Proceedings of Anticancer Research

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ISSN (ONLINE): 2208-3553
ISSN (PRINT): 2208-3545

Proceedings of Anticancer Research

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May 2022, Volume 6 Issue 3

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http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

Progress in the Use of Natural Products for the Treatment of Lung Cancer

Song Teng, Zhuoxuan Liang, Tong Lyu, Yuntao Yang, Mengying Gou, Xiaoqiong He*

School of Public Health, Kunming Medical University, Kunming 650500, Yunnan Province, China

***Corresponding author:** Xiaoqiong He, hexqcn@aliyun.com

Abstract: Lung cancer is a malignant disease with high morbidity and mortality, which affects the quality of life of patients. It has become one of the most serious public health problems in the world. Most natural products have a variety of anti-tumor activities. In recent years, scholars at home and abroad have studied the anti-tumor effect and mechanism of natural products from many aspects, especially in lung cancer. In this paper, the review on the active components of natural products in the treatment of lung cancer will provide a basis for subsequent treatment of lung cancer.

Keywords: Natural products; Lung cancer; Active components

Online publication: May 20, 2022

1. Introduction

Malignancy has been one of the major causes that threaten the lives of residents. It is and will be one of the great challenges in the medical community in this 21st century and in the future. In China and even across the world, the incidence and mortality of lung cancer have ranked first for a long time [1]. According to the 2021 Global Cancer Report, the number of new lung cancer cases was 2.1 million, which had led to 1.8 million deaths [2]. Due to China's developed heavy industry and other factors leading to air pollution, the incidence of lung cancer in China is much higher than the world's average [3,4]. Therefore, treating lung cancer is one of the issues faced by the medical field in China at the present stage.

At present, surgery is the main way to completely eradicate lung cancer, but some lung cancers have already metastasized at an early stage [5]. The probability of relapse is also extremely high in lung cancer. Chemotherapy is one of the most commonly used methods in the treatment of lung cancer [6]. However, existing clinical broad-spectrum chemotherapeutic drugs generally have serious side effects [7]. Therefore, it is urgent to explore for a novel anti-tumor drug with low toxicity but with high efficacy.

In recent years, research has been focusing on natural products. The anticancer effect of natural products derived from herbs, fungi, or marine organisms have been studied to overcome the issues encountered in the treatment of cancer [8]. It has been discovered that about 60% of anticancer drugs come from natural products [9]. Natural products are characterized by low toxicity and side effects. There are reports on the use of natural drugs to treat liver cancer [9], osteosarcoma [10], and other cancers, with positive outcomes. Therefore, this paper summarizes the recent research on the use of natural products in the treatment of lung cancer to explore their therapeutic effects on lung cancer.

2. Paclitaxel

Paclitaxel is a secondary metabolite – diterpenoid alkaloid – extracted from yew. It is widely used for cancer.

As the first approved natural anticancer drug in the FDA, it has a long history. It has played a major role in the treatment of cervical cancer, breast cancer, lung cancer, and other cancers [11-13]. Paclitaxel was first reported in 1993 to be active against advanced non-small cell lung cancer, and it was found to considerably increase the median survival in patients [14]. Lung cancer patients were reported to have a greater in vitro sensitivity to paclitaxel than cisplatin [15]. However, paclitaxel can be safely used in combination with cisplatin. Eighty patients with advanced non-small cell lung cancer were selected in a study to investigate the effects of using paclitaxel and cisplatin [16]. The results found that both $CD4^+$ and $CD4^+/CD8^+$ levels were elevated in the combination-treated group, thus improving their immunity. Moreover, the adverse effects experienced by the combination-treated group were far lesser than those of the two drugs administered separately, indicating that it can significantly improve patients' quality of life.

While paclitaxel is used to treat cancer, people also tend to focus on its gastrointestinal effect, myelosuppression, allergic reaction, and other side effects [17]. Its side effects are likely to contribute to a discontinuation of treatment or treatment failure. The transport of paclitaxel via liposomes can prevent various side effects. Researchers have encapsulated paclitaxel in two different sizes of cationic liposomes (180 to 200 nm and 80 to 100 nm), in which good biocompatibility and anti-tumor effects have been observed in A549 non-SCLC. Through in vivo experiments, paclitaxel incorporated liposomes were reported to reduce tumors in nude mice quicker than paclitaxel alone. No mechanical or thermal allergies were caused [13]. In order to better promote the delivery of chemotherapeutic drugs to lung tumors, chitosan oligosaccharide modified liposomes were introduced to encapsulate paclitaxel, showing a stronger effect in A549 cells in vitro [18]. Paclitaxel is one of the important drugs for lung cancer in clinical practice.

3. Usnic acid

Usnic acid was first discovered as a secondary metabolite with anti-tumor activity. Songluo is a traditional Chinese medicine with high medicinal value and is known to have more than 1,000 secondary metabolites [19]. Most of its active ingrdients play a significant role in the treatment of lung cancer, especially usnic acid. Usnic acid has an inhibitory effect on the growth of human lung cancer cells [20,21]. The growth and cell cycle of lung cancer cells are also affected. The proliferation of A549 cells is suppressed 24 hours and 48 hours following the addition of usnic acid at different concentrations, in which cells are blocked in the G0/G1 phase during both time periods, and CDK4, CDK6, and cyclin D1 are affected [22,23]. It inhibits proliferation while promoting apoptosis in cancer cells. A study discovered that usnic acid can induce the production of reactive oxygen species (ROS) by inhibiting the mitochondrial respiratory chain (MRC) complex I and complex III, which induces mitochondria and disrupts the PI3K/Akt pathway, thus lowering Nrf2 stability (ROS) [24]. The accumulation of reactive oxygen species reduces the viability of lung SCC cells and induces apoptosis. The role of usnic acid in lung cancer has been validated in numerous experiments, but its mechanism has not been fully understood. Therefore, it is critical to continue the investigation.

4. Crocin

Crocin is one of the bioactive compounds extracted from saffron, which is commonly used in the treatment of spasticity, asthma, liver disease, and cancer [25]. Research has shown that crocin is a TMEM16A ion channel inhibitor, and a high endogenous expression of TMEM16A ion channel in lung cancer is closely related to lung cancer cell proliferation and migration [26]. It has been discovered that crocin inhibits proliferation and migration of LA795 and NCI-H1299 in lung cancer cells by inhibiting TMEM16A. Crocin has a substantially lower inhibitory effect on LA795 cell proliferation and migration when TMEM16A expression is reduced intracellularly [27]. Crocin suppresses lung cancer cell proliferation and induces apoptosis [28]. It is concentration-dependent in A549 and SPC-A1 cells, along with an increased G0/G1

phase arrest. Crocin can significantly increase the mRNA levels of p53 and Bcl-2-associated X protein (BAX) and reduce the mRNA expression of B-cell lymphoma 2 (Bcl-2). In addition, the combination of saffron with cisplatin has an additive effect on the cell growth inhibition rate in the two lung cancer cell lines [29]. Crocin can be studied as a lead compound in the development of lung cancer therapies.

5. Ecliptasaponin A

Ecliptasaponin A (ES) has potent anticancer properties in a variety of cancer cells, especially lung cancer. In non-small CLC, ES inhibits H460 and H1975 proliferation, with a dose-response relationship. It suppresses migration and invasion-related proteins (E-cadherin, N-cadherin, and Vimentin) as well as cycle-related proteins (Cyclin D1, CDK6, and P21) in non-small cell lung cancer [30]. It also induces apoptosis and autophagy in both cancer cells, while inducing apoptosis in human lung cancer cells by the ASK1/JNK pathway, apoptosis-related proteins (cleaved caspase 8, cleaved caspase 9, cleaved caspase 3, BAX, and Bcl-2) as well as autophagy-related proteins (LC3a/b, beclin-1, and p62) [30,31]. ES is most likely a promising therapy for lung cancer.

6. Silybin

Silybin is a phenolic flavonoid isolated from thistle. It plays a major role in the treatment of tumors. Silybin inhibits the proliferation, migration, and invasion of various non-small cell lung cancer cells (A549 cells, H1299 cells, and LLC cells) by acting on the Skp2/P27 pathway [32]. The mRNA and protein expressions affecting Capase-3, Caspase-9, and Bcl-2 promote apoptosis in A549 cells [33]. Tumorigenesis and development are regulated by inhibiting PD-L1 expression in lung cancer cells [34]. In a lung cancer mice model, silybin was administered intraperitoneally. At 400 mg/kg, good anti-tumor activity was seen without any renal or hepatic toxicity [32]. It can be deduced that silybin is a compound with low toxicity and minimal side effects for lung cancer.

7. Conclusion

Researchers at home and abroad have confirmed through various investigations that the active components of natural products can affect the multiple links of lung tumor development and metastasis, but there are only a few clinical and mechanism reports. Therefore, further discussions are required in these fields. It is of great significance to investigate the diversity of active components found in natural products for the treatment of lung cancer. This paper provides a direction in the search for new active ingredients and the clinical development of new drugs.

Disclosure statement

The authors declare no conflict of interest.

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Proceedings of Anticancer Research, 2022, Volume 6, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

Treatment of Melanoma: Current Status and Prospects

Zhiyou Ye*

Zhejiang University, Hangzhou 310058, Zhejiang Province, China

***Corresponding author:** Zhiyou Ye, zhiyouye021699@163.com

Abstract: Melanoma is a tumor caused by the deterioration of melanocytes. Both genetic factors and environmental factors contribute to the occurrence of the disease. Melanoma accounts for about 1% to 3% of all malignant tumors, and its prevalence is increasing year by year at the rate of 3% to 5%. With the exception of early surgical resection, there is a lack of specific treatment for patients with melanoma, and many of them have poor prognosis. Treatment strategies for advanced melanoma have been developing rapidly in recent years. Many new therapeutic drugs have emerged and are being tested in recent years. This review focuses on the current development of melanoma treatment (specifically, gene targeted therapy and immunotherapy) and discusses future treatment possibilities.

Keywords: Melanoma; Genetic mutations; Targeted therapy; Immunotherapy

Online publication: May 20, 2022

1. Introduction

Originated from melanocytes, melanoma is the “king of cancer” and has a high level of malignancy. Melanoma mainly affects the skin, mucosa, and internal organs, accounting for about 3% of all tumors. It is commonly seen in middle-aged to elderly people and can affect any part of the body. The incidence of melanoma has risen rapidly with an annual growth rate of 3% to 5% over the last 50 years. Many institutions around the world have been studying the disease’s mechanism and exploring effective treatments for it. The scope of research is not limited to understanding the epidemiology of the disease, but also the discovery and testing of new therapies as well as improving the diagnostic aspect, in order to promote early detection. Except for a few early-stage cases, most patients with advanced melanoma require further systemic treatment to minimize the risk of recurrence. Targeted therapy and immunotherapy are still the two main methods for treating melanoma. The gene targeted therapy has certain specificity, which can cut off the signal transduction induced by mutant genes and inhibit the proliferation of tumor cells. Biological immunotherapy can help resist, inhibit, and kill tumor cells by enhancing or inducing the patient’s own immune response. We will discuss both approved and experimental drugs in gene targeted therapy and immunotherapy.

2. Current drugs and efficacy

2.1. Gene targeted therapy

2.1.1. Gene targeted therapy

The BRAF gene is located on chromosome 7q34, and it encodes serine/threonine protein kinase, which activates the mitogen-activated kinase pathway. Mutations to this gene would lead to unrestricted cell

growth and proliferation. It has been reported that nearly half of all patients with melanomas have mutations in the BRAF gene. Majority of BRAF mutations occur at codon 600 (V600), among which the most common being V600E (90%) (substitute glutamic acid for valine), followed by V600K (5-6%) (substitute lysine for valine) and V600R/M/D (5%) [1]. Vemurafenib (Zelboraf), dabrafenib (Tafinlar), and encorafenib (Braftovi) are the main drugs that directly attack mutated BRAF proteins. Vemurafenib (Zelboraf) is a kinase inhibitor that specifically targets and binds mutated BRAF kinase. In 2011, it was approved as the first targeted therapeutic drug for metastatic melanoma by the FDA. A clinical trial conducted on 43 patients with BRAF mutations showed that the median progression free survival (PFS) was 6.48 months and the median overall survival (OS) was 11.47 months after receiving vemurafenib. Specifically, vemurafenib improved the OS (11.47 months) of patients with V600 mutation [1]. Compared with vemurafenib, encorafenib showed stronger efficacy in the treatment of melanoma in view of its distinct pharmacological properties [2]. As a reversible ATP-competitive inhibitor, dabrafenib can selectively target and inhibit BRAF V600E kinase, resulting in decreased ERK phosphorylation and inhibition of cell proliferation. MEK is a kinase located downstream of BRAF in the MAPK cascade. Drugs that selectively target MEK1/2 include cobimetinib (Cotellic), trametinib (Mekinist), and binometinib (Mektovi). Cobimetinib is an inhibitor of MEK1/2, which is a protein in the cellular signaling pathway that helps control cell growth and survival. Cobimetinib is used in conjunction with vemurafenib to treat cases that are not surgically treatable or have metastasized. A study of 495 patients with BRFA V600 mutation showed that the combination of cobimetinib and vemurafenib (PFS: 9.9 month) significantly improved PFS and OS compared with vemurafenib alone (PFS: 6.2 months). Besides, the rate of complete or partial response was higher in the combination group (68%) than the control group (45%) [3]. Along with encorafenib, binimetinib was also approved by the FDA in 2018 for treating melanoma, especially for patients with BRAF V600E/V600K mutation. A previous study on 577 patients revealed that the combination of encorafenib and binimetinib (PFS: 14.9 months) also improved the progression-free survival compared with encorafenib alone (PFS: 9.6 months). The median OS was 33.6 months in the combination group, whereas that of the control group was 23.5 months [4].

2.1.2. c-KIT gene

KIT is a tyrosine kinase that can promote cell growth and proliferation. KIT mutations are usually present in mucosal and acral melanoma. Thirty-two studies, which included 5,224 patients, reported 497 cases (9.5%) with KIT mutations that are closely related with age, anatomic location, and chronic sun damage (CSD) [2]. KIT exon 11 and exon 13 mutations appear to be highly sensitive to KIT inhibition, whereas KIT exon 17 mutations appear to have relatively less sensitivity [5]. Imatinib is a targeted therapeutic drug, which has shown significant clinical responses among melanoma patients with c-KIT gene mutation. A previous study conducted on 295 patients with melanoma (51 with c-KIT mutation) showed an overall response rate (ORR) of 16%, median PFS of 12 weeks, and median OS of 46.3 weeks [6]. Another study conducted on 25 patients (8 with c-KIT mutations) showed an ORR of 29% and an overall disease control rate of 50% [6]. The findings substantially confirm the efficacy of imatinib. Dasatinib was also approved for treating patients with c-KIT mutations. The E2607 trial conducted on 30 patients with melanoma (KIT+) showed a PFS of 2.1 months and a median OS of 7.5 months [7]. Due to multiple mutation sites on c-KIT gene, the overall effectiveness of the treatment in c-KIT mutations is lower compared with that in BRAF mutations. Researchers have been trying to explore new KIT inhibitors in order to obtain better curative effect.

2.2. Immunotherapy

2.2.1. CTLA4

Cytotoxic T lymphocyte antigen-4 (CTLA-4) is a 223-amino-acid protein receptor that acts as an immune

checkpoint and downregulates immune responses. Anti-CTLA-4 antibody can maintain T cell activation by releasing a signal that inhibits T cell activation. Ipilimumab (Yervoy) is the first anti-CTLA-4 monoclonal antibody approved for the treatment of tumors. A study conducted on 1,861 patients with advanced melanoma treated with ipilimumab revealed that the median OS was 11.4 months, and the 3-year overall survival rate was 22% [8]. Another study found that ipilimumab, as adjuvant therapy, can prolong the 5-year overall survival rate of patients with advanced melanoma (65.4%), compared with 54.4% in the control group [9].

2.2.2. PD-1

Nivolumab is the first PD-1 monoclonal antibody with melanoma indication and was approved by FDA in 2020. The most prominent clinical study (CheckMate 067) confirmed that nivolumab (single drug) is better than ipilimumab (single drug) in the treatment of advanced melanoma (medial OS: 37.6 months in the nivolumab group versus 19.9 months in the ipilimumab group) [10]. The study also revealed the use of nivolumab in conjunction with ipilimumab significantly improved the overall effective rate and prolonged the overall survival rate. Compared with 34% overall survival rate (3 years) in the ipilimumab group, the nivolumab-plus-ipilimumab group showed 58% OSR, while the nivolumab group showed 52% OSR. However, the side-effects were as high as 56% in nivolumab-plus-ipilimumab group, compared with 21% in the nivolumab group and 28% in the ipilimumab group [10].

As one of the PD-L1 immunotherapy, pembrolizumab helps to detect and fight tumor cells by improving the immune system. It blocks the interaction between PD-1 and PD-L1/PD-L2 and relieves the inhibition of immune response mediated by PD-1 pathway. Previous research has revealed that blocking PD-1 activity can inhibit tumor growth in homologous mouse models. In 2014, pembrolizumab (Keytruda) was approved in the treatment of advanced melanoma. A study (KEYNOTE-001) recruited 655 patients with advanced melanoma, and pembrolizumab injections were administered to these patients. The result showed that patients with advanced melanoma can benefit from long-term survival with a 5-year OSR of 34%; the complete remission (CR) rate was 15%, and the median OS time was 23.8 months [11]. In terms of adverse reactions, pembrolizumab was well tolerated by the patients.

3. Prospects

3.1. NRAS gene

The NRAS (neuroblastoma) gene is responsible for encoding N-Ras, which is a protein that regulates genetic transcription by participating in the RAS-RAF-MEK-ERK pathway. This pathway is closely related with cell proliferation. When pathogenic mutations occur in the NRAS gene, the N-Ras protein encoded by the gene will be continuously activated, resulting in uncontrolled cell proliferation and tumor formation (predominantly at codon 61). Tumors with NRAS mutations are known to be aggressive and are closely related with patient survival. Currently, there is no approved targeted therapy specifically designed for NRAS-mutated melanoma. The current therapies for NRAS-mutated melanoma are still limited. Patients with NRAS mutations are usually given MEK inhibitor (binimetinib) or standard dacarbazine chemotherapy. Previous clinical trials have shown that the efficacy of MEK inhibitor (MEKI) in patients with advanced melanoma is about 20% [12]. The study initiated by Professor Reinhard Dummer (NEMO study) that specifically focused on patients with NRASQ61 mutation showed a median PFS of 2.8 months with binimetinib (MEK162) and 1.5 months with dacarbazine; the rates of effectiveness were 15.2% and 6.8%, respectively, while the total disease control rates were 58% and 25%, respectively [12].

3.2. NOL7 gene

The protein coding gene NOL7 was shown to be strongly linked to the development of melanoma. A study

revealed the novel tumor-promoting capacity of NOL7 gene in melanoma. NOL7's expression increases with disease progression from benign nevus to metastatic melanoma. The knockdown of NOL7 is highly associated with decreased levels of certain cell cycle regulators, such as CDK2 and cyclin A/E, and with increased levels of certain cell cycle inhibitors, such as p21 (CDKN1A) and p27 (CDKN1B) [13]. The study also revealed that NOL7 knockout significantly decreased the growth of tumors in mouse models [13]. These results suggest the significance of NOL7 in regulating apoptosis, cell adaptation, and protection against adverse conditions in melanoma. In terms of mechanism, hypoxia inducible factor HIF-1α can promote the transcription of NOL7 and then transduce the signal to PI3K/AKT/ERK signaling pathway through HRAS, thus further activating downstream cyclin, apoptotic protein, and EMT protein, which finally promotes the growth and metastasis of melanoma.

3.3. CKD pathway

Currently, patients with BRAF mutation are generally prescribed with BRAF inhibitors alone or BRAF inhibitors in conjunction with MEK inhibitors. Previous literatures have revealed that many patients with BRAF mutations have CDK pathway abnormalities (such as CDKN2A gene mutation or deletion). It could be more effective if drugs are designed to co-target CKD pathway and BRAF together. The study published at the 2016 ASCO Annual Meeting deduced that CDK pathway could be an important therapeutic target for acral melanoma in the future. The study reported that among 428 patients with acral melanoma, almost 80% of patients have genetic abnormalities in the CDK pathway. Patients with acral melanoma may need individualized targeted therapy.

4. Conclusion

Clearly, despite the fact that many novel medications have been developed and evaluated for the treatment of melanoma, some of them are ineffective. Although gene targeted therapy has high effectiveness, many patients develop resistance to it after use. Immunotherapy has a relatively slow effect, but the overall benefit is relatively longer. Besides, certain drugs may cause serious side effects, including fatigue, rash, and pruritus. Hence, it is difficult for patients to continue taking these medications. Researchers should also pay attention to alleviating the side effects of drugs in future research, which can largely benefit patients. We should expect to see an increasing number of high-efficiency drugs developed and listed in the future.

Disclosure statement

The author declares no conflict of interest.

