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Astragalus membranaceus: A Review of its Protection Against Inflammation and Gastrointestinal Cancers

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Abstract: *Astragalus membranaceus* is a major medicinal herb commonly used in many herbal formulations in the practice of traditional Chinese medicine (TCM) to treat a wide variety of diseases and body disorders. Among its diversified clinical applications, the potential use of this herb and its chemical constituents in treatments of inflammatory diseases and cancers has been actively investigated in recent years. *Astragalus*-based treatments have demonstrated significant amelioration of the toxicity induced by other concurrently administered orthodox drugs (e.g., immunosuppressants and cancer chemotherapeutics). The major components of *Astragalus membranaceus* are polysaccharides, flavonoids, and saponins. Contemporary use of *Astragalus membranaceus* mainly focuses on its immunomodulating, anti-oxidant, and anti-inflammatory, as well as anticancer effects. In this paper, we summarize the properties of *Astragalus membranaceus* and its major constituents in the biological system based on experimental and clinical studies. The antitumorigenic mechanisms of a novel *Astragalus* saponins extract called AST in treating various gastrointestinal cancers are highlighted. We discuss in detail how the *Astragalus* herb and AST influence the immune system, modulate various cancer signaling pathways, and interact with specific transcription molecules during protection against gastrointestinal inflammation and cancers. This information could help clinicians and scientists develop novel target-specific and effective therapeutic agents that are deprived of major systemic side effects, so as to establish a better treatment regimen in the battle against inflammatory diseases and cancers of the gut.

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Introduction

Because of the low response rate and high toxicity of current orthodox Western drugs, there has been an urge to develop chemotherapeutic drugs and active agents that possess increased pharmacological efficacy and reduced systemic side effects. Research studies in recent years have proven that herbal extracts and phytochemicals possess strong antitumor and anti-inflammatory activities (Lin *et al.*, 2002; Baek *et al.*, 2004; Ellington *et al.*, 2005; Kang *et al.*, 2008). Traditional Chinese medicine (TCM) is often considered complementary or alternative due to the fact that TCM has a different basis of treatment approach than does conventional Western medicine. TCM treatments are based on a holistic approach; TCM practitioners will consider the condition of the entire human body when designing an appropriate treatment regimen for their patients, involving complex synergistic interactions between multiple herbs within a herbal formulation (Tang and Eisenbrand, 1992). Thus, the Western concept of "from bench to bedside" may not always fit in the clinical practice of TCM. However, research studies over the past few decades have focused more on scientific criteria when evaluating the effectiveness of TCM. Having more authentications being accomplished with supporting scientific evidence, the use of TCM as a mainstream therapeutic agent has become better recognized.

Astragalus membranaceus (Radix Astragali or "*Huang Qi*") has a long history of medicinal use in Chinese herbal medicine. The Chinese name *Huang Qi* translates as "yellow leader", referring to the yellow color of the root as well as its status as one of the most important tonic herbs in TCM. It was recorded in "*Shen Nong Ben Cao Jing*", the first book of Chinese herbal medicine, as a superior herb, and was classified under the group of "*qi*"-tonifying drugs. It has been formulated as an ingredient of herbal mixtures to treat patients with a deficiency in vitality, which symptomatically presents as fatigue, anorexia, chronic diarrhea, fatigue, and abnormal uterine bleeding (e.g., menorrhagia). It has also been used as a health food supplement in some Asian populations and also serves as a lead herb in many TCM formulations as well as in Chinese ethnic tonifying soups. *Astragalus membranaceus* is native to Northern China and the elevated regions, including the provinces of Inner Mongolia, Shanxi, Gansu, and Heilungkiang. The medicinal portion of the plant is its four-to seven-year old dried root, which is collected during the spring and autumn. There are over 2000 types of *Astragalus membranaceus* worldwide; the types native to China have been tested and listed in the Pharmacopoeia of the People's Republic of China 2010 (Pharmacopoeia Commission of PRC, 2010). The most commonly used genus includes *Astragalus membranaceus (Fisch.) Bge. Var. mongholicus (Bge.)* Hsiao and *Astragalus membranaceus (Fisch.) Bg*e. (Fam Leguminosae). Radix Astragali refers to the special preparation of the herb for decoction (Radix Astragali Preparata or Zhihuangqi): The stir bake of the slices of herb with honey until it is no longer sticky. *Astragalus membranaceus* has been used to treat a wide range of diseases and body disorders.

In recent years, investigations on its application in the treatment of inflammatory diseases and cancers have been conducted.

Traditional Clinical Indications

Traditional indications of *Astragalus membranaceus* focus on "*qi*"-deficiency symptoms, which present as a lack of strength, anorexia, spontaneous sweating, edema, and abscesses. Other indications include frequent cold and spontaneous sweating, shortness of breath, edema, wasting disorder, night sweating (Hong, 1986), chronic ulceration, sores, and mellitus diabetes (Bensky and Gamble, 1993). It can also induce urination and promote the discharge of pus, plus the growth of new tissue. *Astragalus membranaceus* has also been used to treat cancer patients with "*qi*"-deficiencies following chemotherapy and radiation therapy to improve the condition of anemia and to treat albuminuria in chronic nephritis (Wagner *et al.*, 1997; Chen and Chen, 2004). Additional effects other than traditional TCM actions include anti-tumor and anti-osteoporosis activities (Wagner, 1997). Recovery and longevity in cancer patients who have received chemotherapy or radiation treatment can also be improved by using Radix Astragali (Chou *et al.*, 2007). *Astragalus membranaceus* can also function as a cardiotonic agent and is prescribed by TCM practitioners to treat cardiovascular diseases such as myocardial infraction, angina, and congestive heart failure (Miller, 1998). Moreover, it has been reported that *Astragalus membranaceus* possesses hepatoprotective (Zhang *et al.*, 1992; Li *et al.*, 1998) and antiviral (Yuan *et al.*, 1990) properties.

