

Triptolide in the Treatment of Lung Cancer

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Abstract: Today, Lung cancer is the cancer with the highest fatality rate worldwide, which seriously affects physical and mental health. Due to the inadequacy of existing treatment methods, people have launched extensive research on new drugs with potential for cancer treatment. Triptolide, a diterpenoid epoxide derived from *Tripterygium wilfordii*, has emerged as a promising multi-target agent against lung cancer. This review systematically summarizes triptolide's mechanisms including: (1) proliferation inhibition via HnRNPA2/B1-PI3K/AKT suppression; (2) apoptosis induction through mitochondrial (SIRT2/p53/Bcl-2/Bax) and cuproptosis (ATP7A/B-DLAT oligomerization) pathways; (3) Wnt/ β -catenin signaling blockade by p70S6K/GSK-3 inhibition; (4) immunomodulation via Stat3/NF- κ B axis. Despite demonstrating potent anti-tumor efficacy in preclinical models, clinical translation is hindered by its narrow therapeutic window (hepatorenal toxicity) and poor bioavailability. Recent advances in structural derivatives (e.g., Omtriptolide) and drug combinations (e.g., with cisplatin) show potential for overcoming these limitations. Further optimization of toxicity profiles and pharmacokinetics is essential for advancing triptolide into Phase II trials.

Keywords: Triptolide, lung cancer, cancer mechanism, clinical application

1. Introduction

Nowadays, lung cancer has become the number one malignant tumor in the world due to its high incidence of cancer and high mortality. At present, the treatment of lung cancer and other related cancers mostly relies on chemotherapy (60%-70%), radiotherapy (50%-60%) and surgery (30%-40%). However, up to now, the treatment methods of chemotherapy and radiotherapy are prone to drug resistance and inflammation near the tumor. Surgical treatment is limited to the early stage of lung cancer, and postoperative complications such as residual micro metastasis of the tumor may occur. This environment has led to extensive research into novel therapeutic agents, including natural compounds with potential anti-cancer properties.

Among them, triptolide has gradually entered the mainstream research field because of its effective anti-tumor activity. Triptolide originated from *Tripterygium wilfordii*, which has been used in China for hundreds of years, mainly aim at rheumatoid arthritis and other inflammation. Triptolide was isolated from the root bark of triptolide by Chinese chemist Zhang Yi and his team in 1972 and has been involved in anti-tumor mechanism research since the 1990s. At present, triptolide mainly focuses on the treatment of inflammation, although the treatment of tumor is also being carried out simultaneously, but there is still a lack of unified combining in the treatment of lung cancer.

This article focuses on introducing the mechanism of action, therapeutic potential and clinical application challenges of triptolide in lung cancer and further expand its therapeutic potential in cancer and other pathological applications by clarifying its mechanism of action, so as to review its anti-lung cancer research.

2. Classification of lung cancer

Lung cancer is a malignant tumor originating in the alveoli and bronchus, which consists of two main categories, NSCLC and SCLC, both are the main subtypes of lung cancer, with NSCLC being the most prevalent (80-85%). NSCLC consists of three main subtypes: adenocarcinoma (glandular cells originating in the bronchial mucosa), squamous cell carcinoma (squamous cells originating in the bronchial epithelium), and large cell carcinoma (which lacks both lung cancer features). Small cell lung cancer originates in neuroendocrine cells [1, 2]. Up to now, the therapeutic drugs for lung cancer can be roughly divided into three categories: chemotherapy drugs, targeted drugs and immunotherapy drugs (Table1).

Table 1: Types of drugs for lung cancer treatment and the advantages and disadvantages [3]

Advantages and disadvantages Types	Representative drugs	Mode of action	Advantages	Disadvantages
Chemotherapeutic drugs	Cisplatin	Interfere with DNA replication or cell division	Wide range of application	High toxicity and poor specificity
Targeted drugs	Gefitinib	Targets specific genetic mutations or signaling pathways	Has good effects on specific tumors	Suitable for a small number of people and has a high price
Immunotherapy drugs	Pembrolizumab	Activates the patient's own immune system	Has a wide time span of anti-tumor effect	Few applicable people and many adverse reactions

At present, anti-tumor therapeutics generally have problems such as single target, strong drug resistance, poor specificity, etc. At that time, more strengthening and effective cancer therapeutics are needed, and triptolide has gradually entered people's research field with its advantages of broad-spectrum anti-tumor activity, multi-target mechanism of action, low dose efficiency, anti-drug resistance and so on.

3. Cancer mechanism of triptolide

Triptolide, derived from the root bark of triptolide, is an epoxy diterpene lactone compound, also known as triptolide, with the molecular formula $C_{20}H_{24}O_6$; In the treatment of lung cancer, it can kill tumor cells through the following mechanisms: inhibiting cell proliferation, inducing apoptosis, suppressing signaling pathways, and modulating the tumor microenvironment.

