

Traditional Chinese medicine in the prevention and treatment of cancer and cancer metastasis (Review)

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Abstract. Traditional Chinese medicine (TCM) has been a major part of healthcare in China, and has extensively affected medicine and healthcare in surrounding countries over a long period of time. In the fight against cancer, certain anticancer remedies using herbs or herbal formulas derived from TCM have been developed for the management of malignancies. Furthermore, there are clinical trials registered for the use of herbal remedies in cancer management. Herbal medicine has been used as part of combined therapies to reduce the side-effects of chemotherapy, including bone marrow suppression, nausea and vomiting. Herbal remedies have also been used as chemopreventive therapies to treat precancerous conditions in order to reduce the incidence of cancer in high-risk populations. Emerging evidence has revealed that herbal remedies can regulate the proliferation, apoptosis, adhesion and migration of cancer cells. In addition to this direct effect upon cancer cells, a number of herbal remedies have been identified to suppress angiogenesis and therefore reduce tumour growth. The inhibition of tumour growth may also be due to modifications of the host immune system by the herbal treatment. However, the precise mechanisms underlying the therapeutic effects of herbal remedies remain poorly understood and are yet to be fully elucidated. The present study aims to summarize the current literature and clinical trial results of herbal remedies for cancer treatment, with a particular focus on the recent findings and development of the Yangzheng Xiaoji capsule.

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1. Introduction

The use of complementary and alternative medicine (CAM) has been widely accepted as a therapeutic approach for the management of malignancies, particularly in the supportive and palliative care of cancer patients. In 2007, \$33.9 million was spent by adults in the USA on CAM (1). Furthermore, CAM is commonly used by cancer patients; in fact, 25-50% of prostate cancer patients have used at least one CAM modality (2). As a major part of CAM in Western countries, traditional Chinese medicine (TCM) has also been used to treat malignancies. In 2011, a series of correspondences concerning traditional Asian medicines were published, which created interest in investigating the potential use of traditional Asian medicines for cancer management, in particular, the study of relevant therapeutic approaches using current scientific research methods (3). Traditional medicines, including those practiced in China, Korea, Japan and other countries worldwide, may appear mystical and pseudoscientific due to a poor scientific understanding of their actions and a lack of convincing clinical evidence according to the standards of modern science and medicine. Success has been achieved in studies of traditional medicine, including artemisinin for malaria and arsenic trioxide for leukaemia (4,5). To date, the majority of traditional remedies lack the standardisation and scientific evidence required to validate their effect, efficiency and application. As part of traditional Asian medicines, TCM has been used to prevent and treat a number of disorders, with documentary

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evidence, for two thousand years. Over the last few decades, studies of TCM in cancer have focused on molecules extracted and purified from anticancer herbs. Icariside II, purified from the root of *Epimedium koreanum* Nakai, is able to induce the apoptosis of human acute myeloid leukemia U937 cells through the STAT3 pathway (6). Artemisinin, a compound extracted from the Chinese medical herb, Qing Hao, has been widely used in the treatment of malaria, and has also been involved in cancer treatment (7-10). Based upon the evidence from clinical studies, investigations of certain herbal remedies have been further developed into clinical trials to examine their efficacy and safety in cancer management. However, data concerning the effect and mechanisms of TCM in the prevention and treatment of malignancies, particularly anticancer remedies comprising multiple herbs, remain limited.

The Yangzheng Xiaoji (YZXJ) capsule, derived from a TCM formula, has exhibited anticancer actions (11). In a randomized double-blind lung cancer study, patients who received treatment with conventional chemotherapy combined with YZXJ (n=304) exhibited significantly increased levels of complete and partial disease remission compared with those who received chemotherapy alone (n=103) (23.3 vs. 14%, respectively; $P<0.01$) (11). The patients who received YZXJ also presented with decreased bone marrow suppression. Previous studies have established that YZXJ is able to directly inhibit the adhesion and migration of cancer cells. In addition to its direct effect on cancer cells, it is also able to suppress the angiogenic capacities of vascular endothelial cells, which may contribute to the inhibitory effect on *in vivo* tumour growth (12-14).

Although progress has been made in the research and application of herbal remedies for the treatment of malignancies, data from clinical trials are still required in order to understand the mechanisms that underlie the action of these medicines and therefore guide their clinical application appropriately.

2. Chinese herbal medicine in combined therapies for malignancies

At present, common treatments for cancer include surgery, chemotherapy, radiotherapy, immunotherapy and targeted therapy. Surgery remains the first treatment choice for the majority of early-stage solid tumours, whilst other treatments and surveillance may be used in the management of advanced-stage tumours, or as adjuvant therapies for less advanced tumours. The side-effects and toxicity of chemotherapy and radiotherapy often restrict their application and effectiveness. The damage these therapies cause to the normal tissues of patients is usually more severe than that to the tumours. Those patients who must undergo long courses of chemotherapy and radiotherapy may have to end treatment as a result of the accompanied toxicity and additional side-effects. In combination with chemotherapy and/or radiotherapy, TCM has the potential to be used as an assistant therapeutic approach; it can enhance the response rate (RR) to chemotherapy and radiotherapy, reduce the severity of toxic effects and other side-effects caused by chemotherapy and radiotherapy, and improve the quality of life and survival of cancer patients (15).

