

The Mechanism of Action and Research Progress of Xuefu Zhuyu Decoction Against Liver Cancer

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Abstract: Xuefu Zhuyu decoction comes from Wang Qingren's "Medical Forest Correcting Mistakes" which can be used for the treatment of qi stagnation and blood stasis, and is currently widely used in the treatment of liver fibrosis and liver cancer. This article summarizes the mechanism of anti-liver cancer from six aspects, including regulating the cell cycle, inhibiting inflammatory factors, enhancing antioxidant capacity, controlling tumor angiogenesis, regulating related signaling pathways, and regulating the body's immune ability, and finds that Xuefu Zhuyu decoction has the effects of inhibiting cell proliferation, promoting apoptosis, enhancing the body's antioxidant capacity, resisting the occurrence of inflammation, inhibiting liver cancer neovascularization, and improving the body's immunity, and participates in the regulation of signaling pathways, such as Wnt/ β -catenin、TLR4/NF- κ B、p38MAPK/ERK1/2. It's further confirmed that Xuefu Zhuyu decoction has anti-liver cancer effects in the molecular mechanism. It was found that this side has the effect of relieving patients' symptoms, improving patients' survival time and quality of life, from the clinical application of anti-liver cancer. Through the analysis of the current situation of the treatment of liver cancer, the shortcomings of today's research are put forward, which provides the latest ideas and basis for the in-depth research and clinical application of this side in the future.

Primary liver cancer, a cancer of the digestive system that is the third leading cause of death worldwide, accounts for about 906,000 new cases and 830,000 deaths annually. The incidence of liver cancer in China is about twice the world average. Hepatocellular Carcinoma (HCC) is a kind of primary liver cancer, and its survival rate after diagnosis is much lower than that of other cancers. The 5-year survival rate of HCC in China is only 12.1% [1]. In most patients, HCC is associated with chronic hepatitis virus infection, alcohol abuse, or chronic liver damage from non-alcoholic hepatitis. Etiologically, fibrosis and cirrhosis associated with hepatitis B and C virus infection are the most common risk factors for the development of liver cancer. Between 1990 and 2013, Worldwide, the total number of deaths due to viral hepatitis has increased by approximately 63 per cent, which is a serious health hazard and an increased economic burden on countries. Although there are currently relevant therapeutic means, they all face problems such as low effective rate,

drug resistance, postoperative recurrence, and large side effects, which not only reduce the quality of life of patients but also are not conducive to follow-up treatment. In recent years, traditional Chinese medicine has become an important part in the prevention and treatment of liver cancer due to its unique integrity and syndrome differentiation. TCM intervention can play different beneficial roles in different stages of liver cancer, such as reducing postoperative recurrence and metastasis, alleviating adverse reactions of conventional treatment, protecting liver function, relieving symptoms, etc. [2]. Therefore, exploring the mechanism of traditional Chinese medicine is the main direction of liver cancer prevention and treatment in the future.

Xuefu Zhuyu decoction from Wang Qingren's "Medical forest correction", in the prescription to red flower, peony root, peach seeds, Chuanxiong as the king, blood circulation, smooth blood, bupleurum, fructus aurantii, Radix platycodon, Radix oxanthus as subjects, qi stagnation, which platycodon open the chest, xuan lung Qi, to the line of jiao Qi stagnation; Achyranthes bidentum guided stasis down to pass jiao qi stagnation; Shengdi, Angelica as Zuo, nourishing blood and blood, so as to promote blood does not hurt blood; In order to make licorice harmonize various medicines and prevent them from hurting the stomach, various medicines match to play the function of promoting blood circulation and removing blood stasis, regulating qi stagnation and regulating qi and blood [3]. It is widely used in the treatment of qi stagnation and blood stasis because it promotes blood circulation and removes blood stasis without damaging healthy Qi, and dregs liver and regulates Qi without depleting Yin blood. With China's attention to classic famous prescriptions, Xuefu Zhuyu decoction has been widely used in clinical practice, such as liver fibrosis, liver cancer and so on. This paper summarizes the mechanism of action and clinical application of Xuefu Zhuyu Decoction in the treatment of liver cancer, aiming to provide ideas and basis for further research, clinical application and anti-liver cancer treatment of this prescription, as shown in Figure 1.

