

# *Trametes robiniophila* Murr: a traditional Chinese medicine with potent anti-tumor effects

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**Abstract:** *Trametes robiniophila* Murr also known as Huaier, one of the traditional Chinese medicines, has been shown an effective adjuvant of cancer therapy. Accumulating evidence suggests that the anti-cancer effects of Huaier can be briefly divided into two aspects: the direct effects on tumor cells and the indirect effects on immune cells. In vitro and in vivo experiment showed Huaier directly inhibited tumor cell proliferation, induced tumor cell death, prevented metastasis and interfered with angiogenesis via various signaling pathways. The immunomodulatory effect of Huaier is associated with enhancement of the number and function of CD4<sup>+</sup> T cells and NK cells, regulation of the polarization and function of macrophages, and elevated secretion of immune stimulatory cytokines. In this review, the anti-cancer effects and combined treatments of Huaier with other anti-cancer therapies, and the underlying mechanisms are summarized and discussed.

**Keywords:** Huaier, cancer, anti-tumor therapy, traditional Chinese medicine, adjuvant

## Introduction

*Trametes robiniophila* Murr, also called Huaier, a sandy beige mushroom, has been used as a traditional Chinese medicine (TCM) for over 1,600 years. The most common pharmaceutical preparations of Huaier include aqueous extracts and granules. The mainly active ingredient in Huaier is proteoglycan, which consists of polysaccharides, amino acids and water.<sup>1</sup> Increasing evidence highlights that Huaier has a satisfactory clinical effect on nephrosis,<sup>2</sup> colitis,<sup>3</sup> tuberous sclerosis<sup>4</sup> and cancers.<sup>5</sup> In mesangial proliferative glomerulonephritis, Huaier reduces urinary protein excretion and relieves hyperplasia in mesangial cells as well as inhibits platelet-derived growth factor-BB-stimulated proliferation and DNA synthesis of mesangial cells in vitro.<sup>2</sup> In ulcerative colitis, Huaier not only inhibited NLRP3 inflammasome activation-induced IL-1 $\beta$  secretion and caspase-1 cleavage but also promoted NLRP3 degradation through the autophagy lysosome pathway.<sup>3</sup> Moreover, Huaier attenuated JAK2/STAT3 and MAPK signaling to inhibit the proliferation and metastasis in tuberous sclerosis complex.<sup>4</sup>

Previous experimental studies showed that Huaier could exert a potent anti-cancer effect on hepatocellular cancer (HCC),<sup>6,7</sup> breast cancer,<sup>8,9</sup> ovarian cancer,<sup>10</sup> and so on. This was also verified in a clinical research of 53 HCC patients<sup>11</sup> and a meta-analysis in gastrointestinal cancer.<sup>12</sup> Several studies demonstrated that Huaier prolonged survival time of cancer patients and reduced the recurrence rate of HCC.<sup>11,13–15</sup> Moreover, evaluations of serum hepatic and renal function parameters showed that Huaier almost

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had no cytotoxicity to normal liver and kidney.<sup>13,16</sup> All these results show that Huaier is an effective adjuvant in therapy for cancers. In this review, the anti-tumor effects of Huaier and the underlying mechanisms are discussed.

## The direct anti-tumor effects of Huaier and underlying mechanisms

Sustaining proliferative signaling, resisting cell death, inducing angiogenesis and activating invasion and metastasis are four of the hallmarks of cancer.<sup>17</sup> In breast cancer cell line MDA-MB-231, Huaier regulates 387 genes including the genes that control cell proliferation, apoptosis, tumor metastasis and angiogenesis.<sup>18</sup> Among the 387 genes, the top 5 of 226 up-regulated genes were *MMP3*, *TCP11L2*, *IL24*, *CLEC2B* and *LIPH*; while the top 5 of 161 down-regulated genes were *MPP4*, *HIST1H2BM*, *ID1*, *F3* and *SNORD14D*. The direct anti-tumor effects of Huaier work through different mechanisms, including inhibition of cancer cell proliferation, induction of cancer death, inhibition of tumor-induced angiogenesis and suppression of cancer metastasis.