Astragalus membranaceus is usually used along with 3–15 types of other herbs in a single decoction, according to the status of the patient and nature of the disease, and its dosage ranges from 9 g to 30 g in each decoction. For external use, 10% ointment may be applied on a wound surface. It is generally regarded as a safe drug; no incidence of poisoning associated with the use of Radix Astragali, or its main constituents, has been reported *in vitro* or *in vivo* so far (Upton *et al.*, 2011). The LD_{50} of a crude extract of Radix Astragali is 40 g/kg as determined by intraperitoneal injection in rats (Wagner, 1997). Doses as high as 100 g/kg of raw herb were given to rats by lavage with no adverse effects (Bensky and Gamble, 1993). Additionally, rats were injected with the herbal extract (0.5 g/kg, i.p.) for one month, which caused no abnormal changes in food intake, behavior or urine/fecal production (Chang and But, 1987). Recently, the sub-chronic toxicity of the *Astragalus* extract, consisting of its polysaccharides and saponins, was studied to evaluate the safety dosage range in clinical application. The application ranges were found to be 5.70–39.90 g/kg in rats and 2.85–19.95 g/kg in dogs, which are equivalent to 70 and 35 times the doses in humans, respectively (Yu *et al.*, 2007).

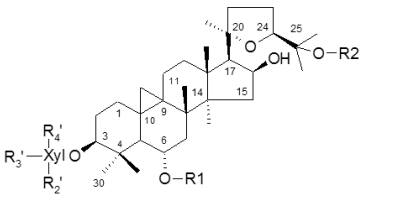
Active Constituents of *Astragalus membranaceus*

Astragalus membranaceus has a complex chemical profile. Its major active constituents include triterpene saponins, flavonoids and polysaccharides (Ma *et al.*, 2002). Other components found in the herb include phytosterols (and other volatile oils), L-canavanine,

(Hong, 1986; Tang and Eisenbrand, 1992), fatty acids (Miyazawa and Kameoka, 1987), sterols, betaine, choline, (+)-lariciresinol, (−)-syringaresinol, lupenone, 3-hydroxy-2-methylpyridine (Subarnas *et al.*, 1991), amino acids (Zheng *et al.*, 1997), bifendatatum (Wang *et al.*, 2003), coumarin (Huang, 1999), and γ-aminobutyric acid (Hikino *et al.*, 1976). Trace elements including zinc, iron, copper, magnesium, manganese, calcium, sodium, potassium, rubidium, silver, chromium, tin, vanadium, and cobalt may also be present in varying quantities (Mills and Bone, 2000). Although investigations on the bioactivities of some of these chemical constituents have been conducted in the past few decades, many of their potential biological functions still remain to be elucidated.

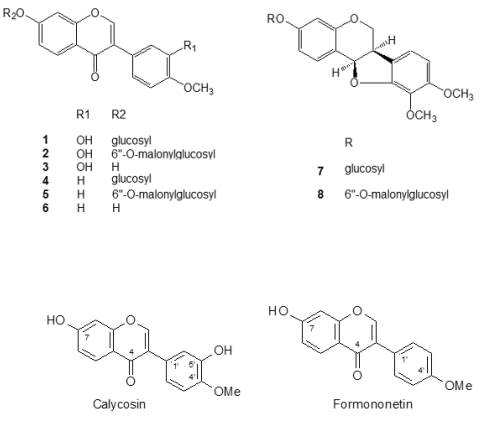
Astragalus Triterpene Saponins

Triterpene saponins, the contents ranging from 0.5 mg/g to 3.5 mg/g, are major constituents of Radix Astragali (Song *et al.*, 2007). The *Astragalus* saponins identified include astragalosides I–VIII, acetylastragaloside, isoastragaloside I, III, astramembrannin II, cycloastragenol, cyclosieversigenis, soyasaponin I, soyasapogenol B, and lupeol (Kitagawa *et al.*, 1983; He and Findlay, 1991; Sinclair, 1998). Among these, there are five major saponins that represent more than 80% of the total *Astragalus* saponins (AST) content of the herb. They are: astragalosides I, II, and IV, and isoastragaloside I and II, all being cycloartane-type triterpenoids (Fig. 1A). Among them, astragaloside IV is known to be the qualitative control biomarker, although its content is relatively low in the crude herb (Qi *et al.*, 2008);



	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

(A)



(B)

Figure 1. (A) General chemical structure of triterpene saponins with aglycone of cycloastragenol bearing the substituents R_1–R_4'. (B) General chemical structure of isoflavonoids (top) with the two isoflavone examples calycosin and formononetin (bottom).

nevertheless, the herb contains an abundant amount of astragalosides I and II (Qi *et al.*, 2006; Yu *et al.*, 2007). Thermal stability is the major concern for these saponins. Acetyls in the xylose of astragalosides are easily removed by heat during sample preparation, converting them to astragaloside IV. Interestingly, a novel compound named malonylastragaloside I was recently reported to be the most abundant but extremely unstable member, even at room temperature (Chu *et al.*, 2010). Both aerial parts and the fibrous roots (the small end roots) contain higher amounts of saponins than the main roots do, but only the main roots are traditionally used (Qi *et al.*, 2008). It is therefore suspected that these saponins may not be the active ingredients responsible for the traditional action as "*qi*"-tonifying drug.

