

Therapeutic and Intervening Effects of Triptolide in Pancreatic Cancer and Their Molecular Mechanisms

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Abstract. Pancreatic cancer is a very aggressive digestive cancer that often appears subtly, is difficult to diagnose in its early stages, has a poor prognosis, is susceptible to chemotherapy resistance, and conventional comprehensive therapy cannot extend the life of patients. Triptolide is an extracted bioactive substance from *Tripterygium wilfordii*; it has shown good anti-tumour activity in pancreatic cancer and is thus considered a promising new natural anti-cancer drug. Review of the research progress and molecular mechanisms of Triptolide in the progression of pancreatic cancer. Triptolide suppresses the growth, movement and spread of pancreatic cancer cells in some ways. It can interfere with the TLR4/NF- κ B pathway to promote the apoptosis of cancer cells and increase their sensitivity to gemcitabine, and at the same time, it may affect the Nrf2/SLC7A11/GPX4 axis to induce ferroptosis. It can regulate the factors of autophagy and is related to the path of apoptosis, ferroptosis and autophagy that suppresses cancer cells. It is highly toxic, has a narrow therapeutic window and poor selectivity; therefore, it has not been used in clinical practice yet. This paper will explore the different anti-pancreatic cancer mechanisms of triptolide and offer theoretical support for the further development and clinical application of natural anti-tumor drugs.

Keywords: Triptolide, pancreatic cancer, programmed cell death, NF- κ B signaling pathway, ferroptosis.

1. Introduction

Pancreatic carcinoma (PC) is mainly pancreatic ductal adenocarcinoma (PDAC), which accounts for more than 90% of all cases, and is one of the most malignant digestive malignancies with a poor prognosis [1, 2]. Given that it has an unknown anatomical position and no early specific symptoms, most patients are diagnosed late in life or at an advanced stage of metastasis and miss the optimal time for surgery. Only 10%-20% of the patients are suitable for primary surgery and have a high recurrence rate after surgery [1, 3]. These pathological features can reduce the penetration of drugs, lead to therapy resistance, promote tumour growth and spread, and are thus urgent problems that need to be solved in the search for new multi-target anti-tumour agents [3, 4].

As the early days of cancer research are getting underway, more and more people are paying attention to natural products. Triptolide (TP) is the main active ingredient in traditional Chinese

medicine *Tripterygium wilfordii*, and it has anti-inflammatory, immunomodulatory and strong anticancer properties. Recently, it has been found through research that TP can inhibit the progression of PC by modulating the NF- κ B, STAT3 and PI3K/AKT pathways, and promotes various types of programmed cell death, such as apoptosis, autophagy and ferroptosis [5]. It can reduce the anti-tumour invasion effect of the TLR4/NF- κ B pathway, disturb the SLC7A11/GPX4/Nrf2 axis, cause ferroptosis, alter the fibrotic tumour microenvironment, and increase the sensitivity of PC cells to gemcitabine chemotherapy [6-9]. At present, most of the research on TP has been conducted in cells and animals, and there is a lack of clinical data [5].

2. Molecular and cellular mechanisms of tripterygium alpiniae radix alkaloid in influencing pancreatic cancer cells

2.1. Molecular nature of triptolide

Triptolide (TP) is a necessary raw material that can be extracted from the root of *Tripterygium wilfordii* in the Celastraceae family. It is an oxidosulfone derivative of diterpenoid lactone. Due to its very reactive epoxy group and numerous oxidative functional groups, it can bind to all kinds of proteins in the body and cause all sorts of harm. Initially, it was believed that TP was the main cause of *Tripterygium wilfordii* toxicity; however, recent pharmacological and toxicological studies have shown that the effects of TP on the body vary with different doses. In a certain concentration range, TP has shown good anti-inflammatory, immunosuppressive and anti-tumor effects, so the best therapeutic window has been the subject of many studies recently [10].

Recently, some studies have found that the target sites of TP are involved in the transcription of various genes by RNA polymerase II, the NF- κ B signalling pathway and other inflammatory regulatory proteins, and by altering the expression of these genes, their stability and cell death pathways can be controlled to inhibit all kinds of cancer cells. TP can directly inhibit the proliferation of pancreatic cancer cells and change the tumour microenvironment to enhance the effect of other drugs; However, due to the problem of significant toxicity in conventional TP, in recent years, the development of low-toxicity derivatives and targeted delivery systems has received much attention.