## Inhibition of cancer cell proliferation

### The cell cycle arrest

Uncontrolled cell proliferation results in tumorigenesis and tumor progression, thus cell cycle regulators are growing potent targets in cancer therapy. Among these regulators, cyclin-dependent kinases (CDKs) play a major role. INK4 and D-type cyclins regulate CDK4 and CDK6 to trigger cells in G0/G1 phase to re-enter and progress into S phase.<sup>19</sup> Cyclin D1 is the dominant cyclin in G1 phase<sup>20</sup> and can be regulated by Huaier.<sup>9</sup> Huaier augmented the tamoxifen-induced inhibition of Akt/mTOR signaling, decreased the downstream element glycogen synthase kinase-3 and cyclin D1 expressions to induce the G0/G1 cell cycle arrest in breast cancer. In another study, Gao et al demonstrated that Huaier could induce G0/G1 arrest in both M7-TR (tamoxifen-resistant cells) and M7-FR (fulvestrant-resistant cells).<sup>21</sup> The results showed that after treatment of Huaier, cyclin D1 and CDK4 were decreased significantly, but ataxia-telangiectasia mutation (ATM) and its downstream target replication protein A phosphorylation were increased. ATM was negatively correlated with tumorigenesis and progression, regulated directly by miR-203.<sup>22</sup> Huaier significantly suppressed miR-203 and up-regulated ATM to inhibit the expression of cyclinD1 and CDK4, resulting in G0/G1 arrest.<sup>21</sup> This was verified in other breast cancer cell lines MCF-7, MDA-MB-468 and MDA-MB-231.<sup>9,23–25</sup> In addition to G0/G1 arrest in breast

cancer, Huaier also induced cell cycle arrest with different mechanisms in other kinds of cancers. In HCC<sup>26</sup> and lung cancer,<sup>27</sup> Huaier repressed the expression of  $\beta$ -catenin and cyclin D1, resulting in remarkable increase of cells arrested in the S phase.<sup>26</sup>

In cervical cancer, Huaier aqueous extract treatment lead to, through activation of JNK/p38 signaling pathway, G2/M phase arrest.<sup>28</sup> This effect of Huaier to induce a G2/M cell cycle arrest has also been proved in studies on gastric cancer cells,<sup>29</sup> brosarcoma cells<sup>30</sup> and melanoma cells.<sup>31</sup> The mechanism may referred to the regulation of the expression of cyclin B1, which binds to Cdc2 to form the complex vital for G2 checkpoint.<sup>29</sup>

Therefore, Huaier can inhibit cancer cell proliferation by cell cycle arrest in G0/G1, S and G2/M phases in different types of cancers.

### Enhancement of I $\kappa$ B $\alpha$ expression to inhibit NF- $\kappa$ B-mediated signaling pathway

NF- $\kappa$ B signaling pathway plays an essential role in the cell proliferation.<sup>32</sup> Normally, NF- $\kappa$ B is kept as an inactive state, which is maintained by inhibitor of NF- $\kappa$ B (I $\kappa$ B).<sup>33</sup> However in tumor microenvironment (TME), NF- $\kappa$ B can be aberrantly activated. After treated with Huaier, proliferation of breast cancer cells was significantly inhibited and mechanism analyses showed that the expression of I $\kappa$ B $\alpha$  was increased, while the expression of p65 and the up-stream c-Met decreased.<sup>23</sup> These data demonstrated that Huaier could down-regulate the expression of c-Met, increase the expression of I $\kappa$ B $\alpha$ , inhibit the activation of NF- $\kappa$ B and finally control the abnormal proliferation of cancer cells.

### Inhibition of NF- $\kappa$ B–estrogen receptor (ER) pathway

Steroid hormones regulate cell growth and differentiation by binding to steroid hormone receptors.<sup>34</sup> ERs are essential members of these steroid hormone receptors. The mechanism of ER action involves forming homodimer and binding to estrogen response elements and other transcription factors, such as NF- $\kappa$ B.<sup>35</sup> There are two subtypes of ERs (ER $\alpha$  and ER $\beta$ ) exerting opposite effects. ER $\alpha$  mediates aberrant proliferation resulting in tumorigenesis and perturbation of ER $\alpha$ /ER $\beta$  ratio has been detected in several types of cancers, especially breast cancer.<sup>36</sup> It was found that Huaier efficiently inhibited the expression of ER $\alpha$  and its downstream genes, abolished the estrogen-induced activation of NF- $\kappa$ B, thus inhibited the estrogen-stimulated proliferation.<sup>37</sup>