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Proceedings of Anticancer Research, 2022, Volume 6, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

Experience in Treating Viral Myocarditis with Master Lei's Huoxue Decoction

Guoxing Sun[1], Hong Fan[2]*

[1]Shaanxi University of Traditional Chinese Medicine, Xianyang 712046, Shaanxi Province, China

[2]Shaanxi Provincial Hospital of Traditional Chinese Medicine, Xi'an 712000, Shaanxi Province, China

***Corresponding author:** Hong Fan, 2236011207@qq.com

Abstract: Modern medical treatment of viral myocarditis by suppressing viral replication and improving cardiomyocyte metabolism has a good effect on the acute infectious period of viral myocarditis, but the curative rate is poor, especially the sequelae of viral myocarditis, which often lingers for years to decades. The use of traditional Chinese medicine can achieve a certain curative effect by promoting the positive and dispelling evil spirits as well as balancing yin and yang. After long-term observation, it is found to have clear effect on improving myocardial injury caused by viral myocarditis.

Keywords: Viral myocarditis; Yangxin Huoxue decoction; Myocardial injury; Traditional Chinese medicine

Online publication: May 20, 2022

1. Introduction

Viral myocarditis is a common condition in clinical practice. It occurs in infancy and adolescence, especially in young adults [1]. It is generally believed that the disease is caused by viral infection, namely Coxsackie B virus, resulting in local or diffuse inflammatory changes in cardiomyocytes, which can be presented as anxiety, chest tightness, fatigue, dizziness, headache, and breathing difficulties. The diagnosis of viral myocarditis depends on endomyocardial biopsy [2], but due to low acceptance rates among patients, clinical reliance is often placed on a history of viral infection and the subsequent diagnosis of viral myocarditis after excluding other factors, such as ischemia-induced cardiovascular disease symptoms. The clinical manifestation of the disease varies; mild symptoms can be cold-like or asymptomatic, whereas severe cases may lead to dilated cardiomyopathy, heart failure, severe arrhythmia, or even sudden death.

2. Understanding viral myocarditis from the perspective of traditional Chinese medicine

Although there is no specific term for viral myocarditis in the classics of traditional Chinese medicine, it is under the category of "chest obstruction," "palpitation," and "consumptive disease" based on its symptoms. Viral myocarditis can also be classified as "epidemic febrile disease." In Ye Tianshi's "Treatise on Febrile Diseases," it is stated that "the warm pathogen first invades the lung and is transmitted retrograde to the pericardium." This refers to a type of pathogenic factor with warm-heat nature that invades the human body from the mouth and nose and is then transmitted to the pericardium meridian of hand-jueyin through the lung meridian of hand-taiyin. The term "retrograde transmission" indicates that it is different from the traditional mode of transmission of infectious diseases [3]. It is mentioned in "Plain Questions on Arthralgia [4]" that "if the pulse is arthralgia, you will feel the evil again, and you will give up in the heart." This

coincides with modern medicine's understanding of viral myocarditis, wherein it is the pathological characteristic of myocardial cells that have been damaged by viral infection.

3. Yangxin Huoxue decoction

Heart-Nourishing and Blood-Activating decoction was introduced by Master Lei Zhongyi [5]. The prescription is composed of 10 grams of ginseng, 15-20 grams of Radix Ophiopogonis, 10 grams of Fructus Schisandrae, 10 grams of Citri Reticulatae Pericarpium, 30 grams of Radix Salviae miltiorrhizae, and 3 grams of notoginseng powder (taken with water). Yangxin Huoxue decoction was originally used to treat coronary heart disease and myocardial infarction, which are due to the injury of both qi and yin, the accumulation of phlegm, and blood stasis. It has the effects of benefiting qi and nourishing yin, resolving phlegm, as well as activating blood circulation [6]. After Master Lei Zhongyi's long-term clinical observation, it is found that Yangxin Huoxue decoction can be used for a variety of heart diseases after flexible addition and subtraction; in addition, it can also nourish the myocardium and improve myocardial metabolism in myocardial injury as a result of myocarditis.

4. Etiology, pathogenesis, and principles of treatment

Suwen "Ping Re Bing Lun" states that "where evil gathers, qi will be deficient." In the early stage, viral myocarditis is often caused by the invasion of heat toxin and dampness toxin into the vessels of the heart due to the six exogenous pathogenic factors and the deficiency of healthy qi [7]. If it lasts for a long time, it will damage the heart yin and consume the heart qi, resulting in the deficiency of heart yin, the deficiency of both qi and yin [8], or the deficiency of both yin and yang [9]. It belongs to the syndrome of deficiency in origin and excess in superficiality. The symptoms are fatigue, shortness of breath, palpitation, chest tightness, and dull pain. The disease is caused by the lack of innate endowment or the imbalance of cold and warm, improper diet, and excessive fatigue, which may lead to the deficiency of healthy qi, thus giving rise to the affliction of warm pathogens. In principle, the treatment should be based on clinical stages. In the early stage, the treatment should focus on clearing away heat and toxic materials as well as eliminating dampness. In the later stage, the treatment should focus on benefiting qi, nourishing yin, and strengthening healthy qi. In the mid-stage [10], both pathogenic factors and healthy qi should be taken into account. Drugs should be selected according to the rise and fall of pathogenic factors and healthy qi, so as to eliminate pathogenic factors without damaging healthy qi or strengthen healthy qi without eliminating pathogenic factors.

5. Example cases

5.1. Case 1

The patient's details are as follows: Wang; male; 26 years old. The date of his first visit was on October 12, 2019. His chief complaint was paroxysmal palpitation, shortness of breath, dizziness, and fatigue for a year. One year ago, the patient developed symptoms due to a cold and went to a local hospital to seek treatment. After examination, he was diagnosed with viral myocarditis. After treatment, his symptoms improved. Over the past one week, he again developed palpitation, chest tightness, dizziness, shortness of breath, and fatigue due to a cold. After self-medicating with cold medication, his symptoms persisted. Although he had good appetite, he did not sleep well and experienced irregular passing of stools and urine. He felt weak most of the time and was intolerant to cold temperatures. From physical examination, his blood pressure was noted to be 110/70 mmHg, his heart rate was 80 beats per minute, with irregular rhythm; early beats were heard, and his heart sounds were slightly low and dull, without any murmur heard; there were no significant findings from lung examination. Troponin I (TnI) was negative, and electrocardiogram revealed ventricular premature beats. Upon examining his tongue and pulse, the patient was noted to have a dark red tongue

with a thin white coating along with deep and thready pulse. From the perspective of TCM, the diagnosis was palpitation (syndrome of deficiency of both yin and yang and blood stasis), whereas the diagnosis of viral myocarditis was made from the perspective of western medicine. The patient was treated based on the method of nourishing yin and tonifying yang, promoting blood circulation, as well as removing blood stasis. The patient was prescribed with a modified Heart-Nourishing and Blood-Activating decoction, which is composed of 18 grams of Radix pseudostellariae, 15 grams of Fructus Forsythiae, 10 grams of Radix isatidis, 15 grams of Radix Sophorae flavescentis, 15 grams of Nardostahyos Radix et Rhizoma, 20 grams of Radix Rehmanniae, 30 grams of Radix Salviae miltiorrhizae, 15 grams of Radix Paeoniae Rubra, 10 grams of Fructus Schisandrae, 10 grams of Radix Angelicae Sinensis, 15 grams of Poria, 15 grams of Cortex Polygalae, 20 grams of Semen Ziziphi spinosae, and Radix Morindae. A total of 7 doses were administered, with a dose each day, decocted with water. The patient was advised to rest and not to tire himself out. On October 12, 2019, the patient came for follow-up. He claimed that his palpitation, chest tightness, fatigue, dizziness, and cold intolerance were relieved. After removing Forsythia, another 7 doses were administered. One week later, the patient's palpitation, chest tightness, and fatigue were resolved. Following that, 10 grams of Atractylodes macrocephala and 10 grams of Saposhnikovia divaricata were added to benefit qi, protect the exterior, and consolidate the curative effect.

Viral myocarditis can be a local or diffuse inflammation of the heart caused by the infection of myocardial cells by Coxsackie B virus. In addition to anti-infection, myocardial nutrition, and symptomatic treatment, modern medicine often has no other effective treatment measures [11]. In traditional Chinese medicine, it is believed that the pathogenesis of viral myocarditis involves pestilences invading the heart through the transmission of defensive qi, nutrient, and blood, while consuming heart yin and heart yang [12], thus resulting in the weakening of healthy qi [13]. The sequelae of myocarditis can be appreciated in this case, in which healthy qi has been damaged and both yin and yang are deficient. Therefore, the symptoms were mainly chest tightness, shortness of breath, and fatigue. The main therapeutic principle is to strengthen the body resistance. The prescription given was a modified Yangxin Huoxue decoction [14]. Pseudostellaria heterophylla can invigorate qi and yin, strengthen the body resistance, as well as eliminate pathogens. Ophiopogon japonicus and Schisandra chinensis are often used as drugs in the treatment of myocardial damage and heart yin consumption to restore heart qi and heart yin. Radix Rehmanniae can nourish yin and blood to make yin and blood have source. Radix Sophorae flavescentis and Nardostahyos Radix et Rhizoma are often used for arrhythmias caused by myocardial cell damage in myocarditis. Studies have shown that both Radix Sophorae flavescentis and Nardostahyos Radix et Rhizoma have anti-arrhythmic effects. They are often used in combination with Concha Margaritifera or Os Draconis and Concha Ostreae to calm the heart and nerves, restore pulse, as well as relieve palpitation. Radix Morindae Officinalis and Herba Epimedii can tonify kidney-yang to warm heart-yang and mobilize the body's yang-qi. Qi deficiency leads to weak blood circulation, while fire deficiency reduces body fluid, refines fluid, and causes blood stasis. Hence, Salvia miltiorrhiza, red paeony root, and angelica were included in the prescription to promote blood circulation and remove blood stasis, nourish yin, as well as tonify deficiency. Studies have shown that Salvia miltiorrhiza has the pharmacological effects of regulating myocardial energy metabolism and restoring damaged myocardial cells, while Radix Glycyrrhizae Preparata has the pharmacological effects of nourishing yin, activating yang [15], as well as warming and tonifying qi. It also has curative effects for palpitation and pulse stagnation caused by the deficiency of heart qi in viral myocarditis. In this case, the patient's heart qi was damaged and heart yin was consumed; hence, the patient found it difficult to fall asleep due to vexation from the deficiency. Polygala tenuifolia, Poria cocos, and Semen Ziziphi spinosae were included in the prescription to relieve restlessness, soothe nerves, and help the patient with his insomnia [16].

5.2. Case 2

The patient's details are as follows: Liu; female; 23 years old. The date of her first visit was on March 17, 2020. Her chief complaint was paroxysmal palpitation, chest tightness, and shortness of breath for 3 years, which aggravated over the past one week. The patient's paroxysmal palpitation, chest tightness, and shortness of breath without exertion started three years ago. At that time, she sought treatment from another hospital. Dynamic electrocardiogram showed sinus rhythm, 28 times of long R-R interval greater than 1.5 seconds, and second-degree type II atrioventricular block. There were 1,922 supraventricular premature beats, 27 paroxysmal supraventricular tachycardia, and 40 paroxysmal supraventricular bigeminy. She was diagnosed with viral myocarditis after a series of biochemical investigations. Subsequent dynamic electrocardiogram showed frequent atrial premature beats, paroxysmal atrial tachycardia, and paroxysmal atrial fibrillation. After four months of antiviral and myocardial nutrition treatment, the patient's symptoms were alleviated, and the antiviral was stopped. Nearly a week after catching a cold, her palpitation, chest tightness, and shortness of breath recurred, accompanied by sore throat, discomfort, and sweating. The patient then sought traditional Chinese medicine therapy. In the past, the constitution was general. She experienced paroxysmal palpitation, chest discomfort, shortness of breath, fatigue, sore throat, feelings of hot and cold, sweating, reduced appetite, and insomnia. Upon examination, she had pale red tongue with thin white coating, and her pulse was thready and weak; her blood pressure was 110/770 mmHg; bilateral lungs were clear, with no significant enlargement of heart boundaries; her heart rate was 78 beats per minute with irregular rhythm and multiple early beats; she had normal heart sounds, with no pathological frame sound in each auscultation area; no abnormal findings from abdominal examination, and no edema in both lower extremities. Her ECG showed sinus rhythm, with multiple atrial premature beats. The TCM diagnosis was palpitation, whereas the diagnosis made based on western medicine was viral myocarditis with arrhythmia (frequent atrial premature beats, paroxysmal atrial fibrillation, and second-degree type II atrioventricular block). The TCM syndrome differentiation was qi deficiency and blood stasis. The therapeutic method was based on supplementing qi, activating blood circulation, nourishing heart, as well as relieving palpitation. She was prescribed with 10 grams of Bupleurum, 10 grams of Scutellaria, 10 grams of Pinellia, 15 grams of Pseudostellaria, 24 grams of Cynanchum paniculatum, 10 grams of Cimicifuga, 10 grams of Sophora tonkinensis, 15 grams of Nardostachys chinensis, 10 grams of Platycodon grandiflorum, 15 grams of Scrophularia, 15 grams of Ophiopogon, 10 grams of Oroxylum indicum, 30 grams of oyster (decocted first), 30 grams of lily, 10 grams of lotus seed, 10 grams of Glycyrrhiza, 15 grams of Acorus calamus, and 30 grams of Salvia miltiorrhiza. She was given six doses, decocted in water for oral administration, and one dose a day. During her second visit on March 24, 2020, her sore throat and feelings of hot and cold resolved, but the patient still felt flustered, with chest tightness, shortness of breath, and fatigue. Lei Zhongyi, a traditional Chinese medicine practitioner, pointed out that the patient's cold symptoms were basically relieved although there were still premature beats. The prescription was changed to Yangxin Huoxue decoction, which includes 18 grams of Radix Pseudostellariae, 15 grams of Radix Ophiopogonis, 10 grams of Fructus Schisandrae, 10 grams of Pericarpium Citri Reticulatae, 30 grams of Radix Salviae miltiorrhizae, 3 grams of Radix Notoginseng powder, 30 grams of Radix Astragali, 15 grams of Radix et Rhizoma Nardostachyos, 30 grams of Concha Ostreae (decocted first), 15 grams of Rhizoma Acori Calami, 30 grams of Bulbus Lilii, and 20 grams of Pericarpium Trichosanthis. She was given six doses, decocted in water for oral administration, and one dose a day. During her third visit on March 31, 2020, she had no palpitation, chest tightness, and shortness of breath, but she still had occasional premature beats, fatigue, anorexia, and abdominal distension. 20 grams of Radix Codonopsis pilosulae, 10 grams of Rhizoma Zedoariae, and 15 grams of stir-fry malt were added to the prescription. Similarly, six doses were given, decocted in water for oral administration, with one dose a day. On April 7, 2020, she complaint of occasional palpitation and chest tightness, with no obvious shortness of breath; her fatigue and appetite

improved. One month later, dynamic electrocardiogram showed 328 supraventricular premature beats without any long intervals. Her condition was stable. In TCM, viral myocarditis belongs to the invasion of toxic heat. If treated in time, it can delay the development of the disease [17]. Otherwise, toxic heat will invade the heart, resulting in chest tightness, palpitation, and other symptoms. In the later stage, various syndromes occur due to the impairment of qi, blood, yin, and yang of the heart. The patient was a young woman who had been ill for a long time and was in the chronic stage of myocarditis. Due to the recurrence of cold, she experienced symptoms of upper respiratory tract infection, exterior excess, and interior deficiency. Therefore, it is important to first relieve the exterior and tonify the deficiency. When the exterior pathogenic factors have been removed, Yangxin Huoxue decoction can then be used to regulate the heart's qi and blood, remove blood stasis, as well as resolve phlegm, so that the toxic pathogenic factors will be removed, with sufficient healthy qi, and eventually leading to recovery [18].

In terms of clinical modification, for patients in the early stage of infection, the decocotion is often combined with Radix Isatidis, Folium Isatidis, Fructus Forsythiae, etc. to clear away heat and toxic materials, or with White Hyacinth Bean and Semen Coicis to eliminate dampness and detoxify. In the mid-stage of the disease, the pathogenic factors compete with each other and invade the heart, so it is necessary to strengthen the body's resistance and eliminate the pathogenic factors [19]. For its sequelae, patients with low immunity, having lingering illness, and high risk of recurrence, Yupingfeng Powder can be used to strengthen qi and consolidate the exterior to prevent infection [20].

Disclosure statement

The authors declare no conflict of interest.

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Proceedings of Anticancer Research, 2022, Volume 6, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

Research Progress in the Application of Lycium Barbarum Polysaccharide in Ophthalmic Diseases

Yanna Lin[1], Huiqin Wu[2]*

[1]Shaanxi University of Chinese Medicine, Xianyang 712000, Shaanxi Province, China

[2]Xi'an First Hospital, Xi'an 710000, Shaanxi Province, China

***Corresponding author:** Huiqin Wu, wuhuiqin65@163.com

Abstract: Lycium barbarum polysaccharides (LBP) are widely used in age-related macular degeneration, glaucoma, diabetic retinopathy, and other eye diseases. With further studies on the pharmacological action of Lycium barbarum polysaccharides in recent years, researchers have found that Lycium barbarum polysaccharides (LBP) play a protective role in the treatment of various eye diseases through anti-oxidation, scavenging oxygen free radicals, and inhibiting inflammatory reaction as well as cell senescence. This paper reviews the application of Lycium barbarum polysaccharides in ophthalmology.

Keywords: Medlar; Lycium barbarum polysaccharide; Ophthalmic diseases; Research progress

Online publication: May 20, 2022

1. Introduction

Wolfberry, also known as citrate thorn, beet, day essence, Digu Dixian, etc., is a commonly used yin-tonic drug, which was first recorded in Shennong Materia Medica. Its nature and taste are "calming" and "sweet," respectively. It belongs to the liver and kidney channels and have the effect of nourishing both the liver and kidney, clarifying eyes, nourishing essence, relieving cough, moistening the lungs, delaying aging, and can be used as both medicine and food [1,2]. Modern pharmacological studies have shown that Lycium bararum polysaccharides (LBP) can scavenge oxygen free radicals, improve microvascular circulation, and increase the activity of superoxide dismutase. It also enhances immune function, lowers blood glucose and lipids, eliminates inflammation, and has anti-tumor, analgesic, as well as neuroprotective properties [2]. In recent years, Lycium barbarum polysaccharide (LBP) has been widely used in ophthalmic diseases, especially in age-related macular degeneration, glaucoma, and diabetic retinopathy.

2. Composition of wolfberry

Lycium barbarum has a complex chemical composition. The leaves, fruits, and bark of Lycium barbarum are rich in polysaccharides, carotenoids, flavonoids, alkaloids, amide compounds, lignin compounds, anthocyanins, essential oils, and sugar lipids, among which the highest content is Lycium barbarum polysaccharide (LBP). It participates in the physiological protective mechanisms of various diseases, such as immune regulation, anti-tumor, antioxidant performance, metabolic effect, neurodegenerative changes, etc. [3-7].

3. Pharmacological effects related to ophthalmology

3.1. Neuroprotection

LBP plays a role in protecting neurons. A study showed that LBP can promote the increase of dendrites and improve the morphology of neurons as well as the density of dendritic spines in elderly rats [8]; the study also found that aging and atrophic neurons still have plasticity, thus delaying the evolution of neural degeneration. It has been suggested that Lycium barbarum not only promotes the proliferation of rat sciatic nerve cells but also the differentiation of glial cells and neurons [9]. These are sufficient to indicate that LBP plays a certain role in nerve regeneration and functional reconstruction and is widely used in various diseases, such as functional injury of the nervous system, which can change tissues and surrounding microcirculation as well as promote the proliferation and division of nerve cells in the damaged area [10,11].

3.2. Antioxidant

Oxidative stress is a phenomenon that damages molecules, cells, and organisms by increasing the production or reducing the clearance of reactive oxygen species (ROS) that accumulates in the body. LBP also has a good antioxidant effect. Zhang Na and another researcher studied the protective effect and mechanism of LBP on diabetic kidney injury [12]; they found that LBP can reduce the level of malondialdehyde (MDA) and increase the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx), so as to alleviate the oxidative stress damage to the kidney of diabetic rats.

4. Application of Lycium barbarum polysaccharide in ophthalmic diseases

4.1. Retinal ischemia-reperfusion injury

Retinal ischemia-reperfusion injury (RIRI) is a common clinical pathology. Factors such as ischemic optic neuropathy, glaucoma, and retinal vascular obstruction can all lead to varying degrees of retinal ischemia, resulting in varying degrees of visual impairment. Several researchers conducted an experimental study on retinal RIRI rat model induced by elevated intraocular pressure and found that LBP has a certain protective effect on the survival of RIRI retinal ganglion cells and non-secretory cells [13]; they also evaluated the protective effect of LBP by detecting retinal cell apoptosis. Studies have confirmed that LBP plays a therapeutic role in the oxidative stress process of retinal ischemia-reperfusion injury model. LBP directly inhibits the production of ROS after RIRI and enhances the activation of Nrf2/HO-1 pathway in RIRI retina through its antioxidant effect, thus inhibiting the apoptosis of retinal ganglion cells and alleviating the damage from oxidative stress. It protects against nerve cell apoptosis and vascular degeneration in retinal ischemic injury.