Astragalus Flavonoids

Radix Astragali contains flavonoids within the range of 0.5–3.0 mg/g (Matkowski *et al.*, 2003). A total of 12 different flavonoids can be isolated (Lin *et al.*, 2000). These flavonoids include isoflavonones, isoflavans, pterocarpans, flavonones, and chalcones (He and Wang, 1990; Song *et al.*, 1997a, 1997b; Bian *et al.*, 2006; Li *et al.*, 2006; Pei *et al.*, 2007; Xiao *et al.*, 2009), of which isoflavones are the major constituents (Fig. 1B). Among these, eight main isoflavonoids, namely calycosin-7-O-β-D-glucoside, calycosin-7-O-β-D-glucoside-6″-O-malonate, calycosin, ononin, formononetin-7- O-β-D-glucoside-6″-O-malonate, formononetin, 6a*R*,11a*R*-3-hydroxy-9,10-dimethoxypterocarpan-3-O-β-D-glucoside and astrapterocarpan-glucoside-6″-O-malonate account for more than 80% of the total flavonoid content (Song *et al.*, 2007). The dominant component, calycosin-7-O-β-D-glucoside, is used as a chemical marker in quality analyses of the herb (Pharmacopoeia Commission of the People's Republic of China, 2010).

Astragalus Polysaccharides

Astragalus polysaccharides have received a great deal of attention in the past few years. In a general study, *Astragalus* polysaccharides A, B, and C were identified as glucans, while polysaccharide D was identified as a heteropolysaccharide (McKenna *et al.*, 2002). Knowledge about the precise chemistry of *Astragalus* polysaccharides is quite limited. Since polysaccharides are macromolecules with complicated chemical structures, it is relatively difficult to isolate and characterize their individual components. Scientists have made strong efforts to characterize the structure of the polysaccharides isolated from Radix Astragali (Shao *et al.*, 2004; Kiyohara*et al.*, 2010). However, mainly crude polysaccharide extracts have been studied so far. When purified polysaccharides were investigated, their chemical properties were usually poorly characterized (Fang and Wagner, 1998; Shao *et al.*, 2004; Wang *et al.*, 2006; Li *et al.*, 2009; Li and Zhang, 2009). Recently, a water-soluble polysaccharide was isolated and purified from Radix Astragali, and its structure was elucidated by monosaccharide composition, partial acid hydrolysis and methylation analysis, with further support from FT-IR, GC-MS and ^{1}H and ^{13}C NMR spectra, SEM and AFM microscopy (Yin *et al.*, 2012). Fourteen polysaccharides can be isolated from the

aerial part of the herb, which have been found to possess immunomodulating activity in intestinal Peyer's patches. Thirteen of the polysaccharides have β-D-(1→3)-galactan moieties branched with β-D-(1→6)-galactooligosaccharide side-chains (Kiyohara*et al.*, 2010). Quality control of polysaccharides is still a big challenge to scientists because of the polysaccharide's complicated structure and macromolecular mass. The structural characterization of polysaccharides and their correlation with their observed bioactivity have not been well established.

Contemporary Use of *Astragalus membranaceus*

Despite the traditional clinical indications, *Astragalus membranaceus* has been widely used in a series of contemporary applications (Table 1).

Table 1. Summary of the Contemporary use of *Astragalus membranaceus*

Category	Herbal Drug Effects	Herbal Constituents	Journal Citation
Immunomodulation	Potentiate therapeutic efficacy of chemotherapeutics	"*Shi-quan-da-bu-tang*" (100 herbs including *Astragalus* and *Ligusticum*)	(Zee-Cheng *et al.*, 1992; McCulloch *et al.*, 2006)
	Stimulate hematopoietic factors and IL production	*Astragalus* herb	(Yoshida *et al.*, 1997)
	Potentiate anti-tumor activity of recombinant LAK cells in cancer and AIDS patients	*Astragalus* herb	(Chu *et al.*, 1988; Wang *et al.*, 1992)
	Enhance body defense; Increase number of stem cells in bone marrow and lymphatic tissues	*Astragalus* herb	(Jiao *et al.*, 1999; Yin *et al.*, 2004)
	Stimulate NK cell activity	*Astragalus* herb	(Zhao, 1992; Mills and Bones, 2000)
	Activate cell proliferation and increase cytokine production	*Astragalus* polysaccharides and flavonoids	(Jiao *et al.*, 1999; Shao *et al.*, 2004)
	Reticuloendothelial system-potentiating activity	*Astragalus* polysaccharides	(Shimizu *et al.*, 1991)
	Antagonize the leukopenic effect of immunosuppressants	*Astragalus* polysaccharides	(Wang *et al.*, 1989)
	Induce cellular and humoral immune responses	*Astragalus* saponins	(Yang *et al.*, 2005)

Table 1. (*Continued*)