### Inhibition of PI3K–Akt pathway

The PI3K–Akt signaling network is closely related to cancer cell proliferation, apoptosis, metastasis and the immune response and angiogenesis in TME.<sup>38</sup> It was demonstrated that Huaier could act as a potent inhibitor of the PI3K–Akt signaling pathway.<sup>29,39</sup> The proteins associated with this signaling pathway include PI3K, AKT1 and PTEN. The phosphorylation of these proteins was significantly down-regulated by Huaier in a dose-dependent manner,<sup>29</sup> but the total Akt expression was not affected.<sup>39</sup>

### Inhibition of Yes-associated protein 1 (YAP1) expression

YAP1 is the first to be activated and drives quiescent epithelial cells re-entry to the cell cycle.<sup>40</sup> Shan et al found that Huaier had an inhibitory effect on YAP1 in HCC cells.<sup>16</sup> The underlying mechanism may due to the Huaier-induced translocation of YAP1. After treated with Huaier, YAP1 locomoted from nucleus to the cytoplasm, phosphorylated, ubiquitinated and finally degraded, losing the ability to promote the proliferation of cancer cells.<sup>16</sup>

### Suppression of cancer stem cells (CSCs)

CSCs play important roles in metastasis, inevitable recurrence and therapy resistance of cancer.<sup>41</sup> It was found that Huaier had a great efficacy to inhibit spheroid formation potential of CSCs<sup>42,43</sup> and could inhibit the viabilities, numbers and sizes of mammospheres in breast cancer.<sup>43</sup> Besides the colony formation, Huaier can decrease the population of aldehyde dehydrogenase-positive cells<sup>42</sup> and CD44<sup>+</sup>/CD24<sup>-</sup> cells, suppress the expression of stem cell marker OCT4, NESTIN and NANOG.<sup>43</sup>

The Wnt signaling pathway and hedgehog pathway have been proved to regulate CSCs stemness and self-renewal.<sup>44</sup> In a study on colorectal CSCs, Zhang et al found that the expression of total  $\beta$ -catenin was down-regulated dose-dependently and the expression of cyclin D1, one of the Wnt/ $\beta$ -catenin target genes, was also decreased after treated with Huaier. The down-regulation of Wnt/ $\beta$ -catenin signaling pathway resulted in the eradication of colorectal CSCs and ovarian cancer cells.<sup>10,42</sup> However in breast cancer, Huaier exerted the inhibitory effect via inactivation of the hedgehog pathway. Among the five proteins (Ptch-1, Smo, Gil1, Gil2 and Gil3) vital in this pathway, Gil1 decreased significantly after Huaier treatment while others remained unchanged.<sup>43</sup> Therefore, Huaier could inhibit the quantity and functions of CSCs via Wnt signaling pathway and hedgehog pathway.

### Induction of cancer cell death

#### Stimulation of autophagy

Autophagy is a highly regulated multistep process to degrade long-lived proteins and damaged organelles in disease, especially cancer.<sup>45</sup> The inhibition of mTOR<sup>46</sup> and mTOR/S6K pathway<sup>47</sup> activated cancer cell autophagy. It was found that Huaier-induced cytotoxicity is partially reacted on cell autophagy.<sup>9,48,49</sup> After Huaier treatment, the expressions of phosphorylated mTOR and its downstream targets p70 ribosomal protein S6 kinase (p70S6K), S6 ribosomal protein and 4E-BP1 were decreased remarkably but the total level of mTOR was unaffected.<sup>48</sup> In a study on hepatoma SK-HEP-1 cells, Yang et al indicated that Huaier could increase the expression of LC3, an autophagy marker, and induce the formation of autolysosome.<sup>49</sup> The activation of autophagy induced by Huaier significantly inhibited the proliferation of cancer cells.

#### Induction of apoptosis

lncRNAs are RNAs that do not encode protein but have a length exceeding 200 nucleotides and play important roles in cancer.<sup>50</sup> Wang et al found that Huaier could induce apoptosis via *lncRNA-H19/miR-675-5p* pathway.<sup>8</sup> The *H19* gene is one of the lncRNA dysregulated in many cancers. There are two conserved microRNAs (*miR-675-3p* and *miR-675-5p*) encoded by *H19* exon1.<sup>51</sup> Wang et al found that *miR-675-5p* was up-regulated in breast cancer. After treatment of Huaier, the expressions of *H19* and *miR-675-5p* were significantly reduced. Furthermore, Huaier-induced apoptosis of breast cancer cells could be reversed by up-regulating *miR-675-5p* or over-expression of *H19*.<sup>8</sup> *miR-26b-5p* was also found to function in Huaier-induced apoptosis.<sup>52</sup>