2.2. TP mediates apoptosis of pancreatic cancer cells by inhibiting the NF- κ B signaling pathway

NF- κ B signalling is one of the required links in the molecular network that controls inflammation, cell growth, resistance to apoptosis, tumour invasion, etc., and is usually kept in check by normal cells. With the occurrence of pancreatic cancer, which is frequently caused by inflammatory factors and genetic mutations, NF- κ B is often in an abnormal and prolonged activated state. It is difficult for cancer cells to survive and resist therapy [11].

Research has found that Triptolide is an anti-pancreatic cancer drug because it can suppress the TLR4/NF- κ B signalling pathway. TLR4 is a kind of natural immune receptor, and when it is overexpressed in pancreatic cancer cells, the MyD88-dependent signalling pathway will be activated. It can activate the IKK complex to phosphorylate and then degrade I κ B α . Therefore, NF- κ B p65 can enter the nucleus to start transcribing some of its target genes [6].

TP can also block the above signal transduction pathway. On the one hand, TP can inhibit the activity of the IKK complex, increase the stability of I κ B α , and thereby block the nuclear translocation of NF- κ B p65; at the same time, TP can also disrupt the binding of NF- κ B to

transcriptional cofactors CBP/p300, reduce the affinity of NF- κ B for DNA, and thus suppress the transcription of target genes [8].

A decrease in the activity of NF- κ B will result in a reduction of the expression of some anti-apoptotic proteins; among them, Bcl-2 is needed to maintain the structural stability of the outer mitochondrial membrane to prevent apoptosis of pancreatic cancer cells, and TP can reduce the level of Bcl-2 and increase the Bax/Bcl-2 ratio by promoting the translocation of Bax to the mitochondria. Bax is in the mitochondrial membrane, and it promotes the release of cytochrome C. As a result, there is also the induction of mitochondrial-dependent apoptosis via caspase-9 and caspase-3 [12].

NF- κ B is another regulator of MMPs, and therefore, it can be increased in the extracellular matrix to promote the spread and metastasis of pancreatic cancer cells through the degradation of the matrix. TP inhibits NF- κ B and thus reduces the expression of MMP-2 and MMP-9, decreases cancer cell migration, alleviates severe fibrosis in the microenvironment of pancreatic cancer, and enhances the therapeutic effect of chemotherapy in tumors [6].

Ma and others used PANC-1 pancreatic cancer cells and a nude mouse xenograft model to show that the combination therapy of TP and gemcitabine can reduce the expression levels of TLR4, NF- κ B p65, Bcl-2 and MMP-2/MMP-9 proteins. At the same time, it has raised both the rate of apoptosis and the effect of tumour suppression; thus, TP can enhance the sensitivity of pancreatic cancer cells to gemcitabine [6]. Therefore, TP can promote the apoptosis of pancreatic cancer cells by inhibiting the NF- κ B signalling pathway and at the same time reverse some chemotherapy resistance mechanisms; thus, it has been proposed that TP may be used to enhance the effect of chemotherapy.

2.3. Triptolide-induced ferroptosis-mediated killing of pancreatic cancer cells

Ferroptosis is a new kind of programmed cell death that has attracted much attention in recent years. It has been widely observed that within the cell, there is the formation of iron-ion-dependent lipid peroxides, which ultimately cause structural changes in the cell membrane and result in cell death; it is different from the old type of apoptosis and is regulated by changes in iron metabolism, lipid metabolism, the antioxidant system, etc.; Therefore, it has been selected as a new treatment strategy for drug-resistant tumours [8].

Pancreatic carcinoma cells have a high metabolic rate and are therefore more susceptible to oxidative damage; as a result, an effective antioxidant system has been constantly studied, and among them, the glutathione (GSH)-GPX4 pathway uses GPX4 to reduce lipid peroxides to alcohols and protects the structure of the cell membrane by preventing permanent damage caused by oxidative stress and ferroptosis in cancer cells [7].

Through research, it has been found that triptolide induces ferroptosis in pancreatic cancer cells by several means. TP can reduce the antioxidant signalling pathway of Nrf2 in the beginning; Nrf2 is a transcription factor that promotes the expression of antioxidant genes in cells. Activation increases the expression of SLC7A11 to enhance cystine uptake and produce GSH; therefore, there is no change in the level of GPX4. At the same time, TP inhibits Nrf2, reduces the amount of SLC7A11 in cells, lowers intracellular GSH, and thus weakens the antioxidant effect of GPX4 [9].