3.1. Inhibition of cell proliferation

In a cell experiment, using CCK-8 methodology, the researchers examined concentration-dependent changes in proliferation rates of triptolide-treated A549 cells. The results showed that the A549 cells showed an IC₅₀ about 50nM when treated with triptolide, indicating that triptolide could inhibit the proliferation of related cells. Researchers used Western blotting to determine the protein content in related cells. Triptolide treatment caused dose-dependent downregulation of multiple proliferative proteins in A549 cells when compared to blank control groups. Further studies showed that triptolide, based on inhibiting the expression of Hn RNPA2/B1, which inhibited the expression of PI3A, AKT cells and related proteins to achieve the effect of inhibiting proliferation [4].

3.2. Induction of apoptosis

3.2.1. Activation of mitochondrial apoptosis pathway

A study showed that when the expression levels of SIRT2/p53 in TP-treated A549 cells were detected using Real-time PCR at various of concentrations, SIRT2 gene expression was also inhibited with the increase of concentration, and the ratio of Bcl-2/Bax in mitochondria decreased, resulting in an increase in mitochondrial permeability. With the increase of the expression level of p53, apoptosis protein enters the cytoplasm and induces cell death [5].

For A549 lung cancer cells with paclitaxel resistance, SRB analysis was used to explore the growth inhibition level of TP under different concentrations of TP. The results showed that the survival rate of cells decreased with the increase of triptolide concentration. In the cell growth cycle analysis, 51% of the cells treated with triptolide were in the S phase. In apoptosis analysis, TP concentration at 40 nm and 60nM could significantly induce apoptosis. Further Western blot analysis showed that after TP treatment, key apoptosis regulators: caspase-3 (cleaved form), PARP, and the pro-apoptotic factor Bax illustrated an increasing trend of its expression, and anti-apoptotic protein Bcl-2 levels showed down-regulated [6].

3.2.2. Copper death pathway induces apoptosis

Other studies have found that triptolide can induce apoptosis through the copper death pathway. In order to investigate the molecular mechanism of the inhibition of lung cancer cell growth by the regulation of copper death related pathway by triptolide, two types of NSCLC cells A549 and H460 were used as research objects in cell experiments, and the intracellular copper ion concentration was detected by increasing the concentration of TP. When determining cell viability by MTT assay, it was calculated that the IC₅₀ ability of both TP-treated cells was 74.67 nmol/L and 34.12 nmol/L, respectively. The following information presented that triptolide inhibited both cells in a concentration gradient way and induced apoptosis. The intracellular copper ion concentration increased with increasing TP concentration. Mitochondrial membrane potential decreased. When conducting in-depth research by using Western bolt technology, the expression of Fe-S proteins (FDX1, POLD1, SDHB, LIAS) and DLAT monomer was down-regulated, and the expression of DLAT oligomers was increased ($P<0.05$). The expression of copper transporter ATP7A/ATP7B was down-regulated ($P<0.05$), but there was no significant change in CTR1. The above results showed that TP can induce cell apoptosis by down-regulating the expression of ATP7A/ATP7B, resulting in intracellular accumulation of copper ions, inhibiting the synthesis of Fe-S proteins, and promoting DLAT oligomerization [7].

3.2.3. Inhibit CHK1 phosphorylation and increase ATM phosphorylation

The cisplatin-drug combination demonstrated marked anti-proliferative activity across multiple lung cancer cell lines. The cell proliferation of A549 and HTB182 NSCLC cells in the medium was tested in the blank control group and the 5ng/ml and 10ng/ml triptolide groups. The apoptosis detection tests of two kinds of cells were performed in three groups using cisplatin alone, triptolide alone, and combination of the two groups. The results showed that: Low dose triptolide alone and cisplatin alone had little effect on the proliferation inhibition of related cells, while 10ng/ml triptolide combined with cisplatin inhibited the growth of related lung cancer cells induced by cisplatin. Further Western blot analysis showed that inhibition of CHK1 phosphorylation and increase of ATM phosphorylation may be an important mechanism of triptolide's enhancement of cisplatin induced apoptosis [8].

3.3. Inhibition of cell signaling pathway activation

Triptolide effectively blocks the activation of Wnt signaling pathway by inhibiting the p70S6K/GSK-3/ β -catenin signaling cascade, thereby reversing the EMT phenotype of drug-resistant lung cancer cells and exerting anti-tumor effects.

An experimental study showed that the expression levels of β -catenin, the key factor of Wnt pathway, and its downstream targets Jagged1 and c-Myc decreased in a concentration-dependent manner after treatment with triptolide. Further immunofluorescence and Western blot analysis revealed that triptolide administration markedly decreased β -catenin expression in both cytoplasmic and nuclear fractions, especially the reduction of β -catenin in nucleus. Inhibition of the Wnt/ β -catenin signaling pathway by triptolide occurs through blockade of p70S6K and GSK-3 phosphorylation, as shown in mechanism studies. Researchers used Western blot to detect the expression levels of different indexes, and its results showed that with the increase of triptolide concentration, the expression of phosphorylated p70S6K (p-p70S6K) and phosphorylated GSK-3 α/β (p-GSK-3 α/β) were significantly decreased. In animal experiments, immunohistochemical analysis of tumor tissues in the 0.8 mg/kg triptolide treated group also confirmed significantly down-regulated expression of p-p70S6K, p-GSK-3 and β -catenin [6].

