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REVIEW

Research Progress on the Anti-Cancer Molecular Mechanisms of Huaier

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Abstract: Huaier (*Trametes robiniophila Murr*), a Chinese traditional herb of medicine, has demonstrated promising curative effects in clinical treatment for various tumors. There are documented experiments showing the biological functions of Huaier with its antineoplastic molecular mechanisms: restraining proliferation and metastasis, arresting cell cycle, inducing apoptosis, pyrosis, and autophagy, anti-intratumoral angiogenesis, attenuating characteristics of tumor stem-like cells, interfering with the function of the tumor-related immune system, reversing drug resistance, and enhancing the sensitivity to chemotherapeutic drugs, etc. In addition, studies suggest that non-coding RNA (ncRNA) acts a pivotal part in cancer occurrence and development, and demonstrates that Huaier adjusts the performance of certain lncRNA (long non-coding RNA) and proceeds to affect the microRNA and its target genes, rendering an anti-tumor effect. Huaier also modulates the expression of lncRNA to attenuate the activity of ncRNA-sponged microRNA and then inhibits the expression of downstream target genes. We summarize and illustrate the experimentally confirmed anti-cancer molecular mechanisms of Huaier, to inspire new ideas for researchers in relevant fields.

Keywords: Huaier, antineoplastic mechanisms, breast cancer, gastric cancer, liver cancer, pulmonary cancer

Introduction

Cancer is a global medical issue threatening people's health. Lung cancer, liver cancer, and pancreatic cancer are lethal malignant tumors in the world. Although the five-year survival rate of most cancers has been increasing in mostly the developed and some developing countries, that of certain cancers such as pancreatic cancer experienced a decline in some areas.[1] The most important cancers causing disability-adjusted life year (DALY) are trachea, bronchus, and lung cancer (TBL cancer) in male, and breast cancer, TBL cancer, colorectal cancer in female.[2] There is no doubt that cancer remains a serious global problem aggravating the burden to the worldwide economy. Clinically, chemotherapy, radiotherapy, and target therapy reduce the tumor recurrence and metastasis rate, but the side effects including drug cytotoxicity and drug resistance all call for new drugs with low toxicity, fewer side effects, and reversible drug resistance.

Huaier has been used in Chinese traditional medicine for thousands of years and its potentials in clinical adjuvant treatment for various diseases are increasingly addressed in recent studies, with reported experimental effects on psoriasis, inflammation, tuberous sclerosis, echinococcosis and cancers.[3–7] Moreover, it shows low toxic side effects and reduces drug resistance in tumor cells.[8,9] In examining the

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anti-tumor effectivity of Huaier, the effective components were extracted using aqueous or alcohol for various in vitro experiments. It is found that Huaier can restrain proliferation and metastasis, arrest cell cycle, induce cell apoptosis, pyrosis, autophagy, anti-intratumoral angiogenesis, attenuate characteristics of tumor stem-like cells, modulate tumor-related immune system function, reverse the drug resistance and enhance the sensitivity to chemotherapeutic drugs.[8–24] The mechanisms of such biological vary depending on types of tumors, thus attracting many scholars to explore the anti-cancer molecular basis of Huaier.

This review is to summarize the anti-tumor molecular mechanisms of Huaier in a variety of tumors, covering the antineoplastic mechanisms of the classical signal pathway mediated by Huaier, as well as the pathways of ncRNAs regulated by Huaier in inhibiting tumors. An increasing number of studies have confirmed that ncRNAs including lncRNA, microRNA, and circRNA play significant roles in tumor progression. For example, lncRNA regulates gene expression within and outside the cell nucleus through chromatin modification, transcriptional regulation, and post-transcriptional regulation among others. This paper is expected to further understand the anti-tumor mechanisms of Huaier with implications for innovative future research.

Breast Cancer

Breast cancer is one of the most common female malignant tumors, simultaneously it is of the most lethal.[2] There was a study estimated that the new 276,480 female breast cancer cases would have a mortality rate of approximately 15% in 2020.[25] In recent years, scientists have invested effort in researching the Huaier mediated mechanisms of inhibition of breast cancer. It is worth noting that researchers have made a breakthrough in terms of ncRNAs regulated by Huaier.

Multi-Target Anti-Tumor Effects

At present, there are many in vitro experimental studies on Huaier's inhibiting breast cancer cells, suggesting multiple targets in breast cancer cells. Kong et al (2015) analyzed by microarray the differential gene expressions in MDA-MB-231 cells. The treatment group was incubated with 8 mg/mL Huaier aqueous extract for 72h followed by enrichment analysis based on GO, KEGG, Biocarta and Realtime databases. The results indicate that the differentially expressed genes were mainly enhanced in DNA transcription, apoptosis, cell cycle arrest, DNA replication, cell proliferation, MAPK signaling pathway, NF-κB signaling pathway, and metabolic pathway, concluding that Huaier leads to multi-target inhibition in breast cancer cells.[26]

P53 Gene

Huaier inhibits the proliferation of breast cancer cells by inducing apoptosis. Huaier induces apoptosis in both estrogen receptor-positive (ER+) breast cancer cell line (such as MCF-7) and estrogen receptor-negative (ER−) cell line (such as MDA-MB-231).[15] The *p53*, an important tumor suppressor gene, encodes a protein that not only interferes with cell cycle arrest but also induces cell apoptosis.[27] Huaier upregulates the *p53* expression in MCF-7 cells, but not in MDA-MB-231 cells, suggesting that Huaier may induce the apoptosis of estrogen receptor-positive breast cancer cells by affecting the *p53* expression gene (Figure 1).[15]

Bcl-2 Family and Caspase Family

Bcl-2 family has great effects on inducing cell apoptosis. This family can be divided into two categories, the one is pro-apoptosis factors, including Bax, Bak, Bad, and the other is anti-apoptosis factors including Bcl-2, Mcl-1, Ced-9. Bcl-2 may prevent the release of mitochondrial cytochrome c, which is a key step in the overall process of apoptosis.[28] Moreover, Huaier could increase the ratio of Bax to Bcl-2 (Bax/Bcl-2) in breast cancer cells, mostly found in MCF-7 cells. It was suggested that Huaier can induce apoptosis through the mitochondrial membrane pathway (Figure 1).[15] In addition, as an executor of the apoptosis pathway, caspase-3 is crucial for chromatin concentration and DNA fragmentation in the process of apoptosis for its catalysis for the specific cleavage of certain cellular proteins.[29] For instance, caspase-3 cleaves the PARP and makes it inactivated toward apoptosis. Huaier can upregulate caspase-3 in both MCF-7 and MDA-MB-231 cells, indicating that Huaier can induce apoptosis of ER+ MCF-7 and ER− MDA-MB-231 through this pathway (Figure 1).

