Role of Huaier Extract as a Promising Anticancer Drug

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Huaier, which is also called Trametes robiniophila murr, has been applied in cancer complementary therapy in mainland China. Many experimental and clinical studies have been conducted to confirm anticancer effect of Huaier extract. Huaier extract could inhibit tumor growth, induce apoptosis and reverse drug-resistance of tumor cells, and performs immunomodulatory and anti-angiogenic functions. Huaier granule, product of Huaier extract, is widely used in the treatment of leukemia, osteosarcoma, malignant lymphoma, breast cancer, lung cancer, rectal cancer, liver cancer, gastric cancer, colon cancer, pancreatic adenocarcinoma and so forth clinically. Multicenter clinical trials are necessary to confirm the clinical uses of Huaier extract for cancer treatment.

Key Words: Huaier, apoptosis, metastasis, angiogenesis

Introduction

Cancer has already become the leading cause of death worldwide. In 2008, there are 7.6 million cancer deaths (around 13% of all deaths) worldwide, and about 70% happened in low- and middle-income countries. Lung (1.37 million deaths), stomach (736 000 deaths), liver (695 000 deaths), colorectal (608 000 deaths), breast cancer (458 000 deaths) and cervical cancer (275 000 deaths) result in the most cancer deaths each year, while the most frequent types of cancer differ between men and women (44). Among women, breast cancer is the leading cause of cancer death, as well as the most frequently diagnosed malignant tumor. In economically developing countries, cervical cancer is the most cancer death reason previously, while now is breast cancer. In male, lung cancer is the leading cancer, and in developing countries it occupies the similar mortality burden to cervical cancer. Liver cancer in male is the second most frequent cause of cancer death, though it is diagnosed the fifth most frequent cancer worldwide (24).

There are multiple strategies for cancer treatment conventionally, including surgery, radiotherapy, chemotherapy, hormonetherapy, transplantation and some complementary therapies. However, current anticancer treatments are not able to completely prevent the recurrence and metastasis for cancer patients, therefore new treatment strategies and new drugs are urgently needed for these patients. As for the complementary therapies, traditional Chinese medicine (TCM) has become more and more popular because of its properties and effects for cancer treatment (37).

Traditional Chinese medicine (TCM) has a long history of three millennia and contains a variety of complex compounds of natural origin. The Chinese patent drugs, products of traditional Chinese medicine, develop quickly and have been widely used in clinical anticancer treatment in mainland China. Nowadays tumor has been defined as chronic disease, and long-term complementary treatments are nearly inevitable. Chinese patent drugs are normally much cheaper and easy to use, compared with the conventional antitumor western medicine, which contributes to the popularity of TCM to some extent. Moreover, traditional Chinese medicine emphasizes on adjusting human body functions, enhancing body immunity then activating anticancer ability, thus improve the quality of life and prevent tumor metastasis and recurrence after operation, radiotherapy and chemotherapy (33, 64).

Traditional Chinese anti-neoplasms herbs have been widely studied worldwide. For example, Wogonin, derived from the traditional Chinese medicine Huang-Qin (Scutellaria baicalensis Georgi), was investigated and showed anticancer effects in human breast cancer (7), human hematopoietic malignancies (1, 28), as well as many other cancers (30). Ginseng was proved to improve the quality of life in breast cancer patients (8), liver cancer and many other gynecological neoplasms (27). Docetaxel could be useful in the treatment of advanced esophagogastric cancer (15), breast cancer (2) and ovarian cancer (48). Huaier (Trametes robiniophila murr) is an officinal fungi in China and has been used for approximately 1600 years (32), many experimental and clinical studies
have been conducted to confirm anticancer effect of Huaier extract.

Huaier and Its Main Components

The effective ingredient of Huaier extract was proteoglycan, which consists of 41.53% polysaccharides, 12.93% amino acids and 8.72% water (19, 20) (Table 1). The proteoglycan is the most effective anticancer element among all of the isolated ingredients of Huaier extract, which was confirmed on breast cancer MCF-7, liver cancer H22, lung cancer Lewis and sarcoma murine S180 cells (unpublished data). However, the inhibitory effect of proteoglycan was less effective than the Huaier extract (19, 20), indicating that the anticancer activity of Huaier extract is associated with the synergistic or additive effect of these fractions. Here we focused on Huaier extract but not the main ingredient, since the components in herbs probably works together, though the ingredient is important for research as well.