3.4. Regulation of tumor immune microenvironment

According to Binnewies, tumor immune microenvironment mainly consists of immune cells, non-immune cells, soluble factors, extracellular matrix and immune checkpoint molecules [9].

In investigating the mechanism by which triptolide inhibits dendritic cell-mediated immune cell chemotaxis, one study found that triptolide significantly inhibits Stat3 protein phosphorylation and NF- κ B signaling pathway activation in dendritic cells, thereby blocking dendritic cell chemotaxis on neutrophil and T cells. The experimental results illustrated that the dendritic cells treated with triptolide showed a significant decrease in the chemotactic ability of neutrophils and T cells, and this inhibitory effect was closely related to the decrease in the secretion of key inflammatory factors. These results revealed the specific molecular mechanism of the regulation of dendritic cell immune function by triptolide targeting the Stat3/NF- κ B signaling axis [10].

4. Application of triptolide

In vitro mouse experiments, mice with H23 NSCLC cells were injected intraperitoneally five times a week with 0.75 mg kg⁻¹ Omtriptolide (a precursor of triptolide derivative), the growth of xenografts in vivo was effectively inhibited, and the survival time of related mice was greatly extended. No subsequent tumor recurrence was found in combination with irinotecan. Omtriptolide has shown effective therapeutic activity in the treatment of advanced solid tumors after a two-week injection of

0.5 mg m⁻² to 18 mg m⁻² content. However, evidence from published clinical studies shows no record of triptolide-based compounds reaching phase II trials for lung cancer indications [11].

5. Limitation of triptolide

At present, triptolide has been confirmed to have good anti-cancer properties, although triptolide has a good anti-cancer effect, but up to now, triptolide is limited to preclinical studies and clinical phase I trials. Its intense toxicity and low bioavailability are key factors hindering its entry into Phase II clinical trials.

5.1. Toxicity

The intense toxicity of triptolide will cause additional side effects in patients during the long cycle of treatment. Studies have shown that when patients use triptolide, they usually experience adverse reactions in intestines, mainly manifested as diarrhea, duodenal ulcer, etc. Also, the skin experience discomfort. It is necessary to strictly control the dosage of triptolide, and the death of animals can be caused by triptolide at 2-4 times the therapeutic dose. This is because triptolide is easy to induce hepatotoxicity and nephrotoxicity, and the onset time of hepatotoxicity is shorter than that of nephrotoxicity. Male sperm can also be attacked by triptolide leading to infertility. This results in a small therapeutic window and hinders the progress of clinical trials [11].

5.2. Low bioavailability

Due to its broad-spectrum anti-tumor activity, triptolide can effectively kill tumor cells in various parts of the body, but if it is used against tumor cells in a single organ, its killing effect on cells is poor, and because of its poor water solubility, the action time of nearly 20h is relatively longer than that of other anti-tumor drugs for several hours, and the half-life is shorter. This makes triptolide treatment cycle longer and need to be used more times [11].

5.3. Optimization

Until now, efforts have been made to develop triptolide and its derivatives to improve its bioavailability while reducing adverse reactions. At present, preclinical studies mainly focus on improving the bioavailability of triptolide by modifying triptolide to mask its toxicity and coupling other compounds to enhance solubility. For example, different substituents are introduced into the B ring of triptolide to reduce the corresponding toxicity, but its action activity is also reduced [12]. Triptolide is coupled with glucose to improve its solubility [13].

6. Conclusion

In recent years, the research of triptolide in the treatment of lung cancer has achieve high accomplishment. As a multi-target anti-tumor drug, its mechanism of action mainly includes: blocking tumor cell proliferation by inhibiting Hn RNPA2/B1 expression and CHK1 phosphorylation; Activation of mitochondrial apoptosis pathway (regulating SIRT2/p53/Bcl-2/Bax pathway) and copper death pathway (down-regulating ATP7A/7B, promoting DLAT oligomerization) induced apoptosis; Inhibition of Wnt/ β -catenin signaling cascade (blocking phosphorylation of p70S6K/GSK-3) to reverse EMT phenotype; And the tumor immune microenvironment is regulated by the Stat3/NF- κ B axis. The synergistic effect of these multiple pathways makes it show remarkable killing effect on lung cancer cells. However, how to inhibit the hepatorenal toxicity and bioavailability of triptolide and put it into clinical phase II is the key point of future triptolide lung cancer treatment research. The current research focuses on the optimization of its structure and the development of

drug combination strategies. During the further research, researchers need to aim at dealing with the problems: the inhibition of toxicity and the improvement of bioavailability of triptolide and its derivatives and determine the drug interaction between various common drugs and triptolide to determine its safe dosage. Its mechanism of action and therapeutic potential in different disease models should be further clarified in clinical live experiments, so as to give full play to its pharmacological effects and improve its drug competitiveness and safety. As the progress of technology, the deeper, safer and more efficient research on the therapeutic power of triptolide will soon make breakthroughs and eventually be deeply applied to the treatment of lung cancer and other diseases.

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