To date, a number of herbs have been reported to be beneficial to cancer patients. These include single herbs, traditional herbal formulations and Chinese medicine preparations. A number of single herbs have been individually adopted for cancer treatment, including Radix Astragali (Huang Qi) (16), ginseng (Ren Shen) (17), blister beetle (also known as Mylabris; Ban Mao) (18), toad venom (Chan Su) (19), garlic (Da Suan) (20) and turmeric (Jiang Huang) (21). Chinese traditional herbal formulations were usually developed by ancestors of TCM, and were based on Chinese medicine theories, including yin and yang, the five elements and meridians. A single formulation often has numerous components. At present, the formulations adopted for cancer treatment always contain certain common major components, which are the popular single herbs with anticancer activities.

Chinese medicine preparations are recently developed herbal remedies that are prepared from either single herb or traditional herbal formulations using modern advanced pharmaceutical technologies. There are a number of different types of preparations, including injections, tablets, pills, capsules and orally-administered liquids. Compared with traditional decoctions, Chinese medicine preparations are safer, more effective and easier to use. With the development of pharmacological research concerning herbal medicines, increasing numbers of effective ingredients have been successfully extracted and their efficacy clarified, which has promoted the development of Chinese medicine preparations. Mylabris has been identified to induce the apoptosis of cancer cells and promote functions of the immune system, but it is greatly restricted in its clinical application due to renal toxicity and bone marrow suppression (22). Subsequent studies have demonstrated that cantharidin is one of its effective ingredients and that a number of artificial analogues of cantharidin exhibit similar effects, but with lower toxicities (23,24). Increased knowledge of herbal medicines will aid in the understanding of Chinese formulations and preparations for the treatment of cancer. The present review summarises the data on single herbs and Chinese medicine preparations that are widely adopted for the treatment of cancer.

The present review describes the commonly used single herbs, Radix Astragali (Huang Qi), ginseng (Ren Shen), Mylabris (Ban Mao) and toad venom (Chan Su), which according to preclinical studies and clinical trials, have anticancer activities. Garlic (Da Suan) and turmeric (Jiang Huang) have demonstrated marked anticancer activities in preclinical and clinical studies, but not in clinical trials (Table I). In addition to the single herbs, there are also certain Chinese medicine preparations being used to treat cancer.

Radix Astragali (Huang Qi). According to a number of pharmacological studies, Astragalus has a potential immunomodulatory role when combined with chemotherapy and radiotherapy; it promotes the activity of lymphocytes, natural killer cells and macrophages (16), which leads to the secretion of interleukin-6 (IL-6) and tumour necrosis factor (TNF). In addition, Astragalus can enhance the biological function of IL-2, whilst also simultaneously reducing its adverse effects (25,26). In the most recent systematic review concerning the combination of Radix Astragali and platinum-based chemotherapy, 34 studies were selected from an initial 1,305, and quantified

Table I. Single herb medicines.

| Common name | Pinyin name | Latin name | Major active ingredients | Evidence of anticancer activity |
|-----------------|-------------|-------------------------------------|---|---|
| Radix Astragali | Huang Qi | <i>Astragalus membranaceus</i> | Polysaccharides, saponins, flavonoids | Immune regulation, increased therapy response, reduced toxicity and improved performance status |
| Ginseng | Ren Shen | <i>Panax ginseng</i> | Triterpene glycosides, ginsenosides | Immune regulation, inflammation suppression, increased therapy response, reduced toxicity and improved performance status |
| Blister beetle | Ban Mao | <i>Mylabris phalerata</i> | Cantharidin | Apoptosis promotion, increased therapy response and improved performance status |
| Toad venom | Chan Su | <i>Bufo bufo gargarizans</i> Cantor | Bufadienolides | Apoptosis promotion, growth inhibition, increased therapy response, reduced toxicity and improved performance status |
| Garlic | Da Suan | <i>Allium sativum</i> | Allicin, alliin | Apoptosis promotion, growth inhibition and induction of cell cycle arrest |
| Turmeric | Jiang Huang | <i>Curcuma longa</i> | Curcumin, demethoxycurcumin, bisdemethoxycurcumin | Inflammation suppression, increased sensitivity to chemotherapy and radiotherapy, and antioxidant properties |

for subsequent meta-analysis. A reduced risk of mortality over 6, 12, 24 and 36 months was reported by 7, 12, 9 and 6 of the studies, respectively, with a significantly increased tumour response observed in 30 studies (risk ratio, 1.34; 95% confidence interval, 1.24-1.46; $P<0.0001$). Although the number of studies concerning chemotherapy-associated toxicity is relatively small, a significant reduction in grade III or IV white blood cell (WBC) toxicity and grade III or IV haemoglobin (Hb) toxicity was identified in patients who had received a combined therapy. An improved or stable performance status was also observed in the majority of the studies (27,28).

Ginseng (Ren shen) and Mylabris (Ban Mao). In addition to Radix Astragali, ginseng and Mylabris are two other single herbs commonly used in combination with chemotherapy and radiotherapy. Ginseng is able to modulate the function of the immune system and reduce the side-effects of radiotherapy. Furthermore, it can dilate small arteries and veins, inhibit oxidation and suppress inflammation (17). According to these anticancer properties, ginseng is a potential candidate for cancer prevention and treatment. It has been used to suppress inflammation, and reduce cancer occurrence and recurrence in several types of tumours, including gastric, liver, pancreatic and colon cancers (17,29). In addition, ginseng has the potential to reduce the toxicity of radiotherapy in normal human tissue, which may be due to its anti-oxidation and immune regulation effects. Cantharidin is the anticancer ingredient in Mylabris (23). In addition to functioning in immune system regulation, cantharidin and Mylabris are able to promote the apoptosis of cancer cells, particularly in liver and oesophageal cancers. However, their application in cancer treatment has been restricted by the occurrence of renal toxicity and bone marrow suppression. However, several cantharidin analogues have been synthesized that exhibit similar anticancer activities, but with lower toxicities (24).