4.2. Diabetic retinopathy

Diabetic retinopathy (DR) is one of the most serious fundus lesions that can cause blindness. High glucose levels thicken the basement membrane of retinal capillaries and increases the permeability of blood vessels, resulting in the damage of blood-retinal barrier and the death of retinal ganglion cells. Although diabetes can induce cell apoptosis through various mechanisms, the mitochondrial pathway still plays an important role. High glucose levels lead to the generation of a large number of reactive oxygen species in cells, which causes changes in mitochondrial permeability, mitochondrial edema, and dissipation of mitochondrial membrane potential, eventually resulting in cell apoptosis. Through intraperitoneal injection of STZ to establish a diabetic retinal rat model, the study found that LBP can effectively remove reactive oxygen species generated in high glucose states and inhibit retinal cell apoptosis induced by high glucose by regulating the expression of retinal-cell-apoptosis-related proteins – caspase-3, Bax, and Bcl-2 [14]. Several researchers observed the ultrastructure of the retina of SD rat DR model after treatment with LBP and found that LBP can improve the disorder of internal and external segments of photoreceptors, reduce the number

of pyretic nuclei, improve Muller cell dysfunction induced by high glucose and GFAP overexpression, upregulate cell mitochondrial membrane potential, as well as alleviate mitochondrial edema changes [15]. It can be concluded that LBP can largely relieve the pathological changes of mitochondria and inhibit neuronal apoptosis by exerting its antioxidant effect [16]. In another study [16], Pan Hong used LBP to detect the expression of body weight, blood glucose, ROS, Nrf2, and HO-1 in a treated rat DR model and found that LBP not only promotes the increase in body weight, but also the decrease in blood glucose levels and the increase in RGCs as well as the number of non-long process cells in rats; in addition, ROS expression can also be reduced by stimulating Nrf2/HO-1 signaling pathway [17]. In short, LBP can regulate the ROS state of diabetic mice's retina, and it is expected to be applied in the early stages of DR in the future, laying the groundwork for the treatment of DR using Lycium barbarum drugs.

4.3. Retinitis pigmentosa

Retinitis pigmentosa (RP) is a clinically inherited optic neuropathy characterized by retinal photoreceptor cell injury and osteocellular pigmentation. Patients with this condition have reduced visual field and night blindness. Its pathogenesis is still unclear. Clinical trials have reported the therapeutic effect of LBP on the cones of RP patients over 12 months, indicating that LBP has a neuroprotective effect on the retina and can delay or reduce the degeneration of RP cones [18]. Zeaxanthin palmitate (ZD) is the main composition of carotenoids in wolfberry. Liu used ZD in a RP mice model and found that it improved the visual behavior of rd10 mice and delayed retinal photoreceptor degeneration; the study also found that ZD improved the light responses of photoreceptors, bipolar cells, and light reaction of retinal ganglion cells; in addition, ZD inhibited STAT3, CCL2, and MAPK pathways, further decreased the activation of signal transduction and transcription activator 3 and chemokine (C-C motif) ligand 2, downregulated the expression of inflammatory factor GFAP, and inhibited the expression of extracellular signal-regulated protein kinase, suggesting that LBP can inhibit the inflammation of rd10 mice and delay RP, with a protective effect on retinal neurons [19]. In a study, the apoptosis and damage of central retinal photoreceptor cells occurred in rats 7 days after intraperitoneal injection of N-methyl-N-nitrosourea (MNU); LBP upregulated the expression levels of PARP and cleaved PARP in retina and downregulated the expression levels of caspase-9, -7, and -3 [20]. These results indicate that LBP can inhibit photoreceptor cell apoptosis and protect retinal damage induced by MNU. Lutein and zeaxanthin are two well-known carotenoids found in Lycium berries. Foreign researchers used lutein and zeaxanthin to intervene in the oxidative damage of human retinal pigment epithelial cells induced by hydrogen peroxide (H_2O_2). The results showed that MMP-2 and TIMP-1 of ARPE-19 cells significantly increased in the H_2O_2 damaged group, suggesting that H_2O_2 can inhibit cell proliferation. After treating with lutein and zeaxanthin, the decrease of MMP-2 and TIMP-1 reversed the inhibition of H_2O_2 on cell viability. These results indicate that lutein and zeaxanthin can restore the inhibited cell activity of ARPE-19 cells under H_2O_2-induced oxidative stress as well as regulate the MMP/TIMP system to protect the retinal pigment epithelium [21].

4.4. Age-related macular degeneration

Age-related macular degeneration (AMD) commonly occurs in middle-aged and elderly people over 60 years old. Clinically, it is a retinal disease, characterized as damage to the macular area of the retina, which eventually leads to the gradual loss of central vision. It is one of the important causes of visual damage. The early features are vitreous warts and changes in retinal pigment epithelial cells. In a study, the ARPE-19 cell line induced by hydrogen peroxide was treated with LBP, and a significant decrease in pro-apoptotic Bax and caspase-3 was detected, along with a significant increase in anti-apoptotic protein Bcl-2, and the activation of Nrf2/HO-1 pathway, thus protecting ARPE-19 cells from H_2O_2-induced damage [22]. These results suggest that LBP-pretreated ARPE-19 cells can reduce oxidative damage and inhibit apoptosis as

well as reduce the risk of AMD-related retinal diseases. Gao Yuanyuan irradiated cells with cold light for up to 12 hours to establish a light-damaged human RPE cell line (ARPE-19) model in vitro [23]. After treating with LBP, it was found that the levels of Akt and mTOR were upregulated, and the PI3K/Akt/mTOR signaling pathway was also activated, thus inhibiting autophagy. It is suggested that LBP can protect retinal epithelial cells in the establishment of photoinduced injury model.

4.5. Other eye diseases

Lycium barbarum is one of the important traditional Chinese medicines in China, which is widely used in eye diseases. Lycium barbarum polysaccharide extract has a good protective effect on various eye diseases. It has been found that the treatment of LBP on a diabetic rat cataract model established by intraperitoneal injection of streptozotocin (STZ) upregulated Sirt1 and Bcl-2, inhibited p53, Caspase3, FOXO1, Bax, and P27 cell death genes, as well as prevented diabetes-related cataract in animals; in addition, it can reduce the body weight and blood sugar levels in diabetic animals [24]. Other research progress has been made in the treatment of corneal xerosis [25,26] and chronic ocular hypertension [27].

5. Conclusion

In conclusion, LBP has the effects of anti-oxidation, inhibiting neuronal apoptosis, and improving retinal inflammation. In recent years, with the deepening of pharmacological research on LBP, it has been known that the drug has multiple biological and pharmacodynamic effects. LBP also has anti-tumor, anti-oxidation, anti-aging, and other biological effects, with good therapeutic effect on various ophthalmic diseases (retinal ischemic disease, glaucoma, diabetic retinopathy, and retinitis pigmentosa). The disadvantage is that these research achievements are basically stalled at the level of animal experiments or cell experiments. Therefore, further research on the clinical application of LBP in ophthalmic diseases and other diseases is still necessitated to ensure LBP plays a greater role in the field of ophthalmology.

Disclosure statement

The authors declare no conflict of interest.

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Proceedings of Anticancer Research, 2022, Volume 6, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

Lei Genping's Experience in Treating Recurrent Urinary Tract Infection

Lin Xia[1], Genping Lei[2]*

[1]Shaanxi University of Traditional Chinese Medicine, Xianyang 712046, Shaanxi Province, China

[2]Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine, Xianyang 712000, Shaanxi Province, China

***Corresponding author:** Genping Lei, 845390563@qq.com

Abstract: In modern medicine, the therapeutic effect in recurrent urinary tract infection is often unsatisfactory. Lei Genping believes that urinary tract infection is mainly caused by the evil of dampness and heat invading the lower jiao (area below the navel). A prolonged disease course would result in the deficiency of spleen and kidney as well as the stagnation of liver and qi. The initial treatment should focus on clearing heat and detoxifying dampness. In the long run, the treatment criteria include tonifying the spleen and kidney, while clearing heat and dampness as well as regulating liver qi.

Keywords: Recurrent UTI; Deficiency of spleen and kidney; Lei Genping; Clinical experience

Online publication: May 20, 2022

1. Introduction

Urinary tract infection (UTI) is a common clinical urinary system disease caused by the growth and reproduction of pathogenic microorganisms, such as Escherichia coli and gram-negative bacteria, in the urinary tract. Its main symptoms include frequent urination, urgency, dysuria, burning sensation in the urethra, turbid urine, suprapubic pain, pelvic pain, and other symptoms. However, a few patients may also present with hematuria. Recurrent UTI can be diagnosed when there are more than two attacks within six months or at least three attacks within a year. According to an epidemiological survey, recurrent UTI is ranked as the second most common infectious disease among the elderly, with an incidence of 16% to 43.3%, only after respiratory tract infection. It is more prevalent in women than in men, and it has an increasing trend with age [1]. Depending on patients' age, deficiency in body elements, comorbidities, decline in visceral functions, estrogen levels, immunity level, susceptibility to pathogens, and the efficacy of treatment, the clinical course may lengthen, and in severe cases, patients may develop chronic pyelonephritis or even renal failure, which will have a negative impact on their daily lives. In recent years, many reports have shown that due to the abuse of antibiotics, pathogenic bacteria have higher resistance to many antibacterial drugs. At present, modern hospital treatment cannot reduce the recurrence rate; instead, it causes many adverse effects, such as double infection, antibiotic resistance, etc. [2]; urinary tract irritation, lumbar pain, abdominal distension, and other typical symptoms cannot be resolved, and the side effects on liver and kidney cannot be underestimated [3]. For this condition, there are many traditional Chinese medicine treatment methods, and after years of clinical experience, the treatment efficacy is more prominent with less adverse reactions [4]. Lei Genping, the chief physician in Shaanxi University of Traditional Chinese Medicine Affiliated Hospital, has been engaged in clinical work for more than 30 years, thus having rich clinical experience and unique insights on the treatment of recurrent UTI.

2. Etiology and pathogenesis

As mentioned above, the pathogenesis of UTI is mainly based on damp-heat evil, kidney deficiency, liver depression, and so on. However, congestion is still one of the main pathogenic factors. Zhu Danxi once put forward that when blood is subjected to heat and humidity, it will become turbid [5]. Due to prolonged and recurrent gonorrhea, damp turbidity accumulates in the body and the mechanism of qi in the three jiao stagnates, which eventually lead to blood stasis. The main cause of congestion is the evil of dampness and heat. Dampness damages yang qi and causes congestion by inhibiting qi with its heavy turbidity and stickiness. The evil of dampness and heat, qi stagnation, and blood stasis are all interrelated, in which they are the pathological products and causation of each other. These factors are the cause of the disease's long-term recurrence and poor recovery. Liu Ziheng's "Clinical Experience Memoir" discussed a blood drench medical case record where 30 grams of Achyranthes Bidentata BI. and 3 grams of frankincense were used to treat blood drench, in which the effect was proven. In the clinical treatment of patients with chronic drench syndrome, drugs are often combined to promote blood circulation and remove blood stasis, such as Achyranthes bidens, Radix paeoniae, Tree Peony Bark, frankincense, peach kernel, myrrh, etc.

3. Syndrome differentiation

3.1. Drench syndrome with hot and humid bladder

Heat is more crucial than wet as the key mechanism. At the early stage of drench syndrome, due to the evil invasion of hot and humid, toxic accumulates in the bladder. As stated in the "Complete Compendium of Zhang Jingyue," "the beginning of the disease is all because of the heat factor, without discrimination [6]; heat is a positive evil, but heat evil itself cannot lead to drench syndrome; therefore, dampness evil is an important pathogenic factor of drench syndrome; whether or not the invasion of dampness evil or internal injury is caused by dampness, it easily leads to the accumulation of heat evil and dampness evil in the lower bladder, resulting in drench syndrome [7]. At the early stage of the disease, the condition is more positive and milder. Clinically, Lei Genping uses the eight-herb powder to clear heat and relieve diarrhea. If the evil of damp and heat cannot be completely removed, the damp and heat will be concealed in the body for a long time, resulting in kidney and spleen injuries. In case of fatigue, emotional changes, and poor diet, repeated attacks may lead to drenching. It can be seen that the essence of the condition has always belonged to the essence of deficiency, and the evil of dampness and heat is a crucial factor in its pathogenesis. In treating the condition, it is important not to disregard hot and humid, as well as factors of the virtual body, so as to avoid choosing products that deviate from hot and humid and those that easily damage the stomach. The commonly used medications are Twotooth Achyranthes Root, frankincense, Agrimoniae herba, tuckahoe, and Alisma, coix seed, in addition to raw Radix rehmanniae, Eucommia bark, Taxillus sutchuenensis (Lecomte) Danser, and others, which not only tonify the kidneys, but also drain dampness without harming the healthy environment.

3.2. Tonifying spleen and kidney

Kidney qi deficiency is inherited; thus, gasification has no right to viscera dysfunction [8]. The most common urinary tract irritation symptoms in patients include stranguria, especially at night, accompanied by fatigue, cold intolerance, as well as lumbar and lower abdominal pain. The foregoing symptoms, as well as recurring infections, are not indicative of renal yang deficiency, kidney qi deficiency, renal failure, water excretion abnormalities, and renal deficiencies. Therefore, in the early stage of drench syndrome [3], there is usually kidney deficiency, which lasts for a long time. In addition, drench syndrome is mainly positive and classified under heat syndrome in the beginning, in which drugs for cold and heat clearing are often used. If the patient is deficient by nature, the patient would be more susceptible to dampness and heat toxic evil, which will most likely cause damage to the spleen, leading to injuries in both the kidneys and spleen

over several days; hence, the disease will not be cured [9]. According to the "True Story of Medicine," kidney deficiency is extremely drench when tonifying kidney and urine, not alone with water medicine [10]. Therefore, Lei Genping believes that tonifying kidney and spleen should be the main treatment method throughout the disease. Lei's treatment of drench syndrome with aconite tablets showed obvious effects. The composition of the drug includes aconite, licorice, Dangshen, Atractylodes [11], and dried ginger to supplement the spleen yang temperature. In addition, the kidney four taste (kidney four taste, also known as Li Ke, which is made from wolfberry, Epimedium, dodder, pku fat) strengthens kidney qi, along with kidney yin and yang. When the yin and yang of the kidney, spleen, and qi are not warmed and toned, tonifying the kidney with peiyuan, reinforcing the deficit, and strengthening the foundation can help stimulate good qi and prevent evil from invading the body [12]. However, when the onset of drench syndrome is mainly to clear heat and detoxify dampness, tonifying spleen and kidney can be used as an auxiliary, in order to avoid excessive tonifying, which retains evil in the body.

3.3. Dredging liver (focus of the treatment)

In traditional Chinese medicine, there is a saying that goes "liver and kidney are of the same origin." Liver and kidney are closely related, and the drainage function of liver also regulates the metabolism of water in the body. If the evil of dampness and heat accumulates in the body over a long period of time [13], yin injury and gas consumption, resulting in the deficiency of kidney yin, liver displacement and nourishment, as well as liver and kidney deficiency may prolong. According to "Huang Di Nei Jing: Lingshu Jizhu," the liver governs the draining system, and enuresis is caused by excessive and hot pathogens in the liver meridian. According to clinical guidelines, the idea of "drenching the liver and gallbladder" has also been put forward [14]. The catharsis function of the liver is abnormal, thus resulting in liver qi stagnation, qi blockage, qi fire over a long period of time, and the accumulation of lower coke; all these eventually lead to urine stagnation and strangury. Early treatment is urgent for patients with heavier burdens, anxiety, and depression; these patients tend to be irritable, heave long sighs, etc. Treating with modern medicine is usually ineffective and requires repetition. The principle of treatment is to select multiple drugs, such as Radix bupleuri, Radix linderae, Atractylodes, Radix paeoniae alba, green husk, etc., to target the liver and spleen as well as to resolve depression and qi. If the liver is hot and the stomach is bitter, Scutellaria baicalensis can be used to clear the bile and stomach [15].

4. Medical record

JM, a 46-year-old female, was first diagnosed with the condition on March 9, 2021. Her chief complaint was abnormal urine test for more than 20 years. Twenty years ago, the patient developed frequent and urgent urination without inducement; she felt a burning sensation over her urethral orifice and had discomfort when urinating. Her condition often recurred. Five years ago, she developed gross hematuria and was treated with levofloxacin-sodium chloride injection, but the effect was poor. She had frequent urination, urgency, cloudy urine, pain over her urethral orifice, discomfort when urinating; soreness, and lumbago; other than that, she had cold intolerance, irritability, sweating, poor appetite, stomach distension upon eating, heartburn, dry mouth, bitter taste in the mouth, sleep disturbances, and dreams; although she defecated once a day, she had abdominal distension along with flatulence. Upon examination, her tongue was red and thin, and her pulse was thin and weak. Upon investigation, she was positive for urine occult blood (++) and urine protein (+++); the urine red blood cell was 296.20/uL, whereas urine white blood cell was 27.50/uL. The TCM diagnosis was drenching syndrome with kidney deficiency and liver depression spleen deficiency type. The treatment was focused on tonifying kidney and liver as well as invigorating spleen and dampness. Chai Ling Tang was prescribed, in which it is made from 15 grams of Grifola, 20 grams of Alisma, 15 grams of bran-fried Atractylodes poria cocos, 30 grams of cogongrass rhizome, 20

grams of salt dodder, 10 grams of cassia twig, 3 grams of vinegar frankincense, 30 grams of plantain, 20 grams of medlar, 20 grams of Bupleurum chinense, 15 grams of Radix scutellariae, 10 grams of jujube, 15 grams of Pinellia, and 10 grams of Dangshen. She was given 15 doses. On March 24, 2021, during her follow-up, she had no frequent urination, urgency, or cloudy urine, with significant improvement in the burning sensation over her urethral orifice. However, she had occasional lumbago. She was relieved of her general fatigue, and her appetite and sleep improved; her bowel movements were normal (once a day). Upon examination, she had red tongue, thin moss, and thin pulse. Investigation showed urine protein +/- and urine occult blood +; urine red blood cell was 58.50/uL. Twenty grams of licorice, 10 grams of soil tuckahoe, and 20 grams of lotus leaf were added to the prescription. She was given a total of 15 doses. After re-examination, her symptoms improved, and all indicators were normal.

According to Lei Genping, Chai Ling Tang is made of Wuling Powder and Xiao Chai Hu Tang (small bupleurum decoction). In the decoction, bupleurum is used as medicine to relieve the evil of shaoyang and qi stasis; it relaxes the liver and strengthens the spleen and stomach; it also improves stomach distension, appetite, and heartburn. Radix scutellariae clears heat, strengthens the gallbladder and stomach, as well as helps with the bitterness in the mouth. Combined with Scutellaria baicalensis, Radix bupleurum not only regulates the qi mechanism, but also harmonizes the liver, bile, spleen, and stomach; it can be used to smooth the qi mechanism and restore the function of the spleen and stomach. Pinellia with ginger [16] disperses cold to remove cold phlegm, dispels the obstruction of qi and blood, as well as improves fatigue. Ginger has the effect of restricting the poison in Pinellia. Wu Ling San formula is used for the infiltration of ze xie in the kidney and bladder. In order to enhance the effect of water infiltration, Poria cocos can be added. Baizhu has the effect of benefitting qi as well as invigorating spleen and dampness. With cassia twig in the bladder, it warms yang and relieves qi; hence, the function of relieving urine is strengthened, which not only solves the surface evil of the sun, but also gasifies the bladder and helps in its recovery [17]. Small bupleurum decoction is used in conjunction with Wuling Powder for the symptoms of dampness in the body, especially for the symptoms of evil. Lei Genping tends to use Li Ke's four kidney flavors: dodder seed, wolfberry seed, Psoraleae, and Epimedium. During the second follow-up, the patient's frequent urination and urgency resolved, with the alleviation of urethral pain and burning sensation; routine urine examination also showed occult blood in urine (+), which took a long time to heal due to dampness and heat toxin trapped in the bladder. Therefore, 20 grams of lotus and 10 grams of soil poria were added to strengthen the heat-clearing effect and the removal of dampness.

Disclosure statement

The authors declare no conflict of interest.

