



Review Article

# Review on anti-tumor activity and underlying mechanism of polysaccharide from *Trametes robiniophila* Murr.

Li-Ying Fang<sup>1,2,†</sup>, Zheng-Hong Wang<sup>1,2,†</sup>, Shuang-Yan Xie<sup>1,2</sup>, Cai-Zhe Ji<sup>1,2</sup>, Jin-Bo Hou<sup>3</sup>, Jun Lu<sup>1,2</sup> (✉), Xue-Li Gao<sup>4</sup> (✉)

<sup>1</sup> Hunan Key Laboratory of Forestry Edible Sources Safety and Processing, Central South University of Forestry and Technology, Changsha 410004, China

<sup>2</sup> College of Food Science and Engineering, Central South University of Forestry and Technology, Changsha 410004, China

<sup>3</sup> College of Biological and Food Engineering, Fuyang Normal University, Fuyang 236000, China

<sup>4</sup> College of Food and Pharmacy, Xuchang University, Xuchang 461000, China

<sup>†</sup> Li-Ying Fang and Zheng-Hong Wang contributed equally to this work.

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**Abstract:** *Trametes robiniophila* Murr., an important medicinal fungus in China, pertaining to Hymenomycetes, Basidiomycotina, is well-known for its therapeutic properties, which has been used in traditional Chinese medicine for thousands of years. Many biological activities, including apoptosis, anti-angiogenesis, drug resistance reversal, anti-metastasis, and systemic immune activation have been identified in *Trametes robiniophila* due to its much primary bioactive components such as proteoglycan, a polysaccharide, amino acids. *Trametes robiniophila* has been discovered and employed as a supplemental drug for cancer therapy in recent decades, especially for its granules and aqueous extracts. They can effectively inhibit disease progression and improve long-term survival rates in patients. Moreover, the water extracts of *Trametes robiniophila* have an impact on several key stages of breast cancer development and spread, including the immune system, DNA replication, and cell proliferation, showing multi-target effects. This paper has summarized the progress of polysaccharide, preparation technology, anti-tumor activity, and mechanism of *Trametes robiniophila* over the last two decades to provide a firm foundation for future research and application of *Trametes robiniophila*.

**Keywords:** *Trametes robiniophila* Murr.; Hymenomycetes; Basidiomycotina; polysaccharide; anti-cancer mechanism; clinical application

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## 1 Introduction

*Trametes robiniophila* Murr. was recorded in the Chinese ancient medical work *Zhou Hou Fang* for the first time before 1,500 years ago<sup>[1]</sup>. Owing to its woody fungus and goose-like appearance, it is also known as the “locust fungus” or “locust goose”. The taste is harsh, bland, and non-toxic. *Trametes robiniophila*, a traditional Chinese medicine that is both edible and medicinal, is known as one of the primary sources of naturally occurring polysaccharides that are safe, effective, and have few negative effects. However, due to the limited availability of wild resources for *Trametes robiniophila*, challenging artificial cultivation, lengthy growth cycles, and low biological efficacy, solid fermentation technology is commonly employed. This technology ferments strains of *Trametes robiniophila* on a fermentation substrate, resulting in *Trametes robiniophila* that contains bioactive ingredients like polysaccharides. *Trametes robiniophila* is then extracted using hot water, ethanol, etc., and further processed

into *Trametes robiniophila* granules. *Trametes robiniophila* can be used in combination with other medicinal plants to exert a therapeutic effect. For example, bi-directional solid fermentation has been shown to improve absorption and enhance medicinal qualities<sup>[2]</sup>. The extract from the bi-directional solid fermentation product of *Trametes* combining *Radix Isatidis* with *Trametes robiniophila* was produced using the recently developed bi-directional fermentation technology of modern Chinese medicine. *Trametes robiniophila* was developed as a therapeutic fungus, and the related *Huaiqi* fungal materials have undergone thorough clinical evaluation, demonstrating a potent anticancer effect<sup>[3]</sup>.

Polysaccharides, the primary bioactive components of *Trametes robiniophila*, possess anti-aging, anti-tumor, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic, and hypolipidemic properties<sup>[4]</sup>. Therefore, the products of *Trametes robiniophila* are often combined with other drugs or medical methods to enhance their medicinal value. For instance,

transcatheter arterial chemoembolization in conjunction with *Trametes robiniophila* granules and radiobioactive  $^{125}\text{I}$  particles can enhance short-term therapeutic efficacy, increase patient survival, and enhance their quality of life while receiving advanced liver cancer treatment<sup>[5]</sup>. The combination of lapatinib and *Trametes robiniophila* granules is an effective treatment for advanced primary liver cancer, which can alleviate symptoms such as fatigue, abdominal distension, and liver pain in patients with advanced liver cancer<sup>[6]</sup>. Therefore, to support the therapeutic effects and health functions of medicinal and edible homologous polysaccharides (MEHPs), the goal of this review is to provide comprehensive and meticulously organized data on polysaccharide, anti-tumor activity, and the potential mechanism. This study aims to establish a theoretical foundation and provide references for the extensive utilization of *Trametes robiniophila* in the fields of food and medicine.

## 2 Polysaccharide and their preparation of *Trametes robiniophila*

Current research shows that there are a large number of polysaccharide compounds present in the *Trametes robiniophila*, which are also its extremely important active components. However, there is very little systematic research on the chemical composition of *Trametes robiniophila*. To facilitate future studies on its chemical components, this paper provides a systematic summary.

### 2.1 Polysaccharide in *Trametes robiniophila*

Currently, many studies have shown that glycoproteins are the main bioactive substances of *Trametes robiniophila*, comprising 8.72% water, 12.93% amino acids, and 41.53% carbohydrates<sup>[7]</sup>. The samples contain a range of organic elements, among which over ten mineral elements are consistent with the entity. The primary bioactive component is polysaccharide-protein (PS-T), which is made up of 18 amino acids (such as glycine, proline, etc.) and six monosaccharides (L-arabinose, L-fucose, D-mannose, D-xylose, D-glucose, and D-galactose), and some protein content in polysaccharide-protein complexes can reach up to 41.23%<sup>[8]</sup>. However, some polysaccharide's purity could reach to  $85.3\% \pm 1.3\%$ <sup>[9]</sup>. Some monosaccharide conclude glucose, xylose, mannose, arabinose, galactose and fucose with the content of 36.94%, 16.88%, 15.85%, 13.11%, 11.40%, and 5.82% respectively were also found in *Trametes robiniophila*.

The compound may attribute to protection against hydroxyl radical, superoxide anion, and ABTS. Moreover, the polysaccharide (TP-1) from *Trametes robiniophila* with a purity of 93.2%, has various critical functions, including anti-tumor and anti-lung metastasis effects in HMCSMMC-7721 tumor-bearing mice, and has no toxicity to normal cells. This unrefined polysaccharide can decrease the expression of proliferating cell nuclear antigen, increase TUNEL-positive cells, and reduce microvascular density, according to an immunohistochemical study of tumors<sup>[10]</sup>.