Category	Herbal Drug Effects	Herbal Constituents	Journal Citation
	Exhibit mitogenic and co-mitogenic activities on mouse splenocytes	*Astragalus* saponins	(Cho and Leung, 2007b; Auyeung *et al.*, 2009b)
	Treatment of autoimmune disorder	*Astragalus* saponins	(Tu *et al.*, 1994; Auyeung *et al.*, 2013)
	Increase proliferation and antibody production from T- and B-lymphocytes	Astragaloside IV	(Wang *et al.*, 2002)
Anti-oxidative and anti-inflammatory actions	Minimize free radical damage to membranes	*Astragalus* ingredients	(Chen *et al.*, 1995; Wang *et al.*, 1996)
	Inhibit glutamine-induced injury in neuronal cells	*Astragalus* flavonoids	(Yu *et al.*, 2005)
	Protection against xanthine oxidase-induced oxidative damage	*Astragalus* flavonoids	(Yu *et al.*, 2009)
	Inhibit lipid peroxidation	*Astragalus* flavonoids and saponins	(Purmova and Opletal, 1995; Shirataki *et al.*, 1997; Toda *et al.*, 2000)
	Relief symptoms and prevent inflammatory bowel disease	*Astragalus* herbal formulations	(Ko *et al.*, 2005; Ko and Chik, 2009)
	Inhibit NF-κB and expression of adhesion molecules in LPS-stimulated endothelial cells	Astragaloside IV	(Zhang *et al.*, 2003)
	Suppress airway inflammation and hyper-responsiveness in chronic asthma animal model	Astragaloside IV	(Du *et al.*, 2008)
	Regulate AGE-induced inflammation in diabetes	*Astragalus* herb	(Qin *et al.*, 2012)
Anti-cancer actions	Potential anticancer therapeutic effects	*Astragalus* herb	(Shen *et al.*, 2008)
	Inhibit growth of cancer cells *in vitro*	*Astragalus* herb	(Lin *et al.*, 2003)
	Inhibit growth of cancer cells *in vivo*	*Astragalus* alone or with *Ligustrum lucidum*	(Lau *et al.*, 1994; Kurashige *et al.*, 1999; Cui *et al.*, 2003)

(*Continued*)

Table 1. (*Continued*)

Category	Herbal Drug Effects	Herbal Constituents	Journal Citation
	Increase sensitivity and reduce side effects of orthodox anticancer drugs	*Astragalus* herbal formulations	(McCulloch *et al.*, 2006)
	Target-specific anticancer potential	*Astragalus* herbal formulations	(Kao *et al.*, 2001; Cho and Leung, 2007b; Na *et al.*, 2009)

Immunomodulating Effects

Astragalus membranaceus is one of the main ingredients in TCM prescriptions for enhancing the immune system and relieving the adverse effects caused by conventional drug treatments. More than 100 TCM herbal formulations have been screened and evaluated. A TCM formulation called "*Shi-quan-da-bu-tang*", comprising *Astragalus* and *Ligusticum*, was proven to improve the therapeutic efficacy of chemotherapy in various animal and clinical studies (Zee-Cheng *et al.*, 1992; McCulloch *et al.*, 2006). The formulation is most effective in stimulating hematopoietic factors and interleukin (IL) production, and is capable of preventing the recurrence of malignancies, prolonging survival, and increasing resistance to the immunosuppression caused by radiotherapy and antineoplastic drugs through stimulation of the macrophages to produce IL-6 and tumor necrosis factor (TNF) (Yoshida *et al.*, 1997). Some researchers have also suggested that *Astragalus membranaceus* have potential antitumor activity of recombinant IL-2 generated lymphokine-activated killer (LAK) cells in cancer and AIDS patients (Chu *et al.*, 1988; Wang *et al.*, 1992).

Astragalus membranaceus has been comprehensively used as a tonic to enhance the body's defenses (Anonymous, 2003; Yin *et al.*, 2004). The herb increased the number of stem cells in bone marrow and lymph tissue, hence facilitating their development into active immune cells (Jiao *et al.*, 1999). In addition, *Astragalus membranaceus* stimulated the natural killer (NK) cell activity of human peripheral blood lymphocytes and restored steroid-inhibited NK-cell activity (Mills and Bone, 2000). The NK cell activity in patients with systemic lupus erythematosis was also enhanced (Zhao *et al.*, 1992). Evidence indicates the importance of the polysaccharide fractions of the herb in the modulation of immune functions both in humans and in experimental animals (Chen *et al.*, 1981; McKenna *et al.*, 2002; Block and Mead, 2003). Modern scientific studies have demonstrated that *Astragalus* polysaccharides exhibit strong immuno-enhancing effects both *in vitro* and *in vivo* (Shimizu *et al.*, 1991; Shao *et al.*, 2004; Lee and Jeon, 2005; Yin *et al.*, 2009; Kiyohara*et al.*, 2010). It was reported that *Astragalus* polysaccharides could activate cell proliferation and increase cytokine production in the B cells and macrophages of mice (Shao *et al.*, 2004), and were capable of stimulating macrophages to express iNOS gene through the activation of NF-κB/Rel (Lee and Jeon, 2005). An acidic polysaccharide obtained from Radix Astragali showed significant reticuloendothelial system-potentiating

activity (Shimizu *et al.*, 1991). Intraperitoneal injection of *Astragalus* polysaccharides into mice increased the weight and number of mouse spleen, elevated the response of mouse spleen against sheep red blood cells, and stimulated the phagocytic activity of peritoneal macrophages (Tang and Eisenbrand, 1992). Leukopenia caused by the immunosuppressant prednisone was antagonized by the polysaccharides of this herb (Chang and But, 1987; Wang, 1989). In addition, *Astragalus* polysaccharides were shown to reduce the side effects of chemotherapy in clinical studies, with a significantly lower degree of myelosuppression in patients (Duan and Wang, 2002).

