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Target-oriented Mechanisms of Novel Herbal Therapeutics in the Chemotherapy of Gastrointestinal Cancer and Inflammation

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Abstract: A prominent group of effective cancer chemopreventive drugs has been derived from natural products having low toxicity while possessing apparent benefit in the disease process. It is plausible that there are multiple target molecules critical to cancer cell survival. Herbal terpenoids have demonstrated excellent target-specific anti-neoplastic functions by suppression of cell proliferation and induction of apoptosis. Transcriptional molecules in the NF-κB, MEK/ERK and PI3K/Akt/mTOR pathways are important molecular targets of chemotherapy that play distinctive roles in modulating the apoptosis cascades. It is recently suggested that NSAID-activated gene (NAG-1), a novel proapoptotic protein, is the upstream anti-carcinogenic target of NSAIDs, PPAR ligands and herbal chemotherapeutic agents that triggers some of the events mentioned above. Besides, angiogenesis, oxidative stress as well as inflammation are important factors that contribute to the development and metastasis of cancer, which could be actively modulated by novel agents of plant origin. The aim of the present review is to discuss and summarize the contemporary use of herbal therapeutics and phytochemicals in the treatment of human cancers, in particular that of the colon. The major events and signaling pathways in the carcinogenesis process being potentially modulated by natural products and novel herbal compounds will be evaluated, with emphasis on some terpenoids. Advances in eliciting the precise cellular and molecular mechanisms during the anti-tumorigenic process of novel herbal therapeutics will be of imperative clinical significance to increase the efficacy and reduce prominent adverse drug effects in cancer patients through target-specific therapy.

Keywords: Herbal therapeutics, phytochemicals, chemotherapy, anti-inflammation, colon cancer, molecular targets.

INTRODUCTION

Cancer remains a major destructive disease worldwide, regardless of the extensive research and considerable effort, in which colorectal cancer is the third most common cancer in men and the second in women [1]. According to the fact sheet of WHO, it has been projected that cancer deaths will rise to over 11 million in 2030. It has been known that about one-third of all cancer-related deaths in the United States could be avoided [2,3]. In 1975, Wattenberg introduced the idea of cancer chemoprevention by plant constituents in animal models [4]. By applying current knowledge, much could be achieved through chemoprevention. Therefore, it is urgently needed to develop more effective therapies for this disease. During early 20thcentury, the development of cancer drug treatment at early stage was hindered by the limitation of appropriate models for testing the potential anti-cancer activity in humans and also the inadequate access to clinical facilities [5]. In 1910, a major breakthrough was made by George Clowes of the Roswell Park Memorial Institute in developing the first tumor xenograft system in rodents [6]. It is believed that the main focus of chemoprevention will be to employ phytochemicals that target the multiple molecular events involved in colorectal carcinogenesis.

Research studies in recent years have proven that herbal extracts and phytochemicals possess anti-tumor activities [7-10]. The underlying mechanisms of action have been intensively studied to evaluate the efficacy and safety of these novel compounds. Clinical trials sponsored by the U.S. National Institute of Health on the use of herbal medicine have been actively conducted, demonstrating a new hope in contemporary cancer chemotherapy. Under experimental conditions, a lot of herbal compounds could delay cancer growth and metastasis [11]. Novel chemotherapeutic cancer agents derived from active phytochemicals could thus be further developed as adjuvants to improve the anti-cancer rates achievable with standard treatments [12]. Among the anticancer drugs developed between 1981 and 2002, those belonging to or derived from natural compounds comprised around 24-28% [13]. Current clinically used phytochemicals can be categorized into four main classes of compounds: vinca alkaloids, epipodophyllotoxins, taxanes and camptothecins [14]. A vast number of other phytochemicals are also under different stages of clinical trials, including curcumin, epigallocatechin and soy isoflavones. Recent studies have shown that herbal terpenoids possess anti-tumor activities and suggest that these components constitute a new class of cancer chemopreventive agents [15]. We have demonstrated in our ongoing investigations that herbal terpenoids could induce cytotoxicity, growth inhibition and apoptosis in human colon cancer cells that are associated with prominent cell cycle arrest and inhibition of cell proliferation [11,16,17].

1. PHYTOCHEMICALS AND DIETARY PRODUCTS IN CANCER CHEMOTHERAPY

Consumption of natural products and vegetables is associated with reduced risk of a series of dreadful diseases, including cardiovascular disorders, diabetes and cancer. More than 250 population-based studies, including case-control and cohort studies, indicate that people who eat above five servings of fruits and vegetables a day have approximately half of the risk of developing cancer, especially for cancers of the digestive and respiratory systems. The National Cancer Institute has identified about 35 plant-based foods that possess cancer-preventive properties, including garlic, soybeans, ginger, onion, turmeric, tomatoes and cruciferous vegetables [18]. Previous epidemiologic studies have also shown that dietary compounds from plant source could play a crucial role in protecting against cancers of the upper alimentary tract and the large bowel [19,20]. Phytochemicals are chemical compounds that exist naturally in plants (“*phyto*” means “plant” in Greek). Although, these substances may have biological significance, they are generally not classified as essential nutrients [21]. It has been proposed that the strong antioxidant and anti-proliferative activities of many fruit and vegetable extracts should be contributed by a combination of phytochemicals inside, leading to potential anti-inflammatory and anti-cancer applications [22,23]. In light of the safety concern on potent chemotherapeutic drugs, innocuous phytochemicals derived from

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the diet are considered potential alternatives in chemoprevention [24]. Knowing the fact that phytochemicals of medicinal value are natural, comparatively safe, and of lower cost, researchers throughout the world have begun to focus on this area of research. To date, a member of cancer patients are looking for cure by using alternative and complementary medicine. The key question is whether a purified phytochemical has the same health benefits as the same constituent when being present in the whole food or a mixture of dietary components. Epidemiological studies have suggested that herbal medicines or fruit extracts play a major role in the prevention and treatment of many types of cancer, including that of the colon [25-27]. Despite these facts, the second WCRF-AICR report published in 2007 found that the evidence of those vegetables and fruits which protect against cancer is less impressive [28]. This lack of convincing evidence is partly because some important contributing factors were not considered in many of the original studies. In some occasions, the levels of phytochemicals being consumed may be below an efficacious threshold and thus provoke no chemopreventive effect. In general, appropriate concentrations of dietary phytochemicals and mechanistic studies are essential to generate conclusive results that are physiologically relevant [29]. Hence, it is imperative to unveil the exact physiological (or nutritional) dose of the phytochemicals or herbal extracts that can be used to improve and maintain optimal health. Other than the need to precisely define working concentrations, clinical trials on dietary substances and phytochemicals mainly focus on single molecules or single classes of compounds that resulted in no or limited effects [30]. In a lot of cases, data from epidemiological studies based on these treatments may not be extrapolated to actual human intervention due to the limitation described above. Since the development of many cancers, in particular colorectal cancer, involves multiple signaling pathways and numerous molecular targets, the use of a single phytochemical molecule may not be good enough to tackle with the complex situation. Nonetheless, it has been proven that mixture of bioactive herbal compounds or phytochemicals, possibly with the synergistic effects of its constituents, can effectively prevent DNA mutation, modulate inflammation and the immune responses and to prevent tumor cell proliferation [31].

Studies have demonstrated that phytochemicals carried in common fruits and vegetables have complementary and overlapping modes of action, such as free radical scavenging and antioxidant activity, gene regulation in cell proliferationand apoptosis, etc. Nowadays, increasing evidence has indicated that a lot of phytochemicals can be used to treat cancers effectively either by reducing the risk of tumor formation or in some cases to be used as an adjuvant in combination with other orthodox chemotherapeutic drugs. Paclitaxel, a mitotic inhibitor being isolated from the bark of Pacific Yew tree, is one of the most successful chemotherapeutic drugs that had been originated from the screening program of natural and synthetic compounds [32]. Some herbal products have possessed distinctive antitumor activity against human colon cancers. A summary of the selected dietary plant extracts or bioactive compounds being found to be effective in the treatment of cancers is listed in Table **1**. Medicinal herbal products that have exhibited anticancer potential by targeting various molecular and cellular mechanisms are summarized in Table **2**. In addition to the use of single herbal compounds, a combination of these agents may provide synergistic or additive effects against tumor formation. This could facilitate a higher chance of complete cancer remission and alleviation of the systemic side effects of the treatment, while a lower dose of other orthodox chemotherapeutic drugs being currently administered could be allowed.

1.1. Herbal Terpenoids

Terpenoids, sometimes referred to as isoprenoids, belongs to a large and diverse class of naturally occurring organic chemicals similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousands of way. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic and other pharmaceutical effects. Well-known terpenoids include citral, menthol, camphor and the cannabinoids found in the Cannabis plant. There are two metabolic pathways in the formation of terpenoids. The first one is the mevalonic acid pathway in which the terpenoids are synthesized through the HMG-CoA reductase mechanism. This reaction takes place in the cytosol. The second pathway is amevalonic acid-independent pathway that takes place in the plastid of plants. The cancer-inhibitory action by a variety of herbal nutrients and non-nutritive plant-derived constituents (phytochemicals) was confirmed in different animal tumor models [66,67], which leads to an increased emphasis on cancer prevention strategies in which these dietary factors are utilized. A number of dietary monoterpenes have anti-tumor activity. For example, *d*-limonene, which comprises >90% of orange peel oil, has chemopreventive activity against rodent mammary, skin, liver, lung and for stomach cancers. Their cancer chemotherapeutic activities are under evaluation in Phase I clinical trials [68].

Geranylgeraniol (GGOH) is the common precursor of all natural diterpenoids. Its chemical name is 3,7,11,15-tetramethyl-2,6,10,14-hexadecatraen-1-ol. It is well documented that GGOH is biosynthesized via the terpenoid pathway and its immediate precursor is geranylgeranyldiphosphate (GGPP). Source of GGOH include a Chinese *material medica*, *Munronia delavayi Franch* and byproducts of *Bixa orellana* seeds. *In vitro* studies utilizing diverse cancer cell lines have demonstrated the pronounced effects of GGOH on the induction of apoptosis. GGOH has been shown principally to be a potent inducer of apoptosis in leukemia [69], lung cancer [70] and hepatoma cells [71]. It was suggested that apoptosis induction by GGOH could be relevant for its chemopreventive activity during hepatocarcinogenesis. Apart from the anticarcinogenic actions, GGOH also has action on the function of osteoclast [72]. It was found that GGOH could prevent the inhibitory effect of alendronate by actingon a rate-limiting step in the cholesterol biosynthesis pathway, which is essential for osteoclast function.