Moreover, a neutral water-soluble polysaccharide (W-NTRP) with a molecular weight of  $2.5 \times 10^4$  Da was extracted from the *Trametes robiniophila* organism, which consist of galactose, arabinose and glucose, and the corresponding  $\text{IC}_{50}$  values were 47.8, 75.9, and 43.7 g/mL, respectively. And it contains 96.5% carbohydrates. It demonstrated strong inhibitory effects on three human cholangiocarcinoma cell lines (QBC939, Sk-ChA-1, and MZChA-1). It did not, however, cause any harm to L-929 normal

cells<sup>[10]</sup>. Additional studies have demonstrated that W-NTRP can upregulate inducible nitric oxide synthase, which significantly enhances macrophage production of nitric oxide. These results indicated that the polysaccharide compound could potentially serve as an anti-tumor drug for treating cholangiocarcinoma.

Meanwhile, a unique component of *Trametes robiniophila* polysaccharides with a purity of 94.35%, somewhat comparable to TP-1, called SP1 (with a molecular weight of 56 kDa) was isolated by Zhou *et al.*<sup>[11]</sup>. The GC/MS results showed that the backbone consisted of 1,4-linked- $\alpha$ -D-Galp and 1,3,6-linked- $\beta$ -D-Galp residues. At the O-3 position of the 1,3,6-linked- $\beta$ -D-Galp unit along the main chain, it ended up with 1-linked- $\alpha$ -D-Glcp and 1-linked- $\alpha$ -L-Araf terminals within the ratio of 1.1:2.0:1.1:1.1<sup>[12]</sup>. *In vitro*, SP1 had the potential not only to prevent cell proliferation but also to inhibit angiogenesis in a hepatocellular carcinoma xenograft model, thereby reducing tumor development and lung metastasis.

In addition, another type of homopolysaccharide (TRP) with an average molecular weight of about  $8.7 \times 10^4$  Da, consisting of glucose, galactose, and arabinose in a molar ratio of 4.2:1.10:1.06, was found in the *Trametes robiniophila* entity. In IR spectroscopy, there was no distinctive absorption at  $1,734 \text{ cm}^{-1}$  (carboxyl group) that suggested TRP was a neutral polysaccharide. The main chain of TRP was analyzed by GC-MS, revealing a 1,3,6- or 1,4-linked Glc unit with 1-linked Gal and 1-linked Ara terminal branches situated in the O-3 position branch of the 1,3,6-linked Glc residue<sup>[13]</sup>. Following oral administration, TRP could inhibit the growth of U-2OS tumors in BALB/c nude mice by significantly inducing apoptosis, increasing the number of TUNEL-positive cells in tumor tissue, and the ratio of Bax/Bcl-2, the expression of caspase-9, caspase-3, and lysed PARP. Also, caspase-8 remained unchanged and reduces Metadherin overexpression, thereby inhibiting the proliferation of human osteosarcoma cancer cells<sup>[13,14]</sup>.

The aqueous extract of *Trametes robiniophila* mycelium, which was the API of *Trametes robiniophila* granules, contained a significant amount of polysaccharide proteins, from which a proteoglycan with a molecular weight of approximately  $5.59 \times 10^4$  Da has been identified, with a total carbohydrate and protein composition of 43.9% and 41.2%, respectively. TPG-1 could enhance immunity by activating the TLR4-NF- $\kappa$ B/MAPK signaling pathway, thereby exerting anti-tumor activity<sup>[15]</sup>. TPG-1's evident anti-cancer effect in hepatoma H22-bearing mice may be explained by TLR4-related up-regulation of TNF $\alpha$  serum levels and leukocyte infiltration.

As science progresses at a faster rate than before, we in fact have cause to think that there may be more active ingredients in the immediate future.

### 2.2 Preparation of polysaccharide from *Trametes robiniophila*

*Trametes robiniophila* polysaccharide is the most important bioactive ingredient. The polysaccharides of *Trametes robiniophila* are always extracted from the entity, cultured mycelium, and filtrate of *Trametes robiniophila*. Nowadays, the extraction process of polysaccharides has reached a relatively mature level. Traditional extraction methods include microwave extraction<sup>[16]</sup>, acid extraction, enzyme extraction, hot water extraction, etc.<sup>[17]</sup>. However, all of them have shortcomings such as low purity, high cost, and easy spoilage of polysaccharides<sup>[18]</sup>.

To increase the yield of polysaccharides, several mechanical

technologies have been applied to the powder production process of the entity of *Trametes robiniophila*, including crushing and grinding. Microwave, ultrasound, and enzyme pretreatment were used to improve the efficiency of extraction and product yield. However, the expected biological activity of natural bioactive polysaccharides may be damaged and lead to antagonism or adverse toxicity by other compounds such as proteins, lipids, and inorganic mineral protons. Therefore, it is necessary to separate polysaccharide substances from other components when studying bioactive polysaccharides so that it can ensure the effectiveness and accuracy of the experiment. Liu *et al.*<sup>[19]</sup> defatted and dried the powder of *Trametes robiniophila* before boiling it in hot water (90 °C) for 2 h and repeated it once, about 28.61% polysaccharide was obtained. The solution was then centrifuged and concentrated at 4,500 r/min for 10 min to completely remove the crushed substrates from the supernatant. The supernatant was concentrated to a viscous consistency, ethanol was precipitated and freeze-dried to obtain the crude polysaccharide.

Furthermore, the content and biological activity of bioactive polysaccharides, which are further purified from crude extracts using techniques such as the precipitation of ethanol, fractional precipitation, ion swap chromatography, gel filtering, and chromatography with affinity are always impacted. The actual yield of crude polysaccharide obtained by precipitating the crude aqueous extract with ethanol was 4.05%, which was second only to the cold alkali technique (6.93%) described by Song *et al.*<sup>[20]</sup>. Generally, most studies have used ethanol precipitation to obtain *Trametes robiniophila* polysaccharides. This is due to the fact that ethanol can break the hydrogen bonds holding polysaccharide molecules in the aqueous solution, decreasing the polysaccharide's solubility in water and causing precipitates to form.

Nevertheless, the polysaccharides produced by precipitating ethanol are impure and have a high capacity to enclose some small compounds, necessitating additional purification. Crude polysaccharides were obtained after ethanol precipitation and combined with proteins, pigments, and other contaminants. Proteins are typically extracted from polysaccharide solutions using the Sevag technique. The denaturation of proteins in chloroform and other organic solvents is the foundation of the Sevag method. The polysaccharide extract was combined in a specific ratio with the Sevag reagent (chloroform: n-butanol = 4:1, v/v), agitated, and the polysaccharide solution with some proteins removed was the supernatant that was produced by centrifugation. A study<sup>[21]</sup> compared the deproteinization effect of three widely used techniques: trichloroacetic acid, hydrochloric acid, and the Sevag method, it was discovered that the rates of deproteinization were 46.1%, 72.5%, and 42.3%, respectively, and the rates of polysaccharide loss were 6.1%, 15.1%, and 14.3%. The hydrochloric acid method had a high deproteinization rate, but it also had a high polysaccharide loss rate; the trichloroacetic acid and Sevag methods were milder, but not as efficient in removing protein.

