

Investigation of the Role of TCM in the Treatment of Hepatic Carcinoma Based on JAK/STAT Signaling Pathway

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Abstract: JAK/STAT signaling pathway is one of the classical signaling pathways. Numerous studies have shown that this signaling pathway provides a suitable microenvironment for tumors by inducing inflammatory responses, and it is closely related to tumorigenesis, development and invasion. Given the fact that targeted therapies for hepatic carcinoma have limited efficacy, perhaps this pathway may serve as an entry point for new targeted therapies for hepatic carcinoma in the future. Due to the above considerations, in recent years, there have been many articles reporting that TCM monomers and compound preparations act on this pathway and have prominent effects in the clinical treatment of hepatic carcinoma with few side effects. This paper focuses on the systematic discussion of Chinese herbal medicines acting on this mechanism, aiming to provide more ideas and basis for the treatment of hepatic carcinoma in Chinese medicine.

Keywords: JAK/STAT signaling pathway; hepatic carcinoma; herbal monomer; herbal compound; treatment of hepatic carcinoma Introduction

1. Introduction

Hepatocellular carcinoma (HCC) is the second deadliest cancer in the world, characterized by high incidence and mortality, and has been on the rise in recent years [1]. In terms of treatment, surgical means mainly include liver transplantation, resection, local ablation therapy, and intravascular chemotherapy. For more than 10 years since 2007, tyrosine kinase inhibition, the agent sorafenib, has been the only option for systemic treatment options. In recent years, lenvatinib, regorafenib and cabozantinib, as well as the VEGF receptor inhibitor ramucirumab, have also been used as advanced systemic therapy for hepatic carcinoma (HCC) [2]. After treatment, these targeted agents failed to improve the overall survival benefit of patients with hepatic carcinoma in a high-quality manner, although the overall survival rate was higher than that of the blank control group. Other entry points are urgently needed for the treatment of hepatic carcinoma. Many studies have shown that many molecular pathways are involved in tumorigenesis, progression, and infiltration and metastasis [3] to varying degrees, such as the VEGF molecular pathway [4], PI3K/Akt signaling pathway [5], MAPK pathway [6], ATR molecular pathway [7], and JAK-STAT molecular pathway. Among them, Janus kinase signaling sensor and activator of transcription (JAK-STAT) signaling mediates almost all immunomodulatory processes, including those involving tumor cell recognition and tumor-driven immune escape [8]. Targeted therapies targeting JAK/STAT signaling pathways are emerging as a hot topic in current tumor therapy.

2. Chinese Medicine's Understanding of Hepatic Carcinoma

There is no record of the name "hepatic carcinoma" in Chinese medicine. In modern times, hepatic carcinoma patients are classified as "accumulation", "obstruction", "dropsy", "jaundice" and "hypochondriac pain" in Chinese medicine according to the clinical manifestations of the patients [9]. In TCM, primary liver cancer (hepatic carcinoma) is a systemic disease, and its pathogenesis is considered to be due to internal and external interactions from a holistic perspective. It is mostly due to deficiency of vital energy, loss of harmony between qi and blood, imbalance of yin and yang, and dysfunction of internal organs, resulting in pathological changes such as stasis of blood and phlegm, which accumulate in the internal organs and become a disease [10]. The clinical manifestations of HCC vary in different periods, and its etiology and pathogenesis are complex. However, in recent years, Chinese medicine has been used to treat patients with hepatic carcinoma with various types of anti-cancer, corrective and liver-

nourishing herbs, and has achieved relatively good results. Therefore, the combination of TCM and modern medical treatment for hepatic carcinoma has gained the attention of medical practitioners while bringing into play their respective advantages and obtaining practical curative effects. With the in-depth research on the mechanism of action, various TCMs that are clinically effective in the treatment of patients with hepatic carcinoma have been discovered and their active ingredients can act on the targets of JAK-STAT molecular pathway to inhibit tumor development and metastasis [11].