The other two main constituents of the herb, flavonoids, and saponins, also exert immunoregulatory effects. A study showed that *Astragalus* flavonoids can promote the proliferation of lymphocytes, raise T-cell count, regulate the T cells subsets and elevate LAK cell-inducing activity induced by IL-2 (Jiao *et al.*, 1999). *Astragalus* saponins can also induce cellular and humoral immune responses with slight hemolytic activity and significantly enhance ovalbumin-specific IgG, IgG1, and IgG2b antibody titers in mice serum (Yang *et al.*, 2005). A broad study on seven *Astragalus* species showed that the IL-2 inducing activity of the triterpene saponins extracted from *Astragalus* roots could be the key mechanism involved in the immunomodulatory and anticancer effects of such species (Yesilada *et al.*, 2005). A recent study also revealed that the bioactive fraction isolated from the roots of *Astragalus membranaceus* could exhibit mitogenic and co-mitogenic activities on mouse splenocytes, both *in vitro* and *in vivo*. It was also found that this bioactive fraction was mitogenic to T-cell depleted populations, but virtually inactive on the B-cell counterpart. Intraperitoneal injection of this fraction into mice significantly amplified the antibody response to red blood cells of sheep (Cho and Leung, 2007b). Experimental studies of peripheral blood mononuclear cells obtained from myasthenia gravis patients have shown that *Astragalus* saponins can reduce the titer of nicotinic acetycholine receptor antibodies significantly. This result has provided an answer to the question of why *Astragalus* saponin was found to be effective in the treatment of autoimmune disorders (Tu *et al.*, 1994). Astragaloside IV, the biomarker of *Astragalus* saponins, could increase T- and B-lymphocyte proliferation and antibody production *in vivo* and *in vitro*. The enhancement of IL-1 at low concentrations *in vitro* also confirmed the immunomodulatory action of astragaloside IV (Wang *et al.*, 2002).

Anti-Oxidative and Anti-Inflammatory Effects

A number of clinical and experimental studies have demonstrated the anti-oxidative effects of *Astragalus membranaceus*. Active ingredients obtained from *Astragalus*' root can minimize free radical damage to membranes (Chen *et al.*, 1995; Wang *et al.*, 1996; Toda and Shirataki, 1999). *Astragalus* flavonoids have contributed largely to the anti-oxidative effects of the herb; they inhibit glutamate-induced injury in PC12 neuronal cells, and significantly increase the activities of anti-oxidant enzymes, including superoxide dismutase and glutathione peroxidase (Yu *et al.*, 2005). *Astragalus* flavonoids can also protect cells from xanthine/xanthine oxidase-induced oxidative damage (Yu *et al.*, 2009). Moreover, *Astragalus* flavonoids and saponins significantly inhibited membrane lipid

peroxidation generated by superoxide, hydrogen peroxide, and ultraviolet rays, while *Astragalus* polysaccharides exerted a weaker protective activity (Toda and Shirataki, 1999). The isoflavones of *Astragalus,* such as afrormosin, calycosin and odoratin, were found to be the key contributors to inhibit lipid peroxidation (Shirataki *et al.*, 1997). Alternatively, *Astragalus* saponins produce a positive effect on heart function by inhibiting the formation of lipid peroxides in the myocardium and decreasing blood coagulation (Purmova and Opletal, 1995).

It is known that traditional herbal formulations containing *Astragalus membranaceus* can be used to treat chronic ulceration and sores (Bensky and Gamble, 1993). Compounds isolated from *Astragalus membranaceus* are capable of minimizing free radical damage to membranes by inhibiting lipid peroxidation (Toda *et al.*, 2000) and by protecting the intestinal endothelium (Hei *et al.*, 2004). There has been a number of clinical studies on the treatment of inflammatory bowel disease (IBD) using herbal formulations with *Astragalus membranaceus* as the leading drug, with results ranging from the relief of symptoms to the prevention of ulcerative colitis (UC) recurrence with increased serum superoxide dismutase activity. We demonstrated that crude extract of *Astragalus membranaceus* possesses both preventive and therapeutic potential in experimental colitis. Such anti-inflammatory actions involve anti-oxidation, along with the inhibition of adhesion molecule synthesis in the colonic tissues (Ko *et al.*, 2005). Another study from our laboratory also showed that both oral and locally administered *Astragalus* extract possesses protective effects against experimental colitis through differential modulation of colonic cytokines (Ko and Chik, 2009). Apart from its beneficial effects in gastrointestinal diseases, the anti-inflammatory properties of the herb could be extended to treatment of other diseases. It has been demonstrated that astragaloside IV exerted the anti-inflammatory potential by inhibiting the NF-κB pathway and regulation of adhesion molecule expression on the surface of TNF-α and lipopopolysaccharides (LPS) stimulated endothelial cells, which is a key process in the pathogenesis of inflammation (Zhang *et al.*, 2003). In addition, astragaloside IV could suppress the progression of airway inflammation, airway hyper-responsiveness and airway remodeling in a murine model of chronic asthma (Du *et al.*, 2008). Other than that, *Astragalus* herbal extract had inhibited advanced glycation end products (AGE)-induced inflammatory cytokine and hence reduced macrophage-mediated inflammation via regulation of the p38 MAPK and NF-κB signaling pathways. These findings indicate that *Astragalus membranaceus* could be an immunoregulatory agent against AGE-induced inflammation in diabetes (Qin *et al.*, 2012). Taken together, we believe that the anti-inflammatory effects of *Astragalus membranaceus* are not organ-specific. By targeting a variety of signaling pathways, the herb and its components can be developed as a broad-based therapeutic agent against inflammation in different diseases.

Anticancer Effects

To date, no medical treatment of most cancers can achieve complete remission, some of which may even cause severe adverse effects. In order to solve these problems, contemporary research has considered integrative and complementary medicine as an alternative

therapy for cancer. It has been suggested that TCM has great advantages to be developed as chemotherapeutics or adjuvant agents by increasing sensitivity and reducing the side effects of orthodox anticancer drugs, aiming to improve the patients' quality of life as well as to increase survival (Konkimalla and Efferth, 2008). TCM products have shown promising antitumor effects with minimal toxicity and systemic side effects (Ji *et al.*, 2009). *Astragalus membranaceus* has demonstrated a potential therapeutic value in cancer therapy (Shen *et al.*, 2008).