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Proceedings of Anticancer Research, 2022, Volume 6, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

A Summary of Lei Genping's Experience in the Treatment of Chronic Nephritis

Yue Zhang[1], Genping Lei[2]*

[1]Shaanxi University of Traditional Chinese Medicine, Xianyang 712046, Shaanxi Province, China

[2]Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine, Xianyang 712000, Shaanxi Province, China

***Corresponding author:** Genping Lei, 2926573834@qq.com

Abstract: Chronic glomerulonephritis involves multiple organs. It has a variety of clinical manifestations and is the major cause of progression to end-stage renal disease in patients with kidney disease. Professor Lei Genping believes that the treatment of this disease in Chinese medicine should be based on the deficiency and reality characteristics of the disease mechanism, distinguishing the priorities and identifying the strengths and weaknesses of the internal organs, as well as advocating the integration of Chinese and western medicine at the right time and in the right manner, in order to achieve fundamental results.

Keywords: Chronic glomerulonephritis; Chinese medicine; Clinical experience

Online publication: May 30, 2022

1. Introduction

Chronic glomerulonephritis is a glomerular immune disease with prevalence ranging from 10.5% to 38.2% [1]. It is the second most common cause for dialysis in kidney disease patients [2], accounting for about 19% of patients with end-stage chronic kidney disease [3]. The main manifestations are proteinuria, hematuria, and oedema, often accompanied by varying degrees of reduced renal function [4]. Chronic glomerulonephritis has various pathological types and relies primarily on renal biopsy to confirm the diagnosis; there are no specific or sensitive biomarkers that can replace this method [5]. Modern medicine has identified immune-mediated inflammation as the initiating factor in the development of chronic glomerulonephritis [6], but there is still a lack of effective therapeutic targets; at present, hormones and cytotoxic drugs are not actively used and their clinical efficacy requires further tests [7]. Non-immune and non-inflammatory factors are the main targets of current pharmacological drugs, such as antihypertensives, diuretics, urinary protein control drugs, and lipid lowering drugs, but some drugs have more side effects with long-term use, such as ACEI and ARB classes [8]. On one hand, these drugs have certain advantages over blood pressure and urine protein control, in which these drugs can improve the glomerular state [9]. On the other hand, especially for patients with kidney damage, they may cause side effects, such as dry cough as well as elevated serum potassium and creatinine [10,11]. In such circumstance, the treatment options for these patients will be limited, or even to the extent of "no drugs available," which may eventually delay the progression to end-stage renal disease. However, there are various methods in Chinese medicine for treating the disease, with better efficacy and fewer adverse effects, as well as in improving symptoms and delaying

the progression of the disease. In regard to this, Professor Lei has his unique insights and rich experience, asserting that the integration of Chinese and western medicine in a proper and timely manner can achieve better curative effect. This paper presents Professor Lei's experience in treating chronic glomerulonephritis.

2. Finding the cause and examining the mechanism of the disease

There is no record of "chronic glomerulonephritis" in Chinese medicine. Most practitioners believe that the disease is under two categories: "oedema" and "blood in urine." Professor Lei believes that chronic glomerulonephritis is caused by wind, cold, dampness, and heat externally, and the damage to vital energy internally due to poor diet and internal injuries from strain and fatigue, especially the damage to the lungs, spleen, and kidneys. In that case, the treatment should focus on the relationship among the three organs [12,13]. From modern anatomical analysis, coordinated renal-pulmonary crosstalk exists in the body under normal conditions to achieve adequate vascular tone, erythropoietin production, and water, electrolyte, and acid-base balance [14], while the spleen is the largest secondary lymphoid organ in the body with extensive immune functions [15]. Therefore, these three organs play a crucial role in the pathogenesis of chronic glomerulonephritis. The basic pathogenesis is an imbalance of yin and yang in the lung, spleen, and kidney, with phlegm and stagnant internal groups as well as water-dampness not being transformed. The pathogenesis is characterized by a deficiency in the body and symptoms. Patients with this disease are often deficient in positive qi and are susceptible to external evil, which in turn damages the positive qi, leading to a vicious cycle that eventually results in the deterioration and prolongation of the disease. Although chronic glomerulonephritis has not progressed to chronic renal failure, the pathological products such as heat, dampness, and stasis are usually produced in the course of the disease due to the deficiency of the internal organs, which is both the result and the cause of the deficiency of positive qi [16].

3. Dialectical treatment

3.1. Lung and spleen qi deficiency, dampness stays

The initiating factor of chronic glomerulonephritis is attributed to an immune-mediated inflammatory response, which can be triggered by an upper respiratory tract infection and present as an acute attack [6]. Repeated episodes of pharyngitis can aggravate the condition by stimulating the immune system to continuously form new immune complexes [17]. In addition, Chinese medicine believes that the external evil binds the lungs, which causes the lungs to lose its ability to declare and descend; the water channels are then blocked, and the skin overflows, resulting in edema. Therefore, it is essential to treat chronic glomerulonephritis in association with the lungs [18]. According to "Jing Yue Quan Shu," "All symptoms such as edema are related to the three zang organs (lungs, spleen, and kidney). Water is the ultimate yin, and its origin is in the kidneys; water is transformed into qi, so it is marked in the lungs; water is only afraid of soil, so it is controlled in the spleen." A deficiency in the spleen's yang energy and the downward drainage of refined substances will eventually lead to proteinuria. By linking the two organs, spleen dampness traps the spleen and offends the lung, congesting lung qi, which in turn causes spleen dampness, leading to a series of symptoms. These symptoms include swelling of the face and lower extremities, or even swelling of the whole body, along with palpitations, shortness of breath, tiredness, abdominal distension, whitish or yellowish face, pale red tongue, with thin white coating, greasy coating on the root of the tongue, and sluggish pulse. The focus should be on tonifying the lung and strengthening the spleen, permeating dampness, as well as promoting water circulation. This formula is based on ginseng and Atractylodes macrocephala with addition and subtraction. This means that the prescription is added and subtracted according to the symptoms. For example, if the swelling is severe, with distended abdomen and the urine is minimal, poria ling and big belly skin should be added; if the chest is stuffy and anorexic, coke malt, coke hawthorn, and fried malt should be added; if the hands and feet are cold, with cold intolerance,

cinnamon branch, dry ginger, and fenugreek can be added.

3.2. Deficiency of kidney and spleen yang and overflow of water-dampness

According to "Essential Readings in Medicine," "He who is a good physician must be responsible for his origin. The essence of the first heaven is the kidney, and the essence of the second heaven is the spleen." It has been pointed out that the kidneys and spleen are the first and second elements of the human being, nourishing each other. According to "Medical Journal," "The vital fire is weak and lacks the power of absorption, so the kidneys cannot seal the blood that eventually comes out with the urine." On one hand, it is argued that the deficiency of kidney yang and the lack of absorption lead to blood in the urine; on the other hand, the deficiency of kidney yang and the weakening of the vital fire cause the spleen to lose its warmth and yang. The symptoms include swelling of the whole body, more below the waist, pale face, cold feet, backache, depression, spermatorrhea, impotence, premature ejaculation in men, thin banding in women, pale, fat and tender tongue with teeth marks and white coating, as well as sunken or sluggish pulse. The formula uses Astragalus and the Kidney Formula (self-formulated) to nourish the spleen and kidney, promote water retention, as well as reduce swelling. The specific herbs used include Astragalus, Radix Astragali, Rhizoma Polygonati, Radix Salviae Miltiorrhizae, and Radix et Rhizoma Pseudostellariae. It is worth noting that Professor Lei believes that diuresis cannot be achieved without Astragalus [19]. In addition, Professor Lei used to use the idea of "prescription instead of medicine." This means that when patients have severe symptoms of blood stasis, Gui Zhi Fu Ling Wan can be used instead of Dan Shen. The overall formula reflects the six methods: cultivating, tonifying, consolidating, declaring, clearing, and promoting [20]. If the urine protein is above ++, fried gold cherry and Astragalus can be reused; ephedra, pseudostellaria, and sophora can be added if the patient feels cold, is edematous, and has urination issues, without any sweating or shortness of breath.

3.3. Deficiency of the liver and kidneys, internalization of water-heat and blood stasis

According to Professor Lei, liver and kidney deficiency is a common pathogenesis of chronic glomerulonephritis. According to Zhu Danxi, "Yang is always in excess, while yin is always in deficiency." Yang refers to fire, which is based on liver and kidney essence and blood. Excessive emotions and passions turn into heat, leading to hyperactive liver fire; excessive sexual desire leads to a deficiency of kidney essence and eventually results in internal heat deficiency. The liver represents wood and blood reservoir, while the kidney represents water and essence reservoir. The evil of fire and heat burns and refines fluid into phlegm and stagnation. The liver is responsible for draining and regulating the qi of the three jiao, promoting the dispersal of water, the transportation of water and grain, as well as the evaporation of water. The symptoms include edema of the face or limbs, tinnitus and vertigo, soreness and weakness of the waist and knees, irritable heat in the five hearts or hot flashes in the afternoon, redness over the cheek and night sweats, seminal emission and spermatorrhea, pale dark purple tongue, with slight yellowish moss or less moss, as well as thin pulse. The formula is based on nourishing the kidneys and softening the liver as well as nourishing yin and clearing heat to promote water retention. If urine protein is above ++, fried cherry seeds and gorgonian can be added; if there is soreness and weakness of the waist and knees, Chuan Xuan Zhuan Zhuan and mulberry can be added; if the spleen is weak and damp, Chen Pi and Coix Seed can be added.

3.4. Yin and yang deficiency

It should be clear that yin mainly refers to kidney yin, but yang does not refer to kidney yang alone. According to Professor Lei, this is an advanced stage of the disease, and the lungs and spleen are in a state of decompensation, so it is important to identify the yin and yang of the internal organs. According to "Su

Wen," "Without yin, yang cannot be generated; without yang, yin cannot be transformed." This shows that yin and yang are mutually dependent on each other. When the warming and propulsive effect of yang is weakened, it will affect the metabolism of water and fluid as well as the functioning of qi, resulting in the accumulation of more pathological products. Patients with chronic glomerulonephritis have a prolonged course of illness and eventually show a state of deficiency of both yin and yang. The symptoms include facial, limb, and periorbital edema in the morning or inconspicuous swelling, fatigue, susceptibility to wind and cold, lack of warmth in the hands and feet, hot flashes in the afternoon or at night, dry mouth and throat, as well as poor urination, reduced volume, or frequent nocturia. The formula should nourish yin and support yang, benefit qi and consolidate the surface, as well as clear the remaining evil. If there is nocturia, codonopsis and jinzhuzi should be added.

4. Case discussion

The patient is a 51-year-old female who complaint of intermittent eyelid swelling for five years, which aggravated over the past two weeks. Her first visit to the hospital was on August 10, 2021. Five years ago, she visited the local hospital for eyelid swelling. She was diagnosed with chronic glomerulonephritis. A renal biopsy was performed at the local hospital, which revealed proliferative sclerosing glomerulonephritis. She was then treated and discharged.

Her symptoms this time were mild puffiness of the eyelids, lumbar pain, drowsiness, cold intolerance, and feelings of anxiousness. She also had nocturia, in which she had to pass urine two to three times a night, and she noted foam in her urine. However, her urine volume was acceptable, and there was no gross hematuria. Upon examination, her tongue was pale, with a white, greasy coating, bordered by teeth marks, and a sunken, thin pulse. Upon investigations (August 6, 2021), her 24-hour urinary total protein (24h-UTP) was 1,670 mg/L; urine protein was +++; serum creatinine was 235 μmol/L, and blood urea nitrogen (BUN) was 10.90 mmol/L. The Traditional Chinese medicine diagnosis was oedema, deficiency of spleen and kidney yang. The goal of the treatment was to warm the kidney yang, strengthen the spleen, and benefit the qi. The formula was based on 90 grams of Astragalus membranaceus, 30 grams of Radix Rehmanniae, 30 grams of Oldenlandia diffusa, 10 grams of Herba schizonepetae, 15 grams of Radix Cinnamomi, 15 grams of Poria, 15 grams of Radix Paeoniae, 15 grams of peach kernel, 15 grams of Mu Dan Pi, 10 grams of Huang Lian, and 15 grams of black shun tablet. A total of 14 doses were prescribed. During the second consultation on August 24, 2021, she was relieved from lumbago and weakness, swollen eyelids in the morning, and frothy urine. Her 24h-UTP was 1,059 mg/L; urine protein was ++; serum creatinine was 211 μmol/L, and BUN was 10.70 mmol/L. On top of the previous prescription, 20 grams of Radix Codonopsis pilosulae, 20 grams of branched Atractylodes Macrocephala, 20 grams of Glycyrrhiza glabra, and 30 grams of Rhizoma Dioscorea were added. A total of 14 doses were given to the patient. During the third consultation on September 14, 2021, her 24h-UTP was 125 mg/L; urine protein was ++; serum creatinine was 124 μmol/L, and BUN was 7.5 mmol/L. On top of the previous prescription, 20 grams of salted bone marrow, 15 grams of fried coix seed, and 5 grams of white lentil were added, with a total of 14 doses given.

Professor Lei mentioned that this patient has passed the age of "7-7" and has a deficiency in the spleen and kidneys. She has not been treated systemically for a long time, which has led to a deficiency of positive qi and the retention of evil qi, resulting in yin and water stagnation. The deficiency of spleen and kidney yang energy as well as the internal stagnation of water and dampness have led to swelling and the aforementioned examination findings in the tongue and pulse. The spleen is responsible for raising qi, and a deficiency in the spleen means that the spleen qi does not rise, which is seen in the downward discharge of essence and microscopic substances, hence the frothy urine. The lumbar region is the capital of the kidneys. With protein leakage, the kidney essence becomes more deficient over time, and the lumbar region loses its moistening, hence the pain in the lumbar region. The kidneys and spleen are the successive

"heavens" of the body. If the kidneys and spleen are dysfunctional, the water and grain essence cannot be dispersed throughout the body, thus causing drowsiness. The formula is based on Ginseng-Qi Dihuang Tang, which has been perfected and clinically tested over the years. In consideration of the patient's pathogenesis, dampness is a yin evil and cannot be dissolved without warmth, so Radix Aconiti was added in this case to promote the warming of the spleen and kidneys; it was then mixed with the bitter cold yellow lotus to combine cold and heat, so that both fire and water can be used to clear the heart and liver fire as well as warm the kidneys. During the second consultation, the patient's symptoms were relieved, but she still had swollen eyelids, weakness, and lumbar pain, so Atractylodes macrocephala and Glycyrrhiza glabra were added to achieve the effect of Ling Gui Zhu Gan Tang. During the third consultation, her symptoms were clearly relieved, and the laboratory test results were also improving, so salt was added to warm the kidneys and improve the yang. If the effect is significant, it can be taken for a long time.

Disclosure statement

The authors declare no conflict of interest.

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Proceedings of Anticancer Research, 2022, Volume 6, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

Are M1 and M2 macrophages Effectual Players in Pathological Conditions?

Elham Abdollahi[1,2]*, Nafiseh Saghafi[1], Maliheh Hasanzade[1]

[1]Department of Gynecology and Obstetrics, Mashhad University of Medical Sciences, Mashhad, Iran

[2]Immunology Research Center, Inflammation and Inflammatory Disease Research Centre, Mashhad University of Medical Sciences, Mashhad, Iran

Corresponding author: Elham Abdollahi, ea6112@gmail.com

Abstract: Pathologic inflammatory conditions are frequently correlated with dynamic alterations through macrophage activation, with classically activated M1 cells associated with promoting and sustaining inflammation and M2 cells implicated in resolving or smoldering chronic inflammation. Inflammation is a common feature of various chronic diseases, and it has direct involvement in the emergence and progression of these conditions. Macrophages participate in an autoregulatory loop characterizing inflammatory process, as they produce a wide range of biologically active mediators that exert either deleterious or beneficial effects during inflammation. Therefore, balancing the ratio of M1/M2 macrophages can help to ameliorate the inflammatory landscape of pathological conditions. This review will explore the role of macrophage polarization in distant pathological inflammatory conditions, such as cancer, autoimmunity, renal inflammation, stroke, and atherosclerosis, while sharing macrophage-driven pathogenesis.

Keywords: M1; M2; Macrophages; Inflammation; Polarization

Online publication: May 30, 2022

1. Introduction

Inflammation acts as a double-edged sword. Inflammatory processes offer a life-preserving and protective response to protect the body against external and internal injuries; however, an unbridled or aberrant inflammation has a negative impact and must be firmly tuned to prevent undue tissue injury [1]. Notably, macrophages, an essential component of non-specific defense (innate immunity) and specific defense (adaptive immunity), have a central role in inflammatory processes [2]. They are found in all tissues and activate in response to various stimuli, such as activated lymphocytes, injured or dead cells, and microbial products. Macrophages engulf and digest foreign substances, cellular debris, and tumor cells, thereby promoting anti-infective immunity, maintaining tissue homeostasis, and protecting the body. Besides, macrophages can modulate immune responses by secreting a wide spectrum of inflammatory cytokines, which may result in inflammation [3].

Macrophages have high plasticity. Their activation leads to differentiation into various subsets with distinct functional phenotypes [1]. Based on environment composition, such as the presence of chemokines, cytokines, and other factors secreted by immune cells, mesenchymal cells, and tumor cells, macrophages polarize into two major phenotypes that play opposite roles in immune defense and immune surveillance. These are called classically (M1) and alternatively (M2) activated macrophages with pro-inflammatory and anti-inflammatory activities, respectively [4]. Pathological inflammatory conditions are frequently

correlated with dynamic alterations through the activation of macrophages. M1 cells are mainly associated with promoting and sustaining inflammation, while M2 cells implicate in resolving or smoldering chronic inflammation [1]. During inflammation, macrophages drive in the autoregulatory loop characterizing this process, as they produce numerous bioactive mediators that incorporate in both harmful and beneficial aspects of inflammation. Therefore, inflammation serves the typical environmental setting, where macrophages possess dual behavior [5-7].

M1 and M2 subtypes have been defined based on the type-1/type-2 helper-T(h) cell polarization scenario [1,8]. Macrophages promote polarization into M1 by Th1 cytokines, such as interleukin-12 (IL-12), IL-18, and interferon-gamma (IFNγ) or by granulocyte-macrophage colony-stimulating factor (GM-CSF), activated toll-like receptors (TLRs), and lipopolysaccharides (LPS) [8,9]. On the other hand, Th2 cytokines, such as IL-4, IL-10, and IL-13, stimulate M2 phenotype [10] (**Table 1**).

Inflammation is a common feature of various chronic disorders, and it directly incorporates in the emergence and progression of these conditions [1]. M1 and M2 macrophages exert distinct roles in the course of different inflammatory diseases [1]. For example, M2 macrophages safeguard adjacent cells via eliminating cellular debris and secreting trophic substances for brain repair, whereas when M1 macrophages are over-activated for a long period of time, they produce neurotoxic substances that can worsen brain damage [1,9]. On the contrary, in the tumor environment, M1 macrophages act as protective killer cells, while M2 macrophages have a major role in driving tumor progression. In response to alterations in the microenvironment, macrophages can reversibly and progressively switch their phenotype [11]. Therefore, balancing a favorable ratio of M1/M2 macrophages can help to ameliorate the inflammatory landscape of pathological conditions.

1.1. M1 macrophages

M1 macrophages participate in Th1 responses to pathogenic microorganisms and tumor cells [12]. These cells produce pro-inflammatory mediators that destroy tumor cells and microorganisms that harm the body. M1 macrophages can also cause tissue destruction, resulting in various clinical complications, such as autoimmunity, renal disease, stroke, and atherosclerosis.

M1 macrophages secrete inflammatory cytokines, including tumor necrosis factor-α (TNF-α), type I IFNs, IL-12, IL-6, IL-1, CCL2, which is also known as monocyte chemoattractant protein-1 (MCP-1), CXCL1-3, CXCL5, and CXCL8-10 (**Table 1**), as well as a large amount of reactive oxygen species (ROS) and inducible nitric oxide synthase (iNOS, an enzyme that converts arginine into the “killer” substance nitric oxide), which all contribute to the induction of inflammatory responses [12,13].

Hypoxia-inducible factors, HIF-1α and HIF-2α, are differentially produced in M1 and M2 macrophages, and they control iNOS2 (M1) and arginase 1 (M2), respectively [14]. Nitric oxide (NO) mediates the elimination of tumor cells by directly inducing apoptosis and cell cycle arrest through the activation of caspases and the downregulation of cyclin D1 [15]. M1 macrophages upregulate interferon regulatory factor 5 (IRF5), which is important for the promotion of cytokines (IL-12, IL-23, and TNF) involved in inducing Th1 and Th17 responses. M1 macrophages are also characterized by the secretion of low levels of IL-10 and high levels of IL-12, as well as their capacity for antigen presentation [16]. IL-12 is one of the most important anti-tumor cytokines [17], and it activates the signal transducer and activator of transcription 4 (STAT4) via stimulating tyrosine phosphorylation of Janus kinases [18].