Restraining the Expression of MTDH

Metadherin (MTDH) has a high expression level in many tumors.[15,30,31] The MTDH expression was found to decrease in MCF-7 cells incubated with polysaccharide extracted from Huaier. The results also show the increase in Bax/Bcl-2 and apoptosis percentage with depleting MTDH. In general, those studies demonstrate that Huaier

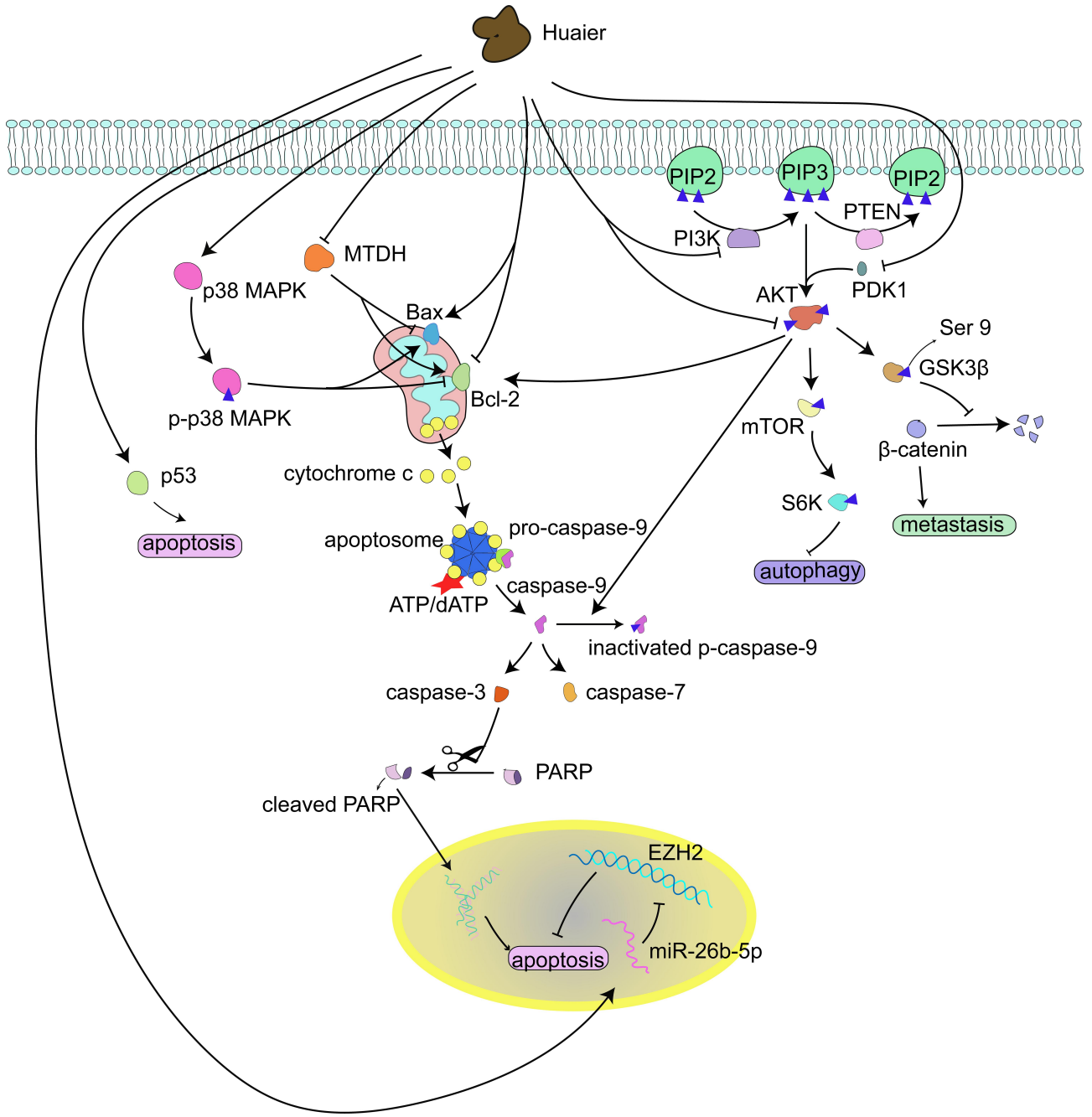

Figure 1 Huaier induces apoptosis by upregulating *p53* expression. Huaier shows direct or indirect effects on increasing the ratio of Bax to Bcl-2 by activating p38 MAPK signaling pathway, decreasing the expression of MTDH, inhibiting PI3K/AKT signaling pathway to promote the release of cytochrome c. It is found to activate caspase cascade reaction, cleave downstream target PARP to induce apoptosis. Huaier may reduce the inactivation of caspase-9 by inhibiting the phosphorylation of AKT to strengthen apoptosis. Huaier can regulate miR-26b-5p/*EZH2* signaling pathway to induce apoptosis, and regulate AKT/mTOR pathway and AKT/GSK3β pathway to induce autophagy and inhibit tumor migration.

could induce apoptosis of breast cancer cells by negatively regulating the expression of MTDH (Figure 1).[13]