Second, TP has an effect on cellular iron metabolism. Research has shown that TP can increase the expression of transferrin receptor TFRC to promote cellular iron uptake, and at the same time reduce the expression of ferritin heavy chain FTH1; therefore, more Ferrostatin-1 is released from ferritin. A large number of free Ferrostatin-1 are generated during the Fenton reaction to produce hydroxyl radicals that promote lipid peroxidation and thus cause ferroptosis [8].

TP increased the amount of intracellular ROS in PANC-1 cells, decreased GSH content, reduced GPX4 protein expression, and increased the levels of lipid peroxides and Ferrostatin-1. Administration of the ferroptosis inhibitor Ferrostatin-1 reduced TP-induced cell death, and therefore, it can be concluded that ferroptosis is involved in the anti-pancreatic cancer effects of TP [13].

Recently, more studies have also shown that ferroptosis can directly kill cancer cells and change the tumour microenvironment of the immune system; oxidized lipids and damaged molecular patterns released during the process of ferroptosis may increase the ability of immune cells to recognise tumours. Therefore, TP-induced ferroptosis may have a good prognosis under the condition of combined immunotherapy.

2.4. The effects of triptolide on autophagy and apoptosis to kill pancreatic cancer cells

Autophagy is a necessary way to break down old organelles and other components of the cell. Autophagy has the following two functions in tumorigenesis and development. First of all, it can make the cells adapt to a bad environment. However, too much may cause cell death, and therefore, regulating the level of autophagy has become one of the important ways to treat cancer [14]. Based on the above results, it was found that Triptolide can induce abnormal autophagy in pancreatic cancer cells and promote their apoptosis and ferroptosis.

TP can modify the expression of the RNA-binding protein PUM1, reduce its inhibitory effect on the translation of ATF5 mRNA, and thus increase the amount of ATF5 protein; at the same time, ATF5 also promotes the expression of autophagy-related genes and increases LC3-II levels and autophagosome formation.

At the same time, autophagy induced by TP can also affect the apoptosis pathway. At the same time, autophagy can degrade the anti-apoptotic protein c-FLIP, restore the function of TRAIL-induced death receptor signalling, promote DISC formation and thus trigger the caspase cascade. At the same time, autophagy can promote NCOA4-mediated ferritinophagy; therefore, FTH1 is degraded and more ferrostatin-1 is released, thereby worsening the signs of ferroptosis.

As a result, TP-induced autophagy is not the only cause of cell death; rather, it works with apoptosis and ferroptosis to induce death in pancreatic cancer cells. Based on the experiment, it was found that the addition of TP to PANC-1 cells increased LC3-II and the number of autophagic structures. 3-MA is an autophagy inhibitor and also suppresses the apoptosis and ferroptosis induced by TP. Therefore, it can be concluded that autophagy is one of the ways TP may suppress pancreatic cancer.

3. Clinical efficacy and combination therapy of triptolide

Although the application of triptolide in the treatment of pancreatic cancer is still in the stage of experimental research, some clinical studies have also explored its efficacy when combined with conventional therapy. Gemcitabine is a typical drug that many people with advanced pancreatic cancer are prescribed. Prolonged use may lead to the development of drug resistance, but at the same time, TP can enhance the sensitivity of cancer cells to gemcitabine by inhibiting the NF- κ B signaling pathway and reducing the expression of anti-apoptotic proteins; thus, the two are sometimes used together [6].

Guo Shaoxian and others have studied the 72 patients with advanced-stage pancreatic cancer (Stages II-IV). Randomly divide the patients into two groups, one group will receive traditional radiotherapy and chemotherapy, and the other will be given combination therapy with Tripterygium

wilfordii extract; based on the results, the overall response rate of the combined treatment group was 63.89%, significantly higher than that of the single-therapy group (50.00%), and the immune function indicator increased and the occurrence rate of side effects decreased [3].

Tripterygium-related drugs can expand the scope of anti-cancer drugs for all stages and improve the tolerability of cancer patients; however, a small number of studies have been conducted so far, and more extensive clinical trials are still needed. In recent years, with the rapid development of immunotherapy, concurrent use of TP and immune checkpoint inhibitors has received much attention. Given that TP can regulate inflammatory responses and optimise the immune microenvironment, it is expected to be employed as an adjuvant therapy to enhance the efficacy of immunotherapy. In addition, in order to solve the problem of toxicity for TP, scientists have developed nanodelivery systems, liposome encapsulation and TP derivatives, etc. For example, tumor-targeted delivery technology can increase the concentration of TP in tumor tissue and reduce its exposure to normal tissues at the same time to reduce side effects.