The removal of polysaccharides also includes the separation of polysaccharides, mainly by graded precipitation, quaternary ammonium precipitation, dialysis, metal salt precipitation, and preparative regional electrophoresis, which are mostly performed by diethylaminoethyl dextran gel or various other different types of gel column chromatography as well as ion exchange chromatography<sup>[17]</sup>. The study by Liu *et al.*<sup>[19]</sup> utilized various methods (deproteinization using a high-temperature, high-pressure alcohol precipitation method; common method; cold alkali method; hot alkaline method; the high-temperature and

high pressure method; high temperature and high pressure graded alcohol precipitation method) to extract the polysaccharide of *Trametes robiniophila* and determine its antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, the results showed that the polysaccharide content of high temperature and high pressure extraction had the highest content (34.59%), the polysaccharide extracted by hot subtraction method had the highest removal rate of DPPH (45.71%), the polysaccharide content obtained from the deproteinized treatment was significantly higher than that of the undeproproteinized group, and both had the highest sugar content after secondary alcoholic sedimentation, at 29.24%, and 41.32%, respectively. Additionally, as the number of alcoholic precipitation phases increased, there was a progressive decline in the undeproproteinized and deproteinized groups' ability to remove the polysaccharide poly-DPPH. In summary, the polysaccharide obtained through high-temperature and high-pressure primary alcohol precipitation with deproteinization treatment exhibited the strongest antioxidant property (54.73%), while the secondary polysaccharide extracted through graded alcohol precipitation with deproteinization treatment had the highest sugar content (41.32%). The deproteinized high-temperature and high-pressure graded alcohol precipitation method was better than the other techniques for determining the antioxidant properties.

### 3 Anti-tumor activity and mechanism of *Trametes robiniophila*

Numerous clinical studies have found that *Trametes robiniophila* can exert corresponding anti-tumor properties in malignant tumors such as liver cancer, breast cancer, and lung cancer with low safety side effects<sup>[22-25]</sup>. According to various mechanisms include stimulating tumor cell apoptosis to prevent vascular neogenesis<sup>[26]</sup>, preventing tumor cell invasion and metastasis<sup>[27]</sup>, boosting the body's immune system<sup>[28]</sup>, and reversing tumor cells' treatment resistance<sup>[29]</sup>. Furthermore, as medication research has gradually advanced, it has been noted that *Trametes robiniophila* can inhibit tumor stem cells and participate in cell autophagy<sup>[30,31]</sup>. For example, the polysaccharide (HP-1), derived from *Trametes robiniophila*, is an official fungal substance in traditional Chinese medicine that can be utilized to treat gastrointestinal tumors with negligible adverse effects<sup>[15,32]</sup>. Renal cancer cells were unable to proliferate, metastasize, or invade because HP-1 halted the cell cycle, triggered apoptosis, and blocked several signaling pathways<sup>[33]</sup>.

Meanwhile, some clinical studies have revealed that *Trametes robiniophila* granules can effectively enhance the long-term recovery rate, reduce the death rate, and have less toxic side effects during the adjuvant treatment of tumors<sup>[34]</sup>. Another research<sup>[35]</sup> discovered that the prognosis of colorectal cancer liver metastases was eliminated when hepatic artery embolization treatment was paired with *Trametes robiniophila* granules. Most of these effects are probably due to its activities of significantly improve immune function, relieve pain, and increase the appetite of the body after chemotherapy.

#### 3.1 Inhibiting inflammatory reactions

It is well known that the body can secrete immune factors to boost immune activity when a pathogen attacks the body. However, the body may suffer damage if the immune response becomes over-activity and results in an inflammatory reaction. Numerous disease conditions emerge in part because of

inflammation, such as cancer or depression. It has been noted that the presence of interleukin-1 $\beta$  (IL-1 $\beta$ ) is so important that it can influence inflammatory cell infiltration and regeneration by increasing the grade of proinflammatory cytokines pulling up. In the response mechanism described above, caspase-1 activation produces IL-1 $\beta$ , whereas the fact that inflammatory vesicles are necessary for caspase-1 activation, which process inflammatory factors, for example, pro-IL-1 $\beta$  and pro-IL-18 for their maturation and release, playing a central regulatory role in the inflammatory response<sup>[36]</sup>. The presence of inflammatory vesicles is dependent on IL-1 $\beta$ , which is a closed-loop stimulatory process that leads to deepening and recurring inflammation, and it is clear that IL-1 $\beta$  is the take-up component of the loop. It has been stated in the literature<sup>[7]</sup> that an aqueous extract of *Trametes robiniophila* can inhibit the induction activity of macrophages by directly reducing the reduction of IL-1 $\beta$  production as well as inhibit the expression of caspase-1 to reduce the level of IL-1 $\beta$  transcription indirectly, and therefore suppressing the inflammatory response (Fig. 1). A multicenter, randomized controlled clinical study applied malefactor to patients with hepatocellular carcinoma has found that the tumor-free survival in the malefactor group is 75.5 weeks, which significantly higher than 68.5 weeks in the control group (risk ratio 0.67, confidence interval 0.55–0.81)<sup>[37]</sup>.

### 3.2 Inhibiting the proliferation of tumor cells and inducing their apoptosis

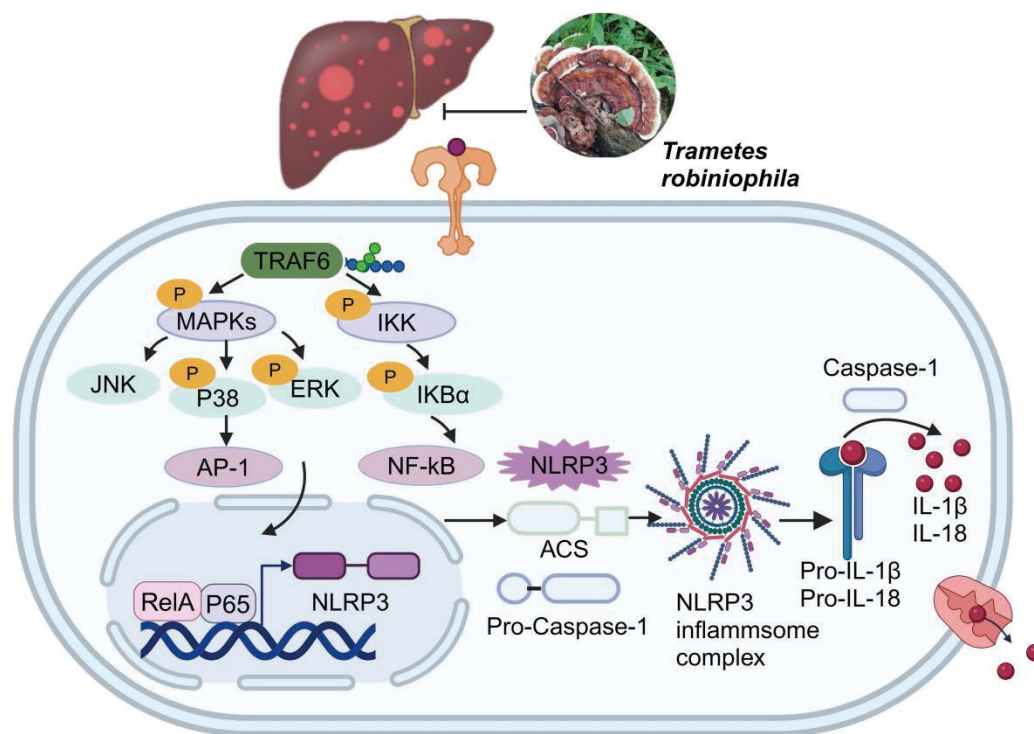
Imbalance of proliferation is a fundamental characteristic of tumor cells<sup>[38]</sup>. Therefore, inhibition of tumor cell proliferation and promotion of tumor cell apoptosis are two important aspects of treating tumors. *Trametes robiniophila* can inhibit the proliferation of breast cancer, lung adenocarcinoma, gastric adenocarcinoma, hepatocellular carcinoma, colorectal cancer,

cervical cancer, and other cancer cells. For example, according to Li *et al.*<sup>[39]</sup> TP-1 could inhibit the growth of transplanted H22 solid hepatic tumour cells *in vivo*, increase the survival period of mice with severe H22 tumours, and prevent H22-bearing mice from lung metastasizing. These mechanisms have been found as the following: regulating cell growth through various micro-RNAs, promoting apoptosis through the caspase-3 pathway, mediating autophagy through the mammalian target of rapamycin (mTOR)/s6k pathway, and blocking cell cycle entry into the G2/M phase. Different tumor cells have their different intrinsic mechanisms, and there is no doubt that one or more mechanisms may be involved in regulation together. Among them, through multi-target effect analysis, *Trametes robiniophila* extract significantly inhibited breast cancer development by promoting apoptosis, regulating DNA transcription and cell cycle blockade (Fig. 2)<sup>[40]</sup>.

