Review

Progress in the expression of P-glycoprotein in brain metastases of lung cancer and related TCM research

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Abstract

Brain metastases are common intracranial tumors, and their occurrence not only represents a high degree of malignancy, but also often is the major factor in treatment failure and poor prognosis. Primary site of brain metastases often occur in lung. P-glycoprotein is a member of the (ATP binding cassette) transporter superfamily, which is closely related to the development of lung metastases. It is the main reason for influencing the drug through the blood-brain barrier into the brain tissue, and it also is an important factor affecting the treatment of brain metastases. According to the theory of traditional chinese mddicine, the pathogenesis of brain metastases is due to phlegm, poison, stasis, virtual and so on. The principle of treatment is to promote blood circulation, remove phlegm turbidity. In recent years, the impact of Chinese herbal medicine on P-glycoprotein is increasing. This paper analyzes the mechanism and components of the relevant Chinese medicine on P-glycoprotein. It provides a reference for clinical rational drug use.
Keywords: Lung cancer; Brain metastasis; P-glycoprotein; Blood-brain barrier; Chinese medicine treatment; Research progress

摘要

脑转移瘤是常见的颅内肿瘤，其发生不仅代表恶性程度高，往往也是治疗失败、预后差的主要因素。脑转移癌原发灶以肺癌最多。P-糖蛋白为 ABC (ATP binding cassette)转运蛋白超家族成员，与肺癌脑转移瘤的发生发展密切相关。并且是影响药物通过血脑屏障进入脑组织中的主要原因，也是影响脑转移瘤治疗的重要因素。传统中医将脑转移瘤的病机归结为痰、毒、瘀、虚等方面，治则以醒脑开窍、活血化瘀、化痰祛浊等为主。近年来，关于中药对 P-糖蛋白影响的报道日渐增多，本文根据已有的国内外研究，归纳了相关中药及其成分对 P-糖蛋白的影响及作用机制，以系统了解并指导该领域的研究，并为临床合理用药提供参考。
关键词：肺癌，脑转移，P 糖蛋白，血脑屏障，中医药治疗，研究进展

Abbreviations: TCM, Traditional Chinese medicine; P-gp, P-glycoprotein.
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Backgroud

Brain metastasis, as a common nervous system tumor, is a poupular complication of malignant tumors, accounting for about 15 to 30 percent of intracranial tumors [1]. The primary focus of brain metastases is lung cancer. The incidence of brain metastasis of lung cancer was 25.4-65.0%, accounting for 40-60% of brain metastases [2]. P-glycoprotein (P-gp) is a member of human ABC (ATP binding cassette) transporter superfamily, which was the first to be discovered. It is a "hydrophobic vacuum scavenging pump" which relies on ATP energy supply and actively to transport drugs or other chemicals out of cells, prevent the body from absorbing harmful substances and mediating the export of substances [3]. It is important in the absorption, distribution, metabolism and excretion of drugs [4]. P-gp is the primary cause of drug entering brain tissue through blood-brain barrier [5], which is the main obstacle in the treatment of brain metastases. However, the efflux of ATP dependent drugs is the important mechanism of multidrug resistance in tumor cells [6].

Inhibitors of P-gp can promote the drug through the blood-brain barrier, increase the concentration of drugs in the brain and the bioavailability of drugs in the central nervous system, which is of great significance for the treatment of brain metastases. It has been reported that many Chinese medicines have inhibitory or inductive effects on P-gp, and have an effect on the combination with P-gp [7]. The purpose of this paper is to systematize and summarize the effects of various traditional Chinese medicine (TCM) on P-gp and the related mechanisms according to the existing research reports. It aims to provide reference for clinical rational use of drugs.

TCM pathogenesis of brain metastasis of lung cancer

TCM has no name for intracranial tumors, but the symptoms and causes of intracranial tumors have been discussed in *Huangdineijing*. As stated in the *Suwen* that the brain is the sea of marrow, and the cold air goes against the brain from the bone marrow, so it makes people have a headache. And as described in the *Lingshu* that the evil spirit in four solar terms and all directions attack the meridians of the human body, which is the cause of tumour disease. Brain metastases cancer belongs to the category of "headache" and "vertigo" in TCM, respectively, also belongs to the disease such as "symptomatic mass" or "rock (cancer)" and so on.