Astragali extract has been shown to inhibit the growth of various colon cancer cells *in vitro* (Lin *et al.*, 2003). A mixture of *Astragalus membranaceus* and *Ligustrum lucidum* significantly reduced the tumor load in mice with renal carcinoma (Lau *et al.*, 1994). Incidence of urinary carcinoma was significantly lower in *Astragalus*-treated mice after N-butyl-N′-butanolnitrosoamine induction when compared to the no treatment control (Kurashige *et al.*, 1999). Hepatocarcinogenesis was also prevented in rats treated with aqueous Astragali extract, with decreased number and area of foci on the liver (Cui *et al.*, 2003). Meta-analysis of randomized trials has also suggested that *Astragalus*-based TCM formula may increase effectiveness and reduce toxicity of standard platinum-based chemotherapy for advanced non-small-cell lung cancer (McCulloch *et al.*, 2006). This implicates that *Astragalus membranaceus* and its constituents could be established as an adjuvant agent to improve efficacy and reduce systemic side effects of conventional chemotherapeutics. For instance, a Chinese herbal formulation "*Bu-Zhong-Yi-Qi-Tang*" (with major ingredients including astragaloside IV, ginsenosides Rb1 and Rg1, saikosaponin A and C, and glycyrrhizin) was able to inhibit the proliferation of human Hep3B, HepG2 and HA22T hepatoma cells that were associated with G1 cell cycle arrest (Kao *et al.*, 2001). The anti-cancer effects of *Astragalus membranaceus* were further examined in gastric cancer-induced mesothelial cell apoptosis (Na *et al.*, 2009). This study has suggested that *Astragalus* treatment could inhibit apoptosis of human peritoneal mesothelial cells induced by gastric cancer cell supernatant, indicating that it can be used an adjuvant chemotherapeutic agent in gastric cancer therapy. Besides, the antitumor effect of *Astragalus membranaceus* was performed in 12 rapidly proliferating tumor cells lines and several transplantable tumors (Cho and Leung, 2007b). In this study, the fractionated extract markedly enhanced the tumoricidal activity of the peritoneal macrophages, primed the tumor-bearing mice for TNF production and induced generation of cytotoxic cells (with LAK-like activity) against tumors *in vitro*. The latter drug effect could play an important role to strengthen the host's defense against primary and metastatic tumors and to restore the depressed immune functions in tumor-bearing mice.

Study of the Novel Agent AST Derived from Total *Astragalus* Saponins

Although the anticancer effects of *Astragalus membranaceus* and its active constituents have been discovered and investigated since the last few decades, the precise mechanisms remain poorly understood. In recent years, our laboratory has investigated the anti-carcinogenic effects of a special extract of the AST in the treatment of gastrointestinal cancers both *in vitro* and *in vivo*. Pilot study using a panel of human cancer cells indicated that AST

caused a universal growth-inhibitory and proapoptotic effects in 7-cell lines (Auyeung *et al.*, 2009a), including those of the colon, stomach, liver, and the breast. AST could promote caspase-dependent apoptosis in HT-29 colon adenocarcinoma cells, and inhibit cell proliferation through cell cycle arrest in the S and G2/M phases, with concomitant suppression of p21 expression and inhibition of cyclin-dependent kinase activity (Tin *et al.*, 2007). In *in vivo* study, the AST-induced reduction of tumor volume as well as the associated pro-apoptotic and anti-proliferative effects in the tumor xenograft were comparable to that produced by the conventional chemotherapeutic drug 5-FU. On the other hand, AST is free from systemic side effects (e.g., body weight drop, neutropenia, and mortality) that were frequently observed when using the drug combo 5-FU+oxaliplatin (Tin *et al.*, 2007). These results indicate that AST could be an effective chemotherapeutic agent in colon cancer treatment, which might also be used as an adjuvant in combination with other orthodox drugs to prevent or reduce the side effects of highly toxic chemotherapeutic compounds. On top of that, we have also revealed that AST could increase the sensitivity of cancer cells to the microtubule inhibitor vinblastine (Auyeung *et al.*, 2014). We confirm that the nonsteroidal anti-inflammatory drug activated gene NAG-1 is a major molecular target of AST in its proapoptotic and anti-tumorigenic activities, which has strong correlation with the PI3K-Akt signaling pathway during its action (Auyeung *et al.*, 2009a). Glucose-regulated proteins (GRP) are induced in the cancer microenvironment to promote tumor survival, metastasis, and drug resistance. GRP in the endoplasmic reticulum (ER) binds to calcium and serves as an ER stress signaling regulator. Elevation of intracellular calcium level activates several pro-apoptotic proteins including a family of calcium-dependent cysteine proteases called calpains. Our current findings exemplify that calpains, in particular calpain II, play a permissive role in the modulation of GRP78 and consequent regulation of ER stress-induced apoptosis. It was shown that AST could inhibit calpain activation upon prolonged ER stress, while combination of calpain inhibitors and AST could exhibit a pronounced pro-apoptotic effect (Wang *et al.*, 2014). This information indeed helps to facilitate future development of a novel target-specific chemotherapeutic agent with known molecular pathway. Other than colon cancer cells, we have also exemplified that AST could induce anti-carcinogenic effects in HepG2 hepatocellular carcinoma (HCC) with downregulation of the HCC tumor marker α-fetoprotein through modulation of an ERK-independent NF-κB signaling (Auyeung *et al.*, 2009b).