Affecting the Expression of DARC and Its Ligands

The seven-transmembrane Duffy antigen receptor for chemokines (DARC) terminates signal transmission by binding chemokines.[32] There is evidence that the expression of DARC ligands is upregulated in breast cancer.[33] The DARC overexpression is accompanied by a decrease in CCL-2 which is one of the chemokines, as well as the inhibition of proliferation and metastasis among breast cancer cells.[34] Chen et al (2018) found significantly higher DARC expressed in primary breast tumors than in metastatic tumors. Because the

expression of DARC is upregulated but that of its ligand is down-regulated among breast cancer cells in Huaier incubation, Huaier may negatively regulate breast cancer by upregulating DARC (Figure 2).[35]

The mTOR/S6K Signaling Pathway

Mammalian target of rapamycin (mTOR) is a group of serine/threonine protein kinases. The environment alteration stimulates the mTOR expression to activate S6K. Inhibiting the mTOR/S6K signaling pathway is related to the initiation of autophagy in tumor cells.[36,37] Autophagy is a metabolic process in which a cell supplies nutrients and energy by degrading its own non-essential components in case of nutrient deficiency, and it is also a mechanism causing tumor cell death.[38,39] Huaier extract is found inducing the autophagy of breast cancer cells and

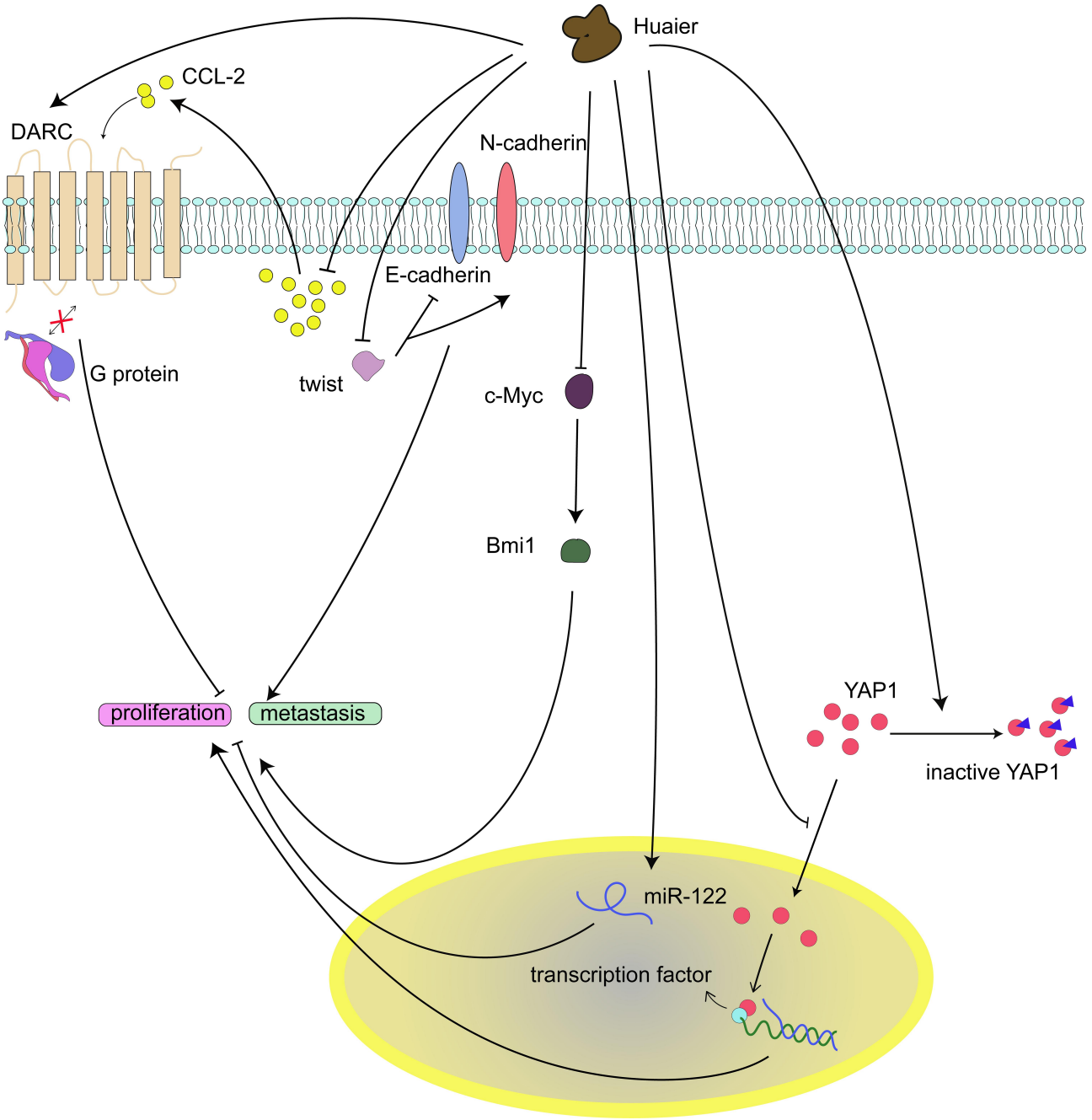

Figure 2 Huaier inhibits cell proliferation and metastasis by upregulating DARC and downregulating its ligands. Huaier suppresses EMT by downregulating twist to inhibit tumor metastasis while the medicine inhibits metastasis by inhibiting c-Myc-Bmi1 signaling pathway. Huaier also upregulates miR-122 to inhibit adhesion and metastasis, and inhibits the proliferation and metastasis by promoting the phosphorylation of Yap1 as well as promoting the transfer of Yap1 to the cytoplasm.

negatively regulating the expression of proteins associated with the mTOR/S6K pathway. It activates the AMP-activated protein kinase (AMPK) pathway, which is antagonistic to the mTOR pathway. The results signify that Huaier may induce autophagy through the mTOR/S6K signaling pathway (Figure 1).[18]