In recent years, many doctors have conducted in-depth studies on the etiology and pathogenesis of brain tumors from the aspects of phlegm, blood stasis, poison, deficiency, etc. They summed up the etiology and pathogenesis of the two types of deficiency and excess, independent or combined pathogenic. Sthenia syndrome is responsible for phlegm, blood stasis, toxin and evil. Wang Guanmin [8] thinks that the brain tumor is caused by the accumulation of phlegm and dampness in the brain, the stagnation of Qi and blood stasis in the brain. which resulted in the blockage of phlegm and stasis, and the condensation of toxic evil.

Qi and blood can not give the brain nutrition causing loss of brain supply, lack of kidney essence. insufficiency of marrow-sea and phlegm endogenous leading to the formation of tumors. Fan Yongping [9] thinks that kidney deficiency, phlegm-heat and stasis-toxin internal resistance are main reasons for the formation of brain tumors.

Cheng Haibo *et al*. [10] summarized the clinical experience and diagnosis and treatment methods of professor Zhou Zhongying, which proposed that the core pathogenesis of the tumor was phlegm stasis, and the basic treatment principle was anticancer, dispelling poison, nourishing and dispelling evil; Anti-cancer antitoxic, phlegm dispersing knot, promoting blood circulation, removing blood stasis and Fuzheng PeiBen often was used in clinic to remove cancer toxin. Therefore, the treatment brain metastases from lung cancer should pay attention to promote blood circulation, removing phlegm and turbid, clearing heat and detoxification.

Expression of P-gp in brain metastases of lung cancer

P-gp is a glycoprotein initially found by Juilano *et al*. [11]. The human *MDR1* gene is located in the arm 21 region of chromosome 7 and contains 28 exons, with a total length of 4.5 kb, encoding 1280 amino acid peptides. After glycosylation, P-gp with relative molecular weight of 170000 was formed, containing two highly conserved ATP binding domains [12].

The expression of P-gp in brain metastases of lung cancer depends on ATP energy supply, and the drugs are pumped out of the cells by active transmembrane transport. The chemotherapeutic failure of brain metastases from lung cancer is closely related to the overexpression of P-gp, and its drug efflux dependent by ATP is an important mechanism of multidrug resistance in tumor cells [13].

In addition to the tumor cells, P-gp was also highly expressed in the microvascular epithelial cells of the brain and the apical membrane of the choroid plexus epithelial cells. The expression of P-gp in the facial membrane was 17 times higher than that in the microvascular endothelial cells of the brain. It is 400 ~ 500 times of the whole brain tissue [14, 15]. Blood-brain barrier is an important structure composed of cerebral microvascular endothelial cells, closely connected basement membrane and astrocyte podocyte, which is the main factor that hinders the entry of drugs into the brain. Studies has shown that [16, 17] P-gp, dependent on the energy released by ATP hydrolysis, pumps some of the drugs diffused into the brain back into the blood, limiting many lipophilic drugs into the brain, becoming the main reason for the influence of drugs to enter the brain through the blood-brain barrier. Therefore, the inhibitor of P-gp has become an important link in the treatment of brain metastases through the

blood-brain barrier.

Recent studies on the inhibitory effect of traditional Chinese herbals on P-gp of brain metastases from lung cancer

At present, the mechanism of TCM affecting P-gp can be summarized as follows: (1) competitive, non-competitive or allogeneic blocking substrate binding sites; (2) regulating the activity of progesterone receptor PXR; (3) affecting cell membrane fluidity; (4) inhibiting or inducing the activity of P-gp ATP hydrolase; (5) affecting the ATP level in intracellular; (6) upregulating or down-regulating the expression of *P-gp* gene [18, 19].