The mitochondrial pathway, which is regulated by Bcl-2 family, participates in regulating tumor cell apoptosis. *Bax* is a pro-apoptosis gene of the Bcl-2 family. Once activated, Bax will bind to Bcl-2 to inactivate the later to promote apoptosis.<sup>53</sup> Treatment with Huaier activated the three MAPK pathways (ERK, JNK but mostly p38), enhanced the expression of Bax, but decreased the expressions of Bcl-2, resulting in the acceleration of apoptosis in cancer cells.<sup>7,16,25,27,54–56</sup> Among the down-stream effectors, caspase-3 acts as the key apoptosis executors to kill cells, recruit macrophages and present an “eat me” signal.<sup>57</sup> Researchers found that the apoptosis of breast cancer cells,<sup>25</sup> melanoma cells<sup>31</sup> and lung cancer cells<sup>27</sup> increased significantly after Huaier treatment. Molecular mechanism analyses showed that increased cleavage caspase-9 and -3 expression but decreased pro-caspase-3

expression were found, indicating that Huaier-induced apoptosis was mainly mediated by caspase-3.<sup>25,31</sup> A study on HCC showed that caspase-7 and its substrate PARP were also involved in the Huaier-induced cleavage.<sup>6</sup>

### Inhibition of tumor-induced angiogenesis

Angiogenesis is a typical characteristic of tumor. Triggering the angiogenic switch is associated with the malignant progression of benign tumors.<sup>58</sup> It had been observed in vivo that Huaier efficiently reduced the microvessel density in tumor.<sup>54,59,60</sup> In vitro experiment demonstrated that Huaier could cause cell skeleton rearrangement in human umbilical vein endothelial cells (HUVECs), resulting in the distortion of vasculature architecture.<sup>59</sup> The progression of angiogenesis can be regulated by numerous pro-angiogenic factors, including VEGF, TNF, matrix metalloproteinases (MMPs) and so on.<sup>58</sup> Among these factors, VEGF, which can be induced via hypoxia inducible factor (HIF), acts as the master regulator.<sup>61</sup> Several studies demonstrated that the expression of VEGF was significantly decreased after Huaier treatment.<sup>54,59,60,62</sup> Wang et al determined the level of HIF in Huaier-treated HUVECs and found that Huaier failed to suppress the level of HIF.<sup>59</sup> However, Li et al<sup>62</sup> and Zou et al<sup>60</sup> used the same detection method but found a totally different consequence in HCC cell SMMC-7721 that Huaier polysaccharide significantly inhibited the expression of HIF-1 $\alpha$ . The possible causes that resulted in this discrepancy may involve the difference in cell type, the purification of Huaier and the different expression level between total HIF-1 and HIF-1 $\alpha$ .

MMPs are also involved in the progression of tumor angiogenesis.<sup>63</sup> It was found that Huaier-treated HCC patients had decreased concentration of serum MMP2, suggesting that the anti-angiogenic effect of Huaier is mediated, at least in part, by inhibition of MMP2 production.<sup>60</sup>

In the progression of angiogenesis, compositions of TME also play vital parts. Huaier also exhibited a inhibitory function on the angiogenesis function of tumor-associated macrophages (TAM).<sup>64</sup> The mechanisms of this regulatory function of Huaier will be discussed in the following section of immunological regulation of Huaier.

### Suppression of cancer metastasis

Degradation of the extracellular matrix (ECM) is an essential step for tumor cells to enter the circulation and invade into the peripheral tissues. MMPs play a crucial role in the degradation and remodeling of ECM.<sup>65</sup> Therefore, MMPs serve as a “helper” to tumor metastasis. It was reported that

administration of Huaier decreased the number of tumor colonies metastasized to the lung.<sup>13,60</sup> Mechanism analyses showed the anti-metastasis effect of Huaier was performed in two ways. One is to directly react on tumor cells and the other is to affect TAM. Generally, Huaier decreased the expressions of MMP-2, MMP-9 and VEGF, helped to antagonize the degradation and remodeling of ECM.<sup>60,64,66</sup>

Another pivotal procedure related to cancer metastasis is the epithelial–mesenchymal transition (EMT). In maintaining the adherence between epithelial cells, E-cadherin plays the major effector and is considered a tumor-metastasis suppressor.<sup>67</sup> However, N-cadherin, AEG-1 and TCF8/ZEB1 are the mesenchymal markers that promote the EMT process. Several studies demonstrated that Huaier could increase the expression of E-cadherin but decrease the N-cadherin, AEG-1 and TCF8/ZEB1,<sup>6,16,60,68–70</sup> suggesting that the anti-metastasis effect of Huaier is mediated, at least in part, by suppression of EMT. Among the transcription factors involved in EMT, Huaier specifically down-regulated the expression of Twist1, while other factors remained unaffected.<sup>68</sup> This suggests that the reverse of EMT induced by Huaier had to be carried out in a Twist-dependent manner.