Pathways Regulating Stem Cells

Cancer stem cells may lead, at partly, to cancer progression, recurrence, and metastasis.[40] Studies suggest that Hedgehog (Hh), Notch, Wnt/β-Catenin, and other pathways were involved in regulating breast cancer stem cells.[41,42] Wang et al (2014) reported that Huaier extract inhibited the level of stem cell markers NESTIN, OCT-4 and NANOG, and reduced the proportion of CD44+/CD24 − cells with tumor stem cell-like characteristics.[43] The result is attributed to the inhibition of Gli1, an important transcription factor in the Hh pathway.[20] It is suggested that Huaier may affect breast cancer stem cells (BCSCs) by regulating the Hh pathway (Figure 3). The AKT/GSK3β/β-Catenin pathway is involved in ERα-36-mediated (estrogen receptor α36) estrogen signal transduction in BCSCs/progenitor cells.[44,45] ERα-36 is a subtype of estrogen receptor α and is specifically related to triple-

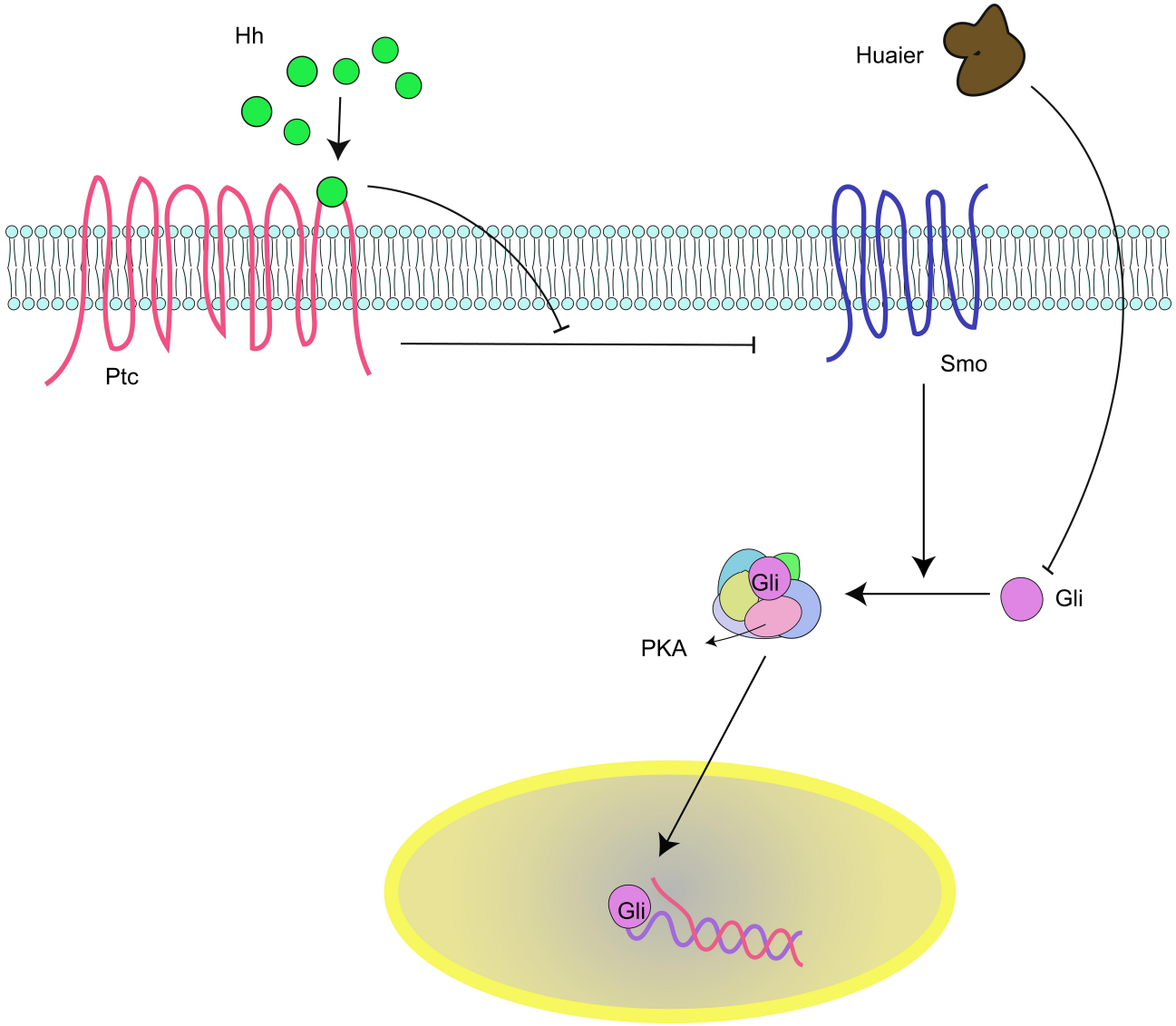

Figure 3 Huaier affects downstream target gene transcription by inhibiting the expression of Gli1 protein.

negative breast cancer stem cells.[44–47] A study of triple-negative breast cancer (TNBC) cells reported that the stem-like characteristics of TNBC could be inhibited by Huaier polysaccharide. The expression of ERα-36 was significantly inhibited in Mb436 cells characterized by highly expressed ERα-36 incubated with Huaier polysaccharide, and its stem-like characteristics were also inhibited. However, no such effect was found in Hs578T cells marked by lower ERα-36 expression. As a contrast, scientists established the Mb436 cell line with low ERα-36 expression and Hs578T with high ERα-36 expression by transfection and gene knockout, respectively, which showed the results are contrary to previous is within expectations. This experiment also confirms that Huaier polysaccharide inhibits breast cancer stem cells by inhibiting AKT/GSK3β/β-Catenin mediated by ERα-36.[19] These research results are very important because around 75% of breast cancer patients are estrogen receptor-positive. Most patients can be alleviated after endocrine therapy.[48] It is generally conclusive that Huaier shows in vitro inhibitive effects on the proliferation of ER+ breast cancer cells.[49] The inhibitory effect of Huaier on TNBC is of potential clinical significance for TNBC patients for the lack of targeted therapy so far. A considerable amount of experimental work is needed.

