact that it is associated with poor prognosis, tumor progression and become targets for many natural chemopreventive and chemotherapeutic agents.

Isoalantolactone inhibits phosphorylation of PI3K/Akt on SGC-7901 cells [98], alantolactone appears to induce detoxifying enzymes via activation of PI3K and JNK signaling pathways [89]. In cervical carcinoma HeLa cell line, oridonin may suppress constitutively activated targets of phosphatidylinositol 3-kinase (Akt, FOXO, and GSK3) [99]. In pancreatic cancer, evodiamine augment the therapeutic effect of gemcitabine through direct or indirect negative regulation of the PI3K/Akt pathway [100] and also in A375-S2 cells [74].

Magnolol protects SH-SY5Y cells against acrolein-induced oxidative stress and prolongs SH-SY5Y cell survival through regulating JNK/mitochondria/caspase, PI3K/MEK/ERK, and PI3K/Akt/FoxO1 signaling pathways [101]. In addition, in SGC-7901 cells, magnolol induces
apoptosis through mitochondria and PI3K/Akt-dependent pathways [102]. Magnolol also suppressed the activation of MAPKs (ERK, JNK and p38) and the PI3K/AKT/mTOR signaling pathway in mES/EB-derived endothelial-like cells (23708970). Honokiol decreases the PI3K/mTOR pathway activity in tumor cells, but not in freshly stimulated T cells [103]. It seems to be mediated by interrupting the early activated intracellular signaling molecule PI3K/Akt, but not Src, the extracellular signal-regulated kinase, and p38 [104]. These reports showed that natural compounds mainly the selected one rapidly induce the phosphorylation of Akt after the stimulation and they can be used as a potent inhibitor against cancer cells.

6.6 Cripto-1 and its allied proteins signaling pathway

In the process of normal cellular function, the dysfunction of activin signaling constituted an active part of tumor formation. To address this phenomenon, activin is blocked in cancer cells by the complex formed by Cripto-1, activin, and activin receptor type II (ActRII). In human colon adenocarcinoma HCT-8 cells, alantolactone performs its anti-tumor effect by interrupting the interaction between Cripto-1 and the activin receptor type IIA in the activin signaling pathway [105].

7. Targeting Cancer Cells by Mitochondria-Mediated Apoptosis Pathway

Mitochondria dysfunction is the key links in the chain of development of pathologies associated with the violation of cellular energy metabolism, including cancer. Mitochondria have become an important component of the apoptosis execution machinery, cytochrome c, initiator in the mitochondrial apoptosis pathway, can be released from the inter-membrane of mitochondria after mitochondria depolarization [106-108].
Recently, many studies reported that the mitochondria play a fundamental role in the processes leading to cell death [109]. Identification of the loss of mitochondrial membrane potential through toxicity is the key piece of natural compounds’ process [110]. Several reports reveal that the effects of selected natural compounds on the intrinsic and extrinsic pathways of apoptosis have been examined in many cell lines, including HL-60, costunolide induces the ROS-mediated mitochondrial permeability transition and resultant cytochrome c release associated with increased expression of Bax, down-regulation of Bcl-2, survivin and significant activation of caspase-3, and its downstream target PARP [111, 112]. Honokiol induced release of cytochrome c into cytosol and a loss of mitochondrial membrane potential (ΔΨm), associated with inhibition of EGFR-STAT3 signaling and downregulation of STAT3 target genes and downregulation of Bcl-2 and upregulation of Bax expression in MDR KB and RASMCs cells [113, 114]. Magnolol induced apoptosis in MCF-7 and HCT-116 cells via the intrinsic pathway with release of AIF from mitochondria accompanied by downregulation of anti-apoptotic protein Bcl-2 and upregulation of pro-apoptotic protein p53 and Bax [115, 116]. To get a better insight into the mechanism of delaying cellular aging by mitochondria targeted natural compounds-induced cytotoxicity, the changes in membrane permeability, mitochondrial membrane potential (MMP) and cytochrome c localization, which influence mitochondrial biological mechanisms, development of mitochondria-addressed compounds highly specific for chemical processes is one of the most promising ways to develop approaches for chemotherapy.