Tumor protein p53 (p53) is the most common mutation gene in malignant diseases, including breast cancer<sup>[41–44]</sup>. According to reports, p53<sup>[45–48]</sup> is an important activator of cell apoptosis and cell cycle arrest. However, another study showed that, in SK-BR-3 and MDA-MB-231 cell lines, TIF-induced bidirectional solid fermentation of *Trametes robiniophila* resulted in enhanced production of caspase-3 and p53. The fundamental mechanisms require more investigation.

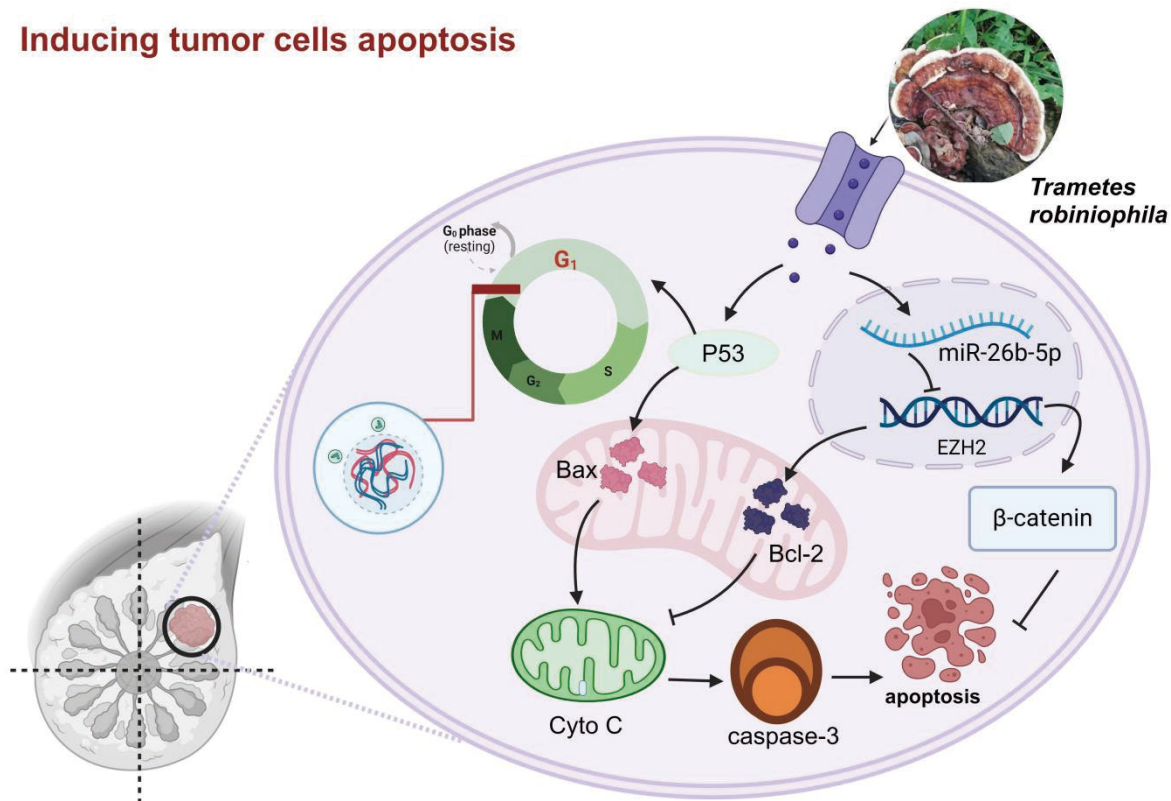
The PI3K/AKT/mTOR signaling pathway regulates some cancer-related processes, such as the growth of the cell cycle, resistance to drugs, survival of cells, metabolic processes, apoptosis, and angiogenesis<sup>[27,49,50]</sup>. Immunohistochemical staining showed that *Trametes robiniophila* could significantly handicap the PI3K/AKT signaling pathway in BT474 breast cancer xenografts. After stimulating HCCLM3 hepatocellular carcinoma cell line with different concentrations of *Trametes robiniophila* ointment<sup>[51]</sup>. The *Trametes robiniophila* ointment 6 mg/mL

### Inhibiting inflammatory response



**Figure 1** *Trametes robiniophila* exerts anti-cancer effects by inhibiting the inflammatory response. It inhibits the expression of Caspase-1 and hinders the maturation of inflammatory factors such as pro-IL-1 $\beta$  and pro-IL-18, thus suppressing the inflammatory response in the liver. Created with BioRender.com.

## Inducing tumor cells apoptosis



**Figure 2** *Trametes robiniophila* promotes apoptosis in breast tumour cells by up-regulating the activity of tumor protein (p53) in SK-BR-3 and MDA-MB-231 cell lines. This causes the Bax protein in mitochondria to function, releasing Cyto C, activating caspase-3, and promoting tumour cell apoptosis. Additionally, p53 blocks the MCF-7 cell cycle in the G0/G1 phase, preventing its entry into the G2/M phase. Created with BioRender.com.