The miR-203/ATM Signaling Pathway

Recent studies confirm that Huaier regulates the level of ncRNA to inhibit breast cancer cells. Ataxia-telangiectasia mutation (ATM) is a key factor in the DNA damage response pathway.[50] In a study of endocrine-resistant breast cancer cells, it was found that Huaier increased the expression of ATM in tamoxifen-resistant cells (M7-TR) and fluvastatin-resistant cells (M7-FR) by down-regulating the level of expression of miR-203, to achieve the anti-tumor effect.[51]

The lncRNA-H19/miR-675-5p/CBL Signaling Pathway

Differential expression of ncRNA incubated with and without Huaier in MDA-MB-231 and MCF7 cells were analyzed using microarray. The results show that Huaier downregulated the expression of lncRNA-H19 and miR-675-5p and increased the level of CBL in breast cancer cells. However, overexpression of H19 or miR-675-5p in breast cancer cells can enhance cell viability and inhibit cell apoptosis, while low expression of H19 or miR-675-5p had the opposite effect. CBL is a direct negative regulatory downstream target of miR-675-5p, and its expression grows with increasing Huaier concentration, showing that the proliferation of breast cancer cells can be inhibited by Huaier through regulating lncRNA-h19/miR-675-5p/CBL pathway.[52,53]

The Linc00339/miR-4656/CSNK2B Signaling Pathway

A weighted gene co-expression network analysis of differentially expressed RNAs in breast cancer cells incubated with Huaier and the control group was made using bioinformatics technology, and linc00339 was screened out as the core gene. The positive correlation mRNAs were then selected from the mRNAs co-expressed with linc00339. Then, scientists analyzed the overall survival (OS) and recurrence-free survival (RFS) and compared the upregulated mRNA in breast cancer tissues against adjacent normal tissues. Finally, through over-expression and under-expression linc00339 in tumor cells, the study identified *CSNK2B* as the most likely downstream target of linc00339. Meanwhile, overexpression of *CSNK2B* could reverse the anti-tumor effect of Huaier. Thus, it is explicit that *CSNK2B* serves as a downstream target of linc00339. Furthermore, in bioinformatics analysis and luciferase experiment, miR-4656 is confirmed as downstream target of linc00339.[54] It is suggested that Huaier can regulate the progress of breast cancer cells through the linc00339/miR-4656/*CSNK2B* signaling pathway.

Regulating the Immune System

Huaier can inhibit the polarization of M2 type macrophages, which promotes angiogenesis and tumor formation and enhances the phagocytic function to interfere with angiogenesis, demonstrating that Huaier extract can inhibit the formation of vascular structure induced by macrophages.[21,36]

Gastric Cancer

The incidence rate of gastric cancer has been decreasing year by year, and gastric cancer ranks as the fifth reason for incidence and the third cause of mortality all over the world.[55] At present, for the patients who suffer side effects after treating with chemotherapy in clinical practice, physicians will consider applying Huaier as adjuvant treatment medicine.

Induction of Cell Cycle Arrest

Some chemotherapeutic drugs inhibit cell proliferation by inducing cell cycle arrest, which is one of the anti-tumor mechanisms. An experimental study reports that the proliferation of gastric cancer cell lines (MGC803 and HGC27) was inhibited with Huaier n-butanol extract by way of arresting the cell cycle at S and G2/M phase.[56] Another study shows that Huaier also induces the arrest of MKN45 and SGC7901 cells at the G2/M phase.[10] In short, Huaier may inhibit the proliferation of various cell line types of gastric cancer cell lines by inducing cell cycle arrest.

Inhibition of EMT

Huaier was found inhibitive to the invasion and migration of gastric cancer cells.[57] Epithelial-Mesenchymal Transition (EMT) refers to the loss of epithelial and acquired mesenchymal properties. EMT is related to the invasion and metastasis of tumor cells. The decrease of E-cadherin expressed in normal epithelial cells corresponds to its weakening adhesion ability, making the cells more vulnerable to invasion. Huaier upregulates the expression of E-cadherin in gastric cancer cells, in contrast to the down-regulation of N-cadherin and vimentin of mesenchymal cell markers.[57] It is suggested that Huaier inhibits the invasive ability of tumor cells by inhibiting the EMT pathway. The twist is a transcription factor that promotes EMT, its expression is inhibited with Huaier.[58] It is observed that the overexpression of twist inhibits the anti-metastasis effect of Huaier but boosts the EMT process (Figure 2).[57]

The c-Myc-Bmi1 Signaling Pathway

The Huaier n-butanol extract is inhibitive to the expression of c-Myc in the c-Myc-Bmi1 signaling pathway, and its downstream target Bmi1. When Bmi1 is overexpressed, the inhibitory effects of Huaier n-butanol extract on proliferation and metastasis of gastric cancer cells are reversed, suggesting that the proliferation and metastasis of gastric cancer cells may be inhibited by Huaier through the c-Myc-Bmi1 signaling pathway (Figure 2).[56]

The PI3K/AKT Signaling Pathway

This pathway is related to multiple biological processes like proliferation, metastasis, apoptosis, and angiogenesis of tumor cells.[59,60] Xie et al (2015) reported that the expression of PI3K and AKT in gastric cancer cells was down-regulated after treatment with Huaier. Meanwhile, PIP3 activated by PI3K and PDK1 are down-regulated, which lowers phosphorylated AKT level, and the activity of AKT is weakened. Also, Huaier reduces the expression of Bcl-2, a substrate of AKT. It is known that Bcl-2 inhibits the apoptosis of gastric cancer cells, decreased Bcl-2 expression thus enhances the apoptosis of gastric cancer cells. And the results also showed that the expression of caspase-9 increased and pro-caspase-9 decreased, besides, the level of p-PTEN was decreased in gastric cancer cells by Huaier.[10] Caspase-9 is a substrate of AKT, and it can be phosphorylated by activated AKT then be inactivated, thus apoptosis is inhibited.[37] In addition, PTEN can dephosphorylate PIP3; thus, the phosphorylation and activation of AKT can be inhibited, and the phosphorylation of PTEN can make itself lose the anti-tumor activity.[61] It can be seen that the PI3K/AKT signaling pathway affects tumor in multiple ways. This experiment confirmed that apoptosis of tumor cells could be induced by Huaier through regulating the PI3K/AKT pathway (Figure 1).