In a systematic review of TCM treatment in hepatocellular cancers, 45 out of an initial 130 studies were included for meta-analysis. The single herbs included Radix Astragali, ginseng, beetle extract (Mylabris), toad skin secretions (bufotoxin), Atractylodes, Bupleurum and Curcuma. The pooled RR of the 37 studies indicated that formulations containing ginseng, Astragalus and Mylabris were significantly correlated with increased tumour responses, including complete response (CR), partial response (PR), stable disease and progressive disease. Among the 45 included studies, 15, 22, 4 and 15 reported a positive correlation between TCM usage and 6, 12, 18 and 24 months survival, respectively. However, just 7 out of the 45 included studies reported improvements in symptoms. There were 6, 4 and 4 studies that reported an improvement in the symptoms of abdominal pain, fatigue and poor appetite, respectively (30).

A further meta-analysis investigated the treatment of colorectal cancer with TCM in combination with the FOLFOX 4 regimen (comprising folinic acid, fluorouracil and oxaliplatin). The single herbs that were used included Radix Astragali, ginseng, Atractylodes macrocephala, Poria cocos, Coix lachryma-jobi and Sophora flavescens. In total, 13 out of an initial 1,487 studies were included. In this meta-analysis, tumour response was defined as a CR or PR. A significant improvement in tumour response was

observed in the combined treatment group compared with the FOLFOX 4 only group. In terms of survival, a positive effect on overall survival was reported in 3 of the studies, but was not supported by the meta-analysis. Although there was a significant difference in favour of the group that received the TCM and FOLFOX 4 regimen, this was the result of only 3 studies. Karnofsky performance status was reported in 10 of the studies, and was significantly higher in the test groups in 9 of the studies, indicating an improved performance status of the test group. In addition, all 13 included studies reported alleviated chemotherapy toxicities. The total grade III or IV events for neutropenia, nausea, vomiting and neurotoxicity were also significantly fewer in the test groups (31).

Toad venom. Chan Su is another single herb with anticancer activity. Bufadienolides, including bufalin, cinobufagin, resibufogenin and telocinobufagin, are the major active constituents derived from Chan Su (32). Chan Su and its constituents have been reported to be able to suppress cellular growth and promote apoptosis in leukaemia, liver, lung and prostate cancers (19). The Chinese medicine preparation, Hua Chan Su, contains bufalin, resibufogenin and cinobufagin, which are derived from Chan Su (33,34). Hua Chan Su can induce the apoptosis and inhibit the growth of hepatocellular carcinoma (HCC) cell lines (35-38). In clinical studies, Hua Chan Su has been identified to improve performance status in HCC, non-small cell lung cancer and pancreatic cancer patients (38). One of the constituents, cinobufagin, when used alone or in combination with other chemotherapy agents, has also demonstrated anticancer activities in HCC, non-small cell lung cancer, pancreatic cancer and gallbladder carcinoma patients (39). The combination of Hua Chan Su and chemotherapy has been used for the treatment of advanced gastric cancer. In total, 14/15 studies reviewed by Xie *et al* (40) indicated that the combination of Hua Chan Su and chemotherapy was unable to increase the tumour RR or 1-year survival rate. However, the performance status was improved by the combined therapy. Further analysis revealed that the combination of Hua Chan Su and chemotherapy significantly reduced the symptoms of chemotherapy-induced toxicity, including leucocytopenia, gastrointestinal side-effects and neurotoxicity (40).

Shenqi Fuzheng injection. The Shenqi Fuzheng injection is extracted from the herbal medicines, Radix Astragali (Huang Qi) and Codonopsis pilosula (Dang Shen). Astragalus has been identified to exhibit marked anticancer activities. Codonopsis pilosula has certain anticancer activities that are similar to those of ginseng. A promising anticancer effect may therefore exist in the Shenqi Fuzheng injection. In clinical trials, Shenqi Fuzheng improved the tumour response and reduced chemotherapy-induced toxicity in the combined treatment of advanced cancers, including lung, breast and colorectal cancers (41,42). In addition, the Shenqi Fuzheng injection has been widely used in the treatment of non-small cell lung cancer. In a meta-analysis, 29 relevant clinical studies were selected, with 27 out of the 29 reporting a tumour RR. A significantly higher tumour RR was observed in the test group, which received Shenqi Fuzheng in addition to platinum-based chemotherapy. Furthermore, a significantly

improved performance status was observed in the test group. The chemotherapy-associated side-effects reported in the meta-analysis included grade III or IV WBC, platelet (PLT) or HB toxicity, nausea and vomiting. Significant reductions in toxicity were also observed in the pooled RR for WBC, PLT and HB toxicity, nausea and vomiting (41).