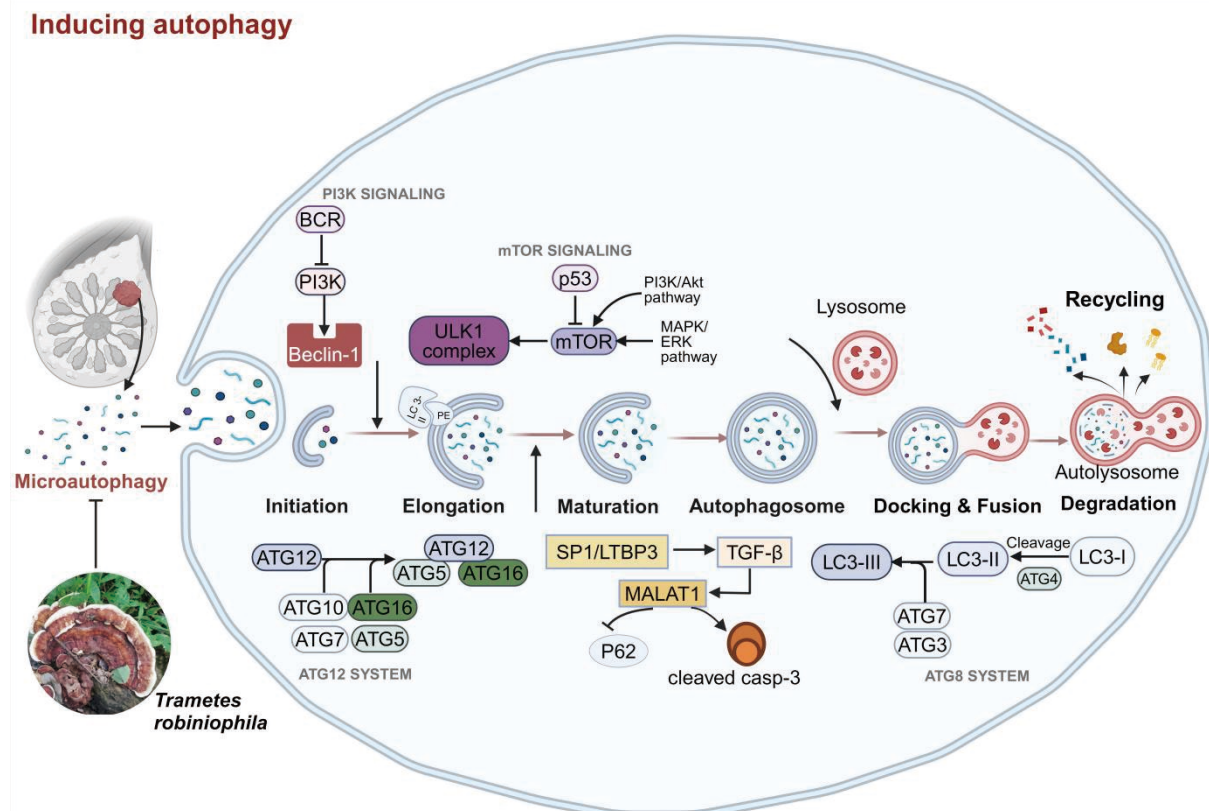
group's cell viability was considerably more inhibited from the third day forward than other groups (3 mg/mL and control groups) based on the results of the CCK-8 cell viability experiment. According to the findings of the clone creation experiment, there were much more cell clones in the 6 mg/mL *Trametes robiniophila* ointment group than in the other groups ( $55.67 \pm 5.03$  vs  $48.33 \pm 4.04$  vs  $37.33 \pm 2.52$ ,  $P < 0.05$ ). High-throughput sequencing was used to detect the expression changes of the coding RNA (Message RNA, mRNA) of HCCLM3 cell lines in the treatment group and the control group. The results showed that 486 different expressed genes were found (multiple changes  $> 2$ ,  $P < 0.05$ ), with 317 genes showing up-regulation and 115 genes showing down-regulation in the experimental group. PCR and western blot tests indicated that *Trametes robiniophila* ointment may dramatically lower the expression of transcription factor A, mitochondrial (TFAM) in liver cancer cells ( $1.00 \pm 0.04$  vs  $0.45 \pm 0.16$ ,  $P < 0.01$ ). Western blot experiments indicated that the protein expression content of p-Akt was significantly reduced after stimulation with *Trametes robiniophila* ointment, while the total Akt content remained unchanged. Experiments<sup>[51]</sup> have shown that *Trametes robiniophila* ointment can effectively inhibit the proliferation and metastasis of liver cancer cells, and this result may be obtained by suppressing the activity of the Akt signaling pathway and lowering the expression of TFAM.

In recent years, the mammalian target of rapamycin (mTOR) has emerged as a promising and important therapeutic target for cancer therapy. The mTOR1 complex is an intracellular molecular nutrient receptor, which can sense the energy and amino acid changes in the cell. Inhibition of the mTOR signaling pathway can induce cellular autophagy. and the study<sup>[52]</sup> has demonstrated through western-blotting experiments that after treatment with

*Trametes robiniophila*, the expression of apoptosis-related cleaved parp and cleaved caspase-3 were elevated, and the expression of protein autophagy markers LC3B-II and Beclin-1 were also increased, meanwhile, the expression of autophagy receptor proteins p62 and p-mTOR decreased. This suggested that *Trametes robiniophila* may promote apoptosis and induce autophagy in breast cancer cells by inhibiting the mTOR pathway (Fig. 3).

### 3.3 Inhibiting the invasion and migration of malignant tumor cells

Invasion and migration are characteristic of malignant tumors and are necessary conditions for the progression of malignant tumors, as well as the main cause of death in patients. Currently, epithelial calcineurin (E-cadherin), tissue inhibitor of metalloproteinase genes, and their expressed products have an inhibitory effect on tumor metastasis. In contrast, over-expression of the adhesion molecule CD44 may be involved in the hematogenous dissemination of some tumors. It has been found that *Trametes robiniophila* ointment can significantly reduce the invasion and migration capacity of PC3 cells in prostate cancer, and can lower the level of expression of neurocalcalis and ZEB1 and elevate the protein expression level of E-cadherin, thereby inhibiting the proliferation and migratory invasion of the PC3 cell in human prostate cancers<sup>[53]</sup>. This effect could be the effects and possible molecular mechanisms for *Trametes robiniophila*'s on hepatocellular carcinoma. It was<sup>[37]</sup> discovered that the *Trametes robiniophila* auriculata 6 mg/mL group had a remarkably stronger inhibitory effect on the ability of hepatocellular carcinoma cells to metastasize than both the 3 mg/mL and the control group ( $67.00 \pm 4.58$  vs  $44.00 \pm 3.00$  vs  $22.33 \pm 2.52$ ,  $P < 0.01$ ). Tumor



**Figure 3** A map of the apoptosis signalling pathway in tumour cells promoted by *Trametes robiniophila*. *Trametes robiniophila* mediates autophagy in breast cancer cells through the PI3K/mTOR/ATG pathway. Following treatment with *Trametes robiniophila*, the expression of the apoptosis-related protein cleaved caspase-3 increased, and the expression of the autophagy marker proteins LC3B-II and Beclin-1 increased, while the expression of the autophagy receptor proteins p62 and p-mTOR decreased. Created with BioRender.com.

metastasis is a complex and dynamic process regulated by multiple factors. Epithelial-mesenchymal transition plays a crucial role in the early stages of invasion and metastasis in many tumors. As the *Trametes robiniophila* inhibited the narrow migration and invasion of kidney cancer cells by regulating the epithelial-mesenchymal transition (EMT) process, and the expression levels of N-cadherin, Vimentin, and Twist decreased in the cells of 786-0 and A498 after 24 h of treatment with *Trametes robiniophila* solution, while E-cadherin's expression level rose, which indicated that *Trametes robiniophila* was able to limit the expression of pro-metastasis-related proteins and suppress the EMT process in a concentration-dependent manner (Fig. 4)<sup>[26]</sup>.

### 3.4 Inhibiting the formation of tumor blood vessels

Tumor growth and metastasis depend on tumor neovascularization, where the proliferation, invasion, migration, and differentiation of vascular endothelial cells are important parts of the neovascularization process and are regulated by vascular endothelial (cell) growth factor (VEGF). *Trametes robiniophila* extract could inhibit the proliferation, migration, and differentiation of related vascular endothelial cells, delaying the growth and metastasis of tumors, and achieving the goal of tumor treatment.

*Trametes robiniophila* extract attenuated VEGF-induced migration and neovascularization of HUVECs, DNA replication, migration, and tubule formation. The ointment can play an anti-colon cancer role by inhibiting the expression of hypoxia-inducible factor-1α and VEGF, thereby suppressing the production of blood vessels<sup>[30]</sup>. In a chicken embryonic chorionic villus allantoic membrane model, *Trametes robiniophila* extract

was also able to inhibit angiogenesis *in vivo*<sup>[54]</sup>. In a study on the inhibition of growth and mechanism of recurrent tumors after resection of hepatocellular carcinoma, it was found that *Trametes robiniophila* reduced the expression of VEGF and PDGF in serum and recurrent tumors and inhibited the growth of tumor blood vessels and lung metastasis<sup>[55]</sup>.