1.2. M2 macrophages

M2 macrophages play an important role in Th2 immune responses, including humoral immunity, tissue remodeling, wound healing, and angiogenesis, in the absence of any infection. Furthermore, M2 macrophages release inflammatory cytokines, such as IL-10, IL-13, and TGF-β, which can promote tumor

progression. Peroxisome proliferator-activated receptor-gamma (PPARγ), a ligand-activated nuclear receptor known as an established marker of M2 macrophages, can regulate M1-related inflammatory responses by suppressing mediators of various signaling pathways, including activating protein-1 (AP-1), STAT, and nuclear factor kβ (NF-kβ) involved in the regulation of genes encoding inflammatory cytokines [19-21]. Moreover, M2 macrophages generate ornithine and polyamines through arginase pathway instead of producing ROS or NO [22-24]. Of note, NO and ornithine are associated with functions such as destruction (M1) and repair (M2), respectively, and have been regarded as the most characteristic molecules associated with macrophages [25].

M2 macrophages present four sub-phenotypes, which include M2a, M2b, M2c, and M2-like [24,26]. M2a macrophages are activated by IL-13 and IL-4, along with Th2 immune response. M2b macrophages, possessing immunoregulatory roles, are activated by immune complex plus TLR or IL-1 receptor ligands. M2c macrophages are induced by IL-10 and transforming growth factor (TGF)-β, and they participate in extracellular matrix (ECM) and tissue remodeling. M2-like macrophages activated by growth factors and cytokines in TME are termed as M2d subtype, which has an immunosuppressive role and pro-tumor property [27].

1.3. Mechanisms underlying macrophage polarization

Polarization and function of macrophages are finely regulated through the activation of a network of transcription factors and signaling molecules, in which the balance between activation of STAT1 and STAT3/STAT6 shows a central impact. Several studies have shown that cytokines play a major role in macrophage polarization [27-32]. M1 polarization usually involves IFN-γ with a TLR agonist, such as LPS, whereas M2 polarization usually involves stimulation with IL-4 or IL-13. This approach is meant to simulate what happens when macrophages are exposed to polarized CD_4^+ T cells in producing their distinctive cytokine combinations (for example, IFN-γ from Th1, or IL-4 and IL-13 from Th2) [33,34]. A predominant activation of NF-κB and STAT1 by IFN-γ and TLR signaling stimulates M1 macrophage polarization, leading to inflammatory and cytotoxic functions. Oppositely, a predominant activation of STAT3 and STAT6 by IL-10 and IL-4/IL-13 signaling skews the function of macrophage towards M2 phenotype, correlated with tissue repairing or immune suppression and tumor progression [35]. When IL-4 engages its type I or type II receptor, STAT6 is activated and promotes expression of typical genes for M2 polarization, such as chitinase 3-like 3 (Chi3l3, Ym1), resistin-like molecule α (Retnla, FIZZ1), and mannose receptor (MRC1) [36]. Moreover, STAT3 is activated by IL-10 and mediates expression of genes (MRC1, TGF-β1, IL-10) involved in the polarization of M2-like phenotype [27,34]. STAT-mediated macrophage activation is controlled by members of the suppressor of cytokine signaling (SOCS) family of proteins. SOCS1 and SOCS3, which are upregulated by IL-4 and IFN-γ, inhibit the function of STAT1 and STAT3, respectively [38,39].

In coordination with or the downstream of STAT/SOCS pathway, several transcription factors direct the polarization of macrophages. The nuclear receptors PPARγ [40] and PPARδ [40,41] regulate a panel of genes mediating the activation of M2 macrophages. Moreover, Krüppel-like factor (KLF)2 and KLF4 are members of a protein family involved in macrophage activity. Notably, STAT6 attunes and synergizes with both KLF4 [42-43] and PPARγ [44]. The collaboration of KLF4 and STAT6 as well as the sequestrating co-stimulators of NF-κB lead to the activation of M2-related genes (ARG1, MRC1, FIZZ1, and PPARγ) and the inhibition of M1-related genes (TNFα, Cox-2, CCL5, and iNOS). KLF2 activates M2 macrophages by suppressing transcriptions regulated by NF-κB/HIF-1α [45].

IL-4 activates c-Myc transcription factor, which controls the expression of M2-associated genes (SCARB1, ALOX15, and MRC1) as well as the activation of PPARγ and STAT6 [46]. IL-4 also activates M2-polarizing IRF4 axis to suppress IRF5-assocated M1 polarization. IL-10 induces M2 polarization by

promoting the activities of STAT3, c-Maf, and p50 NF-κB homodimer. Although p50 NF-κB homodimer is an important mediator for M2 polarization [47], TLR engagement results in the activation of NF-κB and the generation of inflammatory cytokines correlated with M1 macrophages [48]. However, NF-κB also directs a genetic program involved in the resolution of inflammation [49] and polarization of tumor-associated macrophages (TAMs) towards M2 phenotype [50].

2. Conclusion

Balancing the favorable ratio of M1/M2 macrophages can help to ameliorate the inflammatory landscape of pathological conditions. Therefore, it is strongly recommended that future clinical trials should focus on evaluating therapeutic interventions in relation to the polarization of M1 and M2 macrophages in inflammatory conditions.

Table 1. Characteristic future of various subclasses of macrophages

Macrophage subclasses	Stimulators	Surface markers	Metabolic enzymes	Transcription factors	Released cytokines and chemokines	Functions	Ref
M1	LPS PAMPS IFN-γ Modified lipoproteins	CD80 CD86 CIITA CD32 CD16 CD11b CD11c MHC-II	iNOS PFKFB3 PKM2 ACOD1	NF-kB STAT4 IRF-4 HIF1a AP1 STAT6 GATA3 SOCS1 PPAR-γ	TNF-α IL-1β IL-6 IL-12 IL-23 CCL10 CCL11 CCL5 CCL8 CCL9 CCL2 CCL3 CCL4	Bacterial killing Tumor resistance Th1 response	[19,21,51-54]
M2a	L-4/IL-13	CD206 CD36 IL1Ra CD163	ARG1 CARKL	STAT3 IRF4 NF-kβ	IL-10 TGF-β CCL17 CCL22 CCL24	Anti-inflammatory response Tissue remodeling Wound healing	[55]
M2b	TLR ligands IL-1Ra	CD86 MHC II	ARG1 CARKL	STAT3 STAT6 IRF4 NF-kB	IL-10 IL-1b IL-6 TNF-α CCL1	Tumor progression Immunoregulation Th2 response	[55]
M2c	IL-10	CD163 TLR1 TLR8	ARG1 GS	STAT1 IRF3 NF-kB	IL-10 TGF- β CCR-2	Phagocytosis of apoptotic bodies Tissue remodeling Immunosuppression	[55]

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Macrophage subclasses	Stimulators	Surface markers	Metabolic enzymes	Transcription factors	Released cytokines and chemokines	Functions	Ref
					IL-10		[55]
M2d	TLR ligands	CD206	ARG1		VEGF	Angiogenesis	
	A2R/IL-6	CD204	IDO		CCL5	Tumor progression	
		CD163			CXCL10		
					CXCL16		

Disclosure statement

The authors declare no conflict of interest.

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Proceedings of Anticancer Research, 2022, Volume 6, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

Research Progress of Traditional Chinese and Western Medicine in Hyperthyroid Heart Disease

Shuyu Li[1], Mingjun Zhao[2]*

[1]Shaanxi University of Chinese Medicine, Xianyang 712000, Shaanxi Province, China

[2]Affiliated Hospital Shaanxi University of Chinese Medicine, Xianyang 712000, Shaanxi Province, China

***Corresponding author:** Mingjun Zhao, zmj7125@163.com

Abstract: In recent years, hyperthyroid heart disease has become a condition with high incidence rate and high mortality rate. This paper discusses the pathogenesis, treatment, and influencing factors of hyperthyroid heart disease from two different angles – traditional Chinese medicine and western medicine – in hope to provide a reference basis for the treatment of hyperthyroid heart disease.

Keywords: Hyperthyroidism; Heart disease; Research progress; Traditional Chinese and western medicine

Online publication: May 30, 2022

1. Understanding hyperthyroid heart disease from the perspective of western medicine

Hyperthyroidism heart disease occurs when large amounts of thyroid hormones are secreted, causing a series of toxic effects on the heart. Although it has a slow onset, this condition is considered as a serious complication of hyperthyroidism, which can be referred to as hyperthyroid heart in short. A large amount of thyroid hormone increases the secretion of catecholamine, resulting in accelerated heart rate and increased myocardial contractility and systemic vascular resistance, thus further affecting the tissue structures as well as the systolic and diastolic functions of the heart [1]. Arrhythmias, particularly tachyarrhythmias, are the most common clinical manifestations, followed by cardiac insufficiency, which can lead to heart failure or even death in severe cases due to the production of cardiac enzymes that will increase the sensitivity of cardiac β-adrenergic receptors to catecholamine. Epidemiological studies have shown that the incidence rate of hyperthyroid heart disease is 10% to 20% of that in hyperthyroidism [2]. It is most often seen in patients with senile hyperthyroidism. If the condition is not treated promptly, it may lead to various complications or even death. Therefore, this study investigates the pathogenesis, influencing factors, and treatment of this condition from the perspective of traditional Chinese and western medicine, so as to provide a reference basis for the treatment of this disease.

1.1. Pathogenesis

Thyroxine is one of the most important hormones in the human body, and its function is different from that of the thyroid gland. The pathophysiology of hyperthyroid heart disease is related to several factors. First, the thyroid hormone can directly affect the stability of the cardiovascular system, drastically improve myocardial cells' glucose uptake, further strengthen myocardial contraction, and increase vascular expansion capacity. Second, the thyroid hormone can also increase the number of adrenergic receptors in myocardial cells, inhibit the liver function in the degradation of catechu phenolphthalein amine enzymatic

activity, improve heart rate, and increase myocardial contractility. Third, it can activate the renin-angiotensin-aldosterone system (RAAS), thus increasing the heart rate, blood pressure, and myocardial contractility. Several researchers found that when the level of thyroxine in blood exceeds the normal range, it will cause toxic effects on the heart and change the cardiac function and structure [3], which is consistent with the results of another study that investigated the level of myocardial markers in hyperthyroid heart disease [4]. Compared with patients with simple hyperthyroidism, the levels of cardiac troponin (cTnI), creatine kinase (CK), creatine kinase isozyme (CK-MB), and N-terminal-pro brain natriuretic peptide (NT-pro BNP) were all higher than normal. Studies have shown that hyperthyroidism can lead to pseudo-myocardial hypertrophy, which may be reversed with a normalized thyroid state. However, in late stages, it may progress to a pathological state, or even lead to heart failure. Even if thyroid function returns to normal, the cardiac function or structure may not [5]. While exploring the risk factors of this condition, Hao Xiaodong and other researchers discovered that thyroxine can increase glucose uptake by cardiomyocytes, thus improving myocardial contractility and increasing myocardial oxygen consumption; in addition, they also found that thyroid hormone can activate the RAAS system, which might worsen the heart's condition [6]. A study found that a high concentration of FT4 is one of the major risk factors of hyperthyroidism [7]. According to several scholars, there is an increase in Na^+-K^+-ATPase activity in cardiomyocytes in cases of hyperthyroidism, resulting in cardiac electrophysiological activity imbalance. High levels of peripheral FT4 concentrations that cannot be explained by cardiomyocytes may lead to cardiomyocyte necrosis or fibrosis and arrhythmia [8,9].

1.2. Treatment

In western medicine, this condition is controlled through surgery, drugs, and radiation. At the same time, some cardiovascular drugs are given to control complications, and other general measures are taken to improve the prognosis, such as diet and lifestyle modifications. Since surgical treatment has higher risk and incurs more cost, patients tend to opt for drug treatment. Drug treatment mainly includes antithyroid drugs and iodine-131 (^{131}I). The clinical efficacy of ^{131}I in 100 patients with hyperthyroid heart disease was investigated in the study [10], and the study found that the levels of thyrotropin, free triiodothyronine, thyrotropin receptor antibody, and free thyroxine before and after six months of treatment had significant difference; in addition, LVEDD and BNP levels after treatment were lower than those before treatment, whereas LVEF was higher in the experimental group than in the control group, indicating that ^{131}I has high feasibility in treating hyperthyroid heart disease and can effectively improve the disease effect as well as the prognosis of patients. Through clinical observation and research, Huang found that after radical treatment with ^{131}I for hyperthyroidism, arrhythmia can be improved [11]. Another study found that ^{131}I combined with low-dose propranolol can effectively control the occurrence of this disease [12].

1.3. Risk factors of hyperthyroid heart disease

Comparing the data of 112 hyperthyroid patients with 61 hyperthyroid heart disease patients and based on the values calculated according to the Logistic model, it was discovered that older age, a longer course of disease, and high concentrations of FT3 and FT4 were the major influencing factors [6]. Ren Yanru and other researchers found that older age, a longer course of disease, decreased blood lipids, as well as increased FT4, NLR, LDH, and α-HBDH are all clinical risk factors of hyperthyroidism by comparing these clinical indicators in healthy subjects, patients with hyperthyroidism, and patients with hyperthyroid heart disease [13]. Therefore, high-risk groups must be identified using ECG, echocardiography, and serological tests as soon as possible, so as to provide timely treatment.

2. Understanding hyperthyroid heart disease from the perspective of traditional Chinese medicine

2.1. Etiology and pathogenesis

Hyperthyroidism is classified as "gall disease" in ancient Chinese medicine. According to "Authentic Surgery: Discussion on Gall," the pathological basis of this disease is the "mutual beating and knot of qi, phlegm, and blood stasis." Qi stagnation leads to phlegm accumulation. Over time, blood circulation becomes unfavorable and blood stasis occurs, thus blocking channels and collaterals; this is known as gall disease. On the other hand, hyperthyroid heart disease is characterized by a series of heart yin deficiency symptoms, such as palpitation, irritability, sweating, and increased pulse. Therefore, hyperthyroid heart disease belongs to the categories of "palpitation" and "gall disease" in traditional Chinese medicine. Through systematic data analysis and a large number of clinical observations, modern researchers of traditional Chinese medicine attribute the etiology of hyperthyroid heart disease to the deficiency of the five internal organs of the human body and the flourishing fire of Yin deficiency [14]. In the TCM clinical research classification of hyperthyroid heart disease, it has been proposed that blood stasis, qi deficiency, phlegm, yin deficiency, dampness, and heat are the main syndrome elements of hyperthyroid heart disease and various syndrome elements interact to produce a variety of TCM syndromes [15]. The research also found that qi and yin deficiency, phlegm and blood stasis blocking collaterals, damp heat and blood stasis, as well as liver depression and spleen deficiency are the most common TCM syndromes in this disease.

2.2. Syndrome differentiation and treatment

The pathophysiology of hyperthyroid heart disease in traditional Chinese medicine is complex, and there are various etiologies. Modern TCM practitioners have their own traits in the syndrome differentiation and treatment of this disease. Professor Li Zhongnan Li divided the disease into four syndrome types according to their clinical manifestations [16]: liver depression and fire, heart and kidney yin deficiency, heart and kidney yang deficiency, as well as heart and liver yin deficiency. Based on the syndrome types, corresponding treatment methods such as nourishing Yin, tonifying liver and kidney, clearing fire, and promoting blood circulation have been established. In response to the complications caused by this condition, addition and subtraction methods have been proposed. Shengmai powder can be used in those with atrial fibrillation; Chaihu Shugan powder can be used in those with anxiety; Bazhen decoction can be used in those with blood diseases; Danggui Liuhuang decoction can be used in those with sweating syndrome; Schisandra chinensis, Chaihu, and other liver-protecting traditional Chinese medicine can be used in those with liver damage. Zhong Xiaojun and several other researchers believe that the early stage of the disease is due to poor liver qi and the accumulation of phlegm and blood stasis in the heart and pulse over a long period of time [17]; hence, Jianpi Jieyu prescription was formulated to strengthen the spleen and relieve qi as well as soothe the liver and relieve depression. Through clinical trials, it has been discovered that the use of this self-made prescription combined with western medicine can improve cardiac function and regulate the thyroid hormone levels, which provides a certain clinical basis for this prescription. In another study, several researchers investigated the cause of this disease and concluded that the disease is attributed to "gall disease" [18]. In the early stage, it is affected by emotional factors, resulting in abnormal liver catharsis function, stagnation of liver qi and transformation of fire, long-term refining of liquid into phlegm, obstruction of phlegm and qi, poor operation of pulse channel, formation of blood stasis, as well as mind disturbance by virtual fire and palpitation. Therefore, Shugan Yiqi Yangying decoction was formulated and prescribed to several patients in a study. It was found that this prescription can improve heart function and reduce heart rate, and the effect is better than that of western medicine alone. Guoliang Du treated 41 patients with hyperthyroidism after adding and subtracting Xiaoyao powder, a well-known prescription for soothing the liver, relieving depression, and regulating emotions [19]; the results showed that the patients' thyroid function improved significantly with reduced risk of side effects. The curative

effect of Chinese patent medicine on this disease was systematically analyzed in a study; the study found that the incidence of adverse events from using Chinese patent medicine in the treatment of this disease was lower than that of simple antiarrhythmic drugs [20]. For patients whose disease have not developed to the extent of needing long-term oral cardiovascular drugs, Chinese patent medicine may be a better choice. In addition, a large number of clinical researchers have confirmed through experimental research that traditional Chinese medicine combined with western medicine can better improve the prognosis of the disease. In a clinical study [21], 36 patients with hyperthyroidism were treated with Danzhi Xiaoyao powder on the basis of Saizhi. This prescription helps in soothing the liver, nourishing blood, strengthening the spleen, and clearing away heat. After four courses of treatment, the patients' thyroid functions returned to normal, and their ECGs had normal findings. Zhang Zhizhong and several researchers combined Shengmaiyin with western medicine to treat the disease. Shengmaiyin has the functions of nourishing Yin, nourishing heart, and supplementing qi [22]. It improves cardiac function and increases blood flow in the coronary arteries, thus resulting in a better prognosis. In a study [23], 86 cases of hyperthyroid heart patients were randomly divided into a control group and an observation group; the observation group was treated with nerve-calming pill and western medicine; the results showed that the combination of western medicine with is effective, in which the total effective rate of the combined prescription for arrhythmia was significantly higher than that of pure western medicine alone. In addition, the patients showed no obvious adverse drug reactions during the treatment. Therefore, the Anshen Dingji pill (nerve-calming pill) has an important value in the treatment of patients with hyperthyroid heart disease. The use of western medicine combined with TCM has a good therapeutic effect on this disease and can effectively improve the long-term prognosis of patients.

3. Problems and prospects

The pathophysiology of hyperthyroid heart disease is still poorly understood. For a long time, antithyroid and cardiovascular drugs have been used to treat this disease, which can control its progression. According to modern clinical researchers, the clinical efficacy of integrated traditional Chinese and western medicine in the treatment of hyperthyroid heart disease is more optimistic, as it can better improve the thyroid function and cardiac function of patients, along with their prognosis, quality of life, and mortality rate. However, finding a better treatment has become an issue that traditional Chinese and western medicine practitioners should actively deal with.

Disclosure statement

The authors declare no conflict of interest.

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Proceedings of Anticancer Research, 2022, Volume 6, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

Clinical Efficacy of Lenalidomide Combined with Cyclophosphamide and Dexamethasone in the Treatment of Multiple Myeloma

Yudi Miao*

Department of Hematology, Shaanxi Provincial People's Hospital, Xi'an 710068, Shaanxi Province, China

***Corresponding author:** Yudi Miao, miaoyudi26@163.com

Abstract: *Objective:* Multiple myeloma has a great impact on patients; the use of implant denture restorative treatment is ideal, and it is vital to carry out scientific treatment methods. *Methods:* The research subjects were inclusive of 60 patients with multiple myeloma, who were randomly selected from January 2019 to December 2019. The patients were divided into a study group and a control group, with 30 patients in each group. The patients in the control group were treated with conventional treatment, while the patients in the study group were treated with lenalidomide combined with cyclophosphamide and dexamethasone. The effectiveness of treatment, adverse effects, and clinical indices of the two groups were compared. *Results:* Comparing different treatment methods, the differences in the indices between the two groups were statistically significant ($p < 0.05$). *Conclusion:* The use of lenalidomide combined with cyclophosphamide and dexamethasone in the treatment of patients with multiple myeloma increases the effectiveness of treatment and improves patients' clinical indices; thus, it is worthy of promotion.

Keywords: Multiple myeloma; Lenalidomide combined with cyclophosphamide and dexamethasone; Conventional treatment; Clinical efficacy

Online publication: May 30, 2022

1. Introduction

Multiple myeloma, abbreviated as MM, is a malignant plasma cell disease, and its tumor cells originate from plasma cells in the bone marrow, which are a group of B lymphocytes that have developed to their final functional stage. Multiple myeloma is currently classified as a type of B-cell lymphoma, and it is also known as plasma cell myeloma or plasmacytoma. It is characterized by an abnormal proliferation of bone marrow plasma cells with monoclonal immunoglobulin or M protein overproduction and, in rare cases, a gastric differentiated MM without M protein production [1-4]. Patients with multiple myeloma often have multiple osteolytic lesions, hypercalcemia, anemia, and renal damage. Moreover, they are prone to various bacterial infections and pulmonary infections that are not easily controlled due to the suppression of normal immunoglobulin production. Different treatment approaches for these patients have varying effects on their recovery [5-9]. For this reason, this study focuses on the value of lenalidomide combined with cyclophosphamide and dexamethasone treatment as well as conventional treatment in patients with multiple myeloma.

