• Review Article •

Advances in the research of plant-derived natural products against retinoblastoma

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Abstract

● Retinoblastoma (RB) is a highly aggressive ocular tumor, and due to socioeconomic and medical constraints, many children receive treatment only in the metaphase and advanced clinical stages, resulting in high rates of blindness and disability. Although several approaches exist in the treatment of RB, some children with the disease do not have satisfactory results because of various factors. Plant-derived natural products have shown definite therapeutic effects in the treatment of various tumors and are also widely used in the study of RB. We review plant-derived natural products used in the study of anti-RB to provide ideas for the clinical application of these drugs and the development of new therapeutic drugs.

● **KEYWORDS:** plant-derived; retinoblastoma; apoptosis; cell cycle; drug resistance

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INTRODUCTION

Retinoblastoma (RB) is the most common primary intraocular malignancy in childhood[1], with more than 90% of cases presenting before the age of 5 years[2]. It is mainly due to the loss of the double allele of the *RB1* gene in the developing retina[3], as well as the appearance of other genetic and epigenetic alterations eventually leading to the development of cancer[4]. The combination of systemic and local chemotherapy, local condensation, laser therapy, radiotherapy and surgery has significantly improved the survival rate of children. Treatment failure in some children because of the toxic side effects and drug resistance to chemotherapy, increased risk of second primary tumor development with radiotherapy, and the ease of recurrence with local treatment[5-6] has placed a huge burden on families and society, and new therapeutic agents need to be sought to address the problem.

Natural products from plants, animals, microorganisms and minerals have long been a source of drugs for the treatment of human diseases[7]. And studies have confirmed that such products can be used as potential anti-cancer drugs or adjuvants for chemotherapy[8], which are valuable for both cancer prevention and treatment and play an important role in the exploration and development of new anti-cancer drugs. More than 60% of antitumor drugs show a close association with natural products[9] according to the literature, where natural products of plant origin are the main source of anticancer drugs. About 3/4 of the current drugs for cancer treatment are derived from plants[10], such as paclitaxel, camptothecin, etoposide and vincristine have been approved for the clinical treatment of many cancers[11], providing more options for attacking cancer. This class of natural products has been widely used in the research of anti-RB therapy, which can produce anti-RB effects by inducing RB cell apoptosis, inhibiting RB cell invasion, triggering the RB cell cycle arrest, and regulating RB cell resistance in various ways.

PROMOTE RETINOBLASTOMA CELL APOPTOSIS

Apoptosis is the main mechanism regulating cell death, but

apoptosis of tumor cells are being often in a dysregulated state in human cancer[12]. The study of the molecular mechanisms of apoptosis in cancer cells to obtain effective drugs that promote apoptosis in tumor cells is widely used in the current strategies for anticancer drug development and is a key direction of antitumor research.

A variety of plant-derived natural active ingredients have shown good effects in inducing RB cell apoptosis *in vitro* in the current study. These active ingredients exert anti-RB effects by modulating the activity of different signaling pathways in RB cells, altering the expression of downstream related genes, and inducing an increase in intracellular apoptotic signals.

Phosphatidyl Inositol 3-kinase/serine Threonine Protein Kinase Signaling Pathway Phosphatidyl inositol 3-kinase/serine threonine protein kinase (PI3K/AKT) is one of the most important intracellular signaling pathways, which can be activated by insulin, growth factors and cytokines under physiological conditions. This pathway plays an important role in glucose metabolism, synthesis of macromolecules and maintenance of tissue redox homeostasis *in vivo*, supporting the metabolic homeostasis of the system and promoting the growth and metabolism of individual cells[13]. The PI3K/AKT signaling pathway exhibit frequent mutations and enhanced activity in cancer[14], and activation of the pathway induces changes in the expression of a variety of downstream genes that promote tumorigenesis and progression, and has been found to be abnormal in almost all human cancers[15].