4. Controversial challenges and future prospects

Triptolide has demonstrated some anti-cancer effects in the study of pancreatic cancer, but many problems have not yet been solved for its use in clinical practice. At present, most studies on the anti-tumor effects of TP in pancreatic cancer have been conducted in vitro and in animal models; relatively few clinical data have been obtained. Although experiments have shown that TP can inhibit the proliferation of pancreatic cancer cells, induce cell death, enhance sensitivity to chemotherapy, etc., due to the complexity of the pharmacokinetic environment in humans, tumor heterogeneity and individual differences, these results may not be the same as those in experiments. Therefore, more large-scale studies and randomised controlled trials at various centers need to be conducted to determine whether TP can effectively control the disease and improve the quality of life for patients.

Second, triptolide still has inherent toxicity and is thus not suitable for clinical use at present. Although TP shows good biological activity, the effective anti-tumor dose is very close to the toxic dose range, and it may cause side effects such as liver and kidney damage, reproductive toxicity, immunosuppression, etc. [10]. Contemporary research suggests that the pharmacological and toxicological effects of TP may be due to the same molecular targets. Therefore, it is not feasible to increase the dosage solely to improve the anti-cancer effect.

Based on the above analysis, in recent years, more research has been conducted on enhancing the structure of TP and developing delivery systems; for example, TP derivatives have been developed to reduce the toxicity of traditional TP molecules and improve their selectivity. New tumour-targeted delivery methods for nanoparticles, liposomes, other types of drugs have been developed to increase the concentration of drugs in the tumour area and reduce damage to normal cells, thus extending the life of patients suffering from diseases.

In addition, pancreatic cancer is highly heterogeneous, and many factors are different among patients; for example, there are various KRAS mutation statuses, different DNA damage repair capacities and levels of oxidative stress in different cases. Therefore, TP may have different therapeutic effects on these tumors. Therefore, further research should integrate genomics, proteomics, single-cell sequencing, etc., to find predictive biomarkers for TP efficacy and advance the direction of personalised medicine.

Regarding treatment tactics, we need to further explore whether there can be a combination of TP with other treatment methods. Current research shows that the combination of TP and gemcitabine has improved the antitumor effect significantly; additionally, since TP can induce ferroptosis and

regulate the immune microenvironment, it is also possible that it will work synergistically with immune checkpoint inhibitors, PARP inhibitors, and other targeted drugs.

Triptolide has shown some promise for use in the general treatment of pancreatic cancer as an adjuvant therapy. Nevertheless, some problems that need to be solved in the clinical application are the safety and effectiveness of drugs, targeting efficiency and evaluation of treatment outcomes. Looking forward, the development of pharmaceutical engineering technology and the direction of precision medicine will help to further advance the application of triptolide in clinical practice based on basic research.

5. Conclusion

Triptolide is the main active diterpenoid lactone compound in *Tripterygium wilfordii* and has received much research attention for treating pancreatic cancer. It can regulate multiple targets and multiple pathways to inhibit the initiation and development of pancreatic cancer; thus, it offers a new type of treatment that traditional drugs have been unable to achieve against this disease. Research has shown in recent years that triptolide (TP) can inhibit the TLR4/NF- κ B signalling pathway and reduce the expression of some important proteins, such as Bcl-2, MMP-2 and MMP-9. It will thus trigger apoptosis in the pancreatic cancer cells and increase their resistance to gemcitabine. TP can also change the redox state of cancer cells by regulating the Nrf2/SLC7A11/GPX4 antioxidant pathway; therefore, there is an increase in iron ions and lipid peroxidation, which may lead to ferroptosis. TP regulates the autophagy pathway that is associated with PUM1/ATF5 and is involved in several tumour-suppressing effects of autophagy, apoptosis, ferroptosis, etc.

Triptolide and related formulations can be used with conventional chemotherapy drugs, such as gemcitabine, in the clinic to improve the anti-tumour effect and increase patients' tolerance to chemotherapy; at the same time, it is hoped that more research will be conducted to combine it with immunotherapy and targeted therapy based on its multiple anti-cancer mechanisms of action.

Research on triptolide has deepened our understanding of the anti-tumor effects of natural products and provided some ideas for new therapeutic strategies in pancreatic cancer; however, the existing studies still have problems such as a lack of clinical data, high toxicity and an uncertain optimal treatment dose. In the future, research should be carried out to improve safety and efficacy through the development of TP derivatives, nanoscale targeted delivery technology and precision medicine methods, etc., to advance the clinical application of triptolide in the prevention and treatment of pancreatic cancer.

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