2. Materials and methods

2.1. General information

From January 2019 to December 2019, 60 patients with multiple myeloma were recruited as research subjects for this study. In the control group, the male to female ratio, age, and time of admission were 19:11, all around 60, and 3.62 ± 0.96 hours, respectively. In the study group, the male to female ratio, age, and time of admission were 18:12, all around 60, and 3.12 ± 1.06 hours, respectively

Inclusion criteria: (1) patients diagnosed with indications of multiple myeloma [10]; (2) patients who are able to communicate normally; (3) patients whose age is above 18.

Exclusion criteria: (1) patients with other malignancies; (2) patients with cognitive impairment; (3) those with contraindications to medication and poor compliance.

2.2. Methods

2.2.1. Control group

The control group was treated with 0.15 mg/kg/day or 6 mg/m^2 of Mafran for 5 days, combined with 10-60 mg (2-12 tablets) (5-10 mg or 1-2 tablets each time) of oral prednisone (Tianjin Lisheng Pharmaceutical Co., Ltd., State Drug quantification H12020123) per day, and 100-200 mg (4-8 tablets) (25-50 mg or 1-2 tablets each time) per day of oral thalidomide (Changzhou Pharmaceutical Factory Co. Ltd., H32026129).

2.2.2. Study group

The study group was treated with oral lenalidomide (Jing Shuanglu Pharmaceutical Co., Ltd., GZP H20170011), with a dose of 25 mg once daily on days 1-21 of each repeated 28-day cycle until disease progression; intravenous cyclophosphamide (Jiangsu Shengdi Pharmaceutical Co., Ltd., GZP H32024654) at 500-1000 mg/m^2 per dose according to body surface area (1000 mg/m^2 with 20-30 ml of saline, intravenously, once a week for 2 times, repeated after a 1- to 2-week break); and oral dexamethasone (Guangdong Huainan Pharmaceutical Group Co., Ltd., Guodianzhi H44024469), with a starting dose of 0.75-3.00 mg (1-4 tablets) once, 2-4 times a day for adults, and a maintenance dose of approximately 0.75 mg (1 tablet) a day but depending on the patient's condition.

2.3. Observation indicators

(1) Effectiveness of treatment
"Effectiveness" was determined based on patients' physiological indicators and their symptoms. Comparing the treatment effectiveness of both the groups, the patients were categorized into three groups: very effective, effective, and ineffective.

(2) Adverse effects
The adverse effects in terms of changes in blood composition, weakness, and neuropathy were compared between the two groups

(3) Clinical indices
The pain index, serum β2-microglobulin, urine β2-microglobulin, and erythrocyte sedimentation rate before and after treatment were compared between the two groups.

3. Results

3.1. Effectiveness of treatment

The difference in the effectiveness of treatment between the control group and the study group was statistically significant ($p < 0.05$), as shown in **Table 1**.

Table 1. Comparison of treatment effectiveness between the two groups (n, %)

Group	Number of cases	Very effective	Effective	Ineffective	Treatment effectiveness
Study group	30	24 (80.00)	6 (20.00)	0 (0.00)	30 (100.00)
Control group	30	20 (66.67)	5 (16.67)	5 (16.67)	25 (83.33)
X^2					5.4545
p					0.0195

3.2. Adverse effects

The incidence of changes in blood composition, weakness, and neuropathy was significantly lower in the study group than the control group ($p < 0.05$), as shown in **Table 2**.

Table 2. Comparison of adverse effects between the two groups (n, %)

Group	Number of cases	Changes in blood composition	Weakness	Neuropathy	Incidence rate
Study group	30	0	1	0	1 (3.33)
Control group	30	1	3	2	6 (20.00)
X^2					4.0431
p					0.0444

3.3. Clinical indices before and after treatment

Before treatment, there was no significant difference in terms of pain index, serum β2-microglobulin, urine β2-microglobulin, and erythrocyte sedimentation rate between the two groups ($p < 0.05$); however, after treatment, the pain index, serum β2-microglobulin, urine β2-microglobulin, and erythrocyte sedimentation rate of the study group were significantly better than those of the control group ($p < 0.05$), as shown in **Table 3**.

Table 3. Comparison of clinical indices before and after treatment (n = 30, ± s)

Group	Number of cases	Pain index		Serum β2-microglobulin	
		Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Control group	30	6. 55±1.26	4.13±0.57	3.35±0.65	2.26±0.53
Study group	30	6.30±1.58	3.02±0.18	3.30±0.52	1.67±0.53
t		0.6776	10.1711	0.3290	4.3114
p		0.5007	0.0000	0.7433	0.0001
Group	**Number of cases**	**Urine β2-microglobulin**		**Erythrocyte sedimentation rate**	
		Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Control group	30	1.55±0.26	1.03±0.07	35.95±3.05	24.26±3.03
Study group	30	1.50±0.58	0.42±0.08	34.94±3.02	17.27±2.03
t		0.4309	31.4305	1.2889	10.4974
p		0.6682	0.0000	0.2026	0.0000

4. Discussion

Multiple myeloma has no known etiology; however, it might be related to radiation, genetics, or genetic mutations. Patients with multiple myeloma have abnormal M protein in the blood as well as pathological fractures and bone lesions found on imaging [11-14]. Multiple myeloma is a malignant disease of the

hematological system, in which there is substantial increase in primitive and naive plasma cells in the bone marrow as well as inhibition of normal hematopoietic function [15-19]. These plasma cells may secrete abnormal immunoglobulins, which can cause multiple osteolytic lesions and kidney damage. Chemotherapeutic agents or targeted drugs are often used in the treatment of multiple myeloma. Lenalidomide is currently the most common drug used for the treatment of multiple myeloma because it is safe, has a relatively low risk of adverse effects, and is effective in boosting the immune system. Cyclophosphamide is an alkylating agent that, when used, is effective in removing cancer cells from the body. Dexamethasone, on the other hand, has a good anti-inflammatory and anti-toxic effect. Hence, the combination of the three is ideal [20]. In this study, the difference in the effectiveness of treatment between the control group and the study group was statistically significant ($p < 0.05$); the incidence of changes in blood composition, weakness, and neuropathy in the study group was significantly lower than that in the control group ($p < 0.05$); although there was no significant difference in terms of pain index, serum β2-microglobulin, urine β2-microglobulin, and erythrocyte sedimentation rate between the two groups before treatment ($p < 0.05$), the pain index, serum β2-microglobulin, urine β2-microglobulin, and erythrocyte sedimentation rate of the study group were significantly better than those of the control group after treatment ($p < 0.05$).

In conclusion, lenalidomide combined with cyclophosphamide and dexamethasone is effective in the treatment of patients with multiple myeloma and is worth promoting.

Disclosure statement

The author declares no conflict of interest.

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Proceedings of Anticancer Research, 2022, Volume 6, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

Inhibition of RNaseH2A Induces Fas-Regulated Programmed Cell Death in Hepatoma Cells

Xiaowei Chang, Yu Cai*, Pu Yan, Wujun Li

Department of General Surgery, The First Affiliated Hospital of Xi'an Medical University, Xi'an 710000, China

***Corresponding author:** Yu Cai, jxzlkzk@126.com

Abstract: *Objective:* To screen clinically significant potential drug targets in liver cancer and to study the function and potential molecular mechanisms of this target protein in the development of liver cancer. *Methods:* By using the clinical database GEPIA to find genes that are differentially expressed in liver cancer compared to normal tissues, we further screened the genes that are highly expressed in hepatocellular carcinoma and have clinical prognostic relevance. Heat maps were used to sort these genes according to their clinical prognostic relevance, so as to screen for the target gene of interest. The characteristics of target gene expression and clinical prognosis in hepatocellular carcinoma were studied. The target gene was knocked down through siRNA, and cell proliferation experiments and apoptosis experiments were used to verify the importance of the target gene in the occurrence and development of liver cancer. Finally, we elucidated the potential molecular mechanism of the target gene's function in liver cancer based on the mutual regulatory relationship between the target gene and key apoptosis genes. *Results:* 1482 genes were significantly underexpressed in liver cancer, and 725 genes were significantly overexpressed in liver cancer, of which RNaseH2A was significantly overexpressed in liver cancer and had a significant clinical prognosis. Knockdown of *RNaseH2A* inhibited the proliferation of hepatocellular carcinoma cells and induced apoptosis. Knockdown of *RNaseH2A* induced the high expression of *Fas*, a key gene for apoptosis, and liver cancer usually features low expression of *Fas*. After hepatocellular carcinoma cells that were knocked down of *RNaseH2A* continued were subject to *Fas* knockdown, hepatocellular carcinoma cell proliferation and apoptosis returned to normal levels. *Conclusion:* The high expression of *RNaseH2A* regulates the low expression of *Fas*, a key gene for apoptosis, thereby inhibiting apoptosis, promoting cell proliferation, and participating in the development of liver cancer.

Keywords: Liver cancer; RNaseH2A; Fas; Apoptosis

Online publication: May 30, 2022

1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer in adults with high malignancy and poor prognosis [1]. Treatment options and prognosis for HCC depend on many factors, such as tumor size and tumor malignancy. Surgical treatment does not produce obvious therapeutic effect on liver cancer, because only 10–20% of patients are amenable to treatment by surgical resection [2]. Chemotherapy is one of the currently available options for treating HCC but its therapeutic effect is limited. Therefore, the current focus of HCC research is to identify alternative therapeutic drugs with favorable pharmacological effects and low normal cytotoxicity [3].

RNaseH2A is also known as AGS4, JUNB, RNHL, RNIAA and RNaseHI. *RNaseH2A* gene (1148 bp) is located on chromosome 19 [4,5] and is a component of ribonuclease H2 (RNaseH2), mainly responsible for its endoribonuclease activity [6-8]. RNaseH was discovered and isolated from calf thymus [9,10], and is

widely distributed in mammalian cells, yeast, prokaryotes and viruses. It catalyzes the nuclear degradation of RNA in DNA-RNA hybrids, participates in the reverse transcription activity of multifunctional enzymes in retroviruses, and plays an important role in all stages of viral genome transcription [11,12]. In eubacteria, RNaseH is required for several processes, including removal of RNA primers from Okazaki fragments, transcription of primers required for DNA polymerase I to initiate DNA synthesis, and removal of R loops to provide for irregular DNA synthesis conditional start site [13]. In eukaryotes, RNaseH may play a similar role [14]. Recent studies have shown that RNaseH2A mutations cause autosomal recessive neurological dysfunction, Aicardi-Goutieres syndrome, mainly causing microcephaly, mental retardation, brain calcification, increased IF-α and leukocytes in cerebrospinal fluid, fever, thrombocytopenia, and hepatitis [15-17]. RNaseH2A is considered a possible cancer target. Consistently, logistic regression analysis showed that, among other genes, the expression level of RNaseH2A was positively correlated with aggressive prostate cancer [18]. However, the role of RNaseH2A in liver cancer has not been reported. Therefore, in this study, we aimed to evaluate the role of RNaseH2A in liver cancer and explore its underlying mechanism.

2. Materials and methods

2.1. Cell culture

Human hepatoma HepG2 cells were purchased from the ATCC cell bank in the United States, and were cultured in RPMI-1640 medium containing 10% fetal bovine serum, supplemented with 100 μg/mL streptomycin and 100 IU/ml penicillin. Cell culture flasks were placed in a 37°C incubator with 5% CO_2 and 95% humidity.

2.2. Clinical data screening

In the clinical database GEPIA (Gene Expression Profiling Interactive Analysis) [19], the proteins that are highly expressed in liver cancer were identified (tumor/normal>2) , and the proteins closely related to the clinical prognosis of liver cancer were found in the GEPIA database. Differential expression sorting was performed to screen for the target protein.

2.3. Real-time fluorescent quantitative PCR experiment (RT-qPCR)

Primer3 (http://frodo.wi.mit.edu/primer3/) was used to design specific qPCR primers. cDNA was diluted with sterilized pure water to an appropriate concentration, usually 20× dilution, according to the following recipe: qPCR mix 5 μL , primer 1 μL, and cDNA 4 μL. The reagent mixture was then added to a 96-well PCR plate with the film attached. The plate was centrifuged at 2500 rpm for 1 min, and the sample was put into the qPCR instrument for experimentation. After the reaction was completed, the data was copied for analysis.

2.4. Transfection

Six-well plate was used for transfection of siRNA. Two parts of reagent mixture were prepared: 1 μL siRNA mixed with 250 μL Opti-MEM, and 1 μL Lipo2000 mixed with 250 μL Opti-MEM. The reagents mixture parts were left at room temperature for 5 min, and then combined, mixed well, and let stand for 15 minutes.

2.5. Cell proliferation experiment

The overgrown cells were digested, resuspended in 1 ml fresh medium, and then plated with 20 μl at a density that can be plated in a 96- well plate. Each experimental group needed 3 biological replicates, and the required number of experimental wells were plated. The cells were incubated at 37°C with 5% CO_2. siRNA was transfected after cell adherence, and absorbance measurement was conducted after 48 h. The MTS reaction solution was prepared, and the reaction solution was prepared according to the ratio of

MTS:culture solution = 1:20. 100 μl of MTS reaction solution was added to each well, and the culture was continued at 37°C Absorbance was measured every half an hour. The petri dish was shaken at low speed for 10 s on a shaker to fully dissolve the crystals. The absorbance of each well was measured at 490 nm. The daily absorbance values were converted into parameters representing daily cell growth, and cell proliferation curves were plotted.

2.6. Apoptosis experiment

Apoptosis kits (BDscience, New Jersey, USA) were used to detect the apoptosis levels of cells in each group. Cells transfected with siRNA were digested and centrifuged at 1000 rpm for 5 min, washed with phosphate-buffered saline, centrifuged twice, and resuspended in 100μl of 1× Binding buffer. 5 μl of propidium iodide (PI) and 5 μl of Anexin V were added. The cells were incubated at room temperature for 15 min in the dark, and then sent to the scientific research center of a hospital within 1 h for on-machine testing.

2.7. Western blot experiment

The cells were collected for protein quantification and sample preparation. Electrophoresis, electrotransfer device, electrophoresis solution and electrotransfer solution were prepared as per standard procedures. The samples were loaded into the gel, and electrophoresis was conducted at 100 V. The electrophoresis process was terminated as soon as the blue band ran out. The electrotransfer process was carried out at 100 V for 2 h, and the protein was transferred. The membrane was blocked in milk for 1 h, incubated with primary antibody overnight at 4°C, washed 4 times with 5 min each time, incubated with secondary antibody at room temperature for 1 h, washed 4 times with 5 min each time, and eventually developed in the dark room.

3. Result

3.1. Screening of clinically significant target genes in liver cancer

In order to find the target genes with significant clinical significance in liver cancer, we used the GEPIA database to find genes with significant differential expression in liver cancer and adjacent normal tissues, of which 725 were overexpressed and 1482 were underexpressed in liver cancer (as shown in **Figure 1A**). The 725 genes that were overexpressed in liver cancer were ranked according to their clinical prognosis. We looked for genes in liver cancer according to the principle of high gene expression and poor prognosis, combined with the differential expression folds between liver cancer and normal tissues (**Figure 1B**). We found that RNaseH2A has clinical significance.

3.2. Expression characteristics of RNaseH2A in liver cancer

We found a clinically significant target gene *RNaseH2A* in liver cancer. First, we found that the expression of *RNaseH2A* was indeed significantly higher in liver cancer than in normal tissues through the GEPIA database (as shown in **Figure 2A**). At the same time, we found that RNaseH2A has a significant prognosis in liver cancer. As shown in **Figure 2B**, the expression of RNaseH2A also increased with the increase of the malignancy degree of liver cancer (**Figure 2C**), which was significantly higher than that of normal tissues. These characteristics indicate that RNaseH2A has an important role in liver cancer.

3.3. Knockdown of *RNaseH2A* expression can significantly slow down the proliferation of hepatoma cells and induce apoptosis

In order to verify the important role of *RNaseH2A* in liver cancer, we used siRNA interference to reduce the expression of *RNaseH2A* in liver cancer cells (as shown in **Figure 3A**). Apoptosis experiments showed that knocking down the expression of *RNaseH2A* significantly induced apoptosis (**Figures 3C** and **3D**).

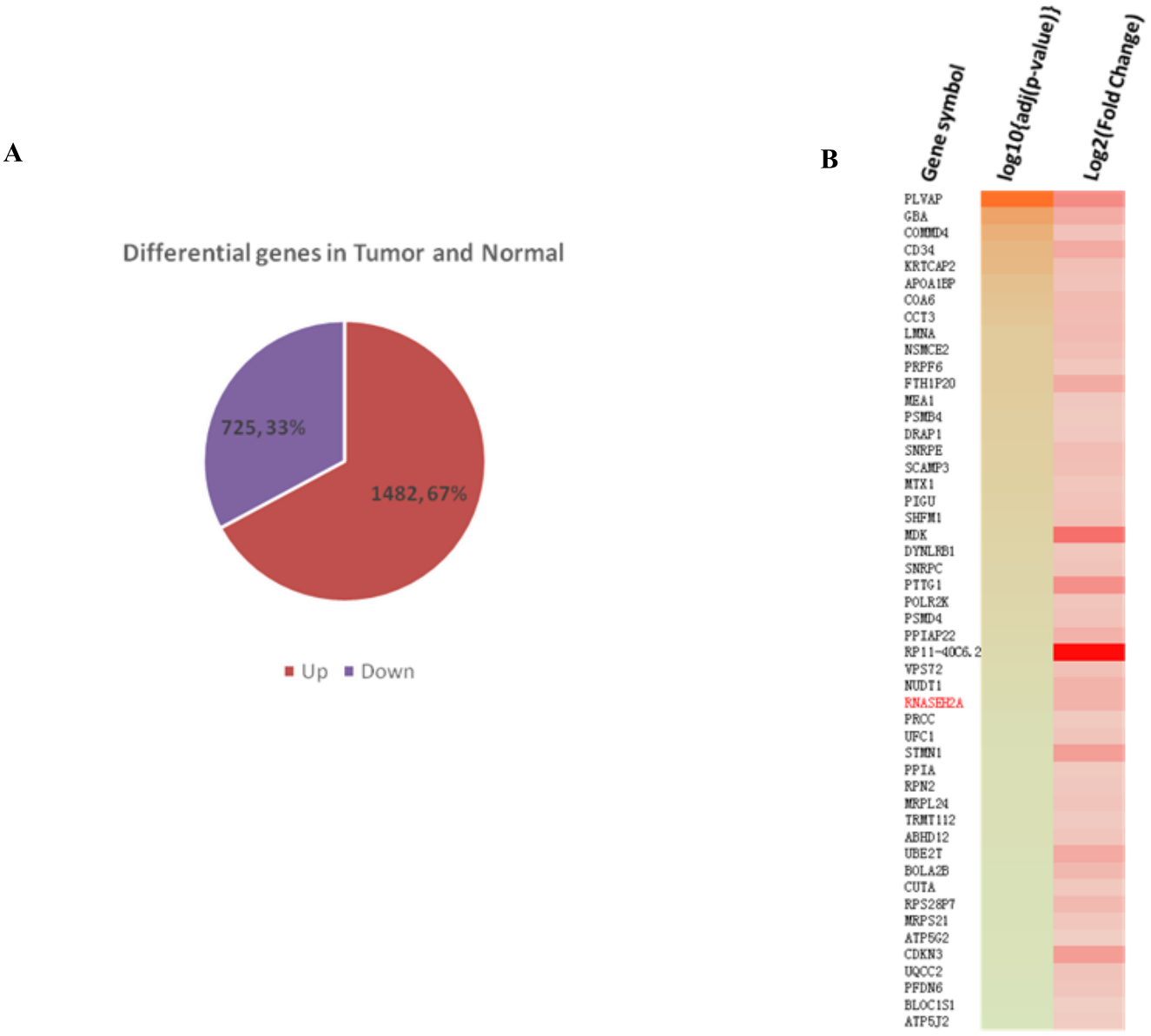

Figure 1. Screening of target genes with significant clinical significance in liver cancer; **A.** The number of differentially expressed genes in liver cancer compared with adjacent normal tissues through GEPIA database; **B.** Heat map analysis of the fold ranking of highly expressed genes in liver cancer