### Regulation of the immune function to support the anti-tumor effect

The TME consists of not only cancer cells but also immune cells, fibroblasts, blood vessels and other ECM.<sup>71</sup> However, these immune cells penetrating in TME somehow have impaired and compromised functions. Therefore, the major goal of immunotherapy is to alleviate the tumor-induced immunological suppression. Huaier extract (mainly polysaccharide) has also been identified as a valid immunopotentiator, hereby exerting indirect anti-tumor effects.

### Promotion on T cell proliferation and enhancement of secretion of immune-stimulating cytokines

After Huaier treatment, the number and functions of CD4<sup>+</sup> T cells and the immune-stimulating cytokines IL-2 and IFN- $\gamma$  in peripheral blood are enhanced. However, the immune-suppressive serum cytokine IL-10 is decreased.<sup>13</sup> In TME, CD4<sup>+</sup> T cells participate in the regulation by secreting different cytokines, whereas CD8<sup>+</sup> T cells exist as a direct “killer” to cancer cells. Both in vitro and in vivo experiments showed Huaier treatment resulted in a promotion on T and B cell proliferation.<sup>13,14</sup> Treatment of mice with Huaier induced an

increase in CD4<sup>+</sup> T cells in HCC microenvironment.<sup>13</sup> Because CD4<sup>+</sup> Th cells exist in various subtypes that may exhibit stimulatory or inhibitory effects on other immune cells and cancer cells within the TME,<sup>72</sup> further studies on how does Huaier affect CD4<sup>+</sup> T cells in TME need to be carried out.

## Induction of “M2” to “M1” polarization of macrophages and enhancement of their anti-tumor functions

Macrophage is one of the indispensable components of innate immunity, and they play an essential part in TME. Generally, macrophages have been classified into classically activated macrophages (M1) or alternatively activated macrophages (M2), depending on several cell surface markers and cytokines produced, such as CD16, CD32, TNF, IL-6, Arg1 and CD206.<sup>73</sup> M1 plays a vital role in promoting Th1 responses and anti-tumor functions. However, M2 exerts the anti-inflammatory and pro-tumor effects by expressing IL-10, Arg-1 and other immunosuppressive cytokines. M1/M2 polarization is mainly regulated by cytokines and TAM often exhibits a pro-tumor M2 phenotype.<sup>74</sup> Polarization from “M1” to “M2” can be inhibited and reversed both quantitatively and functionally by Huaier. Li et al found that Huaier suppressed the motility and M2 markers, including CD206, Mrc-2, Arg-1 and IL-10, of TAM.<sup>64</sup> Immunohistochemistry assay showed that the number of M2 in tumor was declined after Huaier treatment. However, the NO production and the M1 marker iNOS were increased.<sup>14,64</sup> Huaier treatment rescued the repression of phagocytosis of macrophages derived from TME.<sup>14,64</sup> Taken together, Huaier could regulate the polarization and functions of macrophages to antagonize tumor progression.

Besides the immune functions, macrophages also participate in tissue repair and angiogenesis. Huaier could exert an inhibitory effect on macrophage-induced formation of vascular structure through suppression of MMP2, MMP9 and VEGF expression.<sup>64</sup>

## Enhancement of NK cell activity

NK cells are important effector cells of the innate immune system and play a role in immunological surveillance. Low NK cell activity in peripheral blood is associated with the increased risk of cancer.<sup>75</sup> However, both the number and activity of NK cells were enhanced after Huaier treatment,<sup>13,39</sup> emphasizing again the immune-modulating effect of Huaier in cancer therapy.