8. Targeting Cancer Cells by Reactive Oxygen Species-Mediated Apoptosis Pathway

The human body constantly generates free radicals such as superoxide (\(\text{O}_2^-\)), hydrogen peroxide
(H₂O₂), nitric oxide, peroxynitrile, and hypochlorous acid and other reactive oxygen species (ROS) as a result of aerobic metabolism [117, 118]. Reactive Oxygen Species (ROS) are cellular signals generated ubiquitously by all mammalian cells and long-term exposure to physiological or psychological stress is associated with the production of oxidative species through intracellular damage to DNA, RNA, proteins, and lipids but their regulation induced cell proliferation, differentiation, and apoptosis, which are essential for proper cell functioning [119, 122]. ROS are well known mediators of intracellular signaling of cascades. During cellular redox, the excessive generation of ROS can induce oxidative stress, loss of cell functioning, and apoptosis [123].

Induction of apoptosis of cancer cells by n-hexane fraction of sesquiterpene is mediated through activation of proteases, which act on specific substrates leading to the degradation of PARP and other cytoskeletal proteins, responsible for many of the morphological and biochemical features of apoptosis in cancer cells [49, 50, 124-126]. Furthermore, once caspases activated, it might target the permeability of mitochondria, resulting in the loss of mitochondrial membrane potential concomitant with increased production of ROS, and this activity eventually causes disruption of membrane integrity [123]. In addition, several studies revealed that apoptosis induction in chemotherapy depends on many factors like increase in ROS, oxidation of cardiolipin, reduced mitochondrial membrane potential, and release of cytochrome c [124]. To restored cell viability, N-Acetyl Cysteine (NAC), a specific ROS inhibitor blocks completely apoptosis mediated by several natural compounds such as isoalantolactone in PANC-1 cells. The activation of p38 MAPK and Bax is directly dependent on ROS generation.

Cancer chemotherapy involves deregulation of cell proliferation and survival, inducing cell-cycle arrest, cell death and apoptosis by generating ROS and their various enzyme systems,
including the mitochondrial electron transport chain, cytochrome, lipoxygenase, cyclooxygenase, the NADPH oxidase complex, xanthine oxidase, and peroxisomes [127, 128].

Several studies reported that the promising natural compounds influenced the generation of Reactive Oxygen Species. In microglial cells, honokiol and magnolol-induced apoptosis associated with the inhibition of IFNγ ± LPS-induced iNOS expression, NO, and reactive oxygen species (ROS) production [129, 130]. Jaceosidin increased intracellular accumulation of reactive oxygen species (ROS) in MCF10A-ras cells [131]. In HeLa, CasKi, SiHa cell lines, casticin markedly increased the levels of intracellular reactive oxygen species [132, 133]. Parthenolide enhanced geldanamycin-induced changes in the apoptosis-related protein levels, reactive oxygen species formation, nuclear damage and cell death in human epithelial ovarian carcinoma OVCAR-3 and SK-OV-3 cell lines [134].

Induction of apoptosis in T24 and MDA-MB-231 cells by costunolide is associated with the generation of ROS and disruption of mitochondrial membrane potential (Δψm) [112]. In ovarian cancer cell lines (MPSC1 (PT), A2780 (PT), and SKOV3 (PT)), costunolide induced a significant increase in intracellular reactive oxygen species (ROS) [135]. The specific Reactive Oxygen Species inhibitor, N-Acetyl Cysteine (NAC) restored cell viability and completely blocked isoalantolactone-mediated apoptosis indicating that isoalantolactone induces ROSdependant apoptosis through intrinsic pathway in human pancreatic PANC-1 cells [124]. It also induced apoptosis in both androgen-sensitive (LNCaP) as well as androgen-independent (PC3 and DU-145) prostate cancer cells with the generation of Reactive Oxygen Species and dissipation of mitochondrial membrane potential (Δψm) [136]. Alantolactone-induced apoptosis accompanied by ROS generation and mitochondrial transmembrane potential dissipation [49, 137]. In hepatic
stellate, HeLa and U937 cells, oridonin induced biological processes, mainly intracellular ROS generation [138, 139]. Pseudolaric acid B induced reactive oxygen species (ROS) generation and mitochondrial dysfunction in L929 cells [140]. It also caused the elevation of ROS level in DU145 cells [141]. In human malignant melanoma A375-S2 and cervix carcinoma HeLa cells, evodiamine induced apoptotic process associated with ROS release through both extrinsic and intrinsic pathways [142, 143].