Aromatic resuscitation Chinese medicine

Aromatic resuscitation herbs play a role in inhibiting P-gp by acting on the transport sites of P-gp substrates, competitively inhibiting the efflux by P-gp and inhibiting the activity of ATP hydrolase. It has the function of protecting the ultrastructure of brain cells, improving brain metabolism and enhancing the activity of brain cells. It plays a role in three aspects: free penetration of barrier structure, improvement of permeability and promotion of the opening of brain barrier. The mechanism of aromatic resuscitation Chinese medicine in regulating the permeability of BBB is mainly involved in influencing neurotransmitters and its receptors, inhibiting P-gp and regulating the level of nitric oxide. And inhibition of P-gp is a vital aspect of its functioning. The main representative drugs are musk, acorus calamus, heparin, benzoin.

Borneol. Borneol is the crystallization of resin and volatile oil from the plant of *Borneaceae*. The *Compendium of Materia Medica* records that borneol can "Arousing Consciousness, Scattered stasis fire." The borneol can increase the concentration of vincristine and quercetin in brain and play a similar role to verapamil, a classical competitive inhibitor of P-gp [20, 21]. Therefore, one of the mechanisms by which borneol opens the blood-brain barrier may be that the competitive inhibition of substrate affects the function of P-gp. Yuan Zhuo [22] explored the relationship between P-gp and borneol in promoting the physiological opening of BBB. It is believed that borneol has high affinity with P-gp. Adjuvant drug and lead drug can preferentially bind with P-gp, fail to pass blood brain barrier accumulates in cell. Furthermore, it was also reported that borneol could inhibit P-gp by increasing its bioavailability. Lan K [23] also reported that borneol could inhibit P-gp. Further study is needed [24].

Acorus calamus. Acorus calamus is the rhizome of perennial *Artemiaceae*. It has many functions such as eliminating phlegm, removing dampness and appetizing and so on. Acorus calamus contains volatile oil, carbohydrate, amino acid and so on. β -Asarone is the most important component in the volatile oil. Hu Yuan [25] founds that the tight connection between capillary endothelial cells in brain tissue sections of *Acorus calamus* mice was relaxed, which effectively improved the permeability of brain barrier to drugs and opened the bottleneck which could not be broken by traditional chemotherapeutic drugs. Acorus calamus has the ability to inhibit the efflux by P- gp on the membrane of Hela cell to improve the permeability of blood-brain barrier. β-asarone can decrease the expression and activity of P-gp and reverse P-sugar egg, thus increase the intracellular accumulation of antitumor drugs [26]. Many domestic studies have also found that it can cause cell destruction, dissolution and growth inhibition of human lung metastatic cancer cell lines, and ultimately inhibit proliferation.

Yang Yang [27] has found that the main components of aromatic resuscitation drugs, heparin, benzoin and vanillin had obvious inhibitory effects on the efflux function of P-gp through vitro study. It is suggested that inhibition of P-gp function may be the possible mechanism for enhancing BBB permeability of these drugs.

TCM for promoting blood circulation and removing blood stasis:

The TCM of promoting blood circulation and removing blood stasis can inhibit P-gp by affecting the function of cell membrane and inhibiting ATP enzyme. It can protect cells, promote the absorption of hematoma in brain and alleviate inflammatory reaction. Recent studies have shown that [28] it can not only effectively inhibit the invasion and metastasis of malignant tumors, but also have a good preventive and therapeutic effect on brain metastases. Its function is closely related to the reduction of P-gp expression, the promotion of drug entry into the brain, the protection of blood-brain barrier and the anti-free radical injury.

Radix curcuma. The turmeric genus herb is a typical representative of TCM to promote blood circulation and removing blood stasis through blood-brain barrier. Curcumin is a kind of plant polyphenol extracted from Curcuma, and it is the most important active ingredient of Curcuma. Both western blotting and RT-PCR showed that curcumin and KB-V1 cells incubated simultaneously for more than 72 hours could significantly reduce the expression of P-gp in a concentration-dependent manner [29]. Other studies have shown that curcumin inhibits the expression of P-gp by inhibiting the phosphorylinositol -3 carboxyl kinase / serine / threonine kinase (PI3K / Akt) signaling pathway and decreasing the NF- κ B transcriptional pathway [30]. In addition, with the development of research, domestic and international multi-center laboratory studies show that curcumin has excellent anti-tumor effect. It can induce the differentiation of human malignant tumor cells, inhibit proliferation and promote apoptosis of tumor cells [31, 32].