Table 1. The chemical composition of proteoglycan extracted from Huaier

<table>
<thead>
<tr>
<th>A.A.</th>
<th>percent</th>
<th>A.A.</th>
<th>percent</th>
<th>Composition of Polysaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>1.418</td>
<td>Met</td>
<td>0.120</td>
<td>L-fucose 0.51</td>
</tr>
<tr>
<td>Thr</td>
<td>0.731</td>
<td>Ile</td>
<td>0.398</td>
<td>L-arabinose 1.15</td>
</tr>
<tr>
<td>Ser</td>
<td>0.626</td>
<td>Leu</td>
<td>0.569</td>
<td>D-xylose 1.48</td>
</tr>
<tr>
<td>Glu</td>
<td>3.525</td>
<td>Tyr</td>
<td>0.249</td>
<td>D-mannos 1.39</td>
</tr>
<tr>
<td>Pro</td>
<td>0.740</td>
<td>Phe</td>
<td>0.347</td>
<td>D-galactos 1</td>
</tr>
<tr>
<td>Gly</td>
<td>1.073</td>
<td>Lys</td>
<td>0.689</td>
<td>D-glucose 3.24</td>
</tr>
<tr>
<td>Ala</td>
<td>0.662</td>
<td>His</td>
<td>0.267</td>
<td></td>
</tr>
<tr>
<td>Cys</td>
<td>0.160</td>
<td>Try</td>
<td>0.097</td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>0.652</td>
<td>Arg</td>
<td>0.645</td>
<td></td>
</tr>
</tbody>
</table>

Experimental and clinical studies have revealed various anticancer activities of Huaier extract including apoptotic induction, antiangiogenesis, antimetastasis, drug resistance reversal and systemic immune activation (25, 32).

Apoptotic Induction

Apoptosis induced by Huaier extract has been studied in human breast cancer cells (59, 61), pancreatic adenocarcinoma cells (68), ovarian cells (54), hepatocellular carcinoma cells (38, 53), gastric carcinoma cells (51), leukemia cells (47) and so forth. Meanwhile the G0/G1 phase cell-cycle arrest induced by Huaier extract was widely observed (47, 54, 61, 68). We have confirmed that Huaier extract arrests the cell cycle at the G0/G1 phase and induces apoptosis through p53 accumulation and activation in ER-positive MCF-7 breast cancer cells, which express wild-type p53, leading mitochondrial-mediated apoptosis (61). However, in ER-negative MDA-MB-231 breast cancer cells (mutant-type p53), the p53 expression does not increase, and Huaier cannot induce G0/G1 phase cell-cycle arrest, probably because the p53 in this cell line is in mutant form and dysfunctional (61). Meanwhile, we found that Huaier extract inhibits cell viability and mobility in both ER-positive and ER-negative cell lines (61). Ren et al. reported that after the treatment of Huaier extract, the apoptosis of Hep-G2 cells, a human hepatocellular carcinoma (HCC) cell line, is enhanced in a concentration- and time-dependent manner (38). Their study presents that the expressions of p53 and Bcl-2 are significantly decreased and Bax expression is increased after the treatment of Huaier, suggesting that Huaier extract can induce apoptosis of tumor cells by decreasing the p53 and Bcl-2 expressions and enhancing the Bax expression (38). Xu et al. also have reported that Huaier can protect liver from chemical injury and furthermore HCC development, possibly associated with the down-regulation of p53 (53).

Another important element, Caspase-3, plays a central role in the execution-phase of cell apoptosis, and Huaier-induced activation of caspase-3 in cancer cells has been observed (61, 68). Wu and his colleagues (51) found that Huaier extract inhibits the expression of surviving mRNA in human gastric carcinoma cells in a concentration-dependent manner, and may result in the up-regulation of caspase-3, since survivin can inhibit the activity of caspase-3 in direct and indirect ways (39, 60).

Antiangiogenesis and Inhibition of Invasion and Metastasis

Cancer angiogenesis plays an important role in the development of cancer, and angiogenesis inhibition is a promising strategy for the treatment of cancer (14). Much preclinical evidence indicates that com-
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bining antiangiogenic agents with cytotoxic agents results in additive or even synergistic antitumor effects (16). Several studies have reported that Huaier extract can inhibit angiogenesis both in vitro and in vivo. Xu et al. found (52) that Huaier extract significantly inhibits the angiogenic properties, including the proliferation and differentiation, in human umbilical vein endothelial cells (HUVEC). Chen et al. (4) showed that Huaier apparently reduces the proliferative ability, inhibits motility, adherence ability, and the formation of blood vessels. We and others have revealed that Huaier extract can inhibit the mobility and invasion of cancer cells (61, 63) in vitro. Zhang et al. revealed that Huaier extract PT-S can regulate the mRNA expression of some angiogenic-related genes in human high-metastatic large cell lung cancer cells, including the upregulation of β-catenin, E-cadherin, TIMP-1, endostatin and MMP-2, and the downregulation of VEGF and CD44V6 (63). These results indicate that Huaier extract may suppress the invasion and metastasis of cancer cell through inhibiting the expression of cell adhesion molecules (CAM) (14).