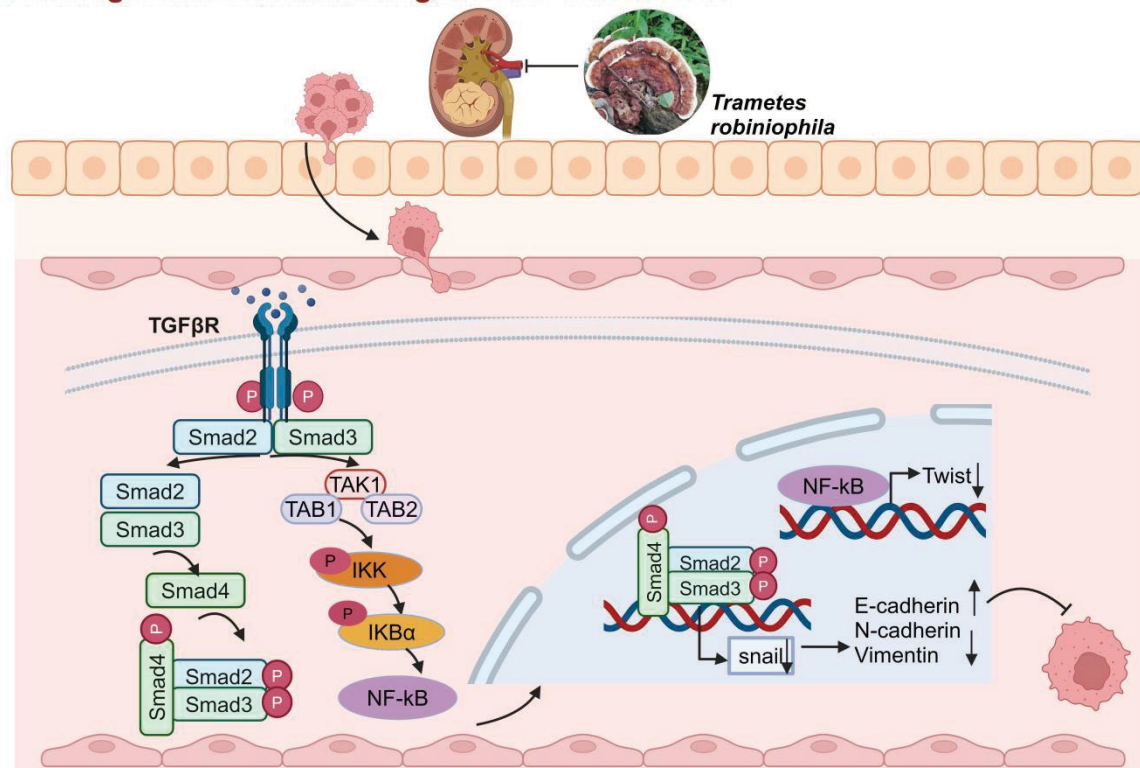
Endostatin is an efficient and specific vasopressor that inhibits the proliferation of vascular endothelial cells and suppresses somatic angiogenesis, and the previous study<sup>[28]</sup> showed that *Trametes robiniophila* ointment upregulated the level of mRNA endostatin in the L9981 lung cancer cell line and exerted an inhibitory effect. It is known that it can act as an anti-tumor by regulating the expression of related genes to inhibit the production of blood vessels and suppressing the migration and invasion of tumor cells.

### 3.5 Reversing the drug resistance of tumor cells

Tumor cell resistance to chemotherapeutic drugs is one of the reasons for the failure of tumor chemotherapy. Studies have shown that during the development of multi-drug resistance (MDR), resistant tumor cells are often accompanied by a significant increase in intracellular glutathione (GSH) levels, and inhibition of GSH activity can effectively reverse chemoresistance to tumor drugs<sup>[56]</sup>. Xu *et al.*<sup>[34]</sup> found that the intracellular GSH content in SGC-7901/FU cells can be significantly reduced within a certain concentration range of *Trametes robiniophila* ointment, while the effect on GSH levels in sensitive cells was relatively minor.

The mechanism of increasing chemotherapeutic drug resistance by *Trametes robiniophila* extract may also be related to the process

## Inhibiting the invasion and migration of tumor cells



**Figure 4** *Trametes robiniophila* inhibits renal cancer cell metastasis. *Trametes robiniophila* inhibits renal cancer cell carvure and invasion by regulating the epithelial-mesenchymal transition (EMT) process. *Trametes robiniophila* inhibits the expression of pro-metastatic proteins N-cadherin, Vimentin, and Twist, while the expression level of E-cadherin increases, preventing malignant tumour cells from continuing to invade and metastasise. Created with BioRender.com.

by which drug-resistant cells undergo apoptosis, and *Trametes robiniophila* extract was found to induce apoptosis in SGC-7901/FU cells and reverse their drug resistance. Li *et al.*<sup>[57]</sup> investigated the reversal mechanism of *Trametes robiniophila* granules on MCF-7 drug resistance in human breast cancer cells and found that *Trametes robiniophila* granules at a concentration of 0.01 mg/mL could reverse the drug resistance in MCF-7/A cells as well as decreasing the expression levels of the drug resistance genes MDR-1 as well as the related P-glycoprotein and MRP proteins. In addition, by blocking the JNK/JUN/IL-8 signalling pathway, *Trametes robiniophila* reduced the cisplatin resistance of NSCLC cells<sup>[58]</sup>. The above study showed that *Trametes robiniophila* can reverse the drug resistance of tumors by inhibiting GSH activity, inducing apoptosis, down-regulating the drug resistance genes of tumor cells, and decreasing the expression of related proteins in various aspects.

### 3.6 Enhancing immune function

Immunomodulatory activity is a prominent activity to enhance the body's defense mechanisms in elderly people and cancer patients<sup>[59]</sup>. Studies have revealed that MEHP modulates the immune action of macrophages with three main approaches<sup>[60-62]</sup>. Firstly, MEHPs increase the activity of macrophage membrane receptors (CD14, TLR4, CR3, and p38 kinase). Secondly, MEHPs could regulate the  $\text{Ca}^{2+}$ /PKC/p38/nuclear factor kappa-B (NF- $\kappa$ B) pathway, upregulate  $\text{Ca}^{2+}$ , p38, and NF- $\kappa$ B activity in macrophages, and then activate PKC and increase NO, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-1 $\beta$ , and IL-6 levels. In addition, MEHPs reduced LPS-induced excessive production of inflammatory mediators by macrophages and decreased the amounts of NO, IL-6, TNF- $\alpha$ , and IL-1 $\beta$  released. The TLR4

signaling pathway is a crucial mechanism by which MEHP regulates the immune system. TLR4, MyD88, along with TRAF-6 expression levels have all been markedly elevated. A cascade reaction raises NO, IL-2, IL-6, IL-17, and IFN- $\gamma$  levels to improve immune modulation both *in vivo* and *ex vivo*. Regarding specific immunity, MEHPs could irritate T cell proliferation as well as regulate T cell cytokine release, which in turn boosts the function of NK cells, killer T cells, and helper T cells involved cellular immunity. Following that, MEHP can enhance B lymphocytes' production of immunoglobulins and strengthen the body's humoral immunity. The body's first line of defense against the introduction of germs and hazardous compounds is the gut mucosal barrier, as it is often known. The intestine is also the body's largest immunological organ. An increasing number of studies have confirmed that the intestinal mucous immune system may be a key target for the role of MEHP. In mice with splenic insufficiency and low immunity models, MEHPs can alter the composition of the intestinal flora and boost the quantity of  $\text{CD3}^+$  T and  $\text{CD19}^+$  B cells in the spleen and thymus.