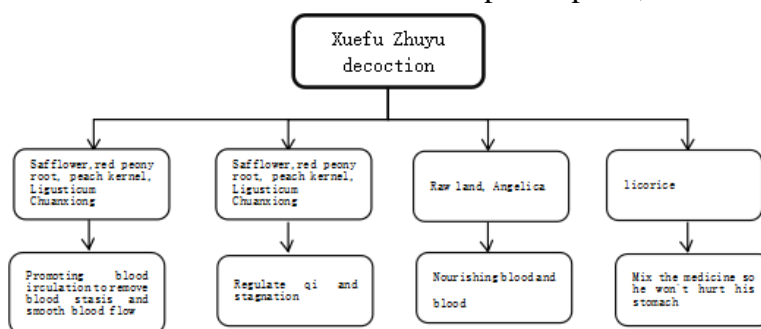


Figure 1: Xuefu Zhuyu Decoction formula solution

1. Xuefuzhuyu Decoction Anti-Liver Cancer Mechanism (Figure 2)

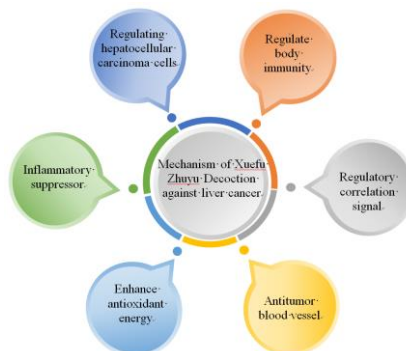


Figure 2: Mechanism of Xuefuzhuyu Decoction against liver cancer

1.1. Regulation of Biological Behavior of Hepatocellular Carcinoma Cells

1.1.1. Regulate Cell Cycle and Inhibit Cell Proliferation

The normal regulation of cell cycle is very important for the health of the body. The abnormal regulation of cell cycle is closely related to the occurrence and development of malignant tumors such as liver cancer. Studies have shown that flavonoids and esters in Xufuzhuyu decoction can block hepatoma HepG2 cells in the early stage of DNA synthesis (G1 phase), and induce cell cycle stagnation in the late stage of DNA synthesis/mitosis (G2/M phase) through AKT signaling pathway, thus inhibiting cell proliferation [4]. Proliferating Cell Nuclear Antigen (PCNA), which is proliferating cell nuclear antigen, is a cyclic homotrimer DNA clip and a key regulatory factor in DNA replication, repair, chromatin metabolism, and gene expression. Compared with healthy normal cells, PCNA expression in hepatocellular carcinoma cells is up-regulated and can be used as a marker of cell proliferation and cancer toxicity. Studies have shown that Xuefuzhuyu decoction can down-regulate the expression of PCNA by inhibiting PI3K/Akt/mTOR pathway, thus inhibiting the growth of liver cancer tumor cells.

1.1.2. Regulate Cell Cycle and Promote Apoptosis

Apoptosis is programmed cell death which is strictly regulated by the body. Inhibiting the escape of apoptosis and promoting apoptosis is the idea of anti-tumor drug development as well as the evaluation index of the efficacy of anti-tumor drugs. Reactive oxygen species (ROS) are important signaling molecules that regulate various cell functions. High levels of ROS can induce cell apoptosis by triggering pro-apoptotic signaling molecules, thus resisting the occurrence of tumors. Hui Liu et al. [4] found that flavonoids and organic acids in Xuefuzhuyu decoction could increase the generation of reactive oxygen species in HepG2 cells, in addition, elevated reactive oxygen species could also reduce mitochondrial membrane potential, thus leading to the release of cytopigment C, activation of caspase and activation of apoptosis signal. Bcl-2 (apoptosis suppressant gene) and Bax (apoptosis promoting gene) are representative genes regulating apoptosis. Studies have shown that red pae hao in Xuefuqiyu decoction can effectively reduce the expression of Bcl-2 and unlysed PARP in tumor cells, increase the level of Bax and lysed caspase3, and thus promote the apoptosis of liver cancer cells.