Liver Cancer

Primary liver cancer (PLC) is the fourth reason for lethal malignant tumors worldwide.[62] And the 5-year survival rate of PLC is extremely low.[63] Hepatocellular carcinoma accounted for 70% - 85% of PLC; therefore, we will focus on the inhibitory effect of hepatocellular carcinoma treated with Huaier in this section.[64]

The YAP1 Signaling Pathway

YAP1 protein promotes tumor progression by binding specific transcription factors in the nucleus. The expression of YAP1 in Hepatoma cells is higher than in normal hepatocytes, and Huaier significantly inhibits the expression of YAP1 and increases p-YAP1 in hepatoma cells. As YAP1 protein overexpresses the proliferation and metastasis of hepatoma cells are enhanced. Moreover, Huaier transfers Yap1 from the nucleus to the cytoplasm.[65] YAP1 in the cytoplasm is phosphorylated and degraded by the ubiquitination pathway. Huaier plays a significant role through the YAP1 signaling pathway (Figure 2).[66]

The P38 MAPK Signaling Pathway

It has been confirmed that Huaier polysaccharide induces hepatoma cell apoptosis through intrinsic and extrinsic mechanisms. After being treated with 100 μg/mL Huaier polysaccharide for 4h, the levels of phosphorylated ERK1/2, JNK, and p38 MAPK significantly increased in cells, especially the active p38 MAPK. However, after the cells

were treated with p38 MAPK inhibitor, the apoptosis induced by Huaier polysaccharide was weakened. Huaier polysaccharide promotes pro-apoptotic proteins such as Bim, Bax and p53 in hepatoma cell and down-regulates the level of Bcl-2, Bcl-xl, Mcl-1 and survivin. However, only if the cells incubated with the specific p38 MAPK inhibitor can increase Bcl-2 and survivin and decreased Bax, as a result, the pro-apoptosis effect of Huaier polysaccharide is inhibited. These observations further confirm that Huaier may induce apoptosis of hepatoma cells through the p38 MAPK pathway.[14] Nevertheless, in another experiment, the phosphorylation levels of ERK1/2, p38 and JNK1/2 in hepatoma cells treated with Huaier aqueous extract were found decreasing. It also shows both Huaier aqueous extract and specific JNK pathway inhibitors may reduce the expression of β-Catenin and cyclin D1 in tumor cells. When they are combined to treat hepatoma cells, the decrease is more obvious than a single drug.[12] MAPK pathway is associated with inducing β-Catenin and cyclin D1 related to the cell cycle.[67] It is suggested that Huaier inhibits the expression of β-Catenin and cyclin D1 through the JNK pathway, inducing cell cycle arrest at S phase (Figure 1).[12]

Regulating the Expression of Lamin B1 and NOV

Besides inhibiting the metastasis of hepatoma cells by attenuating EMT, Huaier can upregulate Lamin B1 protein encoded by *LMNB1* (tumor-promoting gene) and down-regulates the NOV protein encoded by the *Nephroblastoma Overexpressed* (NOV) gene (tumor suppressor gene) to inhibit tumor proliferation to reduce the ability of tumor cell metastasis.[8,11,65,68] In another study, Huaier polysaccharide downregulates mRNA and protein of *AUF-1* and *AEG-1* (both are oncogenes) and upregulates the expression of miR-122 to inhibit the adhesion and metastasis of SMMC-7721 cells (Figure 2).[8] In addition, it is confirmed in vivo that Huaier polysaccharide inhibits hepatoma cell transfer to lung with low toxicity at the concentration of 400 and 800μg/kg.[8,9]

Regulating Caspase Family

Regulating cysteine aspartate specific protein caspase (caspase) cleaves PARP (a DNA repair enzyme) into fragments binding irreversibly with DNA to inhibit the DNA repair function of PARP enzyme, which is also a mechanism of drug-induced cell apoptosis. Huaier polysaccharide treats HepG2 and Huh7 and then upregulates the expression of cleaved-caspase-8 and cleaved-caspase-9, so as the expression of caspase-3. Caspase-3 is the main hydrolase for cleaving PARP, and Huaier polysaccharide can also upregulate the expression of cleaved PARP.[14] It is suggested that Huaier polysaccharide can induce apoptosis through both intrinsic and extrinsic pathways (Figure 1).[12,14]

Interference with Tumor Angiogenesis and Immune System Function

In an experiment in vitro, a nude mouse model was first established with transplanted SMMC-7721 cells. In comparison to the control group, the nude mice treated with Huaier polysaccharide expressed an increasing number of PCNA (cell proliferation marker)[69] positive cells and TUNEL positive cells but decreasing microvessel density (MVD) in tumor tissue and HIF-1α, VEGF, AUF-1 and AEG-1 genes. The results show that Huaier polysaccharide inhibits the proliferation, metastasis and intratumoral angiogenesis of liver cancer in vivo.[9] In another experiment in vitro, Huaier polysaccharide stimulates the secretion of IL-2, IFN-γ and other immune-stimulating cytokines in mouse serum and inhibits IL-10 secretion. It also increases the percentage of CD4+T cells and NK cells, with a decreasing number of CD8+T cells. It is suggested that TP-1 of Huaier shows anti-tumor effects by promoting the function of the immune system.[22]

The AKT/mTOR Signaling Pathway

It is found that Huaier significantly reduces the levels of phosphorylated AKT and mTOR in hepatoma cells.[14] Further confirmation is needed to determine whether the specific AKT/mTOR signaling pathway mediates the inhibitory effect of Huaier on hepatoma cells.