Matrine is an alkaloid isolated from the plant herb bitter ginseng[16]. Zhang[17] used bittersweet to act on RB Y79 lineage cells, they found that apoptosis of Y79 cells increased and cell proliferation was significantly inhibited, and the mRNA expression of PI3K and AKT in the cells was significantly inhibited by assay analysis, and this inhibition was closely related to the concentration of bittersweet, while the protein expression of PI3K and AKT in the cells also showed a dose-dependent decrease. The anti-Y79 cellular effect of matrine was also confirmed in a study by Chen and Ai[18] The expression of p85α subunit protein of PI3K and AKT protein was significantly decreased in Y79 cells treated with matrine, leading to a decrease in the expression of its downstream apoptosis inhibitory protein Bcl-2 protein and a significant increase in the expression of the pro-apoptotic Bax protein, which induced apoptosis in Y79 cells. Liu *et al*[19] used Eucommia chlorogenic acid (CGA), a natural product extracted from *Eucommiaceae*, to treat RB HXO-RB44 cells and found that apoptosis of HXO-RB44 cells was significantly increased after treatment with CGA, and analysis of proteins in the cells revealed that the drug group cells had Ki67, NQO1, TrxR, p-PI3K, and Nrf2 expression were significantly lower in the drug group cells than in the control group, and both p-AKT/AKT ratios were also significantly lower than in the control group, while the inhibitory effect of CGA on HXO-RB44 cells could be reversed after the use of PI3K activator, confirming that CGA has the ability to inhibit the PI3K/AKT pathway in HXO-RB44 cells effect, and that CGA promoted apoptosis in HXO-RB44 cells through the inhibition of this pathway.

Mitogen-activated Protein Kinase/Extracellular Signal-regulated Kinase Signaling Pathway The mitogen-activated protein kinase (MAPK) family is a group of highly conserved protein kinases[20] that *in vivo* can co-regulate extracellular signal-regulated kinase (ERK). Activation of the MAPK/ERK pathway can significantly increase the expression of glucose transporters and a large number of glycolytic genes, resulting in altered cellular metabolism and promoting tumor growth and proliferation[21]. Therefore, blockade of MAPK/ERK signaling pathway can inhibit tumor growth, increase tumor cell apoptosis, and exert anti-tumor effects. Puerarin, a naturally active isoflavone extracted from *Pueraria lobata*, was found by Yang *et al*[22] to significantly inhibit the proliferation of RB Y79 cells and SO-RB50 cells in a dose- and time-dependent manner by MTT assay. Protein blotting assay revealed that p-MEK and p-ERK protein expression in RB cells treated with Puerarin was significantly lower than that in the underage group, and the inhibitory effect of Puerarin on RB cells could be counteracted with MAPK/ERK agonists, confirming that Puerarin exerts its anti-RB effect by inhibiting the MAPK/ERK pathway. Both p38 mitogen-activated protein kinase (p38 MAPK) and c-Jun amino-terminal kinase terminal kinase (JNK) are members of the MAPK family[23]. Yu *et al*[24] found that the p38 MAPK and JNK pathways play an important role in Y79 cell apoptosis induced by the plant polyphenol compound curcumin, and curcumin can promote apoptosis of Y79 cells by activating p38 MAP and JNK in cells and upregulating caspase-9 and caspase-3 activities, while the induction of caspase-9 and caspase-3 protein expression by curcumin was significantly decreased after the use of p38 MAPK or JNK inhibitors, along with a decrease in the apoptosis rate of Y79 cells, thus showing that curcumin produces inhibition of Y79 cells through the p38 MAPK and JNK pathways. Quercetin is a flavonol found in many vegetables. Liu and Zhou[25] found that quercetin induced apoptosis in Y79 cells by a mechanism similar to that curcumin, also by activating the JNK and p38 MAPK pathways in cells to enhance the activity of caspase-9 and caspase-3, producing an anti-Y79 cellular effect, and this inhibition could also be reversed by JNK inhibitors and p38 MAPK inhibitors.

Janus Kinases-Signal Transduction and Activator of Transcription Signaling Pathway The four Janus kinases (JAK1, JAK2, JAK3, TYK2) and seven signal transduction

and activator of transcription *in vivo* (STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6) together constitute the JAK-STAT signaling pathway[26], which is essential for the signaling of various metabolic-related hormones and cytokines in the cells[27]. The JAK/STAT signaling pathway plays a variety of important biological functions in malignant cells[28], and this pathway mediates almost all immunoregulatory processes, including tumor cell recognition and tumor immune escape[29], which are closely related to tumor progression and prognosis. Studies have confirmed that JAK-1 and STAT-3 protein expression levels are independent risk factors for patient prognosis in patients with colon cancer[30], and JAK/STAT was also found to be closely associated with the malignant biological behavior of tumors in a study of RB. Curcumin extracted from turmeric has been shown to have antitumor activity in recent years. Li *et al*[31] used curcumin to act on RB SO-RB50 cells and Y79 cells and found that curcumin significantly inhibited the proliferation, colony formation, migration and invasion of SO-RB50 cells and Y79 cells, while promoting both SO-RB50 and Y79 RB cells apoptosis. Upregulation of miR-99a was detected in curcumin-treated cells, and the phosphorylation levels of JAK1, STAT1 and STAT3 proteins were inhibited. The inhibitory effect of curcumin on the JAK/STAT pathway disappeared after knockdown of miR-99a. It is evident that curcumin can exert anti-SO-RB50 cells and Y79 cells by inactivating the JAK/STAT signaling pathway through the regulation of miR-99a.