1.2. Inhibit Inflammatory Factors

There are a large number of inflammatory factors and inflammatory cells in the tumor microenvironment. These factors and cells are involved in the occurrence and development of malignant tumors. HCC has been proven to be an inflammatory cancer, whose inflammatory response can affect the development of liver cancer by inhibiting apoptosis, promoting angiogenesis and damaging DNA [5]. HCC is associated with changes in transforming growth factor β (TGF- β), and TGF- β signal plays a dual role in HCC, the specific mechanism of which is shown in Figure 3 and Figure 4. TGF- β is a central regulator of chronic liver disease. TGF- β signaling pathway plays a key role in cell proliferation, apoptosis, differentiation, motility, lineage specificity, and normal stem cell homeostasis. This is mainly through the activation of TGF- β receptors (TGFBR1 and TGFBR2) by TGF- β ligands to stimulate downstream SMAD proteins (SMAD2/3/4). TGF- β -SMAD signaling pathway inhibits the occurrence and development of tumors in precancerous cells or in the early stages of cancer. Once tumor cells overcome late-stage cell cycle arrest and apoptosis mediated by tumor, this signaling pathway can up-regulate the expression of active markers such as CD133 and CD90, thus promoting the progression of hepatocellular

carcinoma [6]. Liu Xiaowei et al. [7] showed that Xuefu Zhuyu Decoction could promote the production of TGF- β , which further indicated that the application of Xuefu Zhuyu decoction in the early stage or early stage of liver cancer could effectively curb the progression of tumors, and it also warned us that Xuefu Zhuyu decoction should be prohibited in the middle and late stage of liver cancer.

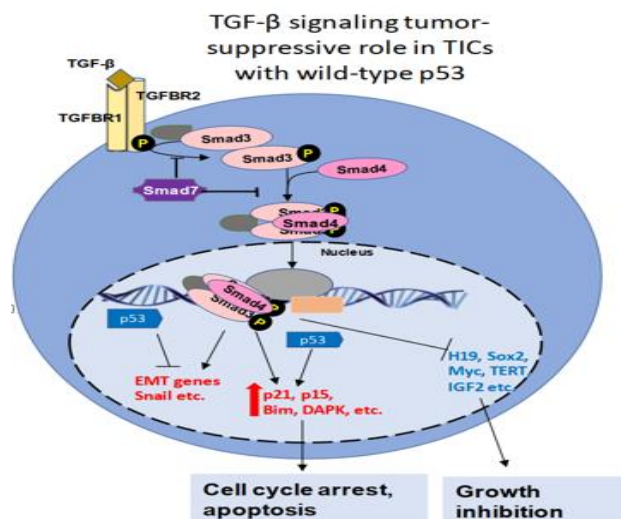


Figure 3: Positive mechanism of TGF- β in HCC

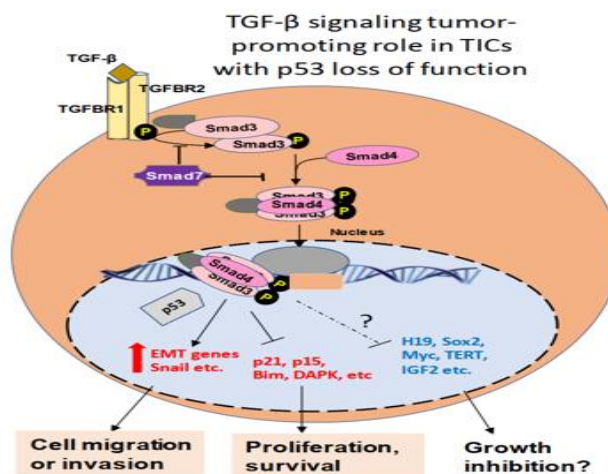


Figure 4: Negative mechanism of TGF- β in HCC

1.3. Enhance Antioxidant Capacity

Under normal conditions, reactive oxygen species (ROS) and active nitrogen (RNS) produced by metabolism in liver cells can be cleared by antioxidant substances such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) in the body. Among them, SOD can catalyze the disproportionation reaction of free radicals, and the generated hydrogen peroxide (H₂O₂) can be removed by GPX or CAT. Therefore, the higher the activity of SOD, the stronger the ability to remove free radicals. When the content of antioxidant substances in the body is relatively insufficient due to external forces or pathological effects, excessive free radicals will lead to liver cell damage, causing inflammation and liver fibrosis. Meanwhile, the accumulation of ROS and RNS produced in liver cells will destroy mitochondrial function and further decrease mitochondrial clearance function, finally making the body enter a vicious cycle. Cause the