Kang Laite (KLT) injection. The KLT injection is extracted from the Chinese herb, coixseed (coicis semen yokuinin). This Chinese medicine preparation has obtained approval from the Chinese government for the treatment of lung and hepatic cancer (43). It has been reported that KLT is able to inhibit cellular mitosis during the G₂/M phase of the cell cycle and promote apoptosis via the Fas/FasL pathway (44). A phase I study indicated that there was no marked toxicity when the KLT injection was administered at the maximum dose of 50,000 mg/day in the first cycle (45). KLT has been widely used in combination with chemotherapy for the treatment of non-small cell lung cancer. In a meta-analysis that included 26 studies, the pooled analysis of 22 of the studies indicated that the combination of KLT and chemotherapy increased the RR. An improvement in the performance status, in addition to a reduction in chemotherapy-induced adverse events, were also observed in the test group. Similar effects were evident in the pooled RR for leukopenia, anemia, thrombocytopenia, nausea, vomiting and phlebitis (46).

YZXJ capsule. The YZXJ capsule is a formula derived from the collateral disease theory. The capsule consists of 16 herbs, including Radix Astragali, ginseng, Atractylodes macrocephala koidz and Poria cocos (Table II) (47). Of these ingredients, Astragalus and Panax ginseng are important single herbs with anticancer activities. YZXJ has been used in the treatment of liver, breast, lung, colorectal and gastric cancers. Preclinical studies have revealed that it exhibits various anticancer activities, including inhibition of adhesion, migration and angiogenesis, and affects the expression of proliferating cell nuclear antigen, p53 and vascular endothelial growth factor (VEGF) (13,14). Recently, its application in the treatment of various tumours was reviewed in a meta-analysis, which included 19 clinical studies. In this meta-analysis, YZXJ was revealed to be able to significantly reduce chemotherapy-induced toxicity, and the pooled odds ratio for bone marrow suppression, leukopenia, thrombocytopenia, gastrointestinal adverse reaction and hepatotoxicity (48).

Despite the promising results of the systematic reviews described in the present review, the majority of the studies have had certain limitations. Firstly, the sample sizes of these studies were relatively small, with 30-40 cases on average. Secondly, the Jadad scale quality scores of the studies were low, with the majority under 3. Thirdly, few studies described the detailed procedure of randomisation. Fourthly, the included studies were not rigorously controlled; for example, the analysis of survival time required the authors to describe how the patients lost to follow up were handled, what percentage were lost to follow-up and whether these patients were censored in the analysis. Therefore, more rigorously controlled, randomized studies are required. However, given the fact that clinical trials consume money and time,

Table II. Sources of the Yangzheng Xiaoji capsule [2010 edition of Chinese Pharmacopoeia (49)].

| Description | Access number | Scientific name | Family name | Part of source |
|------------------------------------|---------------|--|---------------|-------------------------|
| <i>Panax ginseng</i> | 1101 | <i>Panax ginseng</i> C.A. Mey. | Araliaceae | Root and rhizome |
| Membranaceus | 11008 | <i>Astragalus membranaceus</i> (Fisch.) Bge.var. <i>mongholicus</i> (Bge.) Hsiao | Leguminosae | Root |
| Fructus ligustri lucie | 11009 | <i>Ligustrum lucidum</i> Ait. | Oleaceae | Fruit |
| Radices curcumae zedoariae | 11010 | <i>Curcuma phaeocaulis</i> Val. | Zingiberaceae | Rhizome |
| <i>Ganoderma lucidum</i> | 11011 | <i>Ganoderma lucidum</i> | Polyporaceae | Cystocarp |
| <i>Gynostemma pentaphyllum</i> | 11012 | <i>Gynostemma pentaphyllum</i> (Thunb) Mak | Cucurbitaceae | Overground element |
| <i>Atractylodes macrocephala</i> | 11013 | <i>Atractylodes macrocephala</i> Koidz | Compositae | Rhizome |
| <i>Scutellaria barbata</i> D. Don | 11014 | <i>Scutellaria barbata</i> D. Don | Labiatae | Whole plant |
| <i>Oldenlandia diffusa</i> | 11015 | <i>Oldenlandia diffusa</i> (willd.) Roxb. | Rubiaceae | Whole plant |
| <i>Poria cocos</i> | 11016 | <i>Poria cocos</i> | Polyporaceae | Sclerotium |
| <i>Duchesnea indica</i> Focke | 11017 | <i>Duchesnea indica</i> Focke | Rosaceae | Whole plant |
| <i>Solanum lyratum</i> Thunb. | 11018 | <i>Solanum lyratum</i> Thunb. | Solanaceae | Whole plant |
| Herba artemisia | 11019 | <i>Artemisia scoparia</i> (Bge.) Kit. | Compositae | Overground element |
| <i>Cynanchum paniculatum</i> Kitag | 11020 | <i>Cynanchum paniculatum</i> Kitag | Apocynaceae | Root and rhizome |
| <i>Eupolyphaga sinensis</i> Walker | 12003 | <i>Eupolyphaga sinensis</i> Walker | Corydiidae | Dried bodies the insect |
| Endothelium corneum gigeriae galli | 12006 | <i>Gallus gallus domesticus</i> Brisson | Phasianidae | Chicken crop lining |

the included studies can at least identify herbal medicines worthy of further investigation.

3. Molecular and cellular machinery of TCM in the treatment and prevention of cancer

Clinical studies have demonstrated the benefits of certain herbs and herbal medications for the clinical management of malignancies. Studies have been performed in order to identify the mechanisms that underlie the therapeutic effects of these herbal remedies, but at present, have not been able to provide a solid scientific explanation. In the following section, the present review analyses the current literature in order to portray the current understanding of how herbal remedies affect cancer.