A Matrigel invasion assay was employed to demonstrate the effect of AST in the invasiveness of AGS gastric adenocarcinoma cells. Results indicate that the number of AGS cells invaded through the Matrigel membrane was significantly reduced upon AST treatment, with concomitant downregulation of the expression of the pro-angiogenic factor vascular endothelial growth factor (VEGF) as well as the metastatic factors matrix metalloproteinase (MMP)-2 and MMP-9 (Auyeung *et al.*, 2012b). This is the first time when the anti-invasive and anti-angiogenic potential of AST in human cancer cells has been reported. Similar to the case in AGS cells, AST also reduces the number of invaded colon cancer (LoVo) cells through the Matrigel membrane. Following this exciting discovery, we then studied the gene profile of HCT 116 colon adenocarcinoma cells following treatment with 100 μg/ml of AST for 12 h and 24 h of interferon (IFN)-β, a factor that could

Table 2. Alteration of Gene Expression Associated with Angiogenesis, Cell Invasion and Metastasis by AST (100 μg/mL) Treatment for 12 h in HCT 116 Cells

Functions	Genes	Upregulation (⇑) Downregulation (⇓)
Angiogenesis	Interferon β 1 (fibroblast)	⇑
	Thrombospondin 1	⇑
	TEK tyrosine kinase	⇓
Invasion & Metastasis	MMP2	⇓
	MMP9	⇓
	MTA1	⇓
	S100A4	⇓
	TWIST1	⇓

directly inhibit the proliferation of tumor cells of different histological origins and downregulate the expression of proangiogenic molecules such as basic fibroblast growth factor (bFGF) and IL-8. Upregulation of thrombospondin-1 expression, an inhibitor of angiogenesis, as well as downregulation of the VEGF-inducible TEK tyrosine kinase were also observed in AST-treated cells. Furthermore, we also demonstrated that AST down-regulates some genes that are relevant to metastasis and invasion including matrix metallopeptidases (MMP-2 and MMP-9), the metastasis associated genes *mta1* and *Twist*, as well as a novel calcium-binding protein S100A4. It is known that mTOR signaling contributes to promotion of angiogenesis through induction of VEGF and its receptor, which could be mediated by attenuation of COX-2 activation and subsequent HIF-1α downregulation under hypoxic condition. Our findings suggest that AST could modulate tumor progression in colon cancer cells by inhibiting the above-mentioned pro-angiogenic events and invasion-related promoters via diminution of mTOR signaling (Auyeung *et al.*, 2013). Detail action of AST on angiogenesis, tumor invasiveness and metastasis has now been the focus of our current exploration. As summarized in Table 3, the pro-apoptotic, growth-inhibitory and anti-angiogenic potential of AST plus the immunorestorative properties of the agent support its further development as an anti-cancer adjuvant.

Before the closing of the discussion, we have to state that other than the *Astragalus* saponins, we have commenced our study on the potential of individual *Astragalus* isoflavonoids in the anticancer arena. Formononetin, an *Astragalus* isoflavone, was the first one to be explored. We have found that formononetin could also initiate growth-inhibitory and proapoptotic activities in human colon cancer cells (Auyeung and Ko, 2010). It also inhibited angiogenesis and tumor cell invasion by down-regulating the expression of the key pro-angiogenic factors, like VEGF and MMPs, although to a lesser extent than AST. This potential anti-angiogenic effect of formononetin was confirmed in nude mouse xenografts (Auyeung *et al.*, 2012a). This gives us a new insight as we used to believe that mainly *Astragalus* saponins induce diversified anti-tumor effects, while *Astragalus* isoflavonoids are stronger in their antioxidant and anti-inflammatory activities. We now depict

Table 3. Anticarcinogenic Effects of AST in Cancer Cells and Tumor Xenograft

Mechanisms of Action	Involving Pathways/ Target Molecule	Cancer Cell Type	Journal Citation
Growth inhibition and reduction of tumor size		HT-29, HCT 116, DLD-1, Caco 2, LoVo (colorectal); AGS, MKN45 (gastric); HepG2 (liver); MCF-7 (breast)	(Tin *et al.*, 2007; Auyeung *et al.*, 2009a, 2009b, 2012b, 2014)
Inhibition of colony formation		HCT 116	(Wang *et al.*, 2014)
Promotion of apoptosis	Caspase activation, PARP cleavage, Bcl-2, Bcl-x_L, Bid Intrinsic/extrinsic pathways	HT-29, HCT 116, DLD-1, AGS, HepG2, MCF-7	(Tin *et al.*, 2007; Auyeung *et al.*, 2009a, 2009b, 2012b, 2014)
Inhibition of cell proliferation	Cell cycle arrest, cyclins, cdc/cdk, p21, c-myc, NF-κB, α-fetoprotein, IFN-β	HT-29, HCT 116, AGS, HepG2	(Tin *et al.*, 2007; Auyeung *et al.*, 2009a, 2009b, 2012b, 2014)
Inhibition of angiogenesis	VEGF, VEGF-R1, bFGF, HIF-α, thrombospondin-1, VEGF-inducible TEK tyrosine kinase	HCT 116, AGS	(Auyeung *et al.*, 2009a, 2012b, 2013, 2014)
Inhibition of cell invasiveness & metastasis	MMPs, mta1 and Twist, S100A4	AGS, HCT 116, LoVo	(Auyeung *et al.*, 2009a, 2012b, 2014)
Attenuation of chemotherapeutic drug toxicity	Systemic side effects of 5-FU & oxaliplatin; vinblastine	HT-29, HCT 116	(Tin *et al.*, 2007; Auyeung *et al.*, 2014)
Increase sensitivity to chemotherapeutic druog	Potentiation of the anti-carcinogenic activity of vinblastine	HCT 116, LoVo	(Auyeung *et al.*, 2014)
Inhibition of PI3K-Akt signaling	PTEN, Akt, mTOR	HT-29, HCT 116	(Auyeung *et al.*, 2009a, 2013)
Regulation of MAPK signaling	ERK	HT-29, HCT 116, HepG2	(Auyeung *et al.*, 2009a, 2009b)
Activation of NSAID-activated gene (NAG-1)	NAG-1, Egr-1, COX-2	HCT 116	(Auyeung *et al.*, 2009a, 2013)
Induction of ER stress and unfolded protein response (UPR)	XBP-1, CHOP	HCT 116	(Wang *et al.*, 2014)
Regulation of GRPs and calpains	GRP78, GRP94, calpain I and II	HCT 116	(Wang *et al.*, 2014)