Induction of Cell Cycle Arrest

Zhang et al (2015) reported that Huaier aqueous extract inhibits the level of β-Catenin and cyclin D1 associated with the cell cycle in hepatoma cells, and induces cell arrest at the S phase.[12] Bao et al (2016) reported that Huaier arrested the cell cycle of HepG2 and Huh7 at the G0/G1 phase.[14] In addition, Huaier was found inducing arrest at the G0/G1 phase of SKHEP-1 cells by regulating the p18 pathway.[68]

Lung Cancer

Lung cancer possesses the highest incidence rate and mortality rate in China, and the trend of incidence and mortality is still rising.[70,71] Non-small cell lung cancer (NSCLC) is the main type of lung cancer, and its survival rate is very low. However, the current researches founded that Huaier seems to have certain inhibitory effects on NSCLC.

Induction of Pyroptosis

Pyroptosis is known as programmed cell death. By comparing the expression of mRNA and protein levels of pyroptosis-related genes (NLRP3, caspase-1, IL-1 β, IL-18) in non-small cell carcinoma tissue with adjacent normal tissue and detecting the level of them in NSCLC cell lines (H520 and H358) and human lung epithelial cell line (BEAS-2B), the results show that expression of mRNA and protein levels of pyroptosis-related genes in cancer tissue was lower than those in paracancerous tissue, and those in cancer cell line was lower than those in lung epithelial cell line. The expression of NLRP3 inflammasome-mediated inflammatory cytokines (NLRP3, caspase-1, IL-1 β, IL-18) and LDH release significantly increased in H520 and H358 cells incubated with Huaier extract. After knockout NLRP3 gene or using NLRP3 inhibitor-treated cells, the pyroptosis induced by Huaier extract is reversed, so is the inhibitory effect of Huaier extract on cell viability.[16] This result indicates that Huaier may inhibit cell viability by inducing cell pyroptosis.

The miR-26b-5p/EZH2 Signaling Pathway

The miR-26b-5p is down-regulated in many tumors. However, the microarray and RT-PCR analysis on pulmonary carcinoma cell line A549 after incubated in Huaier shows that Huaier upregulated the expression of miR-26b-5p. When miR-26b-5p was overexpressed in the pulmonary carcinoma cell line, the proportion of cellular apoptosis increased, and the proliferation was significantly inhibited, reversing the inhibitory effect of Huaier on cell proliferation. The analysis of the data of the miRBase database identifies that *EZH2* is a direct target gene of miR-26b-5p. *EZH2* is relevant to the proliferation and apoptosis of pulmonary cancer and negatively correlates with miR-26b-5p. The knockout *EZH2* gene decreases the proliferation rate and enhances apoptosis induction. Either cells treated with Huaier or overexpressed the miR-26b-5p in cells can decrease the protein levels of *EZH2*, b-catenin and Bcl-2.[72] It is suggested that Huaier may inhibit the proliferation of tumor cells and induce apoptosis by regulating the miR-26b-5p/EZH2 signaling pathway (Figure 1).

The JAK2/STAT3 and MAPK Signaling Pathways

JAK2/STAT3 and MAPK signaling pathways are important in regulating cell proliferation and metastasis in tumor cells. Chen et al (2018) reported that Huaier inhibited the migration and invasion of pulmonary carcinoma cells and down-regulated the expression of JAK2/STAT3 and the proteins related to the MAPK signaling pathway.[73] It is suggested that the JAK2/STAT3 and MAPK signaling pathway may mediate the Huaier's inhibitive effects on tumor cell metastasis and invasion. Further studies are needed.

Induction of Cell Cycle Arrest and Apoptosis

Huaier was found inhibiting the proliferation of pulmonary carcinoma cells by inducing cell arrest at the S phase of lung cancer cell lines A549 and NCI-H1650, and inducing apoptosis through inhibiting the expression of MTDH and Bcl-2, thus promoting the level of cleaved-caspase-3 and enhancing the activity of caspase-3 (Figure 1).[73]

Kidney Cancer

Renal cell carcinoma is the major tumor type of kidney cancer. Within the range of urological neoplasms, renal cell carcinoma ranks as the third leading cause of cancer-related incidence, but its malignancy is the highest.[74,75] At present, the inhibitory mechanisms of Huaier on renal cell carcinoma are still superficial and needs further study.

PI3K/AKT/mTOR Signaling Pathway

Huaier is reported to inhibit the proliferation, migration, invasion and induce cell apoptosis of renal cancer cell line 786-O. The PI3K/AKT/mTOR signaling pathway is related to tumor growth and proliferation. Furthermore, Huaier reduces the phosphorylation level of PI3K, AKT and mTOR without affecting their total amount and reduces the phosphorylation levels of p70S6K and 4E-BP1 that are the downstream targets of mTOR, and regulates the PI3K/AKT/mTOR/p70S6K/4E-BP1 signaling pathway. However, whether Huaier mediates the inhibition of tumor cells through the PI3K/AKT/mTOR pathway is

unclear yet. There are studies demonstrating that Huaier reversed the EMT process of tumor cells and inhibiting the expression of twist, a stimulator of EMT, to further inhibit the process of EMT, suggesting that Huaier is inhibitive to the metastasis of tumor cells.[76]

Drug Combination Therapy

Huaier polysaccharide combined with sunitinib is reported to significantly enhance the proliferation inhibition of 786-O and A498 cells and to enhance the ability against tumor invasion and migration. Such a combination arrests the cell cycle at the G1 phase. Moreover, it increases the expression of pro-apoptotic factors of Bax and cleaved-caspase-3 and reduces the expression of anti-apoptotic factor Bcl-2 to promote apoptosis. Researchers found that Huaier combined with sunitinib inhibited the proliferation of tumor cells by downregulating the expression of CIP2A. The combination of Huaier and sunitinib also inhibits the metastasis and invasion of tumor cells in vitro by inhibiting EMT and PI3K/AKT/VEGFR signaling pathways.[77]