In vivo study has showed that combination treatment with Huaier extract significantly decreases the microvessel density (MVD) and VEGF expression in HCC tumor tissues (38), indicating that Huaier can inhibit the VEGF expression and tumor angiogenesis. In tumor-bearing HCC model (38) and breast cancer model (59), average tumor volume and growth rate of the implanted tumor are significantly decreased, while the necrosis rate increases significantly after Huaier treatment. These results suggest that Huaier can suppress the tumor growth and stimulate the tumor necrosis, which is highly related to the antiangiogenesis properties and apoptosis induction of Huaier extract on tumor cells.

Drug Resistance Reversal

Drug resistance is one of the main obstacles to successful cancer therapy, and is often associated with multidrug resistance (MDR). Intrinsic or acquired resistance to commonly used therapeutic agents is a major challenge in the treatment of cancer patients, since some cells in cancerous tumors may develop resistance to the drugs used in chemotherapy (21). Many mechanisms are involved in this process, including increased efflux of drug (as by permeability glycoprotein), enzymatic deactivation, decreased permeability, altered binding-sites and alternate metabolic pathways. One of the most studied mechanisms is the up-regulation of multidrug resistance gene-1 (MDR-1) and its product permeability glycoprotein (P-gp), since efflux is a significant contributor for the drug resistance in cancer cells, and cancer cells often acquire the drug resistance due to up-regulation as observed (17).

It is reported that Huaier extract can increase the sensitivity of cancer cells to chemotherapy drugs. Huang et al. (22) found that Huaier extract reverses the cisplatin resistance, and increases cisplatin sensitivity in the chemotherapy of human adenocarcinoma cell A549. Chen et al. (6) revealed that Huaier extract dramatically augments the sensitivity of both human hepatic cancer cells to TRAIL (tumor necrosis factor related apoptosis-inducing ligand), but only shows slight cytotoxicity on human hepatocyte strain. Several studies have been made to explore the mechanisms of drug resistance reversal of Huaier extract on tumor cells (54, 62). Zhang et al. (62) demonstrated that Huaier extract can increase the sensitivity of human gynecologic cancer cells to chemotherapy drug since it lowers the expression of the drug-resistant gene MDR-1 and then reverses the drug resistance. Xu et al. (54) showed that Huaier extract can reverse the tamoxifen-resistance of gynecologic cancer cells in vitro, and the mechanism may relate to the down-regulation of Phospho-P44/42 (ERK1/2) in mitogen-activated protein kinase (MAPK) pathway, since the activation of MAPK pathway and its downstream element AP-1 influences the expression of P-gp (11, 18, 49).

Systemic Immune Activation

Another advantage of Huaier as an anticancer drug is that, it can improve and activate systemic immunity. Normally conventional anticancer therapies rely on killing dividing cells or block cell division, and these therapies have severe effects on normal proliferating cells, so that the treatment causes significant morbidity. Immune responses to cancers may be specific for tumor antigens and will not damage most normal cells, thus immunotherapy has the potential as a promising strategy for cancer treatment (42). The immunity to tumor includes cell-mediated immunity and humoral immunity, and the former plays more important roles than the latter. Immune cells that are capable of killing tumor cells consist of cytotoxic T lymphocytes, natural killer cells, and activated macrophages (3).

