Research progress on synergistic anti-tumor mechanisms of compounds in Traditional Chinese Medicine

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INTRODUCTION

Traditional Chinese Medicine (TCM) treatment for tumors uses multiple components to target multiple sites and optimal efficacy. TCM combines medicines according to their natural odor, meridian tropism, and relationship of monarch, minister, assistant, and guide herbs. The combination of herbs has a network adjustment effect that is not present in Western Medicine. The modernization of TCM focuses on the study of active ingredients in Chinese medicines. This has led to the discovery of a variety of compounds, including artemisinin and tanshinone, which are effectively applied in clinic. The effectiveness of TCM does not lie in one or a few herbs, but in the entire prescription. The toxicity of some Chinese medicine compounds can be abated or gradually offset with extra herbs, but the overall efficacy is strengthened. In this review, we summarized the mechanisms of the anti-tumor effects of effective compounds from TCM, and their synergistic antitumor mechanisms.

RESEARCH PROGRESS ON MOLECULAR MECHANISMS OF ANTI-TUMOR EFFECTS OF TCM COMPOUNDS

The curative effects of TCM compounds result from the coordination of natural compounds. We aimed to review the mechanism of action of Traditional Chinese Medicine (TCM) compounds (TCMC), explore the rationality of formulation theory and synergistic effects in TCM compounds, and analyze the effectiveness of drug compatibility of TCMC in molecular biology. This literature review covers the mechanisms of the anti-tumor effects of compounds, and their synergistic antitumor mechanisms. We aim to provide reference for the effective development and use of natural resources and the organic combination of TCM and modern medicine using molecular biology.

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and nourish Yin. By screening the prescription with high throughput screening, we get more than 20 compounds which have cytotoxic effects on non-small cell lung cancer, including quercetin, isoliquiritinigen, catechin compounds, ophiopogonin, ginsenosides, and bibenzyl compounds.

**Quercetin cytostatic and pro-apoptotic effect on tumor cells**

Quercetin and its derivatives are flavonoids found in plants, and are present in flowers, leaf, and fruit. They have many pharmacological effects including as an anti-oxidant, anti-inflammatory, hypotensive, and anti-coagulant. They also have cancer, aging, mutagenic, and atherosclerosis preventive activities. Jin et al. found that quercetin had an obvious inhibitory effect on the proliferation of A549 human lung adenocarcinoma cells and transplanted mouse Lewis’s lung carcinoma cells. According to immunohistochemistry, quercetin could obviously lower proliferating cell nuclear antigen (PCNA) expression levels. By inhibiting the expression of PCNA, quercetin could reduce the activity of DNA polymerase D, and inhibit the synthesis of DNA in tumor cells. They also found that quercetin induced apoptosis of mouse melanoma Cloudman S91 cells in a dose-dependent manner. The mechanism was via inhibition of the expression of gene protein p53 and BC-I2. This blocked the cell cycle progression from G1 to S phase, and caused the cells in G2 to accumulate, which lowered the PCNA expression level. Lower PCNA inhibited DNA synthesis in the S phase, and inhibited the proliferation of tumor cells. In addition, Cipka et al. reported that quercetin could strengthen the apoptosis effect of cisplatin on human leukemia HL-60 and rat leukemia L1210 cells after joint administration.

**Cytostatic and pro-apoptotic effects of isoliquiritinigen (ISL) on tumor cells**

ISL is an isoflavonoid found in licorice, Guangguogancao (Radix Glycyrrhizae Glabrae), Zhanhuoguancao (Radix Glycyrrhizae Inflatae) dry root and rhizome, Huangqi (Radix Astragali Mongolicus) root, and Da-huangjing (Rhizoma Polygonati Kingiani) root. It has antitumor, anti-oxidative, and anti-inflammatory effects. It also can expand the arteries and protect the heart and brain.