Other Types of Cancers

Cholangiocarcinoma Cells

Huaier combined with 5-FU shows a synergistic anti-tumor effect. The combination of the two drugs is found inhibiting the expression of Mcl-1 and Bcl-2 (anti-apoptotic proteins) and Cyclin A2 and CDK2, for which the S phase is arrested more tightly. Compared with a single drug, the combination of the two drugs strengthens the inhibitory effect on tumor migration and invasion because it inhibits the expression of N-cadherin, vimentin, MP-2 and MMP-9, all relevant to metastasis of tumor cells. STAT3 signaling pathway is closely associated with the occurrence and development of tumors.[78] It is found that the combination of the two drugs has a significant inhibitory effect on the activated form of STAT3, p-STAT3 while the total STAT3 was not damaged.[79]

Prostatic Cancer

The proliferation and metastasis of prostate cancer cell line PC3 are found to be inhibited by Huaier aqueous extract. The protein coded by *Lamin B1* (pro-apoptotic gene) is abnormally expressed in many kinds of tumors. The protein regulates the proliferation and invasion of tumor cells.[80] Huaier aqueous extract significantly inhibits the expression of Lamin B1. Meanwhile, when low-expressing Lamin B1 in prostate cancer cells, cell proliferation, metastasis and invasion were significantly inhibited, and the inhibitory effect of Huaier on cell proliferation and transferability be reversed. It is suggested that the inhibitory effect of Huaier on cells partly depends on the action of Lamin B1. In addition, Huaier can upregulate autophagy-related proteins like Atg3, Atg5, Beclin-1 and LC3-II, and the inhibitory effect of Huaier on cell proliferation is weakened after inhibition of autophagy.[17] It is suggested that Huaier may inhibit prostate cancer by inducing autophagy of PC3 cells.

Cervical Cancer

Huaier is found inhibiting the viability of SiHa and C33A cells, inducing HPV negative C33A cells to become arrested at the G2/M phase, and arresting the cell cycle of HPV positive SiHa cells. After incubation with Huaier, both SiHa and C33A cells showed decreasing metastatic ability, while SiHa showed decreasing invasion ability. In addition, it is reported that Huaier reduced the expression of p-ERK in both cell lines without changing the total ERK. However, p-JNK and p-p38 increased.[81] These results suggest that Huaier may inhibit cell vitality by interfering with the MAPK signaling pathway. So far, it is not fully known whether Huaier affects the proliferation of tumor cells by altering the JNK/p38 pathway.

Ovarian Cancer

Huaier extract is found significantly inhibiting the viability of ovarian cancer cell lines (SKOV3, SKOV3.ip1 and Hey cells), inducing apoptosis and inhibiting cell migration and invasion. Huaier also regulates the AKT/GSK3β/β-catenin signaling pathway to inhibit ovarian cancer cells. Specifically, Huaier inhibits the phosphorylation level of AKT to decrease the phosphorylation of GSK3β in Ser9, thus enhances the activity of GSK3β. The increased phosphorylation of β-catenin accelerates its degradation. When the GSK3β gene is silenced the inhibitory effect of Huaier on β-catenin ceases. It seems that it is necessary for Huaier to go through GSK3β/β-catenin signaling pathway to inhibit cell invasion (Figure 1).[82]

Colorectal Cancer

Huaier aqueous extract is found inhibiting the proliferation of colorectal cancer cell lines T1 and T2, significantly reducing the quantity and size of spheroids in T1 and T2 cells, and decreasing the proportion of ALDH1 positive cells in cancer cells. It is suggested that Huaier reduces the stem-like characteristic of colorectal cancer cells. Besides, the Wnt/β-catenin pathway is known to be relevant to cell proliferation,

and studies show that Huaier reduces the expression of β-catenin protein and cyclin D1, and reduces the downstream target molecules, implying that Huaier may inhibit the growth of tumor cells by inhibiting Wnt/β-Catenin pathway.[83]

Conclusions and Prospects

The microarray gene analysis shows Huaier has multiple targets to achieve anti-tumor effects, including restraining proliferation and metastasis, arresting cell cycle, inducing apoptosis, pyrosis and autophagy, anti-intratumoral angiogenesis, attenuating characteristics of tumor stem-like cells, interfering with the function of the tumor-related immune system, reversing the drug resistance, and enhancing the sensitivity to other chemotherapeutic drugs, etc. In a broader sense, Huaier demonstrates these biological effects by regulating specific signaling pathway-related proteins, targeting oncogenes and tumor suppressor genes, and/or interfering with ncRNA indirectly influencing gene expression. In this review, the analysts summarized the anti-tumor mechanisms of Huaier and discussed in detail the anti-tumor mechanisms of Huaier mediated by ncRNA. It can be drawn that Huaier has great therapeutic potentials for being an effective adjuvant to tumor treatment. It is noted that most studies used Huaier extract from water or ethanol; thus, the research results only reflect the effect of certain components of Huaier. The polysaccharide extracted from Huaier is the main active components identified so far, and the effect of other components has yet been known. At present, there are few in vivo and clinical experiments on the anti-tumor mechanisms of Huaier. The in vitro experiments have shown that Huaier inhibits the growth of transplanted tumors in mice and interferes with tumor angiogenesis with little cytotoxicity, at least in certain types of tumors. More attention should be paid to the role of non-coding RNA in pursuit of the anti-tumor mechanisms of Huaier. Although Huaier has been used as a clinical adjuvant in some tumor therapy, large-scale clinical trials are needed to examine its pharmacological mechanism before common and standardized administration in cancer treatment.

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Disclosure

The authors report no conflicts of interest for this work and declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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