In addition, turmeric curcuma can inhibit the expression of P-gp by affecting the function of cell membrane. The study found that elemene and cepharanthine, the active ingredient of the turmeric root tuber, zedoray rhizome, can modify the phospholipid membrane structure [33], resulting in cell membrane

lipophilic environmental damage. It further induces the change of P-gp function and molecular arrangement to exert P-gp inhibitory effect. Furthermore, 6-elemene and its derivatives such like Elemene hormone, can reduce the *bcl-2* and *P-gp* of anti-apoptotic genes. It can promote drug-resistant cell apoptosis, achieve the purpose of killing the tumor. Elemenum Emulsion is a new anticancer drug developed by China. Its main component is R-elemene, which can inhibit the growth of many kinds of lung cancer cells both in vivo and in vitro [34].

Ligusticum wallichii. Ligustrazine, the effective constituents of Ligusticum wallichii, also known as tetramethylpyrazine (TMP), is one of the alkaloids in the rhizome of Ligusticum chuanxiong, which belongs to Artemisia Umbelliferae. It is widely used in cardiovascular and cerebrovascular diseases. The experimental results from Yang Wen [35] show that Ligustrazine can inhibit P-gp expression significantly. TMP is an inhibitor of P-gP, which affects the function of P-gp by directly inhibiting the activity of P-gp. Prolonged application of TMP can decrease the activity of P-gP by down-regulating the expression level of P-gP. The noncytotoxic dose (300mg / L) of TMP significantly reduced the MCF-7 / dox efflux of vincristine, adriamycin and paclitaxel. And it can inhibit the activity of P-gp ATP in MCF-7 / dox and the expression of P-gp [36]. Tetramethylpyrazine is also the substrate of P-gp, which can compete with other substrates and bind to P-gp to reduce the efflux by efflux gp to intracellular substrates [37].

Salvia miltiorrhiza. Salvia miltiorrhiza is a typical representative of TCM for promoting blood circulation and removing blood stasis, which is a hot research topic in recent years. As the main chemical component of anti-tumor activity, tanshinone has attracted more and more attention. Tanshinone Ⅱ A, cryptotanshinone and dihydrotanshinone are both P-gp substrates and inhibitors. Inhibition of P-gp ATP activity decreased P-gp function [38, 39]. Diterpenoid tanshinone significantly reduces the efflux of cryptotanshinone mediated by P-gp [40]. P-gp is an important factor affecting the entry of Danshensu into the brain, but the specific mechanism is unknown [41].

At present, the effect of P-gp inhibitors is also found in some blood activating drugs, which can inhibit the efflux of other drugs by P-gp and promote the absorption of drugs by brain. Wu Qian [42] found that Bupleurum chinense, cohosh and salvia could enhance the cytotoxicity of vincristine in a time-and dose-dependent manner. It is proved that many drugs can inhibit the efflux by P-gp.

TCM for resolving phlegm and dispersing knot

The application of drugs to dissipate phlegm and dissipate collaterals in the treatment of brain neoplasms is very extensive. Huo Joginger [43] summarized Zhou Zhongying treatments of brain tumors in 25 cases, 11.7% of the total drugs were used to remove phlegm and disperse knot. Removing phlegm and dredging collaterals accounted for 14.35% of the total drug usage. TCM plays a role in inhibiting P-gp by competitively blocking the substrate binding site and down-regulating the expression of *P-gp* gene. Phlegm and blood stasis interknot blocking cerebral collaterals is the main pathogenesis of brain metastasis of lung cancer. Phlegm and stasis are the main pathogenic factors [44]. *Suwen* recorded "Where the intra-abdominal mass lumps and hard physical, available offensive medicine were used." [45]. Therefore, in the treatment of brain metastasis of lung cancer, resolving phlegm and dissipating collaterals should be taken as the primary treatment method. P-gp is widely used in clinic as the key target of anti-tumor treatment of brain metastasis. Its representative drugs are rhubarb, rhizoma alismatis.