Effect of TCM on the immune system. It is believed that the immune system may be regulated by a number of different TCMs. During the development and progression of cancer, the adoptive and adaptive immune responses can be evaded by cancer cells. In addition, radiotherapy and chemotherapy can also impair the immune system, particularly through bone marrow suppression. Patients receiving radiotherapy and chemotherapy may benefit from herbal medicines due to their capacity to improve the functions of the immune system (16,24-26). Firstly, they are believed to enhance the immune response against the tumour. Secondly, they may suppress the immune inhibitory mechanisms and shift the immune system balance. Finally, they may restore the impaired immune system back to its normal condition following the administration of chemotherapy and radiotherapy.

Radix Astragali has been identified to increase interferon and TNF secretion, and activate lymphocytes, natural killer (NK) cells and macrophages against tumours. In addition, Radix Astragali has been revealed to cooperate with IL-2 in order to stimulate lymphokine-activated killer cells against tumour cells (16,24,25). Mylabris and the TJ-41 formulation have been reported to be able to restore antitumour T cells (18,24). In one study concerning TJ-41, the oral administration of TJ-41 in tumour-bearing mice under a stress-induced immunosuppression state, restored the lymphoid cells and cytokine secretion, and in addition, activated cytotoxic T lymphocytes (CTLs). The underlying mechanisms in the stress burdened tumour-bearing mice included stimulated lymphocyte proliferation, restored serum corticosterone levels, recovered surface expression of co-stimulatory molecules, enhanced IL-12 secretion, suppressed IL-4 production, a shift in the TH1/TH2 balance to a TH-1 type response and the restoration of CTLs (50). The TJ-41 formulation has also been reported to enhance immunological function (51,52). Another Chinese medicinal formula known as Juzen-Taiho-To (JTT) contains 10 single herbs and has been reported to reduce the metastatic potential of various solid tumours (53-55). One study reported that the oral administration of JTT could suppress the growth of mouse subcutaneous gliomas and increase the survival rate of the tumour-bearing mice. The potential underlying mechanisms may include the activation of NK cells and the enhanced secretion of TNF (53). Miyagami and Katayama (56) also

reported that the administration of JTT had several effects, including a reduction in the number of suppressor T cells, the promotion of TNF- α secretion and the activation of NK cells. However, the number of cytotoxic T cells was reported to be unchanged.

TCM and the apoptosis of cancer cells. Cancer cell growth depends upon the balance of cell gain and loss. The cell cycle of cancer cells occurs at a much faster rate than that of normal cells, and ultimately leads to cancer cell accumulation. By contrast, cell loss via necrosis, apoptosis and autophagy are much slower. Certain TCMs may also induce cell cycle arrest or apoptosis, and therefore inhibit cell growth and metastasis. Garlic extracts are primarily composed of sulphur compounds, particularly allicin, diallyl sulphide (DAS), diallyl disulphide (DADS), diallyl trisulphide (DATS) and ajoene, all of which exhibit anticancer activities. The antiproliferative effects of the extracts are associated with the induction of apoptosis (20,57). Morphological changes and DNA fragmentation have been observed in cells treated with DADS and DATs (58,59). Furthermore, a previous study reported that DAS induced the apoptosis of solid tumour cells, according to the results of sub-G₁ fraction and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (60). Further research has revealed the mechanisms underlying DAS-induced apoptosis, including elevated p53 and p21/waf1 expression, and a shift in the B-cell lymphoma 2 (Bcl-2)/Bcl-2 associated X protein (Bax) balance (61).

The anticancer activity of cinobufacini (Hua Chan Su) is primarily through the induction of apoptosis. In a previous study, the water-soluble constituent extracted from toad venom, cinobufacini, was revealed to suppress the growth of HCC HepG2 and Bel-7402 cell lines in a time- and concentration-dependent manner. The growth inhibition was due to the promotion of apoptosis through a mitochondrial pathway. Morphological changes, including chromatin condensation, and nuclear and cytoplasmic fragmentation, were clearly observed, particularly morphological changes in the disturbance of the transmembrane potential. In addition, increased Bax and decreased Bcl-2 expression was observed following cinobufacini treatment, which led to an increased Bax/Bcl-2 ratio. Furthermore, poly ADP ribose polymerase cleavage was observed together with cytochrome release and caspase-3 and -9 activation (35).

TCM induces cell cycle arrest. Bu-Zhong-Yi-Qi-Tang (BZYQT) is a mixed formulation, with major constituents of astragaloside IV, ginsenoside Rb1 and Rg1, saikosaponin a and c, and glycyrrhizin. The effects of the formula on liver cancer Hep3B, HepG2 and HA22T cells were analysed in a previous study (62). The results of the study revealed that the whole mixture of BZYQT, or a mixture of the major compounds rather than any single constituent in the mixture, inhibited liver cancer cell growth, particularly in the Hep3B cells. The cell cycle G₀/G₁ ratio increased in proportion following treatment with BZYQT, and finally lead to G₀/G₁ arrest. In addition, apoptotic cells and evidence of DNA fragmentation were clearly observed. Therefore, BZYQT was established to inhibit hepatoma cell growth by inducing apoptosis through G₀/G₁ arrest.