Kanazawa et al. investigated the influence of ISL on the gene expression of cell proliferation, cell cycle control, and cell cycle regulation. They also studied the antitumor activity of ISL in vitro on prostatic cancer, using the prostate cancer cell lines DU145 and LNCaP. They found that ISL had significant dose- and time-inhibitory effects on prostatic cancer cell strains. Fluorescence activated cell-sorting analysis showed that ISL induced cell cycle block in the S and G/M phases, and enhanced the expression of GADD153 protein mRNA. Hsu et al. found that ISL could inhibit the proliferation of human non-small cell lung cancer A549 cells. ISL could not only inhibit the proliferation of A549 cells, but also induce cell apoptosis and block cell cycle progression in G1. ISL realized the anti-proliferative effect on lung cancer cells A549 through the p53 gene and the Fas/FasL cell apoptosis system. ISL could also inhibit the proliferation of human hepatocellular carcinoma cells. With an IC50 of 10.51 μg/mL, ISL can inhibit cell growth, and lead cells to programmed death by activating caspases. ISL can also inhibit the expressions of Bcl-xl and C-IAP1/2 proteins, and reduce the levels of NF-kB and its activities in the nucleus. Hsu et al. also found that ISL could lead to a rise in p53 gene expression, and incrementally regulate p21/WAF1, Fas/APO-1 receptor, Fas ligand, Bax, and NOXA.

**Cytostatic and pro-apoptotic effects of tea polyphenols and their catechins on tumor cells**

Catechins are flavonoids and anthocyanins of flavonoid glycosides. They are widely distributed in plants such as teasleaves, hawthorn, wild strawberries, cocoa fruit, and grape seeds. Catechins have biological activities such as anti-oxidative, anti-cancer, anti-inflammatory, anti-aging, and anti-mutation effects, and liver function improvement.

Gupta et al. found that epigallocatechin gallate (EGCG) could induce cell cycle block and apoptosis of prostate cancer cells. It could increase the expression of WAF1/p21, KIP1/p21, INK4a/p16, and INK4c/p18 protein in a dose- and time-dependent manner, lower the expressions of cyclin D1, cyclin E, Cdk2, Cdk4, and Cdk, except for cyclin D2. It could also increase the combination of cyclin E, WAF1/p21, and KIP1/p21, decrease the combination of cyclin E and Cdk2, and inhibit the adjustment of cyclin Cdk complexes in the G1/S phase.

Sun et al. investigated the influence and mechanism of EGCG on lung cancer cell proliferation. The results showed that EGCG could significantly inhibit the proliferation of A549 cells. After administration of 60 mg/L EGCG, Hoechst staining showed obvious chromatin condensation, and dense or fragmental hyperchromatic cell apoptosis. The apoptotic rate was much higher than that of the control group. The survivin expression in many lung cancer tissues was the strongest apoptosis inhibiting factor found so far. Western blot showed that survivin protein expression in the treatment group was significantly inhibited, which indicated that EGCG suppressed the proliferation of lung cancer cells by inhibiting survivin expression and promoting lung cancer cell apoptosis.

**Inhibition effect of ophiopogonin on tumor cells**

Ophiopogonin is a steroid saponin, and mainly comes from the liliaceous plants Tumaidong (Radix Liriopes Spicatae), Shanmайдong (Radix Liriopes Proliferae), Duantingshanmaidong (Radix Liriopes Musacarli), and Kuoyeshanmaidong (Radix Liriopes Platypyllae). Ophi-
Zhou J et al. / Review

opogonin has extensive pharmacological activities such as those against myocardial ischemia and myocardial infarction, arrhythmias, and tumors.\textsuperscript{16} Zhang et al.\textsuperscript{17} studied the mechanism of ophiopogonin by testing the adhesion and invasion effect of Duantingshanmaidong (Radix Liriopes Musacarli) saponins DT-13 on lung cancer A549 cells and their effects on the expression and activity of matrix metalloproteinases. They found that Ophiopogon DT-13 at 10 and 30 μM could significantly inhibit the adhesion and invasion of A549 cells, and the expression of matrix metalloproteinases-2/9 in A549 cells. Chen et al.\textsuperscript{18} showed that ophiopogonin B had different degrees of inhibition on non-small cell lung cancer cells H157 and H460. Flow cytometry showed that the cells were blocked in the G0/G1 phase. Moreover, ophiopogonin B cells could induce autophagy, transduce the pathways for mTOR signals, and inhibit phosphorylated Akt (Ser473 and Thr380 loci).