occurrence of cirrhosis, liver cancer, etc. [8]. Tang Yafang et al. [9] found that ligusticum Chuanxiong in Xuefu Zhuyu Decoction played an important role in antioxidant, reducing the content of malondialdehyde in endothelial cells and enhancing SOD activity, thus enhancing the antioxidant capacity.

1.4. Inhibit Tumor Angiogenesis

The interaction of vascular endothelial cells, tumor cells and tumor microenvironment can promote tumor angiogenesis. New tumor blood vessels can provide nutrients to tumor cells, and are also an important basis for tumor invasion and metastasis. They are regulated by a variety of factors and signaling pathways. Positive and negative regulatory factors can regulate the formation of tumor blood vessels, and positive regulatory factors such as vascular endothelial growth factor (VEGF) are responsible for the initiation of tumor angiogenesis. Negative-phase regulators include various statins, such as thrombospondin 1 (TSP-1), which inhibit tumor angiogenesis. VEGF is the most specific and important factor in inducing tumor angiogenesis. When tumor tissue grows too fast, oxygen consumption is increased, resulting in hypoxia in tumor tissue and stimulating the expression of hypoxia-inducing factor (HIF-1). HIF-1 can activate VEGF and promote tumor angiogenesis in anoxic environment [10]. Meng Tiantian et al. [11] found that Xuefuzhuyu decoction can effectively reduce the serum HIF-1 α level, inhibit the expression of MMP-9 and MMP-2, and increase the serum TSP-1 level, so as to effectively inhibit the proliferation of tumor cells, control tumor angiogenesis, and curb the progression of liver cancer.

1.5. Regulate Related Signaling Pathways

1.5.1. Wnt/ β -catenin Signaling Pathway

The Wnt/ β -catenin signaling pathway plays an important role in the biological process of tumor development. It has been reported that inhibition of Wnt/ β -catenin signaling pathway can reduce the proliferation and invasion ability of liver cancer cells, while activation of Wnt/ β -catenin signaling pathway can induce the proliferation, migration and diffusion of liver cancer cells and tumor formation. Therefore, abnormal activation of Wnt/ β -catenin signaling pathway is considered to be an important marker of liver cancer and even tumor occurrence. Yang Zhou et al. [12] showed through experiments that paeonia rubra in Xuefuzhuyu decoction could inhibit the expression of 5-HT1D, β -catenin, C-myc and cyclin D1 in HepG2 and SMMC-7721 cells. 5-HT1D can not only activate the Wnt/ β -catenin pathway to promote the progression of liver cancer, but also assist the escape of liver cancer cells by reducing the sensitivity of liver cancer cells to red peony root. The β -catenin gene and AXIN1/2 gene can cause the Wnt/ β -catenin pathway to be consistently hyperactive. Xuefu Zhuyu decoction mainly inhibits the activity of Wnt/ β -catenin signaling pathway by down-regulating the expression of 5-HT1D, so as to prevent the development of liver cancer.

1.5.2. TLR4/NF- κ B Signaling Pathway

TLR4/NF- κ B signaling pathway is the main signaling pathway involved in inflammatory response. Toll-like receptor 4 (TLR4) is a large family of transmembrane proteins that can trigger inflammatory responses by recognizing related ligands, a process involved in various immune responses. Myeloid differentiation Primary response Gene 88 (MyD88) is one of the major adaptor proteins of TLR4 to promote signal transduction. MyD88 activates MAPKs, leading to transcriptional activation of NF- κ B by activation of IKK and degradation of I κ B α by interleukin-1 receptor-associated kinases and tumor necrosis factor receptor-associated factor 6 (TRAF6)

recruited by MAPKs. NF- κ B is believed to be a key link to inflammatory responses, and a transcriptional cascade between NF- κ B and MAPKs ultimately regulates the release of inflammatory cytokines and anti-inflammatory factors[13]. Jiang Hua et al.[14] showed that Xuefuzhuyu decoction could further inhibit the cascade activation of NF- κ B and MAPKs (ERK) by inhibiting the combined action of MyD88 and TLR4, and ultimately inhibit the expression of various pro-inflammatory cytokines downstream of NF- κ B, such as TNF- α and intercellular adhesion molecule 1 (ICAM-1). In turn, it acts as an anti-inflammatory.