A previous study described the effects of the Sho-Saiko-To formulation on a number of ovarian cell lines (63). In the study, all six ovarian cancer cell lines exhibited a response to

Sho-Saiko-To. Glycyrrhizin, baicalin, and baicalein are three constituents of Sho-Saiko-To, and are all able to inhibit cancer cell growth (64-67). In another study, Sho-Saiko-To exerted similar activity, in which apoptosis was involved in the growth inhibitory effect at different concentrations; apoptosis was induced at a concentration of 1,000 $\mu\text{g/ml}$, while cell death via necrosis occurred when the concentration increased to 5,000 $\mu\text{g/ml}$. Although the precise underlying mechanism was unclear, one of the ingredients of Sho-Saiko-To, namely baicalein, was reported to be able to inhibit the activity of type II topoisomerases and induce the apoptosis of the cells (68). The Sho-Saiko-To mixture itself is able to inhibit hepatoma cell growth by inducing apoptosis and cell cycle arrest (63,69).

Constituents of the garlic extract have also been identified to be actively involved in the cell cycle arrest of tumour cells. DATs and DADs are able to induce G_2/M mitotic arrest. In addition, DATs have been demonstrated to induce the Ser(216) phosphorylation of Cdc25c, promote the generation of reactive oxygen species, and increase the cell division cycle inhibition of p21 (70), while DAPs have been established to inhibit p34cdc2 kinase activity and the growth of H-ras oncogene-transformed tumours (71).

Cantharidin is the active component of Mylabris. Previous data has revealed that it inhibits certain PPP family phosphatases, which have antitumour activity, and promotes apoptosis in several cancer cell lines. Flow cytometry analysis revealed that cantharidin slowed the progression of the cell cycle following DNA replication. However, prior to apoptosis, G_1-S or $S-G_2$ phase progression was not delayed. By contrast, cantharidin inhibited cell growth during G_2 or early M phase. However, the cell cycle arrest occurred prior to the end of mitosis and was accompanied by the appearance of abnormal mitotic spindles. Using time-lapse microscopy, cantharidin was observed to interrupt chromosome location in metaphase, extend the mitotic arrest and lead to apoptosis rather than anaphase. Further suppression of phosphatase PP2A α , but not PP2A β , induced the arrest of metaphase. During this period, the chromosomes moved between the spindle and metaphase plate (72).

TCM regulates the adhesion and motility of cancer cells. Cancer cell adhesion and invasion are important steps in the process of cancer metastasis. While relatively few TCMs have been reported to be able to affect these steps, our institute (Cardiff University - Peking University Cancer Institute) has focused on metastasis and angiogenesis for decades, and has recently identified that the YZXJ formulation is effective in inhibiting cancer cell adhesion, migration and angiogenesis *in vitro* and *in vivo* (12-14). The YZXJ capsule consists of 16 herbs, including Panax ginseng, Astragalus, Ligustrum, Curcuma phaeocaulis and Ganoderma lucidum (Table II). Its extract, DME25, has not demonstrated any significant effect on the growth of cancer cells, but it does markedly suppress cell adhesion and migration. The results of an electric cell-substrate impedance sensing assay revealed that YZXJ inhibited the cell adhesion of gastric cancer HGC27 cells (in a concentration-dependent manner), colorectal cancer HRT18 cells, breast cancer MCF7 cells, lung cancer A549 cells and osteosarcoma MG-63 cells, and also the migration of the lung cancer and colorectal cancer cells (12). In addition, it was

verified that the inhibitory effect of YZXJ on the adhesion of cancer cells was associated with the PI3K signalling pathway. Wortmannin, an inhibitor of the activity of PI3K, is able to suppress PI3K/AKT signalling and consequently reduce the adhesiveness of cancer cells. DME25, which also targets the AKT pathway, can enhance this inhibitory effect. The effect of DME25 on the PI3K pathway may not only depend upon the single AKT signalling pathway (13). The identification of the effect of DME25 on vascular endothelial cells demonstrated that YZXJ markedly suppressed the formation of the canaliculi of vascular endothelial cells, so that the cell matrix adhesion and migration was inhibited in a concentration-dependent manner. Cell matrix adhesion is an important indicator of cellular migration. In addition, cellular adhesion and migration are important during angiogenesis, particularly during canaliculus formation by vascular endothelial cells, when they adhere to the cell matrix and subsequently migrate into the extracellular matrix. Hence, the inhibitory effect of YZXJ on the adhesion and migration of vascular endothelial cells may be the principal mechanism for inhibiting canaliculus formation (14).

Previous studies have revealed that the focal adhesion kinase (FAK) signalling pathway is important for cell matrix adhesion and cell-extracellular matrix adhesion (73-75). Following the connection of integrins and matrix, a series of cellular events are triggered and activated, particularly the FAK signal pathway, in the interaction between cells and matrix (76). Subsequently, the interaction between integrins and the cell cytoskeleton system is triggered (76). It has been reported that DME25 exhibited an inhibitory effect on the phosphorylation of the FAK pathway, and that the addition of a FAK inhibitor significantly enhanced the effect of DME25 on the FAK pathway (77). In addition, it was revealed that YZXJ not only inhibited the growth of colorectal and lung cancer cells, but also suppressed the formation of mouse peritoneal tumour nodules in an *in vivo* study. The significant inhibition of tumour growth was observed following either the oral administration or intraperitoneal (i.p.) injection of YZXJ. The results of FAK and pFAK immunofluorescence colony staining revealed that YZXJ lowered the expression of FAK and almost inhibited its activation following the administration of DME25 in combination with a FAK inhibitor. Based on the study of the effect of YZXJ on the adhesion of tumour and mesothelial cells, a further study demonstrated that peritoneal tumour nodules treated with YZXJ through i.p. injection were significantly decreased compared with those of the untreated control group (unpublished experimental data). Therefore, on the basis of the results from previous studies, which demonstrated that YZXJ was involved in the adhesion between tumour cells and mesothelial cells, and in tumour cell migration and angiogenesis through the analysis of an *in vivo* model, it can be hypothesized that YZXJ has an inhibitory effect on peritoneal metastasis.