**Inhibition and pro-apoptotic effect of ginsenoside Rg3 on hormone dependent tumor cells**

Ginsenoside Rg3 is a tetracyclic triterpenoid saponin, which is found in Renshen (Radix Ginseng) root. There are two structural isomers, 20(R)-ginsenoside and 20(S)-ginsenoside. In vitro, Ginsenoside Rg3 has strong inhibition on the infiltration of mouse ascites hepatoma, melanoma B16FE7 cells, human small cell lung cancer (OC-10), and human pancreatic carcinoma. In contrast, the anti-infiltration effects of homolog ginsenosides Rb2 and 20(R)-Rg2 are weaker, and the ginsenosides Rh1, Rh2, 20(R)-Rh1, Rb1, Rc, and Re have no anti-infiltration activities.\textsuperscript{19} Liu et al.\textsuperscript{20} found that LNCaP cells given Rg3 lost their adhesion performance. The expressions of prostate-specific antigen, androgen receptor, 5α-reductase, and proliferating cell nuclear antigen were inhibited. The decrease in the expression of the 5α-reductase gene effectively stimulated the production of double-hydration testicular hormone that promoted the proliferation of prostate cells. This decrease inhibited the expression of cell cycle genes such as proliferating cell nuclear antigen gene, cell cycle protease gene, and therefore inhibited cell proliferation. By increasing the expressions of cell cycle proteinase inhibitor genes p21 and p27, Rg3 blocked LNCaP cells in the G1 phase. By lowering the inhibitor of apoptosis Bcl-2, and activating the apoptosis mechanism mediated by caspase 3, Rg3 could inhibit the growth of cells. The abnormal activation of PI3K/Akt/mTOR signaling pathway caused the proliferation, differentiation, metastasis, and invasion of tumor cells. Ma et al.\textsuperscript{21} investigated the pro-apoptotic effect of ginsenoside Rg3 on the PI3K/AKT pathways of endometrial carcinoma Hec-1-B cells. The results showed that ginsenoside Rg3 had obvious inhibitory effects (P<0.05) on the proliferation of Hec-1-B cells in vitro in a dose- and time-dependent manner. The expression level and activity of cell telomerase PI3K and AKT decreased significantly. Therefore, Rg3 could inhibit cell growth by inducing the apoptosis of human endometrial carcinoma Hec-1-B cells. One of the mechanisms by which Rg3 induces apoptosis of human endometrial carcinoma Hec-1-B cells in vitro is by inhibiting the activity of PI3K and AKT.

**Anti-tumor, angiogenesis, and pro-apoptotic effect of benzyl compounds**

Benzyl compounds are bioactive compounds found in Chinese medicines Shancigu (Pseudobulbus Cremas雄), Shanyao (Rhizoma Dioscoreae Oppositae), Shixian-tao (Pseudobulbus seu Herb Pholidotae Chinensis), Zhuyhulan (Herba seu Rhizoma Arundinae Graminifoliae), Shihhu (Herba Dendrobii Nobilis), and Baiji (Rhizoma Bletillae Sistriatae). Benzyl compounds have anti-tumor and anti-angiogenesis activities.\textsuperscript{22-23} Xu et al.\textsuperscript{24} studied the antitumor effect of bryophyte benzyl compound marchantin M and its mechanism of action on prostate cancer cells. Marchantin M could inhibit the proliferation of human leukemia cells (K562), human hepatoma cells (HepG2), human breast cancer cells (MCF-7), and human prostate cancer cells (LNCaP, DU145, and PC-3). It had especially significant inhibition on the proliferation of human prostate cancer cells PC-3, and weak inhibition on the proliferation of normal retinal pigment epithelial cell line (hTERT-RPE1). Marchantin M blocked the PC-3 cells in phase G0/G1. The PC-3 cell had an obvious sub-G0 peak along with changes in cycle-related proteins. Moreover, 4′, 6-diamidino-2-phenylindole showed characteristic changes in nuclear apoptosis.