1.6. Regulate the Body's Immune Capacity

The immune function is closely related to the occurrence and development of tumor. Immunological indicators such as neutrophils and lymphocytes reflect the dynamic balance between tumor-promoting inflammatory response and anti-tumor inflammatory response in the body, while the imbalance of immune cells and chronic inflammatory response are risk factors for primary liver cancer [5]. Lymphocytes consist mainly of natural killer cells (NK cells), T cells and B cells. NK cells, also known as CD56+, are cytotoxic lymphocytes that play an important role in the early detection of tumors by killing virus-infected cells and also by producing interferon, which activates MI and Th1 immune responses. The role of T cell subsets is important in tumor suppression (see Figure 5), where Th1 cells are responsible for activating and regulating the development of cytotoxic T cells, and cytokines released by Th1 cells activate death receptors on tumor cells, inducing the secretion of IL-1b and IL-6 in antigen-presenting macrophages, leading to the death of cancer cells. T cells are developed from hematopoietic stem cells in bone marrow, in which CD4+T cells assist white blood cells to eliminate pathogens, activate cytotoxic T cells (CD8+T cells) and macrophages, assist B cells to mature into plasma cells and memory cells, and CD8+T cells can directly recognize and kill cancer cells [15]. Therefore, Th1 cells, CD4+T cells, CD8+T cells, and NK cells are essential for the normal functioning of the body.

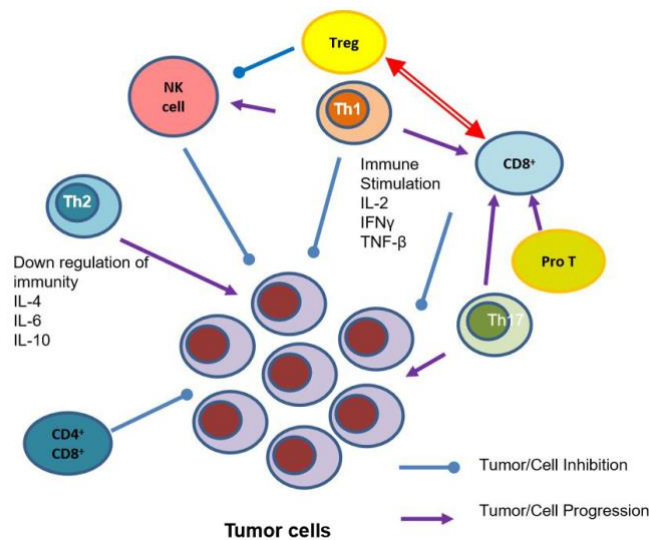


Figure 5: T cell subsets and their role in tumor inhibition

Previous studies have shown that there are a large number of immune cells and inflammatory factors in the tumor microenvironment of liver cancer, among which the number and phenotype of immune cells are related to the occurrence and development of liver cancer. When liver lesions occur, the lymphocyte content is reduced and the ratio of CD4+/CD8+ is unbalanced. The ratio of CD4+/CD8+ can evaluate the immune function of T cells, and the ratio is proportional to the

immune function. Hoffman et al. [15] further found that ligusticum Chuanxiong in Xuefu Zhuyu Decoction can improve the level of Th1 cells and reduce the level of Th2 cytokines in peripheral blood mononuclear cells; safflower can increase the production of CD8+T cells and enhance the activity of NK cells, thus improving the immune capacity and inhibiting the occurrence and development of liver cancer.