Like GGOH, farnesol is a naturally occurring phytochemical which widely exists in flowers, leaves and stems of plants. Many herbs being used in traditional Chinese Medicine and essential oils are abundant in farnesol, including *Cinnamomum tenuipilum* Kosterm, flowers of *Magnolia liliflora* Desr., *Lonicera japonica*, leaves of *Eriobotrya japonica*, *Katsumade galangal* seed, *Abutilon indicum*, *Momordica grosvenori* Swingle, *Cymbopogon* Citratus, Citrus aurantium, *Polianthes tuberosa*, *Myroxylon toluifera*, cyclamen, rose, muskand tolu. However, as farnesol is now broadly applied in various fields, such as pharmaceuticals, agrochemicals, cosmetics, and domestic chemicals, it is synthesized chemically through the mevlonate pathway by enzymatic dephosphorylation of farnesyl disphosphate (FPP). FPP is the last common intermediate for the synthesis of the sterol and non-sterol products, including cholesterol, ubiquinone, heme A, dolichol and prenylated proteins [73]. Protein prenylation is a post-modification of proteins such as *ras*-related small GTP-binding proteins, heterotrimeric G proteins and regulatory proteins nuclear laminins A and B [74], in which many of them are involved in cell proliferation and transformation [75]. Oncogenes like *ras-p21* that has been implicated in the growth of colon tumor cells [76,77] requires this prenylation process to acquire full oncogenic potency [77]. Therefore, alteration of the farnesylation process by farnesol may modulate the formation of metabolites in mevalonate pathway and thus affect the carcinogensis process. Farnesol can reduce the HMG-CoA reductase enzyme activity and also enhance its degradation [78,79]. As a consequence, it leads to the inhibition of intermediate metabolites such as mevalonate that is required for cell cycle progression from mid-G1 phase into late G1 phase. An inhibition of its synthesis slows the progress of tumor cells through the cell cycle with a resultant build up of cells in the G1 phase, leading to suppression of the unregu-

Table 1. Summary of Selected Medicinal Plant Extracts or Natural Compounds in the Treatment of Cancer

Medicinal Plant Extracts or Bioactive Compounds	Sources	Beneficial Anti-Cancer Effects	References
Astragalus Saponins	*Astragalus membranaceus*	• Induces growth inhibition and apoptosis in human liver and colon cancer cell lines and exerts anti-carcinogenic and pro-apoptotic properties in tumor xenograft • Modulates cell invasiveness and angiogenesis in human gastric adenocarcinoma cells	[16,33-36]
Curcumin	*Curcuma Longa*	• Induces growth inhibition and down-regulation of NF-κB in colon cancer cells and xenograft tumors • Causes superoxide anion production and p53-independent apoptosis, and attenuates the Wnt/beta-catenin pathway in human colon cancer cells. • Produces a synergistic preventive effect against colon cancer when combined catechins	[37-40]
Epigallocatechin-3-gallate	Green Tea	• Inhibits cell proliferation, colony formation and the expression of HSP70 and HSP90 in MCF-7 human breast cancer cells. • Suppresses tumor formation and reduce bFGF expression in $APC^{Min/+}$ mice • Induces apoptosis via strong activation of AMP-activated protein kinase	[41-43]
Apple polyphenol	Apple	• Protects intestinal cells from ROS-induced DNA damage in Caco-2 cells • Potentiates the anticancer actions of paclitaxel and reduce HepG2-xenografted tumor volume • Exerts potent demethylating activity and reactivate silenced tumor suppressor genes in colorectal cancer cells	[44-46]

lated cell proliferation [80-82]. More importantly, it results in impairment of the protein prenylation [83] and modulation of molecules such as cholesterol that are required for the tumor cells growth [84,85]. Experimental studies have found that farnesol possess chemopreventive activity in a pancreatic tumor model [82] and significantly suppress colonic ACF formation and crypt multiplicity against colon carcinogensis [86]. Furthermore, farnesol was reported to inhibit the synthesis of phosphatidylcholine (PC), the principal membrane phospholipid in eukaryotic cell. Majority of PC are synthesized through the CDP-choline pathway [87]. Since PC and CDP-choline pathways are important components in controlling cell proliferation and cell death [88-90], some studies proposed that farnesol directly competes with diacylglycerol for binding to the choline phosphotransferase active site and results in inhibition of PC synthesis which leads to apoptosis in HL-60 cells [91] and A549 human lung adenocarcinoma cells [92]. Another study found that farnesol induces growth inhibition and apoptosis of acute leukemia cells (CEM-C1) by decreasing the activity of CDP-choline [81]. These, might all account for the antitumorigenic activities of farnesol. Apart from the chemopreventive action of farnesol, the key role of farnesol in the mevalonate pathway makes it an important role in regulating cholesterol levels, which can also be applied in the cardiovascular fields [93]. Farnesol is a potent inhibitor of vasoconstriction to vascular smooth muscle cells [94]. Exogenous farnesol blocks plasma membrane calcium channels and thus impairs calcium signaling within vascular smooth muscle cells [95].

1.2. TCM Treatment of Cancer

A large amount of data from clinical and experimental studies has provided evidence that consumption of certain medicinal herbal products used in Traditional Chinese Medicine (TCM) can exert chemopreventive and chemotherapeutic effects in human cancers and inflammatory diseases. The underlying principle is to bring the patient back to a healthy state by modifying multiple cancer-causing events [96]. Such "homeostatic" regulation of the body could be able to modulate multiple genes and signaling pathways, which could be superior to agents that merely target a single molecular target. Due to this reason, it is crucial to assess the molecular basis of their chemopreventive actions. A summary of example TCMs with potential cancer preventive properties has been described [97].

1.2.1. Radix Astragali

It is stated in the Chinese Pharmacopoeia that Radix Astragali is the dried root of two plants from the Fabaceae family: *Astragalus membranaceus* (Fisch.) Bunge and *Astragalus membranaceus* Bunge var. *mongholicus* (Dge.) Hsiao, which are produced in Shanxi, Inner Mongolia, Jilin and Hebei provinces. From the perspective of Chinese Medicine, Radix Astragali is a "*qi*"-invigorating drug. It can reinforce "*qi*" and strengthen the superficial resistance, promote the discharge of pus and the growth of new tissue. Traditionally, Radix Astragali is used to treat "*qi*" deficiency syndrome in patients with abscesses that are difficult to burst or heal with anemia and diabetes. Among the different applications, the immunomodulatory and cardioprotective effects of Radix Astragali are most widely studied. It can strengthen non-specific immunity [98] while suppressing hypersensitivity [99], enhance the production of IgM antibody [100] and relieve allergy and asthma [101]. Together with the ability to induce apoptosis [11,16,102], Radix Astragali has also been proven to be effective in treating cancers. Kurashige and colleagues found that its extract significantly lowered the incidence of urinary bladder carcinoma in mice [102]. In the studies concerning its effects on the cardiovascular system, Radix Astragali protected the cardiac muscle from viral myocarditis by inhibiting viral replication, activating interferon system and scavenging free radicals [103,104], and reducing blood pressure [105]. It also has action on the urogenital system by inducing diuresis and improves renal function [106,107]. Animal study even showed its protective effect against Japanese encephalitis [108].

The main constituents of Radix Astragali include flavonoids [109,110], cycloartane triterpene saponins [110,111] and polysaccharides [107]. Other constituents include amino acids, phytosterols

Table 2. Medicinal Herbal Products that Possess Anti-Carcinogenic Properties in Colon Cancer

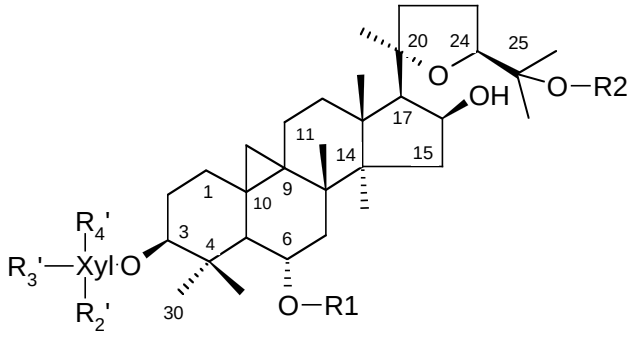
Mode of Action	Functional Part	Original	Cell Lines	References
Cytotoxic	Coptisine	*Chelidonium majus*	HT-29	[47]
Apoptotic	Hesperidin	*Citrus spp.*	SNU-C4	[48]
Anti-proliferation	Water extract	*Coptis chinensis*	HCT 116	[49]
Apoptotic	Berberine	*Coptis chinensis*	SW620	[50]
Anti-inflammatory, apoptotic	Ethanol extract	*Ganoderma lucidum*	HT-29	[51]
Apoptotic	Sulfur-compound	Garlic (*Allium sativum*)	SW620, HCT 116	[52]
Anti-inflammatory	Berberine	Genera *Berberis* and *Coptis*	DLD-1	[53]
Anti-proliferation	Isoliquiritigenin	*Glycyrrhiza uralensis*	RCN-9, Colon 26, COLO320DM	[54]
Apoptotic	Isoliquiritigenin	*Glycyrrhiza uralensis*	Colon 26, COLO320DM	[54]
Anti-proliferative, apoptosis	Polyphenols	Green tea (*Camellia sinensis*)	Human colon cancer tissues, HT-29	[55,56]
Anti-proliferative	Anthraquinones	*Hemerocallis fulva*	HCT 116	[57]
Anti-proliferative, apoptosis	Magnolol	*Magnolia officinalis*	COLO205	[58]
Apoptotic	Thymoquinone	*Nigella sativa*	HCT 116	[59]
Anti-proliferative, apoptotic	Chios mastic gum	*Pistacia lentiscus* L. var. *chia*	HCT 116	[60]
Anti-proliferative, apoptotic	Oridonin	*Rabdosia rubescens*	HT-29	[61]
Apoptotic	Diosgenin	Seeds of *Trigonella foenum graecum* Linn, *Dioscorea spp.*	HCT 116	[62]
Anti-proliferative	Silibinin	*Silybum marianum*	HCT 116	[63]
Anti-proliferative, apoptosis	Genistein	Soy (*Glycine max*)	HT-29	[64]
Anti-proliferative, anti-inflammatory	Saponin	Soy (*Glycine max*)	HT29	[65]