## Application of Huaier in combined cancer therapy

### Huaier combined with surgical therapy Transcatheter arterial chemoembolization (TACE) combined with Huaier

TACE has become one of the most efficient treatments for unresectable HCC. Experiment on rabbit demonstrated that oral administration of Huaier after TACE treatment remarkably suppressed the proliferation and induced the apoptosis of HCC cells.<sup>54</sup> Clinical trials on 62 patients with primary HCC indicated that combined treatment improved clinical efficacy. The 12-month overall survival in experimental group (treated with TACE, lobaplatin and Huaier) and control group (received TACE plus lobaplatin chemotherapy) was 93.5% and 80.6%, respectively. Moreover, the tumor objective response rates were higher in experimental group than control group (72.4% vs 64.3%).<sup>15</sup>

### Liver transplantation combined with Huaier in anti-HCC therapy

A comparison between 53 HCC patients underwent liver transplantation with or without Huaier showed that Huaier treatment led to a higher predictive tumor-free survival rate ( $P=0.02$  when compared with the control group). The 10-month predictive tumor recurrence rate was 17.9% in Huaier group but 60% in control group ( $P<0.05$ ). Although the 30-month predictive tumor recurrence rate accelerated to 35.7% in Huaier group, it was still almost half of that in control group (64%,  $P<0.05$ ).<sup>11</sup>

### Huaier combined with chemotherapy Rapamycin combined with Huaier

Hu et al found that the aberrant activation of mTOR signaling is necessary for Huaier to exert its anti-proliferative effect of tumor cells.<sup>76</sup> Since deficiency in TSC1 or TSC2 caused activation of mTOR, TSC1- or TSC2-null mouse embryonic fibroblasts and ELT3 cells with TSC2 deficiency were used to determine the combined effects of Huaier (2, 4, 6, 8 mg/mL) and rapamycin (10 nM) on cell viability. They found that cell viability in the combined group was significantly lower than that in the rapamycin group ( $P<0.01$ ). Mechanism analyses demonstrated that protein level of p-S6 (a marker of mTOR activity) was increased after Huaier treatment.<sup>76</sup> These data suggest that Huaier could be used as a chemotherapy adjuvant to enhance the sensitivity of cancer cells with activated mTOR signaling pathway to rapamycin.



### Tamoxifen combined with Huaier

Tamoxifen is widely used in treating ER<sup>+</sup> breast cancer. The combination of Tamoxifen and Huaier significantly enhanced the autophagy, apoptosis, proliferation and metastasis of ER<sup>+</sup> breast cancer cells. In the 40-day experiment on mice, the tumor volume in combined treatment group was  $17.41 \pm 8.99 \text{ mm}^3$ , remarkably smaller than that in Huaier group ( $733.45 \pm 55.93 \text{ mm}^3$ ), tamoxifen group ( $503.844 \pm 61.12 \text{ mm}^3$ ) and control group ( $1127.40 \pm 100.07 \text{ mm}^3$ ), suggesting the addition of Huaier did improve the treatment efficacy of Tamoxifen.<sup>9</sup>

### Paclitaxel (PTX) combined with Huaier

Yang et al found Huaier and PTX combined treatments had a greater effect, in a dose-dependent manner, on the inhibition rate of breast cancer cell viability, compared to mono-therapy. In the xenograft model, the tumor volume was  $158.1 \pm 25.2 \text{ mm}^3$  in combined treatment group, while  $367.3 \pm 22.1 \text{ mm}^3$  in Huaier-treated group and  $223.9 \pm 28.3 \text{ mm}^3$  in PTX-treated group, respectively.<sup>23</sup>