9. Targeting Cancer Cells by Cell Cycle-Mediated Apoptosis Pathway

Checkpoint controls function to ensure that chromosomes are intact and that critical stages of the cell cycle are completed before the following stage is initiated. One checkpoint operates during S and G2 to prevent the activation of mitosis-promoting factor (MPF), which is composed of a cyclin and cyclin-dependent kinase (Cdk) that triggers entrance of a cell into mitosis by inducing chromatin condensation and nuclear envelope breakdown; it’s also called maturation-promoting factor. Another checkpoint operates during early mitosis to prevent activation of Adenomatous Polyposis coli (APC) and the initiation of anaphase until the mitotic spindle apparatus is completely assembled and all chromosome kinetochores are properly attached to spindle fibers. Checkpoints that function in response to DNA damage prevent entry into S or M until the damage is repaired [144-146].

When these signals are altered due to various mutations that prevent cells from undergo apoptosis, normal cells are transformed into cancerous cells and undergo high proliferation. Therefore, to arrest cancerous cell proliferation, regulation of apoptosis and its signaling pathways play a critical role [8, 147, 148]. This behavior may lead to cell cycle arrest and upregulation of pro-apoptotic related proteins expression [49-51]. In addition, it also documented
that the selected natural compounds induced cell-cycle arrest either G2/M, or S or G0/G1 phase. We have reviewed the effects of various signaling pathways that have been reported in selected natural compounds-induced apoptosis (Figure 3 and Table 2).

10. Cancer clinical study

Antiangiogenic therapy is at the forefront of drug development. Knowledge of the multiple activities of natural compounds can assist with the development of natural compounds derivatives and the design of preclinical and clinical trials that will maximize the potential benefit of natural compounds in the patient setting for cancer disorders. Thereupon, the natural compounds have been examined in human and recently reported. Parthenolide was found to inhibit the expression of matrix metalloproteinase-9 and urinary plasminogen activator and the migration of carcinoma cells in vitro, as well as osteolytic bone metastasis associated with breast cancer in vivo [149]. Its administration at doses up to 4 mg as a daily oral capsule in the feverfew preparation is not detectable in the plasma [150]. In combination with ciclopirox, parthenolide demonstrates greater toxicity against acute myeloid leukemia than treatment with either compound alone [151].

11. Conclusion and Future Perspectives

Natural products have been, and continue to be, a highly useful source of bioactive molecules. In this review, we have highlighted the recent progress of the natural compounds from Mother Nature with cytotoxic activities. Plants provide a broad spectrum of sources for modern anticancer drugs. Various preclinical findings and results of several in vitro and in vivo studies convincingly argue for potent role of natural compounds in the prevention and treatment of many types of cancer. Many reports on mechanism of actions of the promising compounds target
multiple signaling pathways, which vary widely depending on cancer origin [11, 51].

According to the literature, the major molecular targets that have been characterized are the key challenge for researchers and scientists to use this information for effective cancer prevention in populations with different cancer risks. Moreover, low potency and poor bioavailability of natural compounds pose further challenges to scientists and researchers. The future, full with convergence of chemoprevention and chemotherapy drug development will open new avenues for natural compounds in reducing the public health impact of major cancers. However, additional preclinical studies and clinical trials are certainly yet required to elucidate the full spectrum of cytotoxic activities of the selected natural compounds either alone or in synergistic combination with other small molecules to further validate the usefulness of these agents as potent anticancer agents.
12. Acknowledgements

This study was supported by Ministry of Science and Technology (No. 2010DFA31430), Ministry of Education of China (NCET-10–0316), National Natural Science Foundation of China (No. 30871301, 30700827), Jilin Provincial Science & Technology Department (20130521010JH, YYZX201241), Changchun Science & Technology Department (No. 201114-11GH29), the Program for Introducing Talents to Universities (No. B07017) and the Fundamental Research Funds for the Central Universities (12SSXM005).
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Cancer Prev Res  Published OnlineFirst August 26, 2014.

Updated version
Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-14-0136

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