**RESEARCH PROGRESS ON SYNERGISTIC ANTI-TUMOR EFFECTS OF COMPOUNDS IN TCM**

**Effect of quercetin combined with ligustrazine on growth of mouse Lewis lung cancer**

Quercetin in vitro experiments confirmed that quercetin has inhibitory effects on leukemia, prostate cancer, breast cancer, liver cancer, lung cancer, and colon cancer. Fu et al.\textsuperscript{25} studied the influence of quercetin and ligustrazine on mouse Lewis lung carcinoma growth. They tested the expression levels of tumor tissue microvascular density, vascular endothelial growth factor (VEGF), and PCNA with semi-quantitative immunohistochemistry, and tumor cell apoptosis index with in situ apoptosis terminal-deoxynucleoitidyl transferase mediated nick end labeling. They replicated the model with C57BL mice with transplanted Lewis lung carcinoma cells, and all drug groups had inhibitory effects on transplanted tumor growth. However, the combination group had more obvious effects on tumor inhibition. The tumor growth in the control group was significant.
ly greater than that of the drug group, and the combination group had the slowest tumor growth. Cancer cells in the drug groups had degeneration to different degrees and obvious necrosis according to histopathological observation. In the quercetin and quercetin + ligustrazine groups, the vasculature in the mesenchyma was obviously decreased. In the quercetin, ligustrazine, and quercetin + ligustrazine groups, the growth of tumor cells was significantly inhibited, with inhibition rates of 39.87%, 35.45%, and 54.58%, respectively. The inhibition rate of the quercetin + ligustrazine group was significant higher than that of the single drug groups (P<0.05).

**Effect of platycodin D combined with ophiopogonin, Cnidium lactones, and zedoary on the proliferation and invasion of MDA-MB-231 breast cancer cells**

Han et al. studied the effect of platycodin D with ophiopogonin, platycodin D with Cnidium lactones, and platycodin D with *Rhizoma Zedoariae*, on the proliferation and invasion of 4T1 and MDA-MB-231 breast cancer cells. They found that the inhibitory effect of the platycodin D + ophiopogonin or platycodin D + Cnidium lactones groups on the invasion of 4T1 cells was significantly superior to that of the platycodin D + zedoary group and that of each component used alone (P<0.05 or P<0.01). In addition, the inhibitory effect of the platycodin D + zedoary group or platycodin D + Cnidium lactones groups on the invasion of MDA-MB-231 cells was significantly superior to that of the platycodin D + ophiopogonin group (P<0.01). Therefore, the combination of platycodin D with other active ingredients in TCM could significantly inhibit the invasion and proliferation breast cancer 4T1 and MDA-MB-231. As for the inhibitory effect on the proliferation and invasion, different combinations had different performances.

**Synergistic effect of ophiopogonin, ophiopogonone, and ginsenosides on anti-malignant tumors**

Shennai injection (SM) is produced with equivalent Renshen (*Radix Ginseng*) and Maidong (*Radix Ophiopogonis Japonici*). After observing the inhibition rate of SM on human liver cancer SMMC-7721 cells in vitro, Ye et al. found that at concentrations of 50-250 μL/mL, the tumor cell proliferation inhibition rate was 9.0%-83.1% (P<0.01), in a dose-dependent relationship. The apoptosis index of SMMC-7721 tumor cells increased to 10.91% at a final concentration 200 μL/mL, significantly superior to the 7.57% of the control group. The cell cycle was blocked in the G0/G1 phase and the cells number in G2, M phases increased, while the cell fraction in S and G2/M phase decreased significantly. All these indicate that SM could reduce the synthesis and mitosis of cell DNA, and has significant inhibitory and pro-apoptotic effects on the proliferation of human hepatoma SMMC-7721 cells. Using human colorectal cancer cells injected into nude mice, and immunohistochemistry, Ma et al. found that SM could reduce the cancer tumor MVD, and lower the expression of vascular endothelial growth factor VEGF. However, it had no affect on thrombin-sensitive protein 1.

**Inhibitory effect of honokiol combined with artemisinin on human lung adenocarcinoma SPC-A-1 cells**

Honokiol is a biphenyl phenolic compound isolated from Magnolia officinalis bark and has several pharmacological activities including tumor inhibitory effects. Recent study has confirmed that honokiol can induce apoptosis in many tumor cells. Wang et al. showed that honokiol and artemisinin had synergistic inhibitory effects on lung adenocarcinoma SPC-A-1 cell growth. Honokiol could inhibit SPC-A-1 cell proliferation in a dose-dependent manner, with an IC50 of 8.20 μg/mL, with no obvious inhibition on SPC-A-1 cell growth. The inhibitory effect of honokiol combined with artemisinin on SPC-A-1 cells proliferation was superior to that of the single drug groups. Jin’s formula was used to analyze the effect of the drug combination and found Q>1.15.