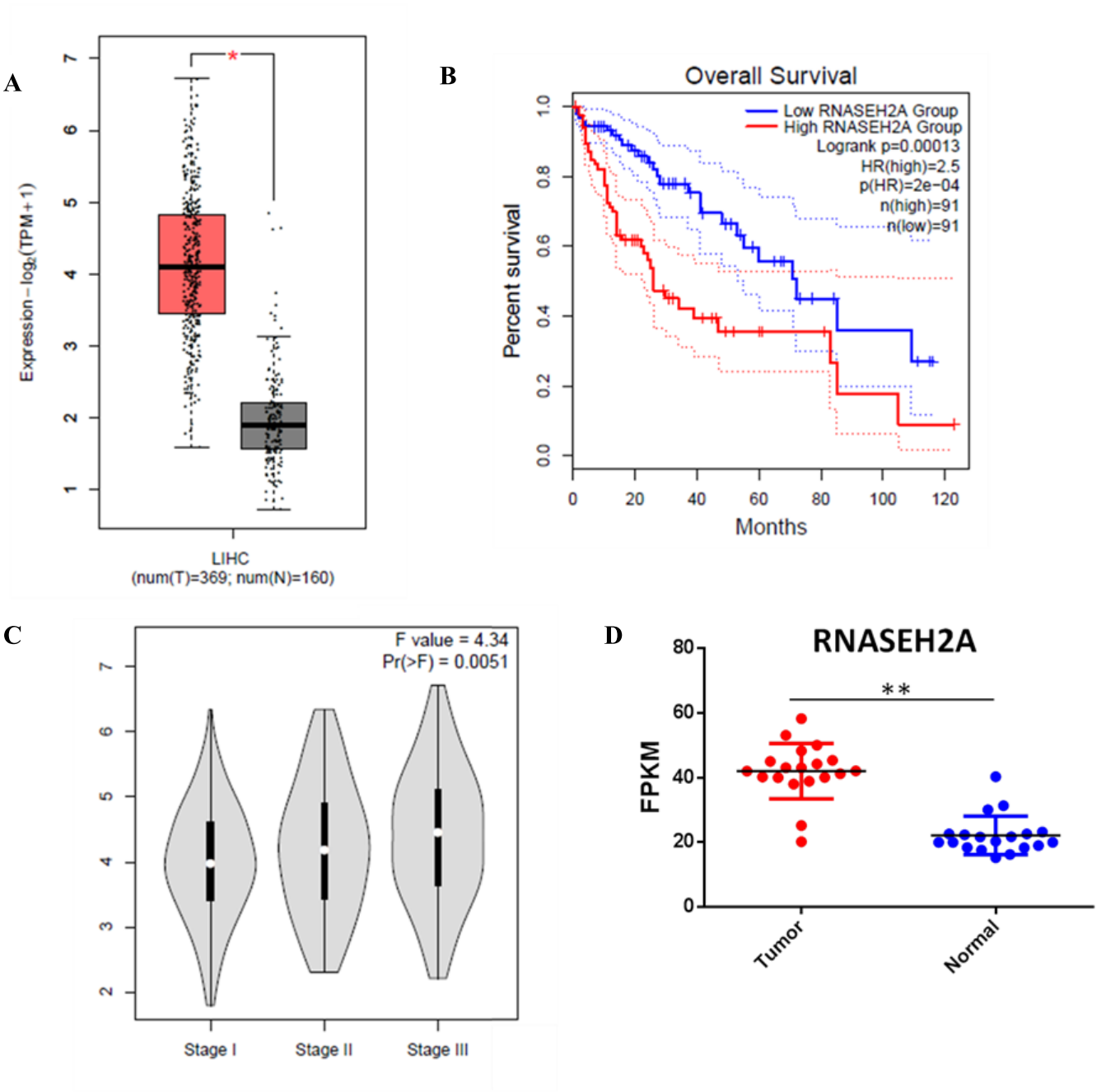

Figure 2. Expression characteristics of RNaseH2A in liver cancer; **A.** The GEPIA database shows the expression of RNaseH2A in liver cancer and adjacent normal tissues; **B.** The correlation between RNaseH2A and the clinical prognosis of liver cancer; **C.** The expression of *RNaseH2A* with the increase of the malignant degree of liver cancer; **D.** The expression of *RNaseH2A* in the sample

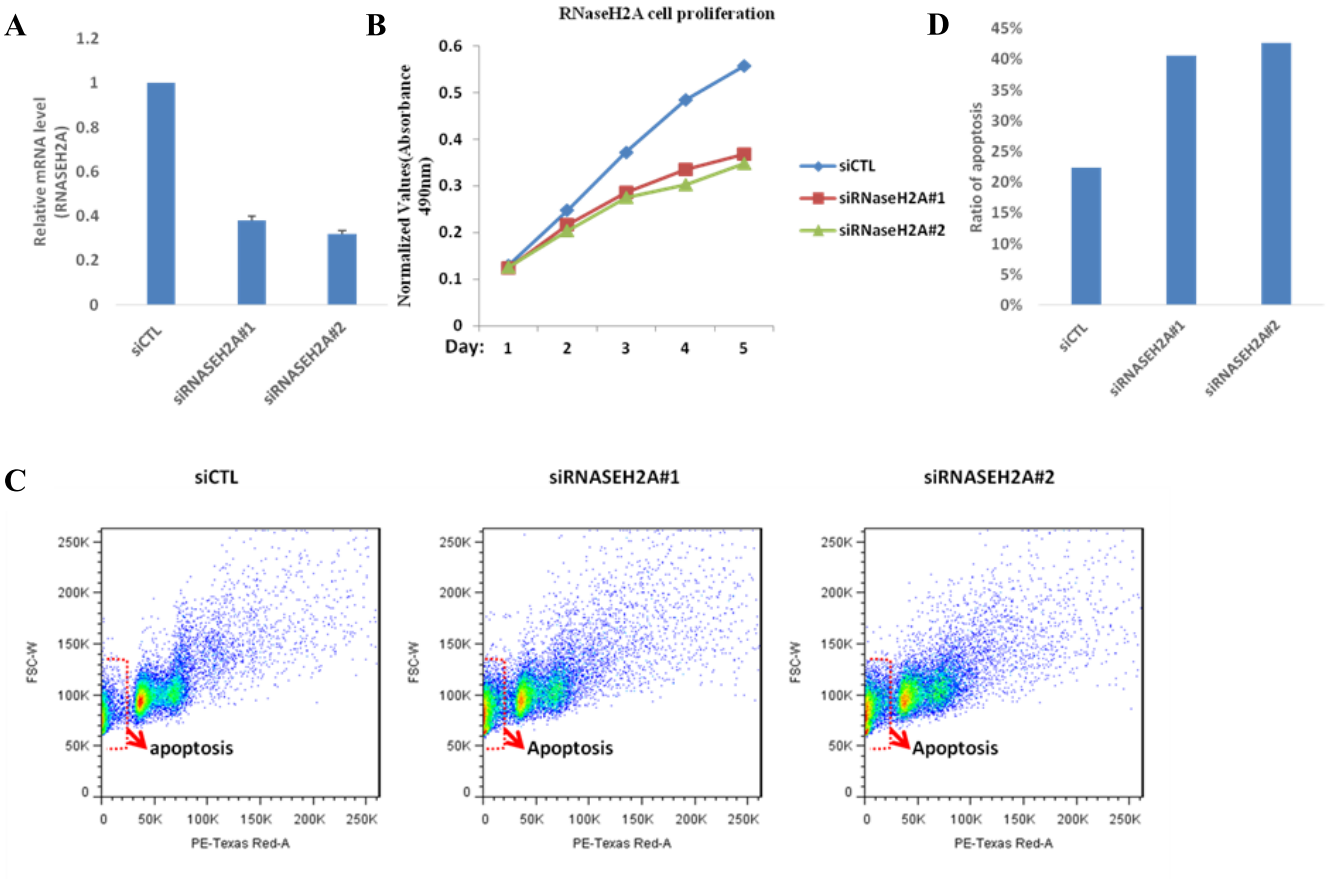

Figure 3. Knockdown of *RNaseH2A* expression can significantly slow down the proliferation of hepatoma cells and induce apoptosis; **A.** The knockdown efficiency of *RNaseH2A* expression by siRNA interference; **B.** The effect of knockdown of *RNaseH2A* expression on cell proliferation rate; **C** and **D.** The effect of knockdown of *RNaseH2A* expression on apoptosis

3.4. Knockdown of *RNaseH2A* can significantly induce the expression of *Fas*

Since the knockdown of RNaseH2A expression can significantly induce apoptosis, we tested the effect on the key gene *Fas* in apoptosis of liver cancer cells which were knocked down of *RNaseH2A* expression. We found that the knockdown of *RNaseH2A* expression significantly increased *Fas* RNA level and Fas protein level (**Figures 4A** and **4B),** showing a negative correlation between *RNaseH2A* and *Fas*. Consistent with this result, *RNaseH2A* was significantly overexpressed in HCC, while *Fas* was significantly underexpressed (**Figure 4C**), and this shows a significant negative correlation with the expression of *RNaseH2A* (**Figure 4D**).

3.5. *RNaseH2A* promotes cell proliferation and attenuates apoptosis by negatively regulating *Fas*

Because of the negative correlation between *RNaseH2A* and *Fas*, knockdown of *RNaseH2A* would induce high expression of *Fas*, thereby promoting apoptosis. Therefore, in order to verify that RNaseH2A directly plays an important role in liver cancer through Fas, we knocked down *RNaseH2A* expression in liver cancer cells. The knockdown of *Fas* expression (as shown in **Figure 5A**) caused the cell proliferation rate returned to the normal level (as shown in **Figure 5B**), and at the same time, abrogated the apoptosis caused by the knockdown of *RNaseH2A* (as shown in **Figure 5C**). These findings all point to the notion that *RNaseH2A* is a direct negative regulator of *Fas*, and it can promote the proliferation of liver cancer cells and slow down the apoptosis of cells to promote the development of liver cancer.

4. Discussion

Gene dysregulation is a hallmark of tumorigenesis and progression [20], and post-transcriptional regulation of mRNA is a key step. Ribonucleases catalyze the breakdown of RNA, thereby affecting mRNA turnover and gene expression; their dysfunction is associated with various types of tumors. For example, a failure to recruit poly(A)-specific ribonuclease (PARN), a polyribonuclease, has been observed in malignant gliomas [21]. In addition, primary osteosarcoma and its derived cell lines also have reduced expression in XRN1, a 5'-3' exonuclease that initiates mRNA degradation [22]. Furthermore, genes encoding RNase L truncations are positively associated with hereditary prostate cancer [23], while modest reductions in enzymatic activity are associated with a higher risk of prostate and colorectal cancer [24] and pancreatic cancer [25]. Inositol-requiring enzyme 1 (IRE1) is a transmembrane endoribonuclease found in the endoplasmic reticulum [26], which acts as a tumor suppressor and determines the fate of cancer cells [27]. Ribonucleases from miRNA pathways, including Drosha, Dicer and Ago2, have also been implicated in tumor biology. For example, Drosha expression was found to be elevated in esophageal cancer [28], and its inhibitory effect resulted in a reduction in cancer cell proliferation. In addition, elevated mRNA levels and genome copy number of Drosha were found in clinical cervical squamous cell carcinoma samples and derived cell lines [29]. Studies report that Dicer is significantly overexpressed in a variety of cancers, including salivary gland, lung, prostate and ovarian cancers as well as Burkitt lymphoma. Ago2 overexpression was also detected in these cancers [30].

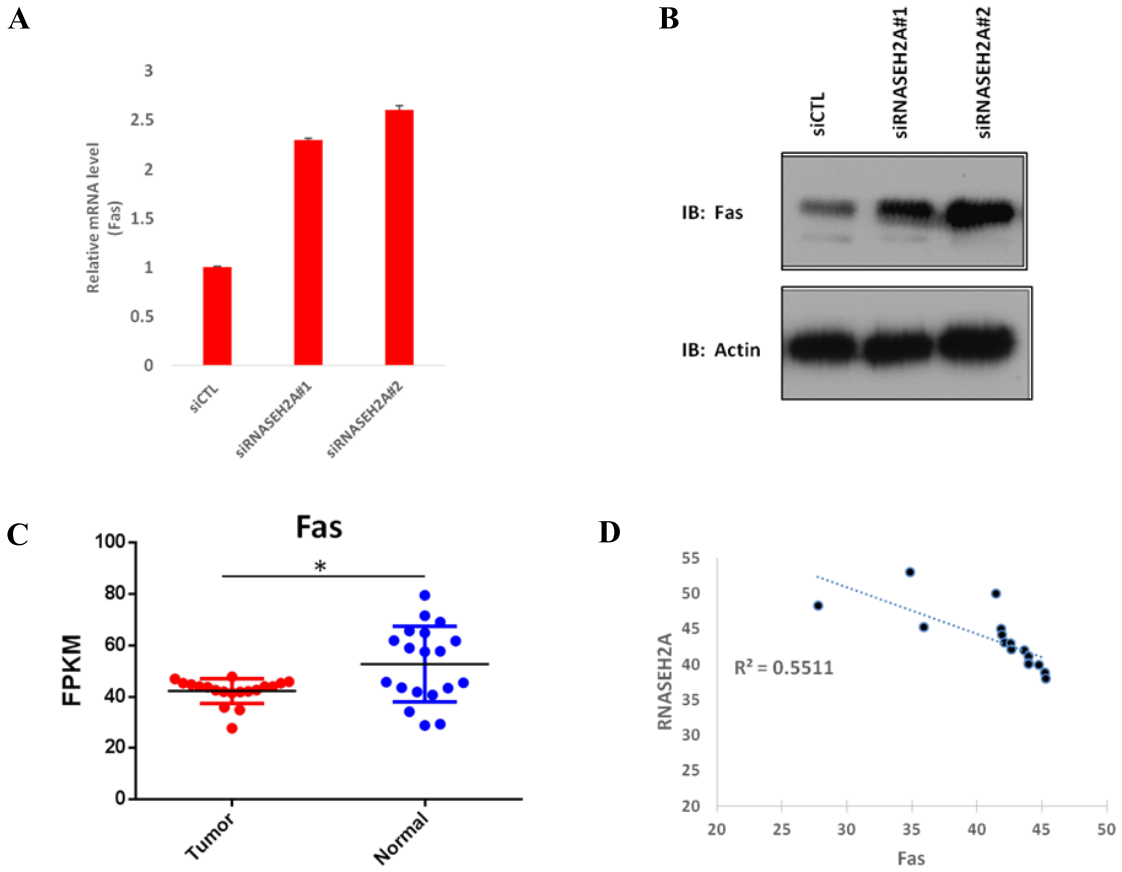

Figure 4. Knockdown of *RNaseH2A* can significantly induce the expression of *Fas*; **A.** Effect of knockdown of *RNaseH2A* expression on *Fas* RNA level; **B.** Effect of knockdown of *RNaseH2A* expression on FAS protein level; **C.** Expression of *Fas* in 19 groups of liver cancer clinical samples; **D.** *RNaseH2A* correlation with *Fas* expression in group 19 of the liver cancer clinical samples

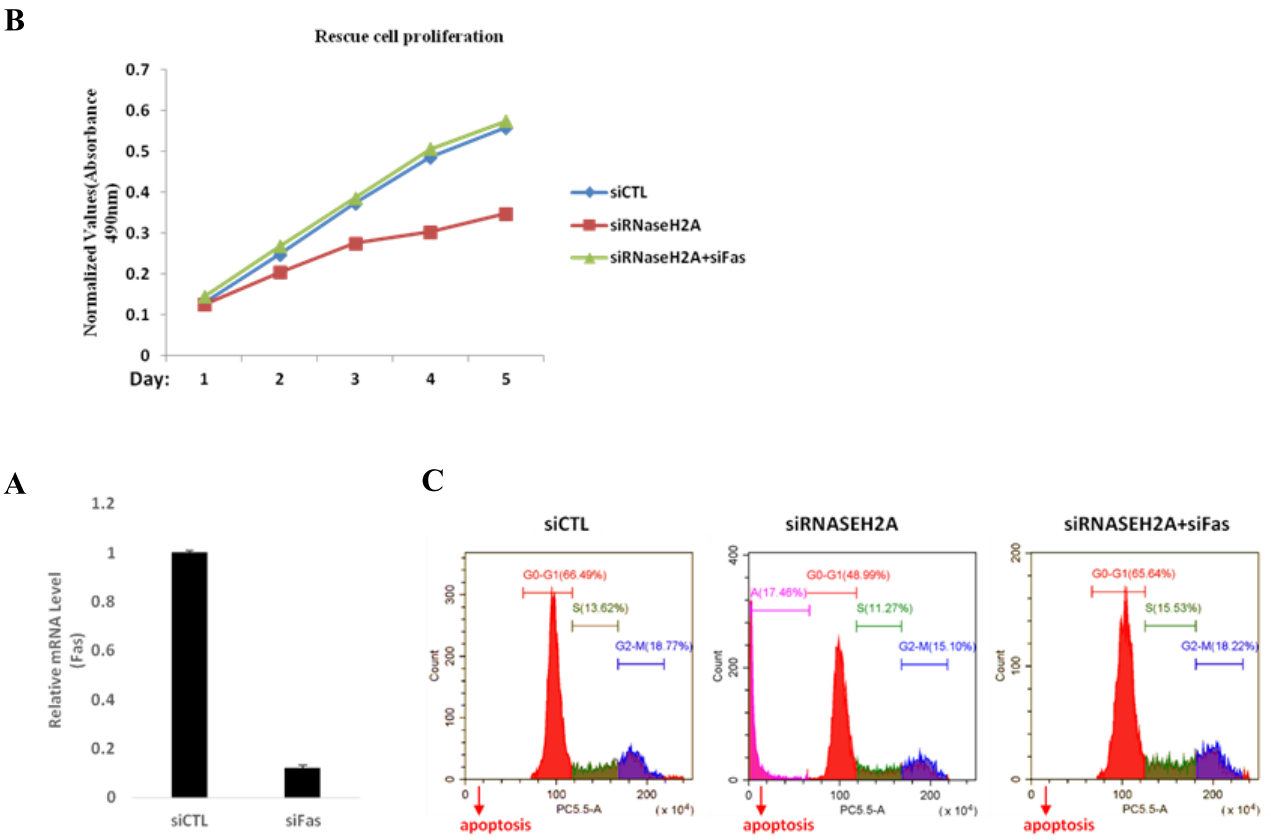

Figure 5. *RNaseH2A* promotes cell proliferation and attenuates apoptosis by negatively regulating *Fas*; **A.** The knockdown efficiency of siRNA interference to knock down *Fas* expression; **B.** The effect of simultaneous knockdown of *RNaseH2A* and *Fas* on the proliferation rate of liver cancer cells; **C.** The effect of simultaneous knockdown of *RNaseH2A* and *Fas* on the apoptosis of liver cancer cells

This article mainly explains the function and molecular mechanism of RNaseH2A in promoting the development of liver cancer. First, we screened *RNaseH2A* based on the fold difference of gene expression between liver cancer and clinically normal tissues in combination with the correlation with clinical prognosis, and further analyzed the database and we found from the clinical samples that *RNaseH2A* was significantly highly expressed in liver cancer, and had a significant clinical prognosis correlation. At the same time, with the increase of the malignancy degree of liver cancer, the expression of *RNaseH2A* increased, and then the expression of *RNaseH2A* was knocked down in liver cancer cells. It was found that it can inhibit cell proliferation and induce cell apoptosis, and cell apoptosis is mainly induced by knocking down the expression of *RNaseH2A*, which can induce the expression of *Fas*, a key gene of apoptosis. This suggests a negative correlation between *RNaseH2A* and *Fas*. *RNaseH2A* is highly expressed in liver cancer, while the expression of *Fas* is significantly lower in liver cancer. In order to further verify the direct regulatory relationship between *RNaseH2A* and *Fas* in liver cancer, we conducted a series of rescue assays and found that knockdown of *RNaseH2A* leads to increased *Fas* expression in liver cancer cells. With further knockdown of *Fas*, the cell proliferation rate and apoptosis returned to normal levels.

5. Conclusion

In conclusion, *RNaseH2A* promotes cell proliferation and slows down cell apoptosis, thereby promoting the progression of liver cancer mainly by negatively regulating the expression of the key apoptosis gene *Fas*.

Funding

General Special Scientific Research Project of Shaanxi Provincial Department of Education “Molecular Mechanism of MiR-122 Competing Endogenous lncRNA in the Development of Liver Cancer” (Project Number: 20JK0887)

Disclosure statement

The authors declare no conflict of interest.

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Proceedings of Anticancer Research, 2022, Volume 6, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

A Study on the Effectiveness of Decitabine Combined with a Half-Dose Priming Regimen in the Treatment of Elderly Patients with Acute Myeloid Leukemia

Ying Gao, Lan Li, Xingxing Hu, Yudi Miao*

Department of Hematology, Shaanxi Provincial People's Hospital, The Affiliated Hospital of Xi'an Medical University, Xi'an 710068, Shaanxi Province, China

***Corresponding author:** Yudi Miao, miaoyudi26@163.com

Abstract: *Objective:* To investigate the clinical effects of combining decitabine with a half-dose priming regimen in the treatment of elderly patients with acute myeloid leukemia. *Methods:* This study was conducted in Shaanxi Provincial People's Hospital from January 2019 to January 2022. Sixty patients were recruited as the research subjects. The patients received different treatments and were randomly divided into two groups, with 30 cases in each group, one of which was treated with conventional priming regimen (control group), and the other was treated with decitabine combined with a half-dose priming regimen (study group). The two groups were compared and analyzed in terms of the effectiveness of treatment. *Results:* The rate of symptom relief in the study group was 96.67%, which was significantly higher than that in the control group (76.67%) ($p < 0.05$). Before treatment, there was no significant difference in the quality-of-life scores between the two groups, with $p > 0.05$. The patients in the study group had significantly longer disease-free survival and overall survival than those in the control group, with $p < 0.05$. The effectiveness of treatment in the study group was also better. *Conclusion:* The use of decitabine in combination with a half-dose priming regimen for the treatment of elderly patients with acute myeloid leukemia is effective in improving patients' quality of life, relieving symptoms, and prolonging their survival.