and trace elements [112]. Astragalus polysaccharides have received a great deal of attention, especially the polysaccharide fraction F3. It has been shown that they possess strong immunomodulatory property, of which polysaccharides A, B and C have been identified as glucans and polysaccharide D – i.e. the heteropolysaccharides [113]. Astragalus root also contains a series of cycloartane triterpene glycosides, including astragalosides I-VIII, acetylastragaloside I, isoastragaloside I and III, astramembrannin II, cycloastragenol, cyclosieversigenis, soyasaponin I, soyasapogenol B, and lupeol. Among these, there is an abundant amount of astragaloside IV, which is commonly used as a qualitative marker for Radix Astragali [114]. In addition, more than ten types of flavonoids have been identified, including formononentin, calycosin, quercetin, kaempferol, kumatakenin and several isoflavone glucosides [115]. Radix Astragali could enhance the body to increase its production of immunoglobulin, to stimulate macrophages and activate T-cells and natural killer cells [116,117]. A clinical trial involving thirty colitis patients had revealed that a herbal combination containing *Astragalus membranaceus* could regulate TNF-α and interleukin IL-8 levels in the subjects [118]. We have also demonstrated the immunomodulating effects of Radix Astragali on cytokines in a colitis animal model [119]. Alternatively, total Astragalus saponins can inhibit cell proliferation by S and G_2/M phase arrest [16]. The cycloartane- and oleanan-type triterpene saponins possess immunomodulatory, anticancer [120], anti-inflammatory [121], cardioprotective [104], antihypertensive [104], gastroprotective [122], anti-oxidative [107,123], analgesic [124], anti-diabetic and nephroprotective effects [125,126]. In our ongoing studies, we have shown that Astragalus saponins (AST; Fig. **1**) extracted from *Astragalus membranaceus* can be used as an adjuvant in combination with other orthodox chemotherapeutic drugs to reduce the side effects of the latter compounds [16]. We also demonstrate that AST could exert anti-carcinogenic effects against colon, liver and gastric cancers through different mechanisms [33,35,36]. Besides, AST also possess anti-angiogenic and anti-invasive properties in colon (unpublished data) and gastric cancer cells [36].

1.2.2. Tripterygium Wilfordii *Hook F. and Triptolide*

Tripterygium wilfordii Hook f. (TWHF) is a TCM plant, which was first recorded in "*Sheng nong ben cao jing*", a Chinese medical herb dictionary thousand years ago. It is a perennial member of the Celastraceae plant family, and the plant has been used for centuries for treating painful obstruction syndrome in a wide range of diseases that comprised of inflammation in China [127]. TWHF has been used in curing autoimmune diseases like systemic lupus erythematosus, rheumatoid arthritis and glomerulonephritis for the recent decades. The extract of TWHF was identified to contain a large numbers of compounds that include diterpenoids, such as triptolide and tripdiolide, alkaloids glycosides, beta-sitosterol and tritoquinones [128,129]. Among these, triptolide remains the chief active component. According to TCM theory, TWHF is characterized to be very toxic. Traditionally, TWHF is usually used to treat

(A)



(B)

	R1	**R2**	**R_2'**	**R_3'**	**R_4'**
Acetyl-astragaloside I	Glc	H	Ac	Ac	**Ac**
Astragaloside I	Glc	H	Ac	Ac	**H**
Isoastragaloside I	Glc	H	Ac	H	**Ac**
Astragaloside II	Glc	H	Ac	H	**H**
Isoastragaloside II	Glc	H	H	Ac	**H**
Astragaloside III	H	H	Glc	H	**H**
Astragaloside IV	Glc	H	H	H	**H**
Isoastragaloside IV	H	Glc	H	H	**H**
Astragaloside V	H	Glc	Glc	H	**H**
Astragaloside VI	Glc	H	Glc	H	**H**
Astragaloside VII	**Glc**	**Glc**	**H**	**H**	**H**

Fig. (1). Chemical structure of Astragalus saponins. (**A**) The general chemical structure of triterpene saponins with aglycone of cycloastragenol (9,19-cyclo lanostane type). (**B**) Table showing the substituents R_1-R'_4 in different triterpene saponins with aglycone of cycloastragenol.

painful obstruction syndrome. TWHF, in addition to its improvement of blood circulation, can relieve pain and reduce swelling in patients who have swollen joints and difficulty moving, and be used in detoxification and anti-parasitic action. Toxicity of TWHF includes local irritation of the gastrointestinal tract, damage to the central nervous system, internal bleeding and necrosis of the organs. In severe cases, overdose of the herb may cause bleeding in the stomach, intestines, liver and lungs. Other symptoms include dizziness, dry mouth, palpitations, necrosis of mucous membranes and irregular menstruation. Hematological disorders have also been noted following chronic use of the herb, with decreased white blood cell and platelet counts due to bone marrow suppression [130]. Because of the toxicity of TWHF, the daily dose should be kept between 5 and 12 grams. In addition, TWHF should be boiled for at least 60 minutes before the addition of other herbs in tonic. It should be used with caution in geriatric, pediatric patients and those with heart, stomach and spleen disorders, and is contraindicated for pregnant women [131].

Alternatively, triptolide (TPL), the active ingredient of TWHF has been reported for its ability to induce apoptosis in different cells [132], and it was reported to inhibit cytokine-mediated activation of NF-κB in immune cells. Recently, TPL has been examined as a novel potential drug to cancer treatment. It was proven to be potent in anti-proliferation and apoptosis induction in certain kinds of cancer cell lines, such as lung cancer, both *in vivo* and *in vitro* [133-135]. Studies showed that triptolide executes anti-neoplastic effect [136-139], and a functional p53 is required for the proapoptotic effect [140]. Other researches did not solely focus on TPL itself, but instead examined its synergistic effect on cancers when used with other drugs. TPL was reported to enhance drugs-mediated apoptosis such as doxorubicin, which means that TPL may have the potential to act in synergy with other drugs to induce tumor cell apoptosis in chemotherapy [141,142]. However, the mechanisms of these therapeutic effects have not completely delineated.

1.2.3. Vinca Alkaloids

Vinca alkaloids belong to a class of drugs that are derived from the plant *Catharanthus roseus* G. Don., under the family of Apocynaceae. More than 100 alkaloids have been isolated from this plant, including vincathicine, vinepidine, vinrosidine and vinleurosine. Among these, natural compounds vinblastine, vincristine, semi-synthetic derivatives vindesine and vinorelbine have been registered to be used in anticancer therapy [143]. New generation of vinca alkaloids is under investigation and the synthesized vinflunine has been regarded as a promising anticancer agent, which is currently in

Phase III trial assessment [144].Vinca alkaloids can cause cell cycle arrest by termination of the mitotic cell division. The most widely studied area is the interaction of vinca alkaloids with tubulin and microtubules [145]. Microtubules are cellular structures that aid the movement of chromosomes during mitosis and tubulins are the proteins that make up microtubules. Binding of vinca alkaloids to tubulin can prevent tubulin polymerization and the formation of the mitotic spindle leading to cytotoxicity [146]. Antitumor effects of Vinca alkaloids are believed to depend on interference with the normal function of microtubules and blockage of cell cycle progression in the G_2-M phase [147]. Furthermore, the cytotoxic and cytostatic effects of these drugs may be related to their ability to inhibit RNA, DNA, protein, and lipid biosynthesis [148]. As vinca alkaloids are regarded as mitotic inhibitors and anti-microtubule agents, they have been widely used in therapies of solid tumors and hematological malignancies. They are regarded as novel anti-angiogenic compounds in recent years [145,149]. Vinca alkaloids are under active clinical trials as anti-angiogenic drugs [150].

The alkaloid vinblastine (VBT) can be found in all parts of *Catharanthus roseus* G. Don. The clinically used drug is the sulphate salt of this alkaloid. The drug is a widely used chemotherpeutic agent in the treatment of breast, testicular, lung and bladder cancers. It is also used to treat patients with lymphomas, leukemias, breast carcinoma, Wilms tumor, Kaposi's sarcoma, Ewing's sarcomaand small cell lung cancer. Furthermore, it is also used in Hodgkin's disease and melanoma. It can be used alone or in combination with other drugs or radiotherapy [152]. Side effects of the drug include anemia, nausea, vomiting, alopecia, constipation and numbness in hands or feet and the mechanism of toxicity are most probably due to cell damage caused by mitotic cell cycle arrest [153]. As a chemotherapeutic drug, vinblastine can bind to tubulin that makes up the cytoskeleton microtubules. Tubulin polymerization is inhibited and mitotic spindle cannot be formed, therefore mitotic cell division is stopped at the G_2-M phase. This leads to cytotoxicity [146]. In recent years, several studies have demonstrated the anti-angiogenic ability of vinblastine. Ribatti *et al*. showed that vinblastine could inhibit angiogenesis by releasing adrenomedullin, a hormone that can stimulate tumor cell growth and angiogenesis, in endothelial morphogenesis *in vitro* and angiogenesis in the chick embryo chorioallantoic membrane assay *in vivo* [154]. Vinblastine at very low dose reversibly impairs certain functions of the endothelial cells and angiogenesis without nonspecific cytotoxic or necrotic damage, which could also produce apparent anti-angiogenic effects by direct cytotoxicity [155].

Similar to vinblastine, vincristine (VCT) can also be found in all parts of *Catharanthus roseus* G. Don. The drug is commonly used in acute lymphocytic leukemia, Hodgkin disease, non-Hodgkin lymphomas, rhabdomyosarcoma, and neuroblastoma. It is used in combination with other drugs due to its limited efficacy. Toxicity of VCT is neurologic and dose-limiting, including loss of deep tendon reflexes, severe pain, muscle weakness, foot or wrist drop, and in rare occasions, paralysis. Abdominal pain and constipation may also manifest [156]. VCT is an anti-mitotic drug, which inhibits microtubule formation in the mitotic spindle and results in the mitotic arrest in metaphase. It may also depolymerize microtubules and interfere with amino acid, cyclic AMP and glutathione metabolisms. Calmodulin-dependent Ca^{2+}-transport ATPase activity, cellular respiration, as well as nucleic acid and lipid biosynthesis may also be affected. Some studies also showed that it can decelerate the onset of apoptosis in association with a delay in poly(ADP)ribose [156]. *In vitro* studies showed that the drug has anti-angiogenic activity at non-cytotoxic concentrations in HUVEC cells cultured on Matrigel gel.