**Synergistic treatment mechanism of tetra-arsenic tetra-sulfide, indirubin, and tanshinone II**

Acute promyelocytic leukemia (APL) is an M3 type of acute myeloid leukemia. Patients with APL accumulate many immature promyelocytes in the bone marrow and have severe bleeding. Two chromosome translocations cause the appearance of a new fusion gene PML-RARα at the joint point of break and exchange. This fusion gene encodes a fusion protein, and can induce a series of gene expression as a nuclear receptor, which ultimately leads to uncontrolled cell growth. Arsenic trioxide (ATO) is a useful drug for the treatment of APL because its target is the PML-RARα fusion protein. ATO can degrade the oncoprotein leading to cancer apoptosis. APL is the first human malignant tumor to be specifically treated at its molecular cause. Chen et al. researched tetra-arsenic tetra-sulfide-tanshinone IIA-indirubin compound (ATI) preparations in TCM and their preparation methods and application in the preparation of antineoplastic drugs. RIF compound preparations include tetra-arsenic tetra-sulfide (A), indirubin (I), and tanshinone II A (T). Among the ingredients, A is the main component of re- algar, I is the active ingredient of natural indigo, and T is the active ingredient of Salvia miltiorrhiza. As with arsenic trioxide, tetra-arsenic tetra-sulfide (A) can also induce the ubiquitination of the PML-RARα fusion protein and the transportation to the nuclear reticulum, and can be degraded by the soluble protein. I or T used alone do not have this function. However, together with A, T and I can promote the ubiquitination of PML-RARα and speed up its degradation. ATI has the most significant degradation effect on cancer pro-
tein PML-RARα. Therefore, it can significantly extend the median survival rate of mice with APL. ATI can induce the differentiation of APL cells in vitro, and enhance the redifferentiation adjustment factor activity of bone marrow cells. Cells need the CDK protein to move from the G₁ phase to the S phase. However, I is an effective CDK inhibitor. Therefore, ATI has a strong effect on blocking cells in the G₁ phase. P27 is a protein that can inhibit CDK, which is produced by the cells themselves. By lowering CDK, reducing protein that can inhibit CDK, which is produced by the cells, and up-regulating P 27 and enhancing the redifferentiation adjustment factor activity of bone marrow cells. Cells need the CDK protein to move from the G₁ phase to the S phase. However, I is an effective CDK inhibitor. Therefore, ATI has a strong effect on blocking cells in the G₁ phase. P27 is a protein that can inhibit CDK, which is produced by the cells themselves. By lowering CDK, reducing protein that can inhibit CDK, which is produced by the cells, and up-regulating P 27 and enhancing the redifferentiation adjustment factor activity of bone marrow cells. Cells need the CDK protein to move from the G₁ phase to the S phase. However, I is an effective CDK inhibitor. Therefore, ATI has a strong effect on blocking cells in the G₁ phase. P27 is a protein that can inhibit CDK, which is produced by the cells themselves. By lowering CDK2, reducing phospho-histone H1, and up-regulating P 27 and P27Rb, ATI can inhibit cell progression from G1 to S, and thereby inhibit tumor cell proliferation. These results are consistent with traditional "monarch and minister and assistant and guide" theory in TCM, which provides a theoretical framework to understand the mechanism of ATI treating APL, and helps TCM practitioners understand the treatment mechanism of other ancient compounds.

RESEARCH PROSPECTS IN TCM

The biggest advantage of treating tumors with Chinese medicines is that they can have a multiple sites and targets. This is why Chinese medicine has clinical effects but multiples effects are obstacles to their popularization, internationalization, and modernization. One Chinese medicine compound is made up of thousands of compounds. Some Chinese medicines do not even have molecular targets, so their mechanisms and effects are uncertain. In contrast, Western Medicine is homogenous and drug factors can be identified, which provides quantifiable parameters for scientific research. However, homogenous drug targets can only control or relieve some symptoms. It is difficult to completely cure complex diseases, such as cancer with one drug. Chinese medicine takes into account the difference between individuals and the relationship between humans and the environment. The accumulation of TCM knowledge provides the conditions for further developing systems biology and integration with modern medicine.

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