Keywords: Decitabine; Half-dose priming regimen; Elderly; Acute myeloid leukemia

Online publication: May 30, 2022

1. Introduction

Acute myeloid leukemia is a malignant clonal disease that involves myeloid hematopoietic stem cells. It can be manifested as anemia, fever, bleeding and infection of the skin and mucous membrane, as well as bone and joint pain, thus having significant impact on patients' health and quality of life [1-4]. In clinical practice, it is important to take into account of the patient's underlying disease condition when formulating a treatment plan that is both effective and safe. This method was first proposed by Japanese scholars, and it consists of two main chemotherapeutic regimens, one of which is a priming regimen using aclarubicin, cytarabine, and granulocyte colony-stimulating factor, and the other is also a priming regimen that uses idarubicin, cytarabine, and granulocyte colony-stimulating factor [5-9]. Since there is a gradual decline in the physical functioning of elderly patients, and they are known to have lower tolerance to chemotherapy regimens, half-dose priming regimens are now widely used in clinical practice [10-13]. In this study, decitabine was used in combination with a half-dose priming regimen to compare and analyze the effects

of this clinical intervention in elderly AML patients.

2. Materials and methods

2.1. General information

This study was carried out in Shaanxi Provincial People's Hospital from January 2019 to January 2022, with a total of 60 cases of elderly acute myeloid leukemia patients. The patients were given different treatments and divided into two groups (study group and control group), with 30 cases in each group. In the study group, there were 16 male patients and 14 female patients, age ranging from 61 to 88, with a mean age of 74.34 ± 4.34. In the control group, the number of male patients and the number of female patients were 17 and 13, respectively, with age ranging from 60 to 88, and a mean age of 74.05 ± 4.55. However, it is known that there are only a few dozens of leukemia cases every year, with older cases accounting for only a handful of them. Statistical methods were used to compare and analyze the general data of the two groups of patients, and the results all showed $p > 0.05$, with no significant differences, thus meeting the criteria for a comparative study.

Inclusion criteria: (1) patients diagnosed with acute myeloid leukemia after clinical examination; (2) patients above 60 years of age; (3) those who agreed to participate in the study with informed consent taken. This study was approved by the hospital ethics committee.

Exclusion criteria: (1) patients who survived for less than 3 months; (2) patients who have severe liver and renal insufficiency or other conditions; (3) patients who withdrew from the study or with poor compliance.

2.2. Methods

In the control group, CAG and IAG regimens were used, of which 29 patients were treated with the CAG regimen, while 21 patients were treated with the IAG regimen. Aclarubicin was administered on the first day of chemotherapy at a dose of 20 mg via intravenous injection and given every other day for a total of four treatments. For cytarabine, 10 mg/m^2 was administered via subcutaneous injection on the first day of chemotherapy every 12 hours for a total of 14 consecutive days. Granulocyte colony-stimulating factor was also administered via subcutaneous injection on the first day of chemotherapy at a dose of 200 mcg/m2, once a day for 14 days, with observation and discontinuation of the drug when the white blood cell count reached 20 x 10^9/L. Under the IAG regimen, the main drugs used were idarubicin, cytarabine, and granulocyte colony-stimulating factor. The dosage and administration of cytarabine and granulocyte colony-stimulating factor were the same as those in CAG regimen. For idarubicin, 5 mg was administered intravenously once a day for six days, beginning on the first day of chemotherapy.

In the study group, decitabine was administered intravenously on the first day of chemotherapy at a dose of 20 mg/m^2 over 3 hours, once daily for 5 days. In addition to that, half-dose priming regimens were used (half-dose CAG regimen and half-dose IAG regimen). Twenty-nine patients were treated with half-dose CAG regimen, while 21 patients were treated with half-dose IAG regimen. The half-dose CAG regimen (cytarabine, aclarubicin, and granulocyte colony-stimulating factor) was used over one cycle of chemotherapy. Aclarubicin was administered on the first day of chemotherapy at a dose of 20 mg via intravenous injection and given every other day for a total of two treatments. Cytarabine, which needs to be started on the first day of chemotherapy, was administered via subcutaneous injection at a dose of 10 mg/m^2; the intervention was given every 12 hours for a total of seven consecutive days. Granulocyte colony-stimulating factor was also administered via subcutaneous injection on the first day of chemotherapy at a dose of 200 mcg/m^2; it was given as a daily intervention for a total of seven consecutive days. Patients on half-dose IAG regimen over one cycle of chemotherapy were treated with idarubicin, cytarabine, and granulocyte colony-stimulating factor. The drugs were administered at the same dosage and in the same

manner as the latter two drugs used in the half-dose CAG regimen. Idarubicin was administered intravenously on the first day of chemotherapy at a dose of 5 mg; the intervention was given once a day for three consecutive days, with patients in both groups receiving two consecutive cycles of chemotherapy.

2.3. Observed indicators

The remission of symptoms in the two groups was evaluated. Complete remission, partial remission, and no remission were the three categories. Complete remission refers to the total disappearance of clinical symptoms and the return of normal neutrophil count, platelet count, and megakaryocyte count after treatment; partial remission refers to the improvement of clinical symptoms and various test indicators after treatment; no remission refers to no significant changes in any symptoms before and after treatment. The exclusion rate is the total effective rate of this study.

The quality of life of the two groups was compared, using the Quality of Life Scale, wherein the higher the score, the better the quality of life [14].

The durations of disease-free survival and overall survival after treatment were recorded and compared between the two groups.

2.4. Statistical analysis

SPSS 20.0 was used to analyze the data. The measurement data were expressed in ($\bar{x} \pm s$), while the calculation data were expressed in [n (%)]. After calculation, validation was achieved using t-values and 2 values, respectively. The results were observed and compared, with $p < 0.05$ indicating statistically significant results.

3. Results

3.1. Symptom relief

The rate of symptom relief in the study group was 96.67%, which was significantly higher than that in the control group (76.67%) ($p < 0.05$), as shown in **Table 1**.

Table 1. Comparison of symptom relief between the two groups [n (%)]

Group	Complete remission	Partial remission	No remission	Rate of symptom relief
Study group (n = 30)	21 (70.00)	8 (26.67)	1 (3.33)	29 (96.67)
Control group (n = 30)	8 (26.67)	15 (50.00)	7 (23.33)	23 (76.67)
χ^2				5.192
p				0.023

3.2. Quality of life

Before treatment, there was no significant difference in the quality-of-life scores between the two groups, $p > 0.05$. After treatment, the scores improved, but the scores of the study group were significantly higher than those of the control group, with a large difference from data comparison, $p < 0.05$, as shown in **Table 2**.

Table 2. Comparison of quality-of-life scores between the two groups ($\bar{x} \pm s$)

Group	Quality of life scores	
	Before treatment	After treatment
Study group (n = 30)	43.13 ± 0.22	90.45 ± 0.32
Control group (n = 30)	43.29 ± 0.54	78.56 ± 0.67
t	1.503	87.710
p	0.138	0.000

3.3. Survival time

When compared with the control group, the patients in the study group had longer disease-free survival and overall survival than the control group, and the differences from the data comparison were all statistically significant, $p < 0.05$, as shown in **Table 3**.

Table 3. Comparison of disease-free survival and overall survival between the two groups ($\bar{x} \pm s$)

Group	Disease-free survival	Overall survival
Study group (n = 30)	12.55 ± 1.43	19.54 ± 2.65
Control group (n = 30)	6.45 ± 2.10	13.22 ± 2.67
t	13.151	9.202
p	0.000	0.000

4. Discussion

Acute myeloid leukemia is a malignant clonal disease of the hematopoietic system with high clinical incidence and a growing prevalence in recent years [15-18]. Age is one of the factors that contribute to the development of this disease. Generally, elderly people have reduced physical functioning, which plays a part in increasing the incidence of this disease. Alcohol abuse and smoking are also major influencing factors in the development of this disease [19]. The current clinical development in the treatment of this condition is directed to the priming approach [20]. Although this method is effective, it is not suitable for elderly patients because their bodies are weak and their functions are declining, which result in a lower tolerance. Furthermore, the use of priming regimens can easily lead to various adverse reactions that threaten patients' health [21,22]. Decitabine is a major drug for the treatment of malignant tumors, and it has effective clinical application. When combining this drug with priming regimens, the dose used in priming regimens can be reduced, for example, a half-dose priming regimen can be used. This will help reduce the adverse effects experienced by patients, promote recovery, and improve patients' tolerance.

In conclusion, the use of decitabine in combination with a half-dose priming regimen in the treatment of elderly patients with acute myeloid leukemia is effective in terms of improving the quality of survival, relieving symptoms, and prolonging the survival of patients, which is significant and should be promoted.

Funding

Basic Research of Natural Science in Shaanxi Province (Grant Number: 2021JM-548)

SPPH Incubator Fund for Development of Science and Technology (Grant Number: 2021YJY-19)

Disclosure statement

The authors declare no conflict of interest.

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Proceedings of Anticancer Research, 2022, Volume 6, Issue 3
http://ojs.bbwpublisher.com/index.php/PAR
ISSN Online: 2208-3553
ISSN Print: 2208-3545

Clinical Value of Peripheral Blood Circulating Tumor Cells and Cell-Free DNA Combined Detection in Triple-Negative Breast Cancer

Jirui Sun[1], Shuangqiu Ma[1], Hong Chen[1], Huijuan Geng[2], Jinku Zhang[1]*

[1]Department of Pathology, Baoding First Central Hospital, Baoding 071000, China

[2]Department of Clinical Medicine, Baoding People's Hospital, Baoding 071051, China

***Corresponding author:** Jinku Zhang, zjkblk@sina.com

Abstract: *Objective:* To determine the clinical value of combined detection of circulating tumor cells (CTCs) and cell-free DNA (cfDNA) in peripheral blood of patients with triple-negative breast cancer. *Method:* 41 patients with breast cancer admitted to the First Central Hospital of Baoding from January 2020 to December 2021 were selected and recruited into the experimental group, 42 patients with benign breast cancer admitted during the same period were recruited into the conditional control group, and 41 healthy patients admitted during the same period were recruited into the blank control group. The positive rate of peripheral blood CTCs, the level of cfDNA, and the diagnostic efficacy of peripheral blood CTCs, cfDNA alone and the combination thereof for breast cancer were analyzed. *Result:* The positive rates of peripheral blood CTCs in the experimental group, the conditional control group, and the blank control group were 43.90%, 11.90%, and 9.74%, respectively, and there was significant difference among the groups. The levels of cfDNA in peripheral blood of the experimental group, the conditional control group, and the blank control group were 0.26 ± 0.08 bp, 0.17 ± 0.03 bp, and 0.15 ± 0.04 bp, respectively, which were statistically significant. The detection levels of 100 bp hTERT/ng·ml^{-1} and 241 bp hTERT/ng·ml^{-1} in the experimental group were significantly higher than those in the conditional control group and the blank control group. The accuracy of peripheral blood CTCs detection in the three groups was 66.21%, the accuracy of cfDA241 bp / 100 bp hTERT detection was 80.41%, and the accuracy of combined detection of peripheral blood CTCs and cfDNA was 94.03%. *Conclusion:* The clinical application of peripheral blood CTCs combined with cfDNA level detection can increase detection accuracy, provide data support for clinicians, and improve the clinical diagnostic effect of triple-negative breast cancer.

Keywords: Peripheral blood circulating tumor cells; Cell-free DNA; Triple-negative breast cancer

Online publication: May 30, 2022

1. Introduction

According to global statistics, the total number of global cancer cases in 2020 has reached 19.29 million, including 2.26 million cases of breast cancer, which surpassed the incidence rate of lung cancer and topped the world's cancer incidence rate. Breast cancer is a serious threat to women's health. Breast cancer is heterogeneous, and there are significant differences in clinical case characteristics, cancer molecular characteristics and cell biological behavior. Triple-negative breast cancer accounts for 15%–20% of the incidence of all breast cancer cases. It is characterized by strong drug resistance, strong invasion ability, and high recurrence and metastasis rates. At present, traditional endocrine therapy and molecular targeted therapy are used in the treatment of triple-negative breast cancer. The effect of these two treatments on

triple-negative breast cancer is not obvious. The patient mortality rate is still as high as 25%, and the survival rate of triple-negative breast cancer patients is only 17%, which is very low [1-10]. B-ultrasound and X-ray are commonly used in the screening of breast cancer, but the detection effect is not high enough to meet the clinical needs. Peripheral blood circulating tumor cells (CTCs) [11] and circulating blood extracellular fragment gene sequence (cell-free DNA, cfDNA) [12] have been proposed and applied to the clinical detection of cancer. This study investigates application value of clinical use of CTCs and cfDNA combined detection in triple negative breast cancer.

2. Data and methods

2.1. General information

41 patients with breast cancer admitted to the First Central Hospital of Baoding from January 2020 to December 2021 were recruited into the experimental group, 42 benign breast patients admitted during the same period were recruited into the conditional control group, and 41 healthy brast patients admitted during the same period were recruited into the blank control group. There was no statistical difference in general data between the three groups.

2.2. Methods

Peripheral blood CTCs and cfDNA were tested and measured in individuals of all three groups. 7.5 ml of blood from the three groups was taken for peripheral blood CTCs test. After the blood was mixed with 6.5 ml buffer, it was centrifuged at the speed of 3000 rpm for 10 minutes, and then the supernatant was taken for test. If ≥ 1.0 CTCs were detected in every 7.5 ml of peripheral blood, the CTC status was considered positive. 4.5 ml fasting blood was taken from the three groups in the morning. Cetyltrimethylammonium bromide was used for DNA extraction, and quantitative polymerase chain reaction was used for DNA detection.

2.3. Observation indicators

The positive rate of peripheral blood CTCs [13], cfDNA level [14], and the single and combined detection of peripheral blood CTCs and cfDNA for breast cancer has diagnostic efficacy.

2.4. Statistical methods

SPSS19.0 software was used for statistical analysis of quantitative data. ANOVA was used for inter-group comparison of normally distributed data. *t*-test was used for pairwise comparison of two independent samples. Non-parametric rank sum test was used for inter-group comparison of non-normally distributed data. The statistical test level was $p < 0.05$. For the measurement data, the normal distribution test was carried out. The data in line with the normal distribution are expressed as mean ± standard deviation. $p < 0.05$ is considered statistically significant.

3. Results

3.1. Comparison of positive rates of CTCs in peripheral blood among the three groups

The positive rates of peripheral blood CTCs in the experimental group, the conditional control group, and the blank control group were 43.90%, 11.90%, and 9.74% respectively, and there was significant difference among the groups, as shown in **Table 1**.

3.2. Comparison of cfDNA levels among the three groups

The levels of cfDNA in peripheral blood circulating blood in the experimental group, the conditional control group, and the blank control group were 0.26 ± 0.08 bp, 0.17 ± 0.03 bp, and 0.15 ± 0.04 bp, respectively,

which were statistically significant. The detection levels of 100 bp hTERT/ng·ml^{-1} and 241 bp hTERT/ng·ml^{-1} in the experimental group were significantly higher than those in the conditional control group and the blank control group, as shown in **Table 2**.

3.3. Diagnostic efficacy of peripheral blood CTCs, cfDNA, and their combination for breast cancer

The sensitivity, specificity, and accuracy of peripheral blood CTCs were 43.90%, 82.25%, and 66.21%, respectively. The sensitivity, specificity, and accuracy of cfDNA 241bp/100bp hTERT were 79.12%, 79.89%, and 80.41%, respectively. The sensitivity, specificity, and accuracy of the combined detection of peripheral blood CTCs and cfDNA were 94.25%, 92.02%, and 94.03%, respectively. It is obvious that the combined detection of peripheral blood CTCs and cfDNA has better effect and higher accuracy.

Table 1. Comparison of positive rates of CTCs in peripheral blood among the three groups

Group	Number of cases	Number of positive cases	Positive detection rate (%)
Experimental group	41	18	43.90
Conditional control group	42	5	11.90
Blank control group	41	4	9.74

Note: Compared with the experimental group, $\chi^2 = 10.6040$, $P = 0.0011$; compared with the blank control group, $\chi^2 = 12.1758$, $P = 0.0005$; compared with the blank control group, $\chi^2 = 0.0015$, $P = 0.9691$.

Table 2. Comparison of cfDNA levels among the three groups

Group	100 bp hTERT/ng·ml^{-1}	241 bp hTERT/ng·ml^{-1}	241 bp/100bp hTERT
Experimental group (n = 41)	35.12±6.25^a	8.45±0.56^a	0.26±0.08^a
Conditional control group (n = 42)	24.36±5.88^b	5.23±0.87^b	0.17±0.03^b
Blank control group (n = 41)	20.69±5.26	2.99±0.51	0.15±0.04

Note: ^{a} $P < 0.05$ compared with the conditional control group; ^{b} $P < 0.05$ compared with the blank control group.

Table 3. Single and combined detection of CTCs and cfDNA in peripheral blood

Index	Sensitivity	Specificity	Accuracy
CTCs	43.90%	82.25%	66.21%
241 bp/100bp hTERT	79.12%	79.89%	80.41%
Combined detection of cfDNA and CTCs in peripheral blood	94.25%	92.02%	94.03%

4. Discussion

Breast cancer is one of the most common malignant tumors in women, and accounts for 23% of the total number of female cancer patients in the world [8,15]. As a special subtype of breast cancer, triple-negative breast cancer has an incidence rate of 23.8% in China. It is characterized by insufficient or null expression of estrogen receptor (ER) and progesterone receptor (PR) and null expression of human epidermal growth factor receptor 2 (HER-2) [16-18]. Traditional endocrine and targeted therapy has no effect on triple-negative breast cancer lacking receptor expression. Therefore, standard cytotoxic chemotherapy combined with surgical resection is still the preferred method for systematic treatment of triple-negative breast cancer, but patients with triple-negative breast cancer may still have a high risk of recurrence and metastasis within 3–5 years after the first treatment. Compared with other breast cancer subtypes, triple-negative breast cancer has strong drug resistance, high recurrence rate, strong tissue invasion and invasion ability, which make

treatment difficult, and there are no effective biomarkers and predictive indicators for this cancer subtype [19, 20]. Therefore, it is very important to study the value of combined detection of peripheral blood CTCs and cfDNA in the clinical diagnosis of triple negative breast cancer.

The molecular fragments of free DNA in peripheral blood is the focus of the current study. It is found that the length of free DNA in peripheral blood in cancer patient is shorter than that in normal people, which may be caused by the proliferation of necrotic cells or tumor cells. The content of cfDNA fragments in the peripheral blood of normal people is low, mainly because the cells have been cleared by the immune system. The cancer cells in the patients undergo rapid proliferation, short growth cycle, and rapid apoptosis. Therefore, the content of cfDNA fragments in the peripheral blood of patients is relatively high, and the autoimmunity of cancer patients is reduced. The peripheral blood CTCs and cfDNA can be used for early diagnosis of breast cancer and evaluation of treatment curative effect.

In this study, we found that both the positive detection rate of peripheral blood CTCs and cfDNA level in the experimental group were significantly higher than those in the conditional control group and the blank control group. The accuracy rate of peripheral blood CTCs combined with cfDNA level in the experimental group was 94.03%, which was significantly higher than that of peripheral blood CTCs alone (66.21%) and cfDNA alone (80.41%).

Therefore, the clinical application of combined detection of peripheral blood CTCs and cfDNA level can increase detection accuracy, provide data support for clinicians, and improve the clinical diagnostic rate of triple-negative breast cancer.

Funding

This present study was financially supported by Baoding City Science and Technology Plan project (2041ZF084), Key Laboratory of Molecular Pathology and Early Diagnosis of Tumor in Hebei Province.

Disclosure statement

The authors declare no conflict of interest.

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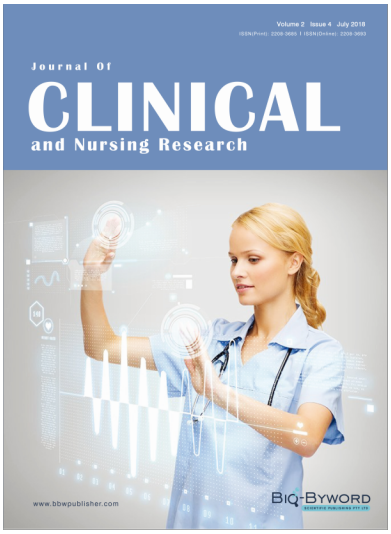

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