1.2.4. Zingiberofficinale (Ginger)

Ginger, the rhizome of *Zingiber officinale*, is a traditional medicine widely used in ginger family (Zingiberaceae). It is usually used for conditions such as nausea, vomiting, cold, etc. Ginger is listed in modern pharmacopoeias and repertories, and has a wide range of confirmed pharmacological properties including hypoglycaemic/antiglycaemic [157], anti-platelet [158] and cardiovascular [159] effects. Recent research suggests that it is promoted for treating headache [160], certain cardiovascular diseases, bronchitis and arthritis [161]. Ginger is commonly used as a seasoning and flavoring material in food, beverage and cosmetics [162].

Ginger contained a number of constituents and active ingredients. Powder ginger will produce ginger oil, which contained a high proportion of sequiterpene hydrocarbonss, predominantly zingiberene, during steam distillation. From the studies of lipophilic rhizome extracts, the major pungent compound in ginger is active gingerols that can be converted to shogols, zingerone and paradol. The compound 6-gingerol appears to be responsible for its characteristic taste. The aromatic, spasmolytic, carminative and absorbent properties of ginger suggest that it has direct effects on the gastrointestinal tracts. In TCM, ginger is characterized as "*warm*" and "*acrid*". It had a long history of medicinal used for resolving vomiting, promoting sweating, and preventing cough. It is an important component in TCM formulae such as *Xiao-Ban-Xia-Tang*, *Gei-Zhi-Tang*, *Er-Chang Tang*, etc.

There has been evidence for the anti-platelet activity of ginger. It was reported that 6-gingerol and 8-gingerol inhibit the COX-1/thromboxane synthase activity. Most recent studies reported that 6-gingerol and 8-gingerol are able to inhibit human platelet aggregation and shown to produce strong COX-1 inhibition without causing gastric ulceration [163]. Ginger was also acclaimed for its high anti-oxidant activity [164]. The extract of zingiber significantly inhibits and scavenges oxygen free radicals like O_2^-, OH and lipid peroxide [165]. Lin *et al* have shown that ginger increased glutathione as well as reduced lipid peroxide level in rats' blood and suggested that ginger could inhibit and scavenge free radicals to different degrees [166]. Ginger has also been reported to have anti-ulcer effects. Zingiberene, the terpenoids, and 6-gingerol are important constituents in contemporary medications against gastric lesion formation [167]. According to al-Yahya, ginger extract exerts highly significant cytoprotection against ethanol, hydrochloric acid and sodium hydroxide induced gastric ulceration. It was suggested that the extract could also prevent the occurrence of gastric ulcers induced by NSAID and hypothermic restraint stress [168]. Pharmacological studies indicate that ginger has the potential in enhancing gastric acid and pepsin secretion, together with increased binding mucus content and inhibition the gastric emptying. This suggests that ginger could act both deleteriously and beneficially in the gastric mucosa. On the beneficial side, ginger was considered to be mucosal protective [169].

2. HERBAL THERAPEUTICS TARGETING ON PRECISE MOLECULAR TARGETS IN CANCER CELLS

Effective new anticancer drugs are required to act on specific molecular targets. However, sometimes herbal therapeutics may exert various anti-carcinogenic actions with unknown mechanistic pathways, which remains a big hurdle in advanced clinical development. In order to more explicitly confirm the promising results from animal and cell culture studies in clinical trials on cancer chemoprevention, identification of validated biomarkers as surrogate endpoints of efficacy is critical, which are somewhat related to the modulation of key cancer signaling pathways during the multiple mechanisms of herbal drug action [24]. Chemopreventive activity is often the result of a combination of several distinct intracellular events, as opposed to a single biological response. Important mechanisms of chemoprevention can be subdivided into antioxidant activity, anti-inflammatory action and induction of apoptosis. These mechanisms of action may provide a useful basis for screening novel phytochemicals or herbal compounds for their chemopreventive potentials. A brief account of the classical modes of action of

phytochemicals in their anticancer activities has been illustrated in (Fig. **2**).

2.1. Apoptosis

Apoptosis or programmed cell death occurs under various physiological and pathological situations and it plays a central role in physiological growth control and regulation of tissue homeostasis. Apoptosis is characterized by typical morphological and biological hallmarks including cell shrinkage, condensation of chromatin, membrane bledding and nuclear DNA fragmentation [170]. The formation of tumor could be due to the imbalance between cell death and proliferation in favor of cell survival. Defects in apoptosis signaling pathway are now thought to be involved in drug resistance of tumor cells and contribute to a number of human diseases, ranging from neurodegenerative disorders to malignancy [171]. The use of cytotoxic drugs in anticancer treatment may act via activating some key elements of the apoptotic pathways. At the molecular level, a number of genes, molecules or signals are often changed in colon cancer and some of these are key regulators of apoptosis, such as p53 mutant, IAP, and COX-2. Direct targeting of the apoptotic pathway offers a novel strategy for treating cancers. Therefore, promoting the expression of pro-apoptotic proteins, like caspases-3 and PARP, and downregulating the expression of anti-apoptotic proteins such as survivin and Bcl-x_L could provide a novel strategy in treating cancer. The two pathways that mediate drug-induced apoptosis are the death receptor (extrinsic) and mitochondria-dependent (intrinsic) cascades [172].

2.1.1. Intrinsic Apoptotic Pathway

The intrinsic (Bcl-2 inhibitory or mitochondrial) pathway of apoptosis functions in response to various types of intracellular stress such as growth factor withdrawal, DNA damage, unfolding stresses in the endoplasmic reticulum and death receptor stimulation. It is mediated by Bax/Bak and the release of cytochrome *c* [173,174]. Following the reception of stress signals, pro-apoptotic Bcl-2 family proteins are activated and Bid is cleaved by caspases-8 in mitochondria to form truncated Bid (tBid). tBid and Bak are then heterodimerized, causing the release of cytochrome *c* from the mitochondria and subsequently to the activation of caspase-3 and caspases-9 [175], which then interact with and inactivate the anti-apoptotic Bcl-2 proteins. Bax-Bax homodimerization or Bax-Bak heterodimerization leads to the translocation of Bax from cytoplasm to mitochondria [176]. Both homodimers and heterodimers interact with a voltage-dependent anion channel (VDAC) in the mitochondrial outer membrane to release cytochrome *c*. Following this, the mitochondrial transition pores are opened and the permeability of mitochondrial membrane is increased [177]. Cytochrome *c* is released and apoptosome is formed, which consists of apoptosis-activating-factor 1 and procaspase 9. dATP then activates caspase 9, leading to subsequent activation of one of the effector caspases, caspase 3 or caspases 7, which eventually cleaves the inhibitor of caspase-activated DNase, poly (ADP-ribose) polymerase (PARP), the enzyme being identified as a substrate for caspases to cause cell death. The release of cytochrome *c* through the VDAC-mediated permeability transition pore can be inhibited by Bcl-2 and Bcl-$x_{L.}$

In addition to cytochrome *c*, Smac/Diablo is also released from the mitochondria when there is a loss of membrane potential. Smac/Diablo binds to X chromosome-encoded inhibitors of apoptosis protein (IAP), cellular IAP-1 (cIAP-1), and cellular IAP-2, as well as surviving, to inhibit apoptosis [178]. IAP blocks apoptosis by binding to and inhibiting caspases, as well as by activating caspases in the mitochondria-dependent mechanism [179]. IAP can also inhibit the activation of executioner caspases activated by both extrinsic and intrinsic pathways. The Smac/Diablo complex induces apoptosis via the apoptosome-dependent pathway [180], and release of Smac/Diablo complex is inhibited by Bcl-2 and Bcl-x_L [181]. Recent study shows that Smac induced cytochrome *c* release and apoptosis in the absence of Bax/Bcl-x_L via the activation of caspase 3 in human HCT 116 and DU145 colon carcinoma cells [182]. Smac is capable of circumventing defects in mitochondrial apoptosis signaling, including the loss of Bax or overexperession of Bcl-x_L, which frequently occurs in tumor cells resistant to anticancer therapy. A recent study suggests that an IAP family protein, apollon, binds to, ubiquitinates, and facilitates the proteasomal degradation of Smac and caspase 9, thereby preventing Smac-induced apoptosis [183]. Translocation of endogenous Smac into the cytosol, and release of Smac/Diablo during anticancer drug-induced apoptosis did not appear to play a major role in cell death after treatment of human lung carcinoma with etoposide [184], because cytochrome *c* and mitochondrial protease Omi/HtrA2 are still detectable in the cytosol in the absence of Smac.

2.1.2. Extrinsic Apoptotic Pathway

Extrinsic apoptotic pathway, also known as the death receptor pathway, involves activation of death receptors such as tumor necrosis factor (TNF)-α, Fas and TNF-related apoptosis-inducing ligand (TRAIL), which belongs to the tumor necrosis factor receptor (TNFR) gene superfamily. *Fas* gene is located on human chromosome 1 and comprises 5 exons. The *Fas* gene encodes a 40-kDa protein that has no signal sequence at the N-terminus, but a domain