that some isoflavonoids may also be potential antitumorigenic compounds. Hence, the anticancer effect of another isoflavonoid calycosin is under our vigorous investigation.

Conclusion

There has been an increase in interest on the use of active herbal compounds in the treatment of human diseases, possibly because herbal formulations and phytochemicals could generate profound effectiveness in treating inflammatory and infectious diseases as well as more complicated health problems such as cancers. More importantly, we may take advantage of the relatively nontoxic nature and immunomodulating property of herbal medicinals. *Astragalus membranaceus* has demonstrated all-round and superb effects when used to treat various human diseases based on its immunoregulatory, anti-oxidative, anti-inflammatory and anticancer properties. Although all three main constituents of *Astragalus* could contribute to all the above-mentioned drug actions, it appears that *Astragalus* polysaccharides possess the strongest ability in promoting the immunorestorative and immunomodulating works, *Astragalus* flavonoids are mainly responsible for the antioxidant and anti-inflammatory effects of the herb, while *Astragalus* saponins are more specialized in facilitating the anti-carcinogenic effects. Despite the promising effectiveness of *Astragalus membranaceus* and its active components in the treatment of different human body disorders, it is crucial to study the precise underlying molecular mechanisms before it

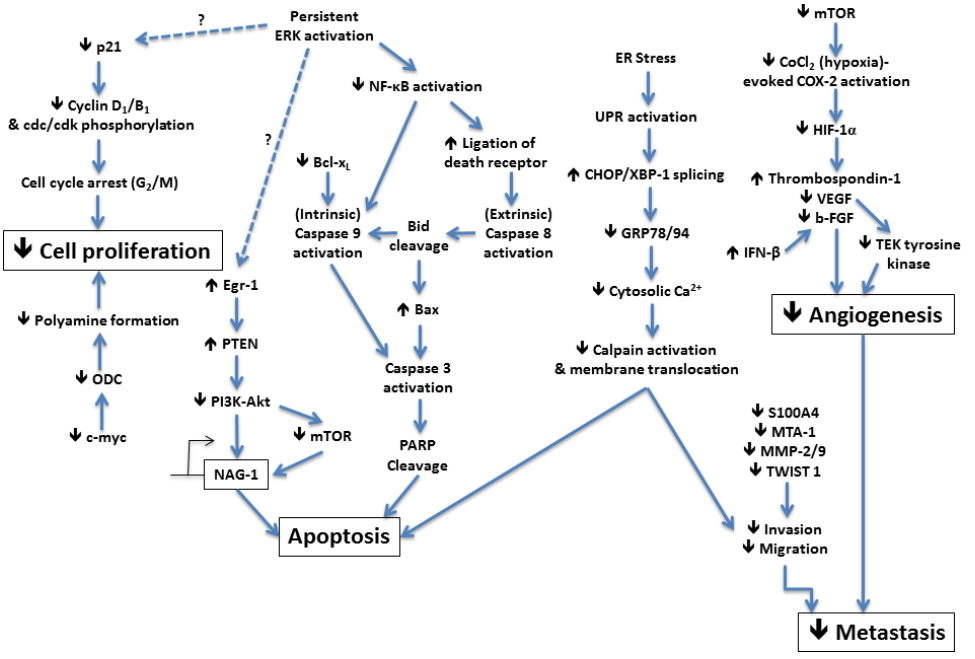


Figure 2. Proposed mechanistic actions of AST in the alleviation of gastrointestinal cancers through regulation of multiple signaling pathways and drug targets.

can be further developed into a target-specific therapeutic agent. In our current research, a specific extract from AST has been extensively investigated on its potential in treating various gastrointestinal cancers, in particular that of the colon. We discovered that AST possess a wide range of antitumorigenic power, from growth inhibition in solid and primary tumor cells to the prevention of cancer metastasis and control of the invasive activity of advanced cancers. It also undergoes a diversified mode of action, from promotion of apoptosis, inhibition of cell proliferation through cell-cycle arrest, to the manipulation of angiogenesis and cell invasiveness. Other than having a good synergistic working relationship with different combinations of chemotherapeutics in its antitumorigenic activities, AST could also alleviate the detrimental systemic side effects of the orthodox drugs by its immunomodulating and organ-protective actions. This is the first review that concisely describes the properties of *Astragalus membranaceus* and its bioactive constituents in the protection against inflammatory diseases and cancers. It also elucidates the systematic modulation of various cancer signaling pathways and specific target molecules by AST (Fig. 2), which could be further developed into an effective therapeutic agent for treating cancers of the gastrointestinal tract that is deprived of major side effects in the human body. Oncologists can now have more options when choosing an appropriate treatment regimen for both primary and advanced gastrointestinal cancers, as novel herbal compounds (Ko and Auyeung, 2013) like AST can be adopted as adjuvant agents to synergistically work with conventional chemotherapeutics.

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