Nuclear Transcription Factor-κB Signaling Pathway Nuclear transcription factor-κB (NF-κB) is an important transcription factor in biological cells and has an important role in the immune system in regulating the expression of a variety of cellular response genes[32]. A growing number of studies are now confirming that inflammation is closely related to the generation and development of human cancers, and NF-κB, as a key molecule linking persistent infection and chronic inflammation to increased cancer risk[33], is a current hot topic in antitumor research. Mu *et al*[34] found that the proliferation and migration ability of cells were significantly decreased after the action of curcumin on WERI-RB1 cells, and the protein expression assay of the cells revealed that the p65 nuclear translocation was significantly inhibited and the protein expression of NF-κB downstream factors matrix metalloproteinase (MMP)-2, MMP-9, and vascular endothelial growth factor (VEGF) was significantly decreased, indicating that the inhibitory effect of curcumin on WERI- RB1 cells was achieved by regulating the NF-κB signaling pathway. Elemene is a natural component with anticancer effects extracted from the ginger family, and Wei[35] found that the proteins of NF-κB and the downstream factor surviving were highly expressed in human RB HXO-RB44 cells, and the expression of both proteins decreased significantly after treatment of HXO-RB44 cells with elemene, indicating that elemene may promote apoptosis in HXO-RB44 cells by inhibiting NF-κB activity, downregulating the expression of Survivin gene and thus promoting apoptosis in HXO-RB44 cells. Thus, in RB, natural active ingredients of plant origin may inhibit tumor progression by down-regulating NF-κB activity and thereby inhibiting tumor progression.

Tumor Protein 53 Signaling Pathway Tumor protein 53 (p53) is an important tumor suppressor gene involved in the regulation of various cellular activities such as apoptosis of tumor cells, genomic stability, tumor angiogenesis, metabolism of cancer cells and tumor microenvironment, and has a role in the prevention of tumorigenesis[36]. The *p53* gene was found to be one of the most frequently mutated genes in tumor cells, and inactivation of this gene is a key driver in the development of several cancers[37], and mutations in the p53 protein are present in approximately half of human tumors, and alterations in the components of the p53-mediated tumor suppressor pathway also occur in the other half[38]. So regulation of the p53 signaling pathway is an important antitumor strategy. Ziyuglycoside I is one of the main active ingredients isolated from the traditional Chinese herb Diel root. Zhu *et al*[39] found that Ziyuglycoside I significantly inhibited the viability of WERI-RB1 cells. The cellular protein assay after the action of Ziyuglycoside I found that Ziyuglycoside I upregulated Bcl-2 and Mito- by inducing the activity of phosphorylated and acetylated p53 proteins in the cells. Cyto c protein expression decreased, while inducing Bax and Cyto-cyto c protein expression in cells, which promoted apoptosis. Inhibition of WERI-RB1 cells by Ziyuglycoside I was significantly decreased after blocking p53 indicating that the p53 pathway plays an important role in Ziyuglycoside I -induced apoptosis of WERI-RB1 cells.

Wnt/β-catenin Pathway The beta-catenin dependent Wnt signaling (Wnt/β-catenin) pathway is an evolutionarily conserved signaling pathway that plays an important role in regulating tissue development and maintaining homeostasis *in vivo*, and its abnormal regulation often leads to the development of a variety of diseases[40]. Abnormalities in this pathway can directly affect many cytokines that are closely related to the anti-tumor activity of T cells[41], leading to tumorigenesis and progression. Abnormalities in the Wnt/β-catenin pathway is present in a variety of tumor tissues, including ovarian, colorectal, and prostate cancers[42-44], and inhibition of this pathway results in varying degrees of suppression of these tumors. 7-methoxyheptaxanthine is an alkaloid component extracted from the rhizomes of *Phellodendron spp*. Chen *et al*[45] found that the alkaloid had a significant inhibitory effect on Y79 cells, and the Wnt/β-catenin

signaling pathway was significantly inhibited in Y79 cells treated with 7-methoxyheptaxanthine, and with increasing drug doses, the wnt and β-catenin protein expression levels also decreased in a gradient with increasing drug dose, confirming that 7-methoxyhepatocarpine could produce anti-Y79 cell effects through the Wnt/β-catenin signaling pathway.