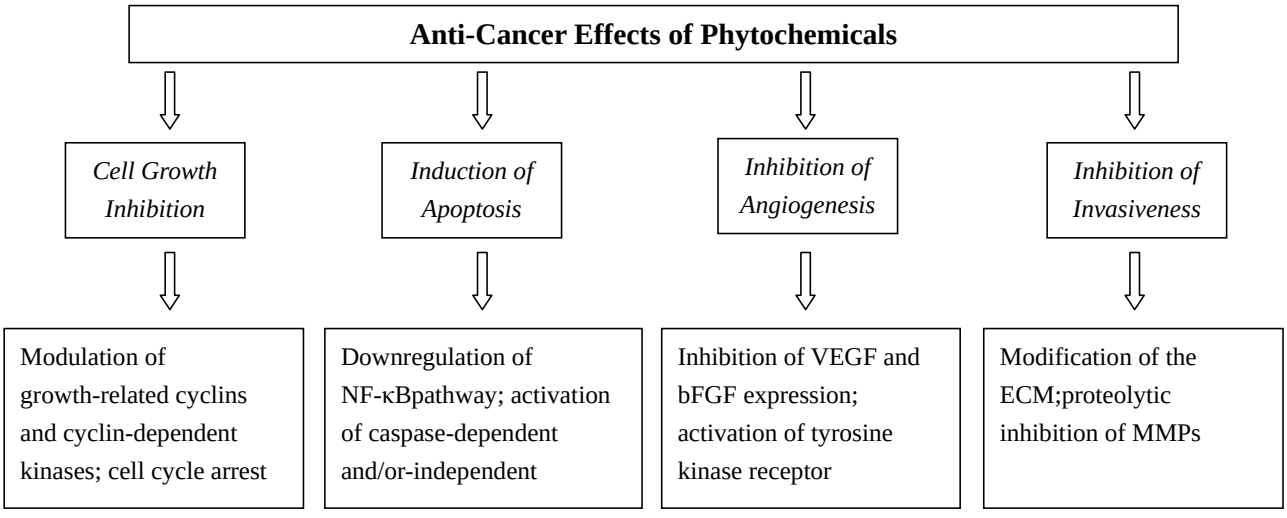


Fig. (2). The classical anti-cancer mechanisms of herbal medicinal compounds and phytochemicals.

of hydrophobic amino acid in the middle of the molecule, indicating that it is a Type II membrane protein with the C-terminal region outside the cell. This extracellular region has significant homology to the corresponding region of other members of the TNF family. FasL is expressed almost exclusively by activating T cells. Expression of FasL can be induced in T cells through activation of the T cell receptor. The Fas receptor was identified in 1989 as a target for antibodies that induce apoptosis in various human cell lines [185]. Fas ligand is a homotrimeric membrane molecule. Each Fas ligand trimer binds three Fas receptor molecules on the surface of the target cell. This results in the clustering of the receptors' death domains (DDs), which then recruit the cytosolic adapter protein FADD by binding to FADD's death domains. FADD not only contains a DD but also a Death-Effector-Domain (DED) that binds to an analogous domain repeated in tandem within the zymogen form of caspase 8. The complex of Fas receptor (trimer), FADD and caspase 8 is called the Death-Inducing-Signaling-Complex (DISC). Upon recruitment by FADD, caspase 8 oligomerization drives its activation through self-cleavage [186]. The death receptors are activated by the death-inducing signaling complex, which is formed by recruitment of a Fas-associated death domain and procaspase 8 to the death receptor, with caspase 8 being activated [173,174]. This directly activates caspase-3, and would subsequently lead to apoptosis.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL, also called Apo-2L), is a recently characterized member of the family of molecules that induce programmed cell death [187]. Like TNF-α and Fas ligand, TRAIL has both membrane-bound and soluble forms and acts through Type I membrane receptors that signal programmed cell death through a cytoplasmic death domain [188]. The regulation of TRAIL-mediated cell death, however, appears to be complex and at least five receptors for TRAIL have been identified and characterized to date [189]. TRAIL can modulate an apoptotic response by binding to two of these receptors, DR4 and DR5, which have two cysteine-rich extracellular ligand-binding domains and cytoplasmic death domains that signal downstream caspase activation and induce apoptosis [190]. Additionally, two decoy receptors called DcR1 and DcR2 are also involved in the induction of apoptosis. The extracellular domain of DcR1 shares homology with DR4 and DR5, but it does not have a cytoplasmic death domain, and it is anchored to the membrane through a glycosylphosphatidyl inositol linkage. Unlike DR4 and DR5, DcR1 is unable to transduce an apoptotic signal, and it is possible that DcR1 acts as an inhibitor of TRAIL-mediated apoptosis by competing with DR4 and DR5 for binding to the TRAIL ligand. DcR2 is a classical Type I transmembrane protein with an extracellular domain characterized by two cysteine-rich regions showing 58-70% identity to the ligand-binding domains of DR4, DR5 and DcR1. The carboxy-terminal cytoplasmic domain of this receptor lacks 52 of the 76 amino acids that used to encode the predicted DR4 and DR5 death domain, suggesting that it may not be able to signal cell death. A soluble TRAIL-DcR2-Fc fusion protein efficiently blocks TRAIL-mediated killing of sensitive cell lines [189,191]. Osteoprotegerin, a secreted TNF receptor homologue that inhibits osteoclastogenesis and increases bone density *in vivo*, has been identified to be the fifth TRAIL receptor. Unlike other members of the TNF receptor family, osteoprotegerin does not possess a transmembrane domain [192]. Osteoprotegerin can inhibit TRAIL-induced apoptosis of Jurkat cells. Conversely, TRAIL blocks the anti-osteoclastogenic activity of osteoprotegerin [193]. TNF and Fas ligands have potent cytotoxic activity against many types of tumor cells. The application of these death ligands to cancer therapy has been restricted by their severe toxicity to normal tissues [194]. TRAIL induces apoptosis in a wide variety of transformed cell lines, but does not seem to be cytotoxic to normal cells *in vitro*. Because of this, TRAIL might be more suitable than TNF-αor Fas ligand for systemic cancer therapy [195,196]. Thus, the family of TRAIL proteins and receptors has been studied by a considerable number of laboratories. During colorectal carcinogenesis, there is a marked increase in sensitivity to TRAIL-induced apoptosis associated with progression from benign to malignant tumor [197]. The outgrowth of human colorectal tumors grown in mice was completely blocked by transduction with AAV.TRAIL *in vitro*, while *in vivo* transduction significantly inhibited the growth of established tumors [198]. The administration of soluble recombinant TRAIL by vein infusion also resulted in the elimination of metastatic colon cancer in the liver [199].

Recent studies have demonstrated that many phytochemicals possess apoptotic effect against various types of cancer [16,33-37,43]. Our laboratory has demonstrated that AST promotes apoptosis in HepG2 cells through modulation of an ERK-independent NF-κB signaling pathway [33] and NAG-1 is a potential molecular target of AST in its proapoptotic actions [34]. Other than that, a recent study revealed that the rate of apoptosis in the small intestine and colon was increased by treatment with genistein via the down-regulation of the oncogene transformation-related protein 63 and the activation of caspase 3 [200]. The information obtained could facilitate future development of a novel target-specific chemotherapeutic agent with known molecular pathway.

2.2. NF-κB

The transcription factor nuclear factor (NF)-κB was first discovered as a major activator of immune and inflammatory function via the induction of several genes encoding cytokines, cytokine receptors, and cell-adhesion molecules. However, it has recently been linked to the control of cell growth and oncogenesis. In fact, the roles of NF-κB in the pathogenesis of cancer appear to be complex, but it is likely to involve with its transcription factor and hence controlling apoptosis and cell-cycle progression, and possibly cell differentiation, angiogenesis and cell migration [201,202]. In addition, NF-κB is constitutively expressed in tumor cells and its key role as an up-regulator of inflammation that it is involved in the early stage of progression [203]. As such, it is one of the molecular targets for the prevention and therapy of cancer that a number of studies have been focused on it. For example, curcumin could suppress NF-κB activation inducedby TNF, phorbol ester, and H_2O_2 through suppression of IκBα degradation and it can alsoinhibit TNF-induced Akt activation and its association with IKK. This suppression of NF-κB by curcumin plays a major role in its ability to prevent cancer [37,204]. Another well-known example is isothiocyanates, which can be found abundantly in cruciferous vegetables such as broccoli and watercress, could significantly inhibit LPS-induced NF-κB dependent gene expression in human carcinoma HT-29 cells [205].

NF-κB is one of the pivotal regulators of pro-inflammatory gene expression and is highly activated at sites of inflammation in diverse diseases, such as inflammatory bowel disease (IBD) and asthma. In many cases, NF-κB activation induced by pro-inflammatory signals is short-lived. However, if the signal is prolonged, it could lead to a sustained stimulus [206]. Increased NF-κB activity with nuclear localization is detected in patients with the abovementioned diseases. Enhanced recruitment of inflammatory cells and production of pro-inflammatory mediators are also found. However, it is unclear whether increase in pro-inflammatory cytokine production is the cause or result of NF-κB activation [207]. Studies have shown that patients with IBD have a higher risk of developing colorectal cancer. Schottelius & Dinter suggested two pathways that link IBD to colon cancer [208]. First, following epithelial injury, the NF-κB pathway in macrophages was triggered and pro-inflammatory factors were secreted, which promote survival of epithelial cells. Together with the oxidative stress caused by inflammation, the epithelial cells were more likely to undergo transformation and carcinogenesis. This pathway is complemented by another cell autonomous pathway, in which the NF-κB pathway was activated through Toll-like receptors (TLRs) on the surface of

the transformed epithelial cells, causing increased expression of anti-apoptotic genes. The combined effects of the two pathways are increased in cell proliferation and survival. Greten *et al*. showed the link between inflammation and tumorigenesis in a mouse model of colitis-associated cancer [209]. IKKβ-drived NF-κB contributes to the development of colorectal cancer through two different cell-type-specific mechanisms: it activates anti-apoptotic genes and suppresses the apoptotic elimination of pre-neoplastic cells in enterocytes, while concurrently promotes production of cytokines that act as growth factors for pre-malignant enterocytes in myeloid cells. Besides, IKKβ-dependent NF-κB in enterocytes contributes to tumor initiation or early promotion rather than growth and progression.

2.2.1. NF-κB and Inducible Nitric Oxide Synthase (iNOS)

Inducible nitric oxide synthase (iNOS) is important in the immune response to infection. It is one of the isoforms of the NOS enzyme that generates NO. Overexpression of this gene can be seen in many diseases under inflammatory stimuli (e.g. multiple cytokines and/or lipopolysaccharides), producing large amount of NO, which exerts beneficial effects such as anti-microbial, anti-atherogenic and anti-apoptotic activities, whereas its overproduction is disastrous, resulting in direct cellular injury and pro-inflammatory effect. [210,211]. A constitutive level of iNOS expression has been shown to prevent apoptosis in hepatocytes-treated TNF-α/β[212]. Whereas overexpression of this protein together with high output of NO concentrated in the tumor microeviroment induced apoptosis in several cell types [213]. In most tumors, there is higher expression and activity compared to adjacent normal tissue. However, its expression strongly depends on the cell types of tumor and tumor stage [214].