2. Clinical Application of Xuefu Zhuyu Decoction Against Liver Cancer

2.1. Xuefu Zhuyu Decoction Single Drug Anti-liver Cancer Treatment

Hepatic fibrosis is a kind of chronic liver injury, which is an important pathological process of cirrhosis and liver cancer. Therefore, the treatment of hepatic fibrosis is a key step to effectively curb the formation of liver cancer. Fang Yuanmeng et al. [16] found that the effective rate of Xuefu Zhuyu Decoction in the treatment of hepatitis B fibrosis was 90.00% in the treatment group, while that of the control group was 60.00% after the treatment of compound Biejiruganan Tablet, and the effective rate of the two groups was $P < 0.05$, proved that Xuefu Zhuyu decoction can effectively prevent the occurrence and development of liver fibrosis, and then inhibit the formation of liver cancer.

2.2. Xuefu Zhuyu Decoction Combined Therapy

Tian et al. [11] randomly divided 70 patients with advanced liver cancer into control group and observation group. Patients in the control group were given XELOX chemotherapy consisting of oxaliplatin (OXA) and the new fluorouracil drug capecitabine (CAP), and patients in the observation group were treated with Xuefuzhuyu Decoction on the basis of XELOX treatment. Compared with the two groups, it was found that the serum CD3+, CD4+, HIF- α and adverse reactions in the observation group were lower than those in the control group ($P < 0.05$), which proved that Xuefuzhuyu decoction assisted XELOX chemotherapy could effectively improve the quality of life of patients with advanced liver cancer and alleviate the occurrence of the disease. Gao Yingyan et al. [17] divided the subjects into test group and control group. The experimental group was treated with oral Xuefu Zhuyu decoction combined with oxycodone hydrochloride sustained-release tablets, while the control group was treated with oral oxycodone hydrochloride sustained-release tablets. The efficacy was evaluated by three aspects of efficacy index (KPS, NRS score), TCM symptom score and safety index, and it was found that Xuefu Zhuyu decoction could improve the health status and self-care ability of primary liver cancer patients with Qi-stagnation and blood-stasis type.

2.3. Postoperative Adjuvant Therapy for Liver Cancer

Transcatheter arterial chemoembolization (TACE) is the preferred treatment for patients with advanced liver cancer, but varying degrees of hepatic pain is a common postoperative adverse reaction. Lu Dongyan et al. [18] included 30 patients with pain of qi stagnation and blood stasis after TACE operation for primary liver cancer into the treatment group, and the other 30 patients with the same condition into the control group. The treatment group was given Xuefu Zhuyu decoction, while the control group was given oral Chinese herbal decoction placebo. The results showed that the score difference before and after treatment in the treatment group was significantly greater than that in the control group ($P < 0.05$); Karnofsky functional status score (KPS) in the treatment group was significantly better than that in the control group ($P < 0.05$), thus confirmed that Xuefu Zhuyu decoction can effectively reduce the pain after TACE surgery for liver cancer,

and can be used for adjuvant therapy after liver cancer surgery to improve the quality of life.

3. Summary

This paper summarizes the mechanism of action of Xuefu Zhuyu Decoction against liver cancer through experimental research and clinical application. It is found that Xuefu Zhuyu decoction can regulate cell cycle, inhibit inflammatory factor infiltration, enhance antioxidant capacity, inhibit tumor angiogenesis, regulate Wnt/ β -catenin and other signaling pathways, and regulate the body's immune capacity, so as to prevent the progression of liver cancer. However, there are still shortcomings in the current studies: first, the anti-tumor effect of Xuefu Zhuyu Decoction involves multiple signaling pathways, but most of the current studies only study a single pathway, and ignore the connection and regulation between each pathway; Second, compared with the clinical research on cardiovascular diseases, Xuefu Zhuyu Decoction has relatively few clinical applications on liver cancer and postoperative prevention and treatment, and relevant research should be strengthened. Third, liver cancer needs long-term drug treatment, but the safety of Xuefuzhuyu decoction still needs rigorous research; Fourth, it has been clear that Xuefu Zhuyu decoction has the effect of anti-liver cancer, but which kind of liver cancer patients is the most effective, which period of use is the most appropriate, and how much dose should be used need to be further studied and thought.

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