Rhubarb. Rhubarb is first contained in *Shennong Herbal Classic*. It has the functions of purging heat toxin, breaking accumulation and stagnation, and removing blood stasis. Emodin, an effective component of rhubarb, is a hydroxy anthraquinone derivative with anti-tumor and renal protection effects. Emodin is a strong inhibitor of P-gp [46], which can decrease the expression of mitogen-activated protein kinase by blocking the binding site of AP-1DNA, and down-regulate the expression of P-gp [47].

Alisma. Alisma, typical Chinese Medicine for treating vertigo of phlegm and drink, can clear damp and promote diuresis. Alisma Decoction, from Zhang Zhongjing's *synopsis of the Golden Chamber*, can treat vertigo caused by branching drink. The results showed that [48], 23-acetylalisol B, an active component of Rhizoma Alismatis, was the substrate and inhibitor of P-gp, and activated the activity of P-gp ATP enzyme in a dose-dependent manner. The expression of P-gp was inhibited. It can increase the accumulation of adriamycin and decrease the output of R123 in resistant cells, which is also the important reason for reversing multidrug resistance. Alisma can significantly inhibit the spontaneous metastasis of Lewis lung cancer, and its mechanism may be related to the changes of some protein components in serum [49].

Stephania tetrandra. Stephania tetrandra is a commonly used Chinese medicine to dissipate phlegm and remove turbidity, among which many alkaloids have inhibitory effect on P-gp.

Tetrandrine and fangchinoline can significantly reduce the expression of P-gp in a concentration-dependent manner and inhibit its function by blocking the P-gp substrate binding site [50]. In addition, Tetrandrine has the effects of calcium antagonism, membrane regulation, inhibiting drug efflux and reversing P-gp-mediated MDR in vivo and in vitro. In addition, hanfangchin A, as the representative of TCM, has higher specificity and lower toxicity. As a third-generation inhibitor, it is in the stage of clinical research and development [51, 52].

Stemona. Stemonae extracts can significantly increase the accumulation of vinca alkaloids, paclitaxel and colchicine in KBV-1-overexpressing P-gp resistant cells in a dose-dependent manner. The inhibition of the extract for P-gp may be to interfere with the substrate binding

site, so that P-gp can not bind to the substrate [53].

Antipyretic and detoxifying TCM

In recent years, professor Zhou Zhongying's theory about carcinogenesis is one of the most important innovations in the theory of tumor pathogenesis in TCM. "Poison of cancer" is the specific pathogenic factor of tumor, and cancer toxin, phlegm and blood stasis form tumor. Therefore, phlegm, blood stasis stagnation and toxin are the main pathogenesis of the tumor. Antitumor and toxin dispelling method commonly used in clinic [11]. Most of the antipyretic and detoxification herbs inhibit the expression and function of P-gp by regulating the activity of *P-gp* gene and related signal pathway. It regulates the activity of the GP receptor PXR and plays a role in inhibiting the P-gp.

Licorice. Licorice has the effect of reducing fire and detoxification, moistening lung and relieving cough. The extract of liquorice activates, PXR, promotes the metabolism and excretion of poison in vivo, and has the effect of detoxification. The composition of Glycyrrhiza uralensis is complex and its effect on P-gp is different. Some studies showed that Glycyrrhiza uralensis was a mild inhibitor of P-gp [54]. But glycyrrhizin monoamine can induce the function of P-gp and enhance the expression level of P-gp to promote P-gp-mediated efflux. It is believed that Glycyrrhiza uralensis has effect on P-gp activity [55].

Cordate houttuynia. Quercetin, an effective component of Herba Houttuynia cordata, can not only reduce the expression of P-gp in MDR cells, but also inhibit the expression of P-gp by inhibiting the activity of heat shock protein. And it can inhibit the function of P-gp [56]. By double inhibition of P-gp expression and function, the efflux in brain metastases was reduced.