Activation of NF-κB is an intracellular signal transduction pathway of these stimuli. In biochemistry level, the enhancer elements of NF-κB were found to be located at the promoter gene of iNOS, thus NF-κB can induce the iNOS gene, consistent with its role in regulatory inflammation- associated genes. Studies showed that cytokine-induced iNOS expression in human liver and lung epithelial cell lines is dependent on NF-κB. The active response elements are localized in the 5' flanking region upstream of -4.7 kb. The cytokine-responsive region from -4.7 to -7.2 kb functions in aposition-independent fashion, thereby exhibiting the characteristic of an enhancer element [215]. The iNOS gene expression is also contributed via the mitogen-activated protein kinases (MAPK) pathway, which leads to the activation of transcription factors such as activator protein (AP1), cAMP-responsive elements and NF-κB [216]. Based on the phenomenon that only a combination of multiple cytokines was able to exhibit a prominent result, two or more signal transduction pathways are required to alter iNOS expression fully [217].

2.3. MEK/ERK

MEK/ERK pathway is an important mediator in some homeostatic mechanisms including cell growth, differentiation, and apoptosis in normal intestinal mucosa. However, MEK1 is also an important downstream candidate in oncogenic Ras signaling [31,218,219]. Constitutive activation of MEK1 results in transformation and colon cancer [220], whereas a small molecule inhibitor of MEKs suppresses transformation and tumor growth in cell culture and mouse models [221]. Recent studies also revealed that activation of extracellular-signal-regulated kinase (ERK) protects certain cells from undergoing apoptosis and that the MEK/ERK pathway modulates the expression and activity of some members of the Bcl family [222,223]. Other studies also show that ERK may induce site-specific phosphorylation of IκB-α in HeLa cells and directly activate the IKK complex (205). On the other hand, ERK signaling involves a cascade of phosphorylation events and is generally regarded to be related to cell proliferation and differentiation (218). However, the precise role of ERK remains controversial. Some stimuli may act through the ERK pathway to cause apoptosis, although the exact mechanism of ERK-caused apoptosis has yet to be elucidated. It has been suggested that transient ERK activation may be linked to cellular proliferation while strong and persistent activation may lead to programmed cell death (219). It is also proposed that ERK will mediate NF-κB during its proapoptotic action. Our data show that protein expression of both phosphorylated form of ERK-1 and ERK-2 is increased without any change of total ERK during the 12 to 72 hours of drug exposure, which indicates the crucial role of ERK in NF-κB signaling and the resulting apoptotic process. A recent report showed that indole-3-carbinol (I3C), a common phytochemical in the human diet, down-regulated the expression of MAP2K3, MAP2K4, MAP4K3 and MARK3. These results indicated that I3C has inhibitory effects on the p38 mitogen-activated protein kinase (MAPK) pathway, resulting in the abrogation of cancer cell survival [224]. Another studies demonstrated that EGCG regulated MAPK signaling, in which EGCG induced MAPK phosphorylation and hence negatively regulating tumorigenesis [225], and JNK is also involved in EGCG-induced apoptotic cell death of HT-29 human colon adenocarcinoma cells [226]. Therefore, it is believed that alterations in MEK-ERK signaling cascade may affect the tumorigenic potential of cancer cells.

2.4. PI3K/Akt/mTOR

The PI3K/Akt/mTOR cascade is important in growth factor-dependent signaling [227,228], of which Akt and mTOR are two key mediators in re-programming the metabolic pathways in cancer cells [32]. It was found that several components of the PI3K/Akt pathway are dysregulated in a wide spectrum of human cancers, whereas its activation confers resistance to the cell death caused by chemotherapy and radiation [229]. In general, activation of PI3K and the serine/threonine Akt contributes to competitive growth advantage, metastatic competence and drug resistance. Hence, regulation of PI3K/Akt signaling has been regarded as an important goal for new chemotherapeutic drug development [230]. Phosphatidylinositol 3-kinase (PI3K) belongs to a ubiquitous lipid kinase that is involved in receptor signal transduction through tyrosine kinase receptors. Upon stimulation at the cell membrane, PI3K phosphorylates phosphatidylinositol-4,5-bis-phosphate (PIP_2) to form phosphatidylinositol-4,5-tri-phosphate (PIP_3) and recruits other downstream molecules such as the serine-threonine kinases, in particular Akt and PDK. PTEN can prevent the signal transduction by opposing the conversion of PIP_3. The *pten* gene can be mutated both very early in tumorigenesis and much later in advanced cancers. Loss of PTEN function will promote malignancy [231]. Akt is partially activated through phosphorylation at threonine 308 in its activation loop by PDK1. Full activation can be achieved by additional phosphorylation at serine 473 in the C-terminus of Akt, which in turn regulates a wide range of target proteins that control cell proliferation, survival, growth and other processes [232,233]. In addition, activation of Akt also results in the activation of mTORC1 via the inhibition of negative regulator tuberous sclerosis (TSC) complex TSC1/2, leading to the translational initiation via the phosphorylation of two best studied downstream effectors, p70 ribosomal protein S6 kinase 1 (S6K1) and eIF4E binding protein (4E-BP1). TOR is regarded as a central regulator of cellular responses, achieving in part by the regulation of translation initiation [234]. mTOR can be activated at the downstream of the PI3K/Akt pathway, of which hyperactivation has been found in a wide range of tumors including colon cancer [235]. Other growth stimulations, such as low energy, nutrient and stress can also affect the mTORC1 activation [236]. The rapamycin-insensitive complex mTORC2 placed in parallel of PI3K/Akt signaling and can directly phosphorylate Akt at Ser473 [237]. mTOR inhibitors have been actively studied as potential chemotherapeutic agents for cancer since they showed high therapeutic index and can restore the sensitivity of some existing anti-cancer agents [238-240].

Recent studies suggested that the PI3K/Akt/mTOR pathway could interact with many phytochemicals. Genistein inhibits proliferation of colon cancer cells by attenuating a negative effect of epidermal growth factor on tumor suppressor FOXO3 activity, in which PI3K/Akt pathway is involved in such effect [241]. According to the X-ray crystallography, both quercetin and myricetin (a flavonoid found in many grapes, berries, fruits, vegetables, herb) can directly bind and suppress PI3K activity [242]. Myricetincan also induce pancreatic cancer cell death via the inhibition of the PI3K signaling pathway [243].

2.5. NSAID-activated Gene

NSAID-activated gene (NAG-1) was identified as a member of the transforming growth factor TGF-β superfamily. Studies showed that it could induce apoptosis in cells and suppress tumor progression in a transgenic mouse, providing the evidence that it has anti-tumorigenic activity. NAG-1 is highly expressed in mature intestinal epithelial cells, but is reduced significantly in human colorectal carcinoma samples and neoplastic intestinal polyps on Min mice [173]. Moreover, it was reported that treatment of prostate cancer cells with recombinant NAG-1 could induce apoptosis [166]. Nonetheless, high expression of NAG-1 is frequently occurred in primary tumors, suggesting that it may act as a tumor suppressor at the early stages of tumor development but as a pro-progressive gene in malignant tumor, similar to other members of the TGF-β superfamily. In general, NAG-1 induction in colon caner cells by NSAID treatment would induce apoptosis and lead to a cyclooxygenase (COX)-independent anti-tumorigenic effect [244]. It was suggested that overexpression of the death receptor would couple with NAG-1 induction to cause apoptosis [245]. NAG-1is now regarded as a novel therapeutic target that contributes to the anti-tumorigenic and proapoptotic effects in various cancer cells. Initially, NAG-1 was found to be induced in a p53-dependent manner and therefore considered as a biomarker for p53 activation [246,247]. Nevertheless, later discovery indicated that basal transcription of NAG-1 can also be regulated by several other regulatory elements in its promoter region, of which its expression requires the transcription factor early growth response gene (Egr-1) [248,249]. Current study further suggests that NAG-1 could be a downstream target of the PI3K-Akt signaling pathway [250]. All these information indicate that NAG-1 induction may involve multiple mechanisms. At present, both its biological activities under normal physiological condition or its role involved in the changes of expression are not fully understood. Only under certain pathological and/or extreme physiological conditions (e.g. acute tissue injury, inflammation and cancer), the secreted form becomes predominant in the plasma. Regulation of NAG-1 expression was believed to be through multiple steps, with the precise mechanisms remain unclear. Although, there are many conflicting findings of its expression in tumors, its anti-tumorigenic activities have been well-documented [251]. As previously reported, the anticancer activities of several compounds obtained from natural sources could be associated with NAG-1 induction, such as resveratrol, a compound found in grapes and red wine [252] and diallyl disulfide, a lipid-soluble compound from garlic [253]. It had been reported that genistein, a naturally occurring isoflavanoid, possesses anti-carcinogenic properties through a p53-dependent induction of NAG-1 [254]. Besides, epicatechin gallate was also reported to induce NAG-1 expression in colorectal cancer cells via a p53-independent activation of its transcription factor ATF-3, leading to a G_1 to S phase growth arrest and subsequent induction of apoptosis [255]. Based on the findings of our recent investigation, we discovered that NAG-1 is a molecular target of a herbal diterpenoids pseudolaric acid B and triptolide as well as Astragalus saponins in its growth-inhibitory and proapoptotic activities, with close correlation with the PI3K-Akt signaling pathway [11,34].

2.6. PPARs

Peroxisome proliferator-activated receptors (PPARs) are transcriptional factors that belong to the steroid hormone nuclear receptor gene superfamily. PPARs bindto and are activated by fatty acids, eicosanoids and a group of xenobiotics called peroxisome proliferators. Each of these receptors binds to specific peroxisome proliferator response elements (PPRE) as a heterodimer with a retinoid X receptor (RXR). Binding to these receptors has been proven to control the pathological conditions associated with obesity, aging- related diseases, inflammation, immune disorder, cell cycle regulation as well as cancer. There is a strong mechanistic basisfor PPAR and RXRtargeting, as these nuclear receptors are transcriptional factors that modulate gene expression relevant to the control of blood glucose and lipids, as well as theprocesses of inflammation and carcinogenesis. Since PPARs play an important role in lipid metabolism, the search for natural ligands had begun with fatty acids and eicosanoids. In fact, the discovery that many natural dietary compounds, especially phytochemicals, are PPAR activators is of great significancefor human health. PPARs were classified as three isoforms, PPARα, PPARβ and PPARγ recently. Each type plays an important role in cellular differentiation, apoptosis, and anti-inflammatory response [256-258]. Among the isoforms, PPARγ is of our greatest interest as it is expressed in colon cancers as well as in cancers of the breast, stomach and bladder. Many researches showed that PPARγ activation could lead to regulation of COX-2 expression [259,260]. PPARγ ligands regulate colon cancers cells proliferation via two ways: down-regulate the expression of pro-inflammatory genes and inhibit the tumor cells growth [261,262] by triggering cell cycle arrest through induction of terminal differentiation. Activation of PPARγ by troglitazone, ciglitazone has been reported to inhibit cell growth and induces apoptosis in human HepG2 hepatoma and HT-29 colon cancer cells [259, 260], whereas activation of PPARγ in APC^{Min} mice causes increase in both frequency and size of the polyps being formed. The role of PPARγ in coloncancer development remains skeptical. It appears that the action of PPARγ depends on the cell type and/or the contingence of mutational events that predispose tissues to cancer development.