4. Targeting angiogenesis in solid tumours

The process of angiogenesis, whereby new blood vessels develop from pre-existing ones, is important during normal development and homeostasis. Angiogenesis is a key process involved in a number of physiological events, including wound

healing, development, reproduction and growth. Under normal circumstances, this process is tightly regulated, however, abnormal levels or poor control are apparent in a number of different disease states, including cancer. Angiogenesis is important for the progression of cancer and the development of tumours where, without an independent blood supply, tumour size is limited by its reliance on simple diffusion in order to obtain resources and remove metabolic waste. This has long been realised, and has led to the development and implementation of anti-angiogenic strategies for the treatment of cancer (78,79). As discussed in the present review, the use of traditional medicines or herbal extracts in the treatment of diseases, such as cancer, has been long established and practiced. Scientific scrutiny of these medicines and extracts has begun to demonstrate their efficacy in important physiological processes, such as angiogenesis, and therefore highlight their importance as treatment regimes.

TCM/herbs implicated in tumour angiogenesis. The mechanisms through which various herbal medicines exert their anticancerous roles have begun to be elucidated. In addition to their direct effect on cancer cells, a number of herbal medicines have also been demonstrated to exhibit an impact upon tumour progression through their effects on angiogenesis. One such remedy displaying anti-angiogenic effects is the standardised *Ginkgo biloba* extract, EGb 761[®]. Extracts from *G. biloba* have demonstrated inhibitory effects or chemopreventative properties *in vitro/in vivo* in ovarian cancer (80), breast cancer (81), hepatocellular carcinoma (82) and oral cavity cancer (83) cells. A study by Koltermann *et al* (84) identified an anti-angiogenic role for the standardised extract of *G. biloba*, EGb 761. In this study, a marked anti-proliferative effect of EGb 761 on endothelial cells was observed, in addition to a significant inhibition of endothelial cell migration and tubule formation on Matrigel following treatment with EGb 761. These anti-angiogenic *in vitro* effects were mimicked *in vivo*, where the induction of angiogenesis in a chorioallantois membrane assay was inhibited following EGb 761 treatment (84). A role was established for EGb 761 in enhancing the activity of tyrosine phosphatases, including SHP-1, which in turn prevented signal transduction through the Raf/MEK/ERK pathway in response to growth factor stimulation (fibroblast growth factor or VEGF) (84).

Scutellaria barbata D. Don is another herb frequently used in TCM. Extracts from *S. barbata* have demonstrated inhibitory effects on a number of cancer cells *in vitro* (85-87). The therapeutic potential of extracts from this herb have been further demonstrated through a phase 1B dose elevation clinical trial for an orally delivered aqueous extract, BZL101, in metastatic breast cancer patients, in which it was revealed to be well tolerated and demonstrated promising clinical evidence (88).

Extracts from this herb have also exhibited anti-angiogenic roles. In a recent study by Dai *et al* (88), the potential of the total flavonoids of *S. barbata*, TF-SB, to inhibit angiogenesis *in vitro* and *in vivo* were analysed. In this study, TF-SB was identified to have dose-dependent inhibitory effects on the proliferation, migration and tubule formation of human umbilical vein endothelial cells *in vitro*. Similar effects were observed *in vivo* using a chorioallantois membrane assay, whereby treatment with TF-SB significantly decreased the number of blood vessels formed compared with the control groups. The study further established that TF-SB treatment affected the expression of and

decreased the levels of VEGF, a trend that was also correlated with the treatment concentration (89). This result is consistent with that of an earlier study, which revealed an inhibitory effect of *S. barbata* extract on endothelial cell migration and proliferation (90). This study also demonstrated that *S. barbata* significantly decreased the transcriptional activity of hypoxia inducible factor-1 α and the expression of VEGF in lung cancer cells, delayed the tumour burden, and reduced the capillary density in an *in vivo* model (90). An ethanol extract of *S. barbata* has also proven to be effective in reducing tumour volume in colorectal xenograph mice, a finding that was accompanied by a reduction in tumour microvessel density (91). The treatment of mice with this extract also reduced the expression of a number of molecules associated with the sonic hedgehog (SHH) signalling pathway in tumour tissues, and downregulated the expression of VEGF-A and VEGF receptor 2, which indicated that the inhibition in colorectal cancer cell growth *in vivo* following treatment with *S. barbata* may be due to its inhibitory effects on SHH-mediated angiogenesis (91). Quercetin is a type of flavonoid that is generally extracted from the *G. biloba* leaf it reduces the viability of vascular endothelial cells and the formation of tubules, in which the ERK pathway may be involved (92).