It has been found that during the development of most infectious diseases and tumor formation, the  $\text{CD4}^+/\text{CD8}^+$  T lymphocyte ratio is reduced or even inverted, causing immune dysfunction in the body and further promoting disease development<sup>[63-68]</sup>. *Trametes robiniophila* helps to maintain a healthy ratio and improve the immune function of the body<sup>[69]</sup>. Numerous studies have shown that *Trametes robiniophila* promotes lymphocyte proliferation and increases the percentage of T-cell subsets and  $\text{CD4}^+/\text{CD8}^+$  T-lymphocyte ratios in tumor patients. According to the study<sup>[39]</sup>, TP-1, a polysaccharide component of *Trametes robiniophila*, was initially thought to be an immunological enhancer due to its ability to increase

immunostimulatory serum cytokines and lymphocyte proliferation *in vivo* (Fig. 5). CD4<sup>+</sup> based on their mediated functions and released cytokines, T cells are classified into two functional subpopulations: Th1 cells and Th2 cells. Th1 cells primarily produce IL-2 and IFN- $\gamma$ . IL-2 is a key T cell growth factor that encourages T cell proliferation and differentiation. Several studies have demonstrated that *Trametes robiniophila* can enhance IL-2 production, preserve T cell homeostasis, and support the body's immune system<sup>[70]</sup>. Th2 cells mainly secrete IL-4, IL-10, etc. One of the biological components that define Th2 cells is IL-4, which can sustain Th2 cell proliferation<sup>[66]</sup>. Meanwhile, a cytokine with a broad spectrum of immunological effects, IL-10 suppresses the immune system and promotes the spread of tumor cells by impeding the development of anti-tumor immunity and altering the body's immune system through several different mechanisms; it also increases the expression of angiogenic factors and increases the density of tumor blood vessels<sup>[71]</sup>, thus promoting tumor growth<sup>[64]</sup>. Under normal conditions, Th1 and Th2 cells maintain a dynamic balance in the immune regulation by regulating each other, and an increase or decrease in either side will cause abnormalities in immune response<sup>[72]</sup>.

The immune system and its organs are where immune cells develop and function. The most significant organs are the bone marrow, thymus, tonsils, spleen, lymph, and appendix. Furthermore, the thymus and spleen indices are significant markers of the body's immune function<sup>[73,74]</sup>. In a study involving

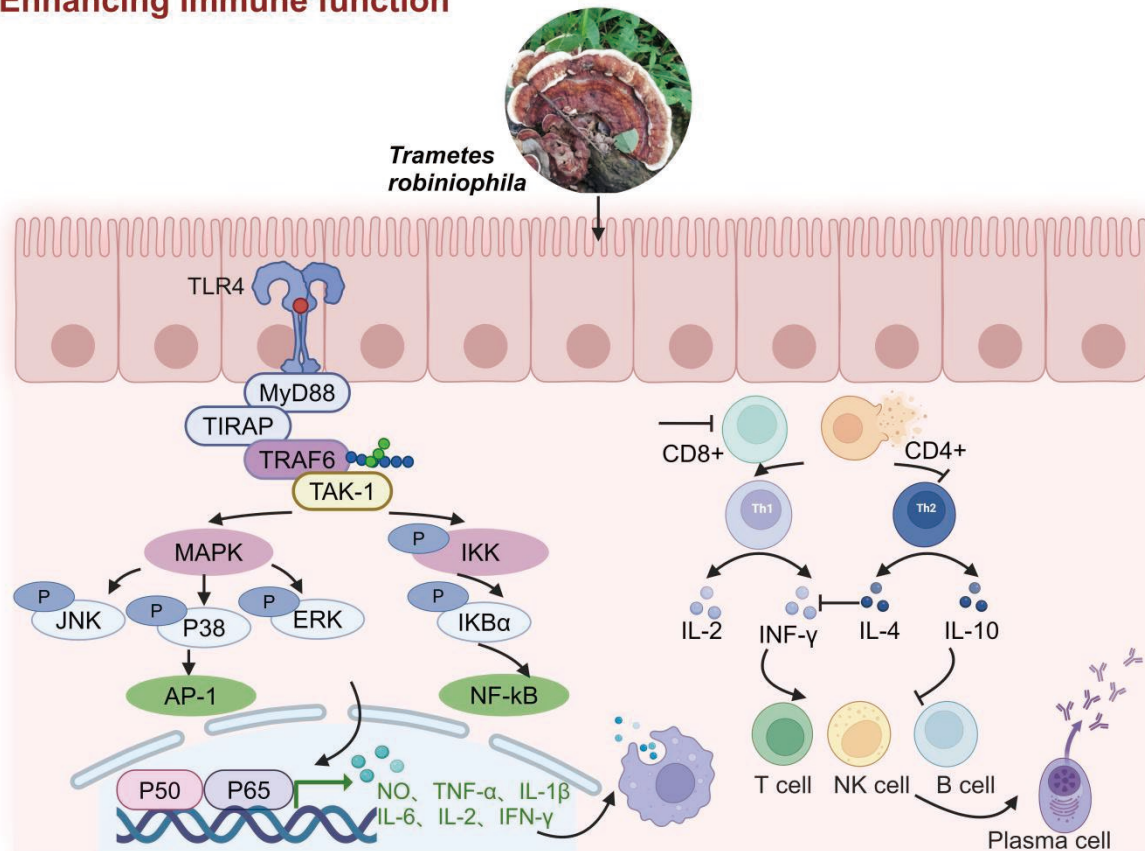
H22 tumor-bearing mice was injected intraperitoneally by TP-1, a polysaccharide from *Trametes robiniophila*. The results revealed that the mice's relative weights increased and even exceeded the normal level when treated with TP-1<sup>[39]</sup>. Huaier polysaccharides inhibited hepatocellular carcinoma via gut microbiota and related metabolites to mediate M2 macrophage polarization<sup>[75]</sup>. Splenocytes of male BALB/c mice were taken and treated with different concentrations of W-NTRP, a neutral water-soluble polysaccharide isolated from the seeds of *Trametes robiniophila*, and the results showed that W-NTRP significantly promoted the proliferation of murine splenocytes, and the promotion effect was normally distributed with the polysaccharide concentration, with the strongest promotion effect at 100  $\mu\text{g/mL}$ <sup>[59]</sup>.

### 3.7 Exerting synergistic effect with chemotherapy drugs

Chemotherapy drug treatment, as one of the three major treatments for tumors, ultimately achieves the effect of killing tumor cells by stopping their proliferation, infiltration, and metastasis.