2.7. Wnt Signaling

Wnt signaling plays a crucial role in mammalian embryogenesis and human diseases including cancers, and contributes to the maintenance of normal gut homeostasis in adult [263,264]. In view of the fact that recognition of chromosome 5q abnormalities as early events in the carcinogenic process for sporadic and hereditary (i.e. familial adenomatous polyposis, FAP) tumors, activation of the Wnt pathway has been linked to colon cancer [265,266]. Changes in the composition of the cadherin-catenin complex are believed to play a role in some cases of cadherin regulation [267]. In fact, activation of the Wnt signaling pathway and the result of increase in levels of β-catenin (or plakoglobin) could promote the complex formation at the plasma membrane and enhance cadherin-mediated adhesion in certain cell lines [268,269]. However, the levels of cadherin expression, rather than catenin levels, seem to be more important for complex formation and cell adhesion in many cell types [270]. Due to its important role in the development and progression of cancer, Wnt signaling is now recognized as one of the molecular targets for phytochemicals. In the past few years, several laboratories have identified numerous phytochemicals that may have the ability to modulate the Wnt signaling, in which a recent study showed that resveratrol inhibited proliferation and induced cell cycle arrest and apoptosis in Waldenstrom's macroglobulinemia cells by down-regulating the Wnt signaling pathways [271]. It has also been found that resveratrol exerts its anti-proliferative and pro-apoptotic properties through suppression of IGF-1R/Akt/Wnt

pathways and activation of p53 signaling [272]. Another well-studied phytochemical, quercetin, has also shown to have growth inhibitory effect on colon cancer cells via the down-regulation of Wnt pathway [273].

2.8. Angiogenesis, Invasion and Metastasis

Angiogenesis is a tightly controlled process involving the interactions among tumor cells, endothelial cells, phagocytes and the balance of the secreted angiogenic-promoting and -inhibiting factors, which is required at almost every step of tumor progression and metastasis [274]. Among different identified angiogenic factors secreted by tumor cells, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are the key regulators for vasculogenesis and are more important for sustaining tumor growth [275]. VEGF is a secreted angiogenic factor with four alternatively spliced isoforms (VEGF 121, 165, 189 and 145), which is overexpressed in colon cancer cells. Alternatively, bFGF is a heparin-binding protein monomer that can induce the proliferation and migration of endothelial cells. Other effects include stimulating the plasminogen activators and collagenases [276]. These two growth factors contribute synergistically to the regulation of blood vessel formation [277]. However, bFGF has been shown to be a critical survival factor during early vasculogenesis, whereas VEGF is an essential maturation factor of endothelial development and appears to determine the initiation of morphogenesis in forming the vascular pattern [278]. Apart from the involvement of angiogenic factors, the spread of cancer through metastasis represents one of the inherent dangers of the disease [279]. Remodeling of the extracellular matrix (ECM) is essential for the formation of new blood vessels. It involves the breakdown of sub-endothelial basement membrane and turnover of the interstitial matrix, resulting in the stimulation of endothelial cell migration. The process is tightly regulated by the balance of positive effectors (proteinases) and negative regulators (proteinase inhibitors). These enzymes are expressed at low level in physiological conditions but many of them are overexpressed in tumor cells. ECM degradation can be initiated by action of the proteolytic enzyme metalloproteinases (MMPs) in tissues surrounding the tumor, a critical event in the process of cancer invasion and metastasis [280]. MMPs are responsive to cytokines, growth factors and hormones, and are generally induced by interleukin (IL)-1β, tumor necrosis factor (TNF)-α, epidermal growth factor (EGF) and bFGF, but repressed by tumor necrosis factor (TGF)-β. Their proforms are capable of binding the tissue inhibitors of metalloproteinases (TIMPs), which in turn regulating the conversion of pro-MMPs into active form. MMPs activation is a key event in endothelial cell invasion because it participates in the initial phase of tumor-associated angiogenesis [276]. Hence, MMP could be one of the potential targets in the treatment of tumor cell invasion and metastasis.

Targeting on tumor angiogenesis has been the focus in modern cancer treatment regimens, since it is essential for tumor progression, of which tumor vasculature has been identified as a strong prognostic marker for tumor grading. Furthermore, angiogenic factors have been found to correlate with constitutive mitogenic and cell survival signaling in tumors. For example, the activation of receptor tyrosine kinases, such as EGFR, IGF-1R and platelet-derived growth factor receptor β (PDGFR-β), leads to the phosphorylation of important signaling molecules (e.g. ERK1/2 and PI3K), and also induces the expression of VEGF and MMPs in tumor cells [281]. Inflammation has been found to be one of the important processes in mediating angiogenesis. Inflammatory/angiogenic cytokines secreted from leukocytes, such as neutrophils, mast cells and macrophages, play crucial roles in tumor angiogenesis and vascular remodeling [282]. Therefore, cancer therapeutic strategy may involve targeting angiogenic molecules in tumors cells and inflammatory system, such as VEGF, bFGF, TNFα, IL-8 and their associated receptors on endothelial cells, MMPs, cyclooxygenases (COX), lipoxygenases (LOX), etc [281]. At present, several angiogenesis inhibitors are tested in advanced clinical trials for possible approval for cancer treatment. They can act indirectly by clearing the angiogenic growth factors from the circulation or by blocking/preventing the receptors/signaling pathways of the growth factors. They may also possess angiostatic activity acting directly on endothelium, affecting cellular regulatory processes that are distinctive in tumor cells. Many phytochemicals have been shown to inhibit tumor angiogenesis by targeting mitogenic and survival signaling associated with the production of angiogenic factors in tumor cells, and therefore capable of regulating endothelial cell growth, proliferation, migration, invasion and capillary organization that are required for tumor angiogenesis. Since many dietary and non-dietary phytochemicals possess anti-angiogenic and anti-tumorigenic activities but do not affect the survival of normal cells, there could be rationale approaches to examine their inhibitory effects in tumor angiogenesis [283]. Vayalil *et al.* reported that proanthocyanidins from grape seeds inhibited metastasis-specific MMPs (MMP-2 and MMP-9) in tumor cells, which was associated with the inhibition of activation of the MAPK and NFκB pathways [284]. Our laboratory also demonstrated that AST significantly inhibits the expression of both MMP-2 and MMP-9 in human gastric adenocarcinoma cells. These observations have supported our hypothesis that the anti-carcinogenic activities of AST could act via inhibition of cell migration and invasiveness [36]. Our unpublished data also showed that AST down-regulates the expression of the two key angiogenic factors VEGF and bFGF in human colon cancer cells. Besides, other recent study simultaneously showed that genistein can suppresse the invasive potential of breast cancer cells by down-regulating the transcription of all MMP genes [285].

2.9. Oxidative Stress, Inflammation and Cancer

The generation of excessive reactive oxygen species (ROS) and a drop in the intrinsic antioxidant capacity of cells leads to a state of oxidative stress, which contributes to inflammation and carcinogenesis. A variety of dietary and non-dietary phytochemicals have been shown to exert anti-inflammatory and/or chemopreventive effects by inducing cellular anti-oxidative and cytoprotective capacity through activation of different signaling pathways, aiming to suppress inflammation, tumor cell proliferation and growth-related signaling [286]. Tumor hypoxia was reported to be associated with tumor propagation, malignant progression and resistance to therapy and is correlated to patient mortality [287]. Hypoxia induces a series of proteome changes in tumor and/or stromal cells so that they can adapt to nutritional deprivation or to escape their unfavorable environment. However, such changes will result in promoting tumor propagation. Hypoxia can trigger neovascularization, proliferation and remodeling of the vascular wall by upregulating the gene expression of VEGF, VEGF receptor flt-1, bFGF, platelet-derived growth factor (PDGF), nitric oxide synthases and angiopoietin 2 [288]. Hypoxia also stimulates the transcription of glycolytic enzymes and glucose transporters (GLUT1 and GLUT3), tumor invasion-associated proteins (e.g., urokinase-type plasminogen activator) and other resistance-related proteins [287,289].

Hypoxia-inducible factor-1 alpha (HIF-1α) is a key regulator of the cellular response to oxygen deprivation which is composed of O_2-regulated HIF-1α and constitutively expresses HIF-1β subunits [290]. It functions as a master regulator of oxygen homeostasis in virtually all tissues [236,291]. Under hypoxic condition, the ubiquitination of HIF-1α is inhibited. Accumulation of HIF-1α dimerizes the constitutively expressed HIF-1β subunit and activates the transcription and stabilization of VEGF gene [34]. HIF-1 plays important roles in tumor progression. First, HIF-1α expression increased in multiple types of primary and metastatic human cancers relative to adjacent normal tissue, and that the level of expression is correlated with tumor angiogenesis and patient mortality [290]. HIF-1α overexpression has been demonstrated in human colon cancer biopsies, and forced overexpression of HIF-1α in HCT 116

human colon cancer cells increased tumor growth and angiogenesis in nude mice [292]. Second, genetic alterations in tumor suppressor genes (e.g. p53, Von Hippel-Lindau (VHL) and PTEN) and oncogenes also induced HIF-1 activity. Third, HIF-1 controlled the gene expression attributed to angiogenesis (e.g. VEGF) and to metabolic adaptation to hypoxia (e.g. glucose transporters and glycolytic enzymes), providing a molecular basis for tumor growth and angiogenesis [290]. The PI3K/AkT/mTOR pathway is possibly involved in the hypoxic response induced by HIF transcription factor in transformed cells [288]. A report has shown that hypoxia-induced upregulation of HIF-1α and its downstream targets GLUT1 and stromal cell-derived factor-1 (SDF-1) in colorectal cancer are closely related to an active PI3K/AKT/mTOR pathway [293]. Another report also showed that the inhibitory effect of rapamycin on HIF-1-dependent transcription in PC-3 prostate cancer cells was mediated through the suppression of mTOR function. Furthermore, rapamycin could interfere with HIF-1 activation in hypoxic cells by increasing the rate of HIF-1α degradation. This study demonstrated the anticancer activity of rapamycin being attributed to the inhibition of the hypoxic response program in developing tumors. Drugs targeting on HIF-1 could be a novel and tumor-specific approach for cancer therapy [294].