3. JAK-STAT Pathway

JAK-STAT is composed of two genes: the Janus kinase and the STAT (signal transducer and activator of transcription). Janus kinase is a non-receptor type tyrosine protein kinase that mediates cellular signaling. STAT is an important substrate of JAK and consists of seven members, including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6. SH2, on the other hand, is an important structural region of STAT proteins [12]. In this pathway, cytokines bind to JAK tyrosine protein kinases on the signal transduction chain of their corresponding cytokine receptors, causing dimerization of the receptors and activation of the receptor-bound JAK proteins. Activated JAK proteins attract each other close to free phosphate binding thereby phosphorylating both the receptor and themselves. These phosphorylated sites become binding sites for STAT proteins and junctional proteins with SH2 structure, i.e. "mooring sites", which are separated from the receptor, expose their nuclear localization signals and enter the nucleus, bind to target genes and regulate gene transcription [13].

4. Relationship Between JAK-STAT Pathway and HCC

The JAK-STAT pathway plays a very important role in the proliferation and infiltration of hepatic carcinoma cells, and the signaling transcription factor STAT is essential [14] in this pathway. He G, et al [15]. showed that STAT3 was highly expressed in nearly 60% of human hepatic carcinoma tissue samples. In addition, a large number of studies have shown [16-18]. that the activation levels of STAT1, STAT3, and STAT5 were found to be significantly higher in human hepatic carcinoma (HCC) tissues than in other cells. The enhanced expression of STAT3 protein RNA had a positive correlation with the clinical grade of hepatic carcinoma. P-STAT is also involved in many aspects of tumorigenesis, and increased levels of P-STAT or enhanced expression of P-STAT in hepatocytes have been shown to be associated with the development of hepatic carcinoma.

5. Fundamentals of TCM Intervention in JAK/STAT

The active ingredients of traditional Chinese medicine are complex, and accordingly, its antihepatic carcinoma treatment has multi-effects, multi-targets and multi-level characteristics. In clinical practice, their effects are obvious, and the side effects of traditional Chinese medicines are minimal compared with those of sorafenib and levatinib, which are the first-line recommended treatments for hepatic carcinoma mentioned above. Therefore, these proprietary Chinese medicines have become the focus of research by scholars both at home and abroad. The mechanism of action of the main active ingredients of TCM against hepatic carcinoma mainly includes: inhibition of cancer cell proliferation, invasion, migration, inhibition of receptor pathway and regulation of related signaling pathways [19]. The active ingredients of herbal medicines that are clinically effective against hepatic carcinoma tend to activate the receptor family of JAK/STAT pathways widely distributed in cells of various tissues and inhibit these pathways.

5.1. Chinese medicine monomer intervention in JAK-STAT pathway

5.1.1. Baicalin

Baicalin [20] is a flavonoid extracted from the root of *Scutellaria baicalensis*, which has significant antibacterial, diuretic, anti-inflammatory, and anti-metabolic effects and, according to modern studies, has strong physiological effects such as anti-cancer responses. The effect of baicalin on STAT3 and P-STAT3 expression in the JAK-STAT pathway was investigated by RTPCR and Western blot techniques at the gene and protein levels. The results showed that baicalin could down-regulate STAT3 mRNA expression and inhibit STAT3 protein expression, and also significantly inhibit the further conversion of STAT3 into P-STAT3 to block the JAK-STAT signaling pathway and inhibit the proliferation of hepatic carcinoma cells.

5.1.2. *Hedyotis Diffusa*

Hedyotis diffusa is an annual herb of the genus *Erythrinaceae* [22], which is bitter, sweet, and cold in nature, and has the ability to reduce swelling and detoxification, relieve pain, and diuretic and laxative effects. At present, 30 organic components have been isolated from *Hedyotis diffusa*, including terpenoids, anthraquinones, flavonoids, organic acids, volatile oils, and polysaccharides. According to modern clinical research, it has been found to have good anti-tumor effects, especially for hepatic carcinoma, lung cancer and cervical cancer. However, it has been found to also have immunomodulatory, antitumor, and bactericidal effects [23]. Sun Chao et al [24] detected STAT3 and phosphorylated STAT3 protein expression levels by Western Blot method. The active ingredient in *Hedyotis diffusa* promotes apoptosis in hepatocellular carcinoma (HCC) cells by downregulating IL-6/STAT3 activity, which can downregulate the expression of anti-apoptotic gene Bcl-2 mRNA and upregulate the expression of pro-apoptotic gene Bax, caspase-9 mRNA.