Chemotherapy drugs, however, are not very selective, so while they kill tumors, they also invariably damage normal tissues. This deteriorates the body's overall health and lowers immune function, increasing the risk of infection and tumor development in tumor patients. On the other hand, chemotherapeutic medications become ineffective against tumor cells, thus leading to the failure of chemotherapy. These techniques are a double-edged sword due to their limited effectiveness and evident negative

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**Figure 5** *Trametes robiniophila* enhances immune function by upregulating macrophage p38 and NF- $\kappa$ B activity in the intestinal mucosa through the TLR4 signalling pathway and the NF- $\kappa$ B pathway. This leads to increased levels of NO, TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, which enhances immune regulation both *in vivo* and *in vitro*. Additionally, *Trametes robiniophila* promotes the proliferation of CD4<sup>+</sup> lymphocytes and the secretion of IL-2 and IFN- $\gamma$  by Th1 cells, improving lymphocyte proliferation and differentiation and enhancing the body's immune function. Created with BioRender.com.

effects<sup>[76]</sup>. As is generally known, therapy-related side effects such as nausea, vomiting, peripheral neuropathy, pain, and sleeplessness significantly lower quality of life<sup>[77-79]</sup>. TCM (Traditional Chinese medicine), usually used as an adjunct or alternative medicine, also provides an effective treatment option for cancer<sup>[80,81]</sup>. In recent years, there have been growing evidences that TCM is essential in the treatment of cancer<sup>[80]</sup>. Several clinical research using complementary and alternative therapies have revealed that, Herbal medicines can considerably increase patients' quality of life and survival time when taken in conjunction with complementary and alternative medicine, these trials include patients with breast cancer<sup>[82-84]</sup>. There is a study reporting a case of HCC-related pulmonary transplantation, in which patients received combined therapy with BACE and *Trametes robiniophila* and maintained good hematological and radiological complete relief for 60 months. *Trametes robiniophila* showed effective anti-tumor therapeutic capabilities<sup>[85]</sup>. Moreover, *Trametes robiniophila* cream also enhances the sensitization of tumor cells to chemotherapeutic drugs, thus enhancing the antitumor effects by combining chemotherapeutic drugs with *Trametes robiniophila* cream.

#### 4 The medicinal value and application of *Trametes robiniophila*

It is recognized that polysaccharide-protein is the primary bioactive ingredient. Because polysaccharides and their derivatives are biodegradable and biocompatible, they are superior to synthetic polymers<sup>[86,87]</sup>. Currently, polysaccharides have been crucial to conventional disease prevention and treatment. Additionally, a wide range of novel application domains are being investigated, including tissue engineering, drug administration, internal and external wound care, cancer prevention, detection, and medical treatment, and functionalization of polysaccharides and their derivatives for the treatment of bacterial and viral diseases.

Since 200 AD, Chinese medicine has been utilized to cure many illnesses<sup>[88]</sup>. Numerous studies have shown that *Trametes robiniophila* and its products have important effects in the fight against cancer, among others. For example, *Trametes robiniophila* used liquid fermentation and solid fermentation two production processes after the extraction of the bioactive ingredient components of the Dutch s180 tumor animals within the tumor inhibition experiments, the results show that the former tumor inhibition rate of 12%, the latter tumor inhibition rate of > 30%. Huang *et al.*<sup>[89]</sup> treated different parts of *Trametes robiniophila* with systemic solvents and proved that petroleum ether, ethyl acetate, n-butanol, and ethanol parts of *Trametes robiniophila* were the effective anti-tumor parts, and the n-butanol part was the most effective, and the inhibition rate of the half of MKN-45 cells (IC<sub>50</sub>) was 56.142 µg/mL. Li<sup>[90]</sup> showed that *Trametes robiniophila* granules, a proprietary Chinese medicine preparation, had an anti-tumor effect on hepatocellular carcinoma cells, with the effect of activating blood circulation and eliminating symptoms, resolving stasis and stagnation, and improving patients' symptoms such as pain in the liver area and liver function impairment. Huang *et al.*<sup>[91]</sup> used patients with liver metastases of colorectal cancer undergoing radical resection as the treatment group and adjuvant chemotherapy alone as the control group, and the treatment group was treated with *Trametes robiniophila* granules associated with adjuvant chemotherapy. When Shi *et al.*<sup>[92]</sup> separated 826 cases of HCC patients into Huaier and control groups and

compared the three-year overall survival rates of the two groups, they discovered that Huaier granule, a medication that contains sophora mycelium polysaccharides, significantly improved the OS rate and had a positive prognostic effect on patients with HCC. Qiu *et al.*<sup>[93]</sup> observed the efficacy of *Trametes robiniophila* granules in the treatment of chronic granulocytic leukemia (chronic phase), the results showed that the complete remission rate of *Trametes robiniophila* granules for patients in the chronic phase was 36.37%, the partial remission rate was 43.33%, and the total effective rate reached 80%, and the treated cytokine levels such as interleukin 2 and tumor necrosis factor. The cytokine levels such as interleukin 2, tumor necrosis factor and interferon-gamma were significantly different from those before treatment, which concluded that *Trametes robiniophila* granules were effective in the treatment of patients with chronic illnesses.

In addition, pharmacological experiments confirmed that polysaccharide-protein is the main bioactive component of *Trametes robiniophila*. Therefore, the quality control index of *Trametes robiniophila* fermentation and the fermentation endpoint of the fungus can be developed accordingly. *Trametes robiniophila* can also play itself or improve the value of other substances through two-way fermentation technology. The key to the "new (two-way) solid fermentation of medicinal fungi" technology is to use herbs with bioactive ingredients as a medicinal substrate for fungal growth, which can meet the growth needs of fungi and can be changed by the growth and metabolism of fungi, thus possibly producing new bioactive ingredients. Bidirectional fermentation has the potential to decrease, increase, or even triple the efficacy of traditional Chinese medicine. That is, if the fermentation combination is appropriately planned and the production method is rational<sup>[94]</sup>. Furthermore, Liu *et al.*<sup>[95]</sup> found that the effects of *Trametes robiniophila* on MDA-MB-231 cells were enhanced by bidirectional solid fermentation, suggesting that the granules of this species could be a useful adjunctive medication for the treatment of cancer.

#### 5 Conclusion

Polysaccharides derived from natural sources has the advantages of good biocompatibility, low immunogenicity, and low cytotoxicity, and has good prospects for drug delivery and controlled release applications. In modern medicine, *Trametes robiniophila* polysaccharide is often used in combination with other drugs or medical treatments to combat various types of cancer, including liver, lung, breast, and stomach cancers. However, some issues, such as how polysaccharide content and structural type affect reaction mechanisms and therapeutic benefits, the impact of *Trametes robiniophila* on the survival rate of organ transplants, and the solutions, require more study. At the same time, the number of clinical trials for some cancers are still limited, which hinders its application in patients. Further research on human pharmacokinetics and influencing factors is necessary. Additionally, while the bioactive ingredients of *Trametes robiniophilas* are diverse, the mechanism of action of *Trametes robiniophilas* remains unclear. Further research is needed on the pharmacokinetics, influencing factors in the human body, and expanding the practical application of *Trametes robiniophila*.

#### Data availability

All data needed to support the conclusions in the paper are presented in the manuscript.

## Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

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## Author contribution statement

Liying Fang: data curation, writing manuscript, writing - review & editing, formal analysis; Zhenghong Wang: visualization, software and writing - review & editing; Shuangyan Xie: conceptualization, validation and resources; Jinbo Hou: conceptualization and validation; Caizhe Ji: investigation, visualization; Jun Lu: project administration, writing - review & editing, supervision; Xueli Gao: conceptualization and investigation.

## Informed consent

Not applicable.

## Ethics statement

Not applicable.

## Use of AI statement

None.

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