YZXJ in tumour angiogenesis. A previous study assessed the efficacy of the TCM formula, YZXJ, for its potential to affect the angiogenesis profile (14). In this study, the impact of YZXJ on key angiogenic traits, including cell growth, matrix attachment, migration and tubule formation, were examined in the human endothelial HECV cell line. YZXJ was identified to have an inhibitory effect on endothelial tubule formation, and significantly inhibited this process at sub-cytotoxic concentrations (dilution, 1:1,000) compared with untreated control cells. Similarly, YZXJ was also identified to inhibit endothelial cell migration, another important process involved in the angiogenic cascade, in a concentration-dependent manner, and to significantly inhibit cell-matrix attachment. Therefore, this study demonstrated an anti-angiogenic role for YZXJ in these model systems. A further study was also conducted in order to investigate the mechanistic side of these effects and to identify potential associations with FAK. The inhibitory effects of YZXJ on cellular migration and matrix adhesion were synergistically increased following the inhibition of FAK, bringing about stronger inhibition of these key processes when YZXJ treatment was combined with a small molecule FAK inhibitor. Furthermore, treatment with YZXJ, whilst not having a substantial impact on FAK staining intensity, substantially reduced the staining intensity of p-FAK upon immunofluorescence staining analysis. p-FAK staining was further decreased through a combination of YZXJ treatment and FAK inhibition. Furthermore, western blot analysis demonstrated a decrease in tyrosine-phosphorylated FAK in HECV cells that had been treated with YZXJ. These results identified an anti-angiogenic role for YZXJ, and suggested that this may be induced, at least in part, by its impact on the FAK signalling pathway (14).

Anti-angiogenic therapies have marked potential for the treatment of cancer. A number of these therapies are currently implemented and demonstrate efficacy in the treatment of various cancers (93). The potential of herbal extracts and TCMs to exhibit anti-angiogenic properties, together with their ability to affect tumour cell function directly, further highlights the

importance and potential of these remedies in the development of novel therapeutic strategies to target cancer progression.

5. Herbal therapy for precancerous disorders

Cancer may develop from the progression of precancerous lesions and conditions. Precancerous lesions, for example, high-grade squamous intraepithelial lesions caused by infection with human papillomaviruses (HPVs) 16, 18, 45, 31 and 33, can develop into squamous cell carcinoma, whilst another strain of HPV, HPV 18, predominates in adenocarcinomas (94). Precancerous conditions or premalignant conditions, including actinic keratosis, Barrett's oesophagus, atrophic gastritis and oral submucous fibrosis, are generally considered to possess a significantly increased risk of cancer. Two decades ago, investigators began to examine the preventative effects of herbal formulas on different precancerous conditions. An anticancer 2 tablet, composed of six TCM herbs, was assessed for the treatment of epithelial dysplasia in the cheek pouches of hamsters that had been exposed to a carcinogen (95). *Radix curcumae* is one of the commonly used TCM herbs for the treatment of malignancies. Its extract is able to reduce precancerous lesions of the stomach induced by N-methyl-N'-nitro-N-nitrosoguanidine (96). Herbal supplements, consisting of quercetin, curcumin, silymarin, ginseng and rutin, that are added to the diet can be beneficial to patients with precancerous lesions of the large intestine (97). This herbal mixture has been shown to suppress aberrant crypt foci in an azoxymethane-induced rat colon cancer model, and also to induce apoptosis via the regulation of Bax (proapoptotic) and Bcl-2 (anti-apoptotic), ultimately leading to caspase-9 activation. Clinical trials using Zeng Sheng Ping (ZSP) to treat oesophageal epithelial dysplasia have been performed in two Chinese districts with a high risk of oesophageal carcinoma (98,99). The results from these clinical trials have revealed the benefits of ZSP, which can reduce the incidence of cancer in high-risk groups with severe oesophageal epithelium dysplasia by 34.5-47.79% over a follow-up period of up to 9 years. A further study demonstrated that ZSP exhibited chemopreventive effects in patients with oral leukoplakia, and also affected tumour development in two animal models of oral cancer (100). YZXJ has also been reported to be able to improve atypical dysplasia of the stomach (47). There have been a number of clinical studies that have established effects on precancerous conditions or lesions. However, the quality of these clinical studies was such that they were unable to validate the findings and therefore, further clinical trials are warranted. In addition to the requirement for improved clinical studies, the underlying mechanisms, for example, the effect on oncogenes, tumour suppressor genes and key pathways involved in tumorigenesis are yet to be investigated.

6. Perspectives

Increasing evidence has demonstrated that herbal remedies from TCM have the potential to be developed into therapeutic approaches for the clinical management of various malignancies. The corresponding effects and benefits include its ability to act as an effective therapy for certain malignancies, reduced side-effects when combined with chemotherapy/radiotherapy, a chemopreventive effect for certain precancerous

conditions/lesions, improved quality of life and prolonged survival. However, a number of limitations and challenges exist that reduce the progress of studies on the use of TCM remedies for the treatment of cancers. These limitations include the liability and variability of the herbal medications, a lack of reliable biomarkers for clinical and laboratory-based studies, a lack of relevant pharmacokinetic data for standardising dosage, and a lack of reliable data from well-designed clinical studies to further establish subsequent clinical trials. For example, one previous clinical trial was terminated following the contamination of a patented mixture of eight herbs with warfarin and indomethacin (101). An improvement in the set-up of clinical studies is critical in order to provide solid evidence for subsequent clinical trials. The Best Case Series programme from the National Cancer Institute has provided a standardised process to validate results using rigorous scientific methods, particularly for the studies of conventional medicine (102,103). In addition, a requirement exists to consider the side-effects and toxicities of herbal medications (104) in the evaluation of any clinical studies.

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