In addition, the active ingredient of Scutellaria baicalensis, thousand layer A, down-regulated the expression of P-gp by inhibiting NF- κ B signaling pathway [57]. The activity and expression of *P-gp* gene were down-regulated by inhibiting the activity of c-Jun amino terminal kinase [58]. By down-regulating the activity of *P-gp* gene and the expression of related signaling pathway, P-gp was inhibited.

Yiqi Fuzheng TCM

The effect of tonic Chinese medicine on P-gp can also be used to increase the efficacy of drugs. Its effective components play a role in inhibiting P-gp by stimulating immune cells to proliferate or induce immune effector molecules. Improving the therapeutic effect of chemotherapeutic drugs is of great clinical significance.

Ginseng. Ginseng has enhancing immune and cognitive function and preventive effect on a variety of diseases. The main effective components were ginsenosides and ginseng polysaccharides. The interaction between ginsenoside and P-gp azidopine site can inhibit P-gp and increase the intracellular accumulation of anti-tumor drugs [59]. Song Juan *et al* [60] found ginsenoside Rg1 is an inhibitor of P-gp. The other components of ginseng, Rg2, Rg3, Rh1, Rh2 and Rh3, inhibit P-gp only at high concentrations [61].

In addition, the active constituents of Panax notoginseng, Rg1, ginsenoside Rh1 and notoginsenoside R1 have obvious P-gp substrate properties and inhibit the efflux of P-gp substrate [62].

Astragalus mongholicus. Astragalus saponin Ⅱ and Astragalus saponin Ⅳ have the function of inhibiting P-gp. Western blotting and RT-PCR showed that astragaloside Ⅱ could inhibit the expression and function of P-gp by inhibiting the extracellular phosphorylation pathway and regulating the expression of N-terminal kinase of kinases 1/2, p38 and c-Jun [63]. Astragalus polysaccharide down-regulated the expression of *P-gp* mRNA in a dose-and time-dependent manner [64]. Astragalus polysaccharide itself has no antitumor activity, but it can increase the cytotoxicity of chemotherapeutic drugs such as cyclophosphamide and doxorubicin on mouse hepatoma 22 drug-resistant cells (H22 / ADM) in a dose-dependent manner. This is closely related to the regulation of human immune.

Schisandra chinensis. Schisandra chinensis belongs to *Magnoliaceae*. Its main medicinal components, lignans, can inhibit P-gp. DeoxySchisandrin, Angelica Gomisin, Gomisin A, crotonyl Gomisin H and Gomisin C inhibited the extracellular excretion of R123 cells in a concentration-dependent manner, among which deoxyschisandrin had the strongest inhibitory effect. Schisandrin A increased the accumulation of adriamycin and down-regulated the transcription and expression of P-gp [65]. Schisandrin B significantly inhibited the expression and function of P-gp and reversed the response of K562 / Adr-KBv200 MCF-7 / ADR cells to paclitaxel, adriamycin and vincristine [66].

Fructus psoraleae. Psoralen, a TCM of tonifying kidney and nourishing marrow, could increase the sensitivity of MCF-7/Adr cells to ADR and increase the content of R123 in cells. The expression of MCF-7 / Adr, P-gp decreased in a time-dependent manner after R3 treatment. It suggested that R3 inhibited P-gp expression by increasing the content of R123 in cells.

Brief summary

P-gp, as the main reason of blocking drug passing through the blood-brain barrier into brain tissue, has become an important obstacle to the treatment of lung cancer brain metastases. The effect of TCM on the inhibition of malignant tumor invasion and brain metastasis has become an important base point for western pharmacists to accept Chinese herbal medicine. TCM or active components which can inhibit P-gp can reduce the efflux of chemotherapy drugs in blood brain barrier and tumor cells. It has important clinical significance in treating brain metastases of lung cancer and reducing drug resistance of tumor cells. And some effective ingredients of TCM, such as hanfangchin A, have higher specificity and lower toxicity to P-gp. It is at the stage of clinical development of the third generation inhibitor study. However, both the active components of TCM and the mechanism of action on P-gp are complex.

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