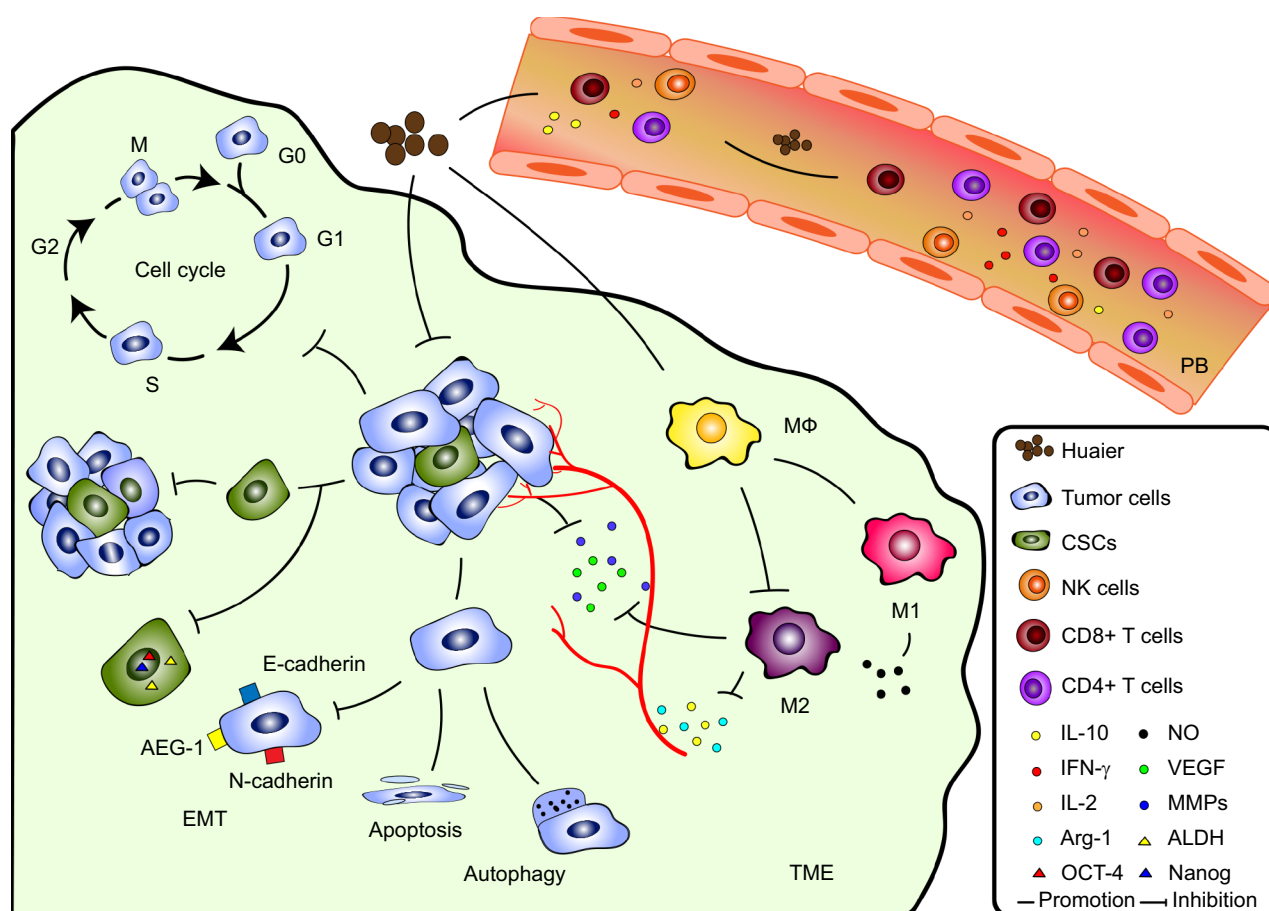
The enhanced effects of combined treatment of Huaier and PTX were also proved by Chen et al.<sup>77</sup>

### Huaier combined with radiotherapy

Huaier has also been found to alter the radiosensitization on breast cancer cells. The survival fraction at 2 Gy (SF2) was used to verify the regulatory effects of Huaier on the radiosensitivity on breast cancer. In MCF-7 cells, the SF2 was  $28.55\% \pm 0.03\%$  for cells pretreated Huaier (4 mg/mL), a significant reduction of  $\sim 47.65\%$  compared to control group ( $P < 0.01$ ). The underlying mechanisms were due to the alteration on cell cycle-regulating proteins and the progression on homologous recombination.<sup>24</sup>

### Huaier combined with dendritic cells and cytokine-induced killer cells (DC-CIK) immunotherapy

Compared with the mono-therapy, combined treatment of Huaier and DC-CIK exerted a restrained tumor growth effect



**Figure 1** The mechanisms of anti-cancer effects of Huaier.

**Notes:** In TME, Huaier can directly inhibit progression of tumor by cell cycle arrest in different phases, promotion of tumor cell death through apoptosis and autophagy, inhibition of EMT and CSCs stemness as well as tumor-induced angiogenesis. Huaier can also exert indirect anti-cancer effects by immune regulation. Instead of the superior amount of M2 in TME, Huaier helps Mφ to polarize towards M1 and inhibits the expression of M2-derived Arg-1, IL-10, MMPs and VEGF that promote angiogenesis and progression of cancers. On the other hand, Huaier can exert systemic immunomodulatory effects by enhancement of the number and function of CD4<sup>+</sup> T cells as well as NK cells, and by promoting production of immunopotentiating cytokines, including IFN-γ and IL-2, and inhibiting secretion of immunosuppressive cytokine IL-10 in peripheral blood. **Abbreviations:** ALDH, aldehyde dehydrogenase; CSC, cancer stem cell; EMT, epithelial-mesenchymal transition; MMP, matrix metalloproteinase; TME, tumor microenvironment.

in nude mice transplanted with HT-29 colon carcinoma cells.<sup>78</sup> After 3-week treatment, tumor weight and size were significantly suppressed in combined treatment group when compared to two mono-therapy-treated groups and control group.