CONCLUDING REMARKS

Colorectal cancer represents a significant cause of morbidity and mortality worldwide. Despite its prevalence, current chemotherapy for the disease remains unsatisfactory and lead to serious side effects. Contemporary treatment of colorectal cancer includes adjuvant chemotherapy that involves combined use of conventional antitumor drugs and novel target-oriented agents that act by modulating specific cellular and molecular targets of carcinogenesis. In animal and cell culture studies, the growth and metastasis of cancer can be decelerated by many nutrients and herbal extracts. Novel chemotherapeutic agents derived from active phytochemicals could actually be used as adjuvants and improve the anticancer rates achievable with standard treatments. Herbal extracts containing terpenoids and other bioactive phytochemicals exhibit growth inhibition and induce apoptosis in cultured tumor cells and lead to tumor regression in nude mice xenograft [16]. Apoptosis plays a central role in tumor growth control. Activation of either the death receptor-mediated or mitochondrial cascade in apoptosis signaling has been the mode of action of many antitumor drugs. In recent years, several major signaling pathways have been identified as important regulators of cell proliferation and survival. It was found that components of many of these pathways are dysregulated in a wide spectrum of human cancers, while its activation confers resistance to cell death induced by chemotherapy and radiation. Hence, therapeutic strategies that target at these pathways are now under active development, including the practical use of specific inhibitors and gene silencers of the key signal transduction factors as anticancer drugs. It is also a challenge to unveil the properties of such drug inhibitors and the outcome of their combined use with conventional chemotherapeutic drugs. The best way that we could start to head our course is to make use of the biomarkers of these signaling cascades during drug-induced apoptosis and growth inhibition. As a matter of fact, inhibition of NF-κB by orthodox or novel (herbal) chemotherapeutics could contribute to their induction of apoptosis [35]. Modulation of the MEK/MAPK signaling is responsible for the drug-induced regulation of NF-κB. Other than this, we have recently proven that induction of the proapoptotic protein NAG-1 is a crucial step that determines the anticarcinogenic activity of anticarcinogenic herbal compounds [11]. It is also evident that PI3K/Akt/mTOR signaling could be the upstream regulator of NAG-1 in the action of potent herbal compounds such as AST [34]. Modulation of their protein and/or gene expression has been found to contribute to the promotion of apoptosis in colon cancer cells. Besides, ligands for the nuclear hormone receptor PPAR(γ) have also proven effective in pre-clinical models of colorectal cancer, including those from herbal origin [17,295]. Its anti-carcinogenic actions involve several conventional and novel transcriptional pathways. Contemporary studies have indicated that PPARγ has direct interactions with both NF-κB and TGF-β signaling pathways. Family members of TGF-β are multifunctional mediators that regulate cellular growth, migration and apoptosis, of which MAPK have been implicated in mediating downstream TGF-β signaling. The loss of TGF-β-mediated apoptosis may permit selective accumulation of premalignant cells. Apart from apoptosis, there is abundant evidence that ROS are involved in carcinogenesis. In fact, inflammatory cells produce a range of ROS that is associated with cancers in various tissues along the gastrointestinal tract. Natural compounds such as curcumin could attenuate oxidative DNA damage in various animal tissues by suppressing the generation of ROS such as superoxide and hydrogen peroxide in peritoneal macrophages. Under the circumstances that NO plays an important role in carcinogenesis, experimental results have indicated that curcumin is a NO scavenger in addition to its ability to inhibit the gene expression of iNOS in mouse peritoneal macrophages. There is no doubt that the anti-oxidative property of curcumin may contribute to its cancer chemopreventive ability, of which the idea could be extrapolated to most herbal therapeutics.

Epidemiological evidence indicates that many pharmacologically active constituents of herbal formulations have protected against a variety of cancers. The ability of many of them in stimulating the bone marrow and improving the peripheral white cell counts, as well as reversing other immunosuppressive effects caused by orthodox chemotherapeutic drugs, would be crucial determinants to use them as modifiers of existing anti-tumor agents. These substances possess the ability to alleviate the side effects and systemic toxicities induced by cytotoxic chemotherapeutic drugs in cancer patients. In general, the plant-derived medicinal compounds exhibit strong antioxidant and anti-inflammatory activities, and may attenuate tumor formation and growth by processes such as antiproliferative and/or pro-apoptotic activities, modulating metabolic enzymes and modifying the pharmacokinetics of concurrently used chemotherapeutic drugs. *Astragalus memebranaceus* has been used as immunomodulating agent in treating immunodeficiency diseases and to alleviate the adverse effects of chemotherapeutic drugs. In recent years, it has been proposed that *Astragalus* may possess antitumorigenic potential in certain cancer cell types. We have demonstrated from our current investigation that the total saponin extract from *Astragalus* possesses anti-carcinogenic effects in human colon cancer cells and tumor xenograft. Our findings have shown that AST inhibit cancer proliferation by inducing cell cycle arrest and promotion of apoptosis in HT-29 cells. Besides, the antitumorigenic effects of AST in nude mice xenograft are comparable to that produced by the conventional chemotherapeutic drug combo 5-fluorouracil (5-FU) and oxaliplatin, with reduced toxicity. These results indicate that AST could be an effective chemotherapeutic agent in colon cancer treatment, which might also be used as adjuvant in combination with orthodox chemotherapeutic drugs. A proposed multi-target mechanism of action for AST has been summarized in (Fig. **3**). To conclude, the discovery of effective chemotherapeutic phytochemicals or potential adjuvant herbal agents is crucial in the battle against colorectal cancer and other gastrointestinal cancers. More importantly, advances in eliciting the precise cellular and molecular mechanisms during the anti-tumorigenic process will be of imperative clinical significance to increase the efficacy and reduce prominant adverse drug effects in patients through target-specific therapy.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

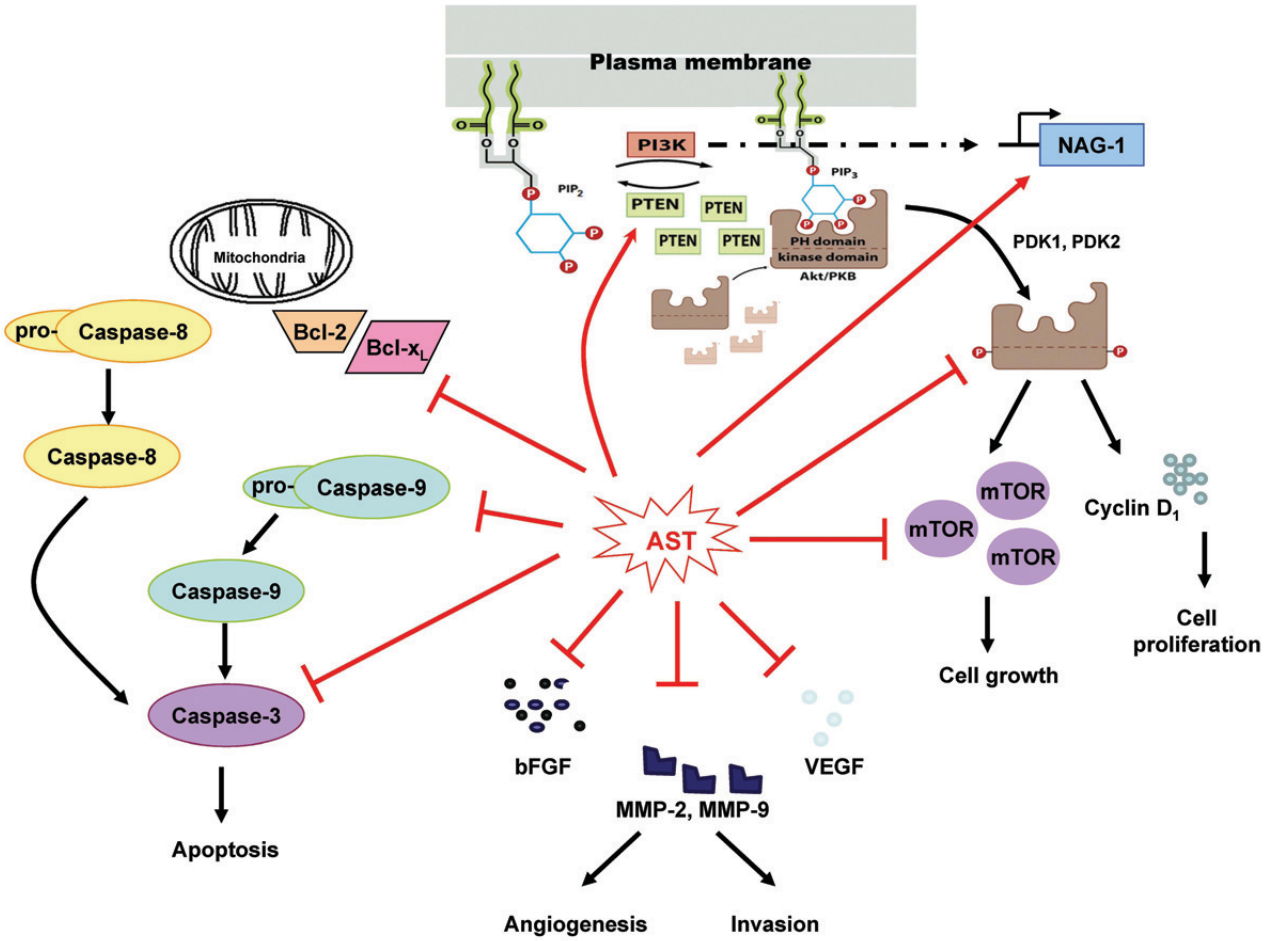


Fig. (3). Illustration of the multiple molecular targets of AST in its modulation of colon cancer.

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