5.1.3. *Verbena*

Verbena is a perennial herb [25] belonging to the *Verbenaceae* family, with its main origins in Hubei and Jiangsu. Its main efficacy is to clear heat and detoxify the blood, cool the blood and promote blood circulation, promote water retention and reduce swelling. Its main chemical components are cyclic enol ether terpene glycosides, phenylpropanoid glycosides, triterpenoids, flavonoids, sterols, volatile oils, organic acids and so on. Combined with modern clinical research, its main components have obvious anti-tumor effects. Li Yongming et al [26]. used different mass concentrations of total flavonoids of *Verbena officinalis* to treat hepatic carcinoma cells cultured in vitro. The expression levels of IL-6, p-JAK2 and p-STAT3 were detected by Real time PCR, and the expression levels of JAK2 and STAT3 were analyzed by Western blot method. The results showed that the RNA expression of P-STAT3 and P-JAK2 was significantly reduced after the action of flavonoids in *Verbena officinalis* on hepatic carcinoma cells, thus inhibiting the value-added of hepatic carcinoma cells.

5.1.4. *Sophora Flavescens*

Sophora flavescens belongs to the genus *Sophora* in the family *Leguminosae*, and its main origin is in Inner Mongolia and Hefei. Its main components are flavonoids and other alkaloids. Its function is to clear heat and detoxify, anti-inflammatory and analgesic, and anti-tumor. Its antitumor effect is mainly [26] based on Matrine and Oxymatrin. Matrine and Oxymatrin can inhibit tumor cell proliferation and induce apoptosis [27]. Zhao Junyan et al [28]. examined the effects of bittersweet and oxidized bittersweet on hepatic carcinoma cells and the effects of STAT3 and SRAR5 on their corresponding mRNA synthesis in hepatic carcinoma cells. The results showed that both Matrine and Oxymatrin could down-regulate the expression of STAT3 and SRAR5 on their corresponding mRNAs in hepatic carcinoma cells. In addition, the effect of bittersweet and oxidized bittersweet was significantly better than that of oxidized bittersweet on the above effects. Therefore, it can be concluded that bittersweet and oxidized bittersweet can significantly inhibit the proliferation of hepatic carcinoma cells and promote their apoptosis.

5.1.5. *Turmeric*

Turmeric [29] is a plant from the rhizomes of some plants in the ginger family, *Tennantaceae*, mainly distributed in the tropical and subtropical regions of China. It is pungent, bitter, warm, and belongs to the spleen and liver meridians, and its main effects are to break up the blood and mobilize Qi, and relieve menstrual pain. The main active chemical components are phenols, terpenoids, alkaloids and sterols, etc. According to recent clinical and experimental studies, it has been found to have tumor preventive and therapeutic effects. The effect of curcumin on the growth of human hepatic carcinoma cells was detected by MTT method after treatment [30] with curcumin in experimental and control groups. The expression of STAT3, phosphorylated STAT3 protein and mRNA in human hepatic carcinoma cells were detected by Western blot and RT-PCR respectively. The results showed that curcumin could down-regulate the expression of STAT3 and phosphorylated STAT3 protein, which could significantly inhibit the proliferation of human hepatic carcinoma cells.

5.1.6. *Chan Su*

Chan Su is a white dried secretion from the postauricular and skin glands of the *Bufo gargarizans* or *Bufo nigriorbitalis* of the toad family, with its main origin in Hebei and Shandong. It has the function [31] of detoxifying, relieving pain, dispersing knots and opening orifices. The drug belongs to the valuable Chinese medicine. According to the clinical research in recent years, *Chan Su*'s mainly has analgesic, anti-inflammatory, anti-cancer and cardiogenic effects [31]. Among them, the inhibition of

hepatic carcinoma by the main anticancer component, huachanin, was found to be related to the JAK-STAT pathway. Shen Fangxue et al [32]. used Western Blot to investigate the expression of JAK/STAT3 pathway proteins and to verify the IL-6/JAK/STAT3 inflammatory pathway. Compared with the model group, the expression of P-STAT3 and Jak2 could be reduced in the Huachanin group, which could significantly inhibit the expression of IL-6/JAK/STAT3 pathway and reduce the inflammatory response, thus having a significant effect on the treatment of hepatic carcinoma.