Taken together, Huaier can be used as an adjuvant to improve the therapeutic efficacy of surgical therapy, chemotherapy, radiotherapy and immunotherapy.

## Conclusion

TCM has been widely used in clinical practice due to its cure without significant side effects. Huaier is one of the TCM and has been shown an effective adjuvant of cancer therapy. The anti-cancer effects of Huaier can be briefly divided into two aspects: the direct effects and the indirect effects (Figure 1). Huaier directly regulates the cell cycle, apoptosis of tumor cells, resulting in the repressed proliferation. Besides, it also directly inhibits tumor cell motility and secretion, suppresses tumor metastasis and angiogenesis. The indirect effect refers mainly to the immunomodulatory effect. As have discussed

above, Huaier can enhance the number and function of CD4<sup>+</sup> T cells, as well as NK cells, regulate the polarization and function of macrophages, increase secretion of immune stimulatory cytokines to antagonize the tumor progression (Table 1).

However, despite the aforementioned promising effects, there are also limitations. Firstly, although there are two forms of the drug (aqueous extract and granule) used in clinic, and it is generally accepted that the bioactive substance is proteoglycan, the detailed effective components of Huaier remain elusive. Secondly, although there is increasing studies focused on the direct effects on cancer cells, only a few studies explored the immunomodulatory effects of Huaier. It has been extensively demonstrated that immunosuppression exists in almost all TME. Detailed examination of the *in vivo* modulatory effects of Huaier on immune cells in the TME becomes urgently needed. Thirdly, although there are studies on the combined therapy of Huaier with surgical therapy, chemotherapy, radiotherapy and immunotherapy, most of them were carried out in animal cancer models. Only

**Table 1** Mechanisms of Huaier to exert anti-tumor effects

Tumor origin	Related mechanisms	References
Breast cancer	Induction of G0/G1 phase cell cycle arrest	9, 23–25
	Inhibition of NF- $\kappa$ B	23, 36
	Suppression of CSCs	43
	Stimulation of cancer cell autophagy	9, 48
	Induction of cancer cell apoptosis	8, 25, 52, 56
	Inhibition of cancer-induced angiogenesis	64
	Regulation of tumor-associated macrophage polarization	64
	Inhibition of metastasis by suppression of ECM degradation	64, 66
Lung cancer	Induction of S phase cell cycle arrest	27
	Induction of cancer cell apoptosis	27
Hepatocellular carcinoma	Induction of S phase cell cycle arrest	26
	Inhibition of Yes-associated protein 1 expression	11
	Stimulation of cancer cell autophagy	49
	Induction of cancer cell apoptosis	6, 7, 11, 54
	Inhibition of cancer-induced angiogenesis	54, 60, 62
	Inhibition of metastasis by suppression of ECM degradation	12, 60
	Suppression of metastasis and EMT	6, 11, 69, 70
Cervical cancer	Promotion of T cell and NK cell proliferation, and enhancement of immune-stimulating cytokines secretion	12
	Induction of G2/M phase cell cycle arrest	28
Gastric cancer	Induction of G2/M phase cell cycle arrest	29
	Modulation of PI3K/AKT signaling pathway	29
	Suppression of metastasis and EMT	68
Brosarcoma	Induction of G2/M phase cell cycle arrest	30
Melanoma	Induction of G2/M phase cell cycle arrest	31
	Induction of cancer cell apoptosis	31
Colorectal cancer	Suppression of CSCs	42
Ovarian cancer	Suppress CSCs	10

**Abbreviations:** CSC, cancer stem cell; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition.

a very small numbers of patients were enrolled in clinical studies. Therefore, large-scale clinical studies are needed in future to verify the mechanisms and efficacy of Huaier in the combined therapy. With solution of these limitations, Huaier will be used in clinic more efficiently and safely.

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## Disclosure

The authors report no conflicts of interest in this work.

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