NK cells are considered as a major component of the anticancer immune response and are involved in regulating cancer progression and metastases in animal models (13, 43). Studies have reported that Huaier granule can enhance NK cell activity in gastric cancer patients undergoing postoperative concurrent radiochemotherapy (25) and breast cancer patients received Huaier combined chemotherapy (10). It was noted that CD4+/CD8+ T cells are significantly increased in Huaier groups than control groups, and the
changes may be related to the enhancement of immunity in cancer patients (10, 25). It is widely known that CD4^+ T cells are divided into two subgroups, Th1 and Th2. Th1 cells secrete IFN-γ, IL-12, TNF-β and others, mediating cell-mediated immunity and antitumor activities. Th2 cells can secrete IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13, mediating humoral immunity and immune tolerance. The antitumor effect of CD4^+ T cells mainly relies on some cytokines such as IFN-γ, which are secreted by Th1 cells, and the Th1/Th2 balance is quite important for normal intrinsic antitumor activities (12, 40). You et al. (57) found that Huaier extract PS-T improves the IFN-γ and Th1 cell levels meanwhile decreases the IL-4 and Th2 cell levels, compared to the control group in the patients with non-small cell lung cancer. Wang et al. (46) showed that Huaier extract promotes the secretion of Th1-type cytokines IL-2, IL-4, IL-10 and IFN-γ, in breast cancer patients. Huaier extract PS-T increases the number of IL-2R^+ cells in liver, strengthens cell-mediated immunity and restrains cancer effectively (35). Another study also revealed that Huaier extract contributes to the Th1/Th2 shift in primary hepatic cancer (PHC) patients after surgical resection (45). All of these studies demonstrate that Huaier extract displays positive anticancer immunomodulatory effect by improving the expression levels of Th1-type cytokines. The results suggest that Huaier extract is hopeful to be a comprehensive therapy to improve the anticancer immunological function in cancer patients.

**Clinical Practice**

Huaier granule (trade name: Jinke), the product of Huaier extract, has been widely used in clinic in mainland China and proved to be effective in improving the prognosis and quality of life of patients with various human cancers.

**For Lung Cancer Treatment**

Research have showed that Jinke can significantly improve the quality of life, prolong the life time in elderly patients with advanced non-small cell lung cancer (34), and enhance the immunity in patients during chemotherapy (56) or after surgery (66) as an adjuvant.

**For Breast Cancer Treatment**

In a cohort of breast cancer patients before operation, Chen et al. showed that Huaier granule combined with chemotherapy significantly improves the remission rate in patients with breast cancer compared to control group with chemotherapy alone (5). After operation, Huaier granule has also been demonstrated to significantly improve the quality of life and prolong the life time in patients with breast cancer (50, 67).

**For Liver Cancer Treatment**

Jiang et al. reported that Huaier treatment works well in patients with intermediate and late primary liver cancer, and may demonstrate more clinical value in liver cancer patients with a history of hepatitis B (26). Huaier granule can increase the 2-year tumor-free survival rate, restrain the recurrence and metastasis of hepatocellular carcinoma (HCC), and meanwhile does not increase the incidence of immune rejection, in postoperative patients with liver transplantation, suggesting that Huaier treatment is safe and effective for HCC patients with liver transplantation (23, 36).

**For Gastric Cancer Treatment**

As an effective adjuvant during the postoperative chemotherapy, Huaier granule improves the chemotherapy endurance, enhances the immunity and prolongs the life time in patients with gastric cancer, (25, 55). Tang et al. also found that Huaier Granule obviously relieves the symptoms and improves the quality of life in elderly patients with advanced gastric cancer (41).

**For Other Cancer Treatment**

Zhao et al. have reported that Huaier shows good efficacy on re-chemotherapy in recurrent non-Hodgkin’s lymphoma (NHL) (65). Studies investigated the impact of Huaier granule on several thoracic malignancies including esophageal carcinoma, cardiac carcinoma and adenocarcinoma, and the results revealed that Huaier granule can promote apoptosis of cancer cells (31) and modulate the immunity in these patients (66). Huaier granule significantly enhances the immunity, improves the hematopoietic function and reduces the toxicity when combined with chemotherapy in postoperative patients with colorectal cancer (58).

**Discussion**

Traditional Chinese medicine (TCM) has a history of thousands of years, and some Chinese Herb have showed favorable anticancer activities with high efficiency and low toxicity (9, 29). Huaier (Trametes robiniophila murr) belongs to officinal fungi with a history of 1600 years, which is of natural origin. The anticancer properties of Huaier extract have been investigated and applied in complementary therapy for decades (32). Preliminally experimental studies
indicate that Huaier extract shows effective anticancer activities, including apoptotic induction, antiangiogenesis, anti-metastasis, drug resistance reversal and systemic immune activation (25, 32). Clinical practice also confirms that Huaier treatment is effective and safe for various cancers (32), and the adaptive changes such as altered immunity are also observed after Huaier administration as mentioned above. Traditional Chinese medicine (TCM) is an important player in adaptive medicine, and we believe that Huaier granule is a promising antitumor TCM that contributes to the integrated control of body functions, and will work as an effective adjuvant in complementary anticancer therapy. Intensive studies are expected to investigate the potential active constituents and the anticancer mechanism of Huaier extract, and multicenter studies are necessary to fully explore the clinical value of Huaier treatment for human cancer.

Conflicts of Interest

The authors declared that no competing interests exist.

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