5.2. Traditional Chinese Medicine Preparation Intervene in the JAK-STAT Pathway

5.2.1. Yiguan Decoction

Yiguan Decoction is derived from the book named *Xu Ming Yi Lei An Xin Wei Tong Men*, Volume 18. Its formula is added or subtracted with Radix Ginseng, Radix Ophiopogonis, Radix Rehmanniae, Radix Angelicae Sinensis, Fructus Lycii, and Rhizoma Toosendanae (35). Yiguan Decoction uses Zishuihanmu, Zuojin Pingmu, and other methods. [35]. According to modern research, Yiguan Decoction can improve the activity of immune cells and immune factors, enhance the body's anti-tumor immune response, regulate the body's internal environment and exert anti-tumor and anti-metastatic effects [36]. In clinical practice, Yiguan Decoction can improve clinical symptoms, accelerate tumor regression, shorten the treatment course, reduce the incidence of radiotherapy adverse reactions, and improve the quality of life of patients with hepatic carcinoma. Xie Bin et al [37]. used 50 male Kunming mice and randomly divided them into model group, cyclophosphamide group, and Yiguan Decoction high, medium and low dose group. The mice were injected with hepatic carcinoma cells under the axilla and treated with CTX, Yiguan Decoction high, medium and low dose gavage and saline gavage for two weeks. The tumor tissues were weighed, and the tumor tissues were examined for JAK1 and STAT1 protein phosphorylation levels and protein expression. The results showed that Yiguan Decoction high, medium and low doses and CTX had significant tumor suppressive effects compared with the model group. The high and medium doses of Yiguan Decoction significantly decreased the phosphorylation level of JAK1 protein and increased the phosphorylation level of STAT1, protein. It was concluded that Yiguan Decoction could inhibit the proliferation of hepatic carcinoma cells. The mechanism may be related to the control of JAK1/STAT1 phosphorylation and promotion of downstream protein expression, thus promoting apoptosis in hepatic carcinoma cells.

5.2.2. Biejiajian Pill

Biejiajian Pill [38] is derived from Zhang Zhongjing's Synopsis of the Golden Chamber, and its effects are mainly to invigorate blood circulation, resolve blood stasis, soften hardness and disperse knots, and is used for lowering lumps under hypochondrium. The ancient people believed that the lumps in the body are called "zheng", such as modern Western medicine's liver and spleen enlargement of uterine fibroids, etc., which are hard when pressed and do not move when pushed. It is used clinically in the treatment of liver cirrhosis, liver fibrosis, uterine fibroids, breast hyperplasia and tumors and other solid lesions [39]. Xiao Kunmin et al [40]. Firstly established an in vitro experimental model of IL-6-induced epithelial mesenchymal transformation of HCCLM3 by culturing HCCLM3 cells and applying IL-6 (100ng/ml) for 48h after wall induction. Then the intervention was performed with Modified Formula of Biejiajian Pill low, medium and high doses of drug-containing serum and JAK2/STAT3 inhibitor JSI-124 (0.5umol/T). Western blot was performed to detect the JAK2/STAT3 signaling pathway-related proteins JAK2, p-JAK2, STAT3, p-STAT3 and the expression of TWIST, a key transcription factor of EMT. The results showed that after the in vitro model was established, the IL-6-induced group could activate JAK2 and STAT3 compared with the Modified Formula of Biejiajian Pill blank serum group, and the expression of STAT3 phosphorylation level was significantly increased.

6. Conclusions and Outlook

The JAK-STAT pathway plays an important role in the development, invasion and metastasis of hepatic carcinoma (HCC). By interfering with this pathway, Chinese herbal monomers and their compound preparations inhibit the proliferation of HCC cells, promote their apoptosis, and inhibit hepatic carcinoma migration and invasion. The therapeutic effect on hepatic carcinoma is obvious, and it has the advantages of less side effects and milder effect compared with western medical treatment, and there is still great potential and research space. There are still a large number of herbal monomer and compound preparations for the treatment of HCC which are still limited to animal experiments.

The toxic side effects and specific mechanisms of action are still unclear, and further research is

needed in order to provide more methods and approaches for the treatment of HCC with Chinese medicine treatment combined with Western medicine treatment from various aspects, so as to improve the five-year survival rate of HCC.

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