In addition, extracts derived from plants can also exert antitumor effects on RB through signaling pathways such as PERK-ATF4-CHOP pathway and miR-937/FOXQ1 pathway[46-47]. The above studies indicate that there are abnormalities in the regulation of a large number of signaling pathways in RB cells, and the study of signaling pathways is an important direction for the development of antitumor drugs. Since this class of active ingredient drugs can produce antitumor effects on RB in different pathways, they have great potential in the treatment of diseases.

INHIBITION OF RETINOBLASTOMA CELL INVASION AND MIGRATION

Invasion and migration is an important hallmark of malignant tumor progression, which involves various factors such as degradation of extracellular matrix, epithelial-mesenchymal transition, tumor angiogenesis, and the development of inflammatory tumor microenvironment[48], and is closely related to poor prognosis of cancer. Inhibition of tumor metastasis is important for the treatment of tumors, which can buy time for the treatment of tumor patients and improve their survival and cure rate.

Degradation of the Extracellular Matrix MMPs are key cytokines in the process of cell invasion, which can make tumor cells metastasize easily by degrading the extracellular matrix in tissues, and are a popular target in the current research of antitumor drugs. The results of Li *et al*[49] showed that Astragalus polysaccharide extracted from the root meridian of *Astragalus pterocarpus* could significantly reduce the number of RB44 cells penetrating the basement membrane of Transwell chambers, and the detection of MMPs in the cells treated with Astragalus polysaccharide revealed that the expression of MMP-9 and MMP-2 proteins in the cells was significantly down-regulated, and the degradation ability of extracellular matrix was inhibited, leading to a decrease in cell invasiveness.

Inhibition of Epithelial-mesenchymal Transition Epithelial to mesenchymal transition (EMT) is a biological process in which epithelial cells are transformed into cells with a mesenchymal phenotype through a specific program[50] and has an important role in tumor metastasis. in which transforming growth factor-β1 (TGF-β1) is the main inducer of tumorigenesis EMT and can play an important role by TGF-β1 is the main inducer of tumorigenic EMT, and can exert EMT effects by regulating small molecule proteins such as N-calmodulin, waveform protein and E-calmodulin. In the process of EMT, the polarized form of epithelial cells is destroyed and gradually transformed into mesenchymal cells, which makes the invasiveness and metastasis of tumor significantly enhanced. Therefore, the inhibition of the EMT process of tumor cells can effectively reduce the chance of tumor metastasis. Pinocembrin is a flavonoid extracted from plants such as nut pine, eucalyptus leaves and acacia gum, and Chen *et al*[51] used Pinocembrin in an experimental study of the invasiveness of Y79 cells and found that the invasive migratory capacity of Y79 cells was reduced after the use of Pinocembrin, and the detection of relevant proteins in the cells revealed that Pinocembrin caused the N-calmodulin, αv and β3 integrins and wave proteins in Y79 cells to expression significantly, resulting in a blocked TGF-β1-induced EMT process in Y79 cells. Berberine is an alkaloid extracted from the buttercup plant Huanglian, and in an anti-RB study using Berberine, Wang *et al*[52] found that it inhibited PI3K/AKT and p38 pathways and decreased E-cadherin protein amounts, while upregulating the expression of waveform proteins and α-SMA, resulting in a decrease in the invasion and migration of RB cells.

Inhibition of Retinoblastoma Angiogenesis Angiogenesis is an important event in the process of tumor growth and hematogenous metastasis[53], where tumor cells induce the sprouting and generation of new capillaries by secreting high levels of pro-angiogenic factors to form an abnormal vascular network. The newly formed blood vessels provide oxygen and nutrients to tumor tissues and provide channels for their distant metastasis, which is essential for tumor growth and spread[54]. At the same time, the immaturity and high permeability of newly formed vessels lead to a hypoxic microenvironment in parts of the tumor due to poor perfusion, and this microenvironment can promote the invasion of tumor cells, enhance the anti-immune response of tumors, and increase the drug resistance of tumor cells[55-57]. Therefore, conducting research on anti-treatment angiogenic drugs will help to improve the efficacy of clinical anti-tumor therapy. VEGF and its receptor (VEGFR) are the key regulators identified to promote tumor angiogenesis, and previous studies have demonstrated that inhibition of their expression can effectively reduce tumor angiogenesis. Fisetin is a small molecule compound extracted from plants of the *Lacrimalaceae* family, and Wang *et al*[58] found that the pro-angiogenic ability of Fisetin-treated Y79 cells was decreased, and further studies revealed that both VEGFR protein and mRNA expression in the cells were significantly inhibited, and there was a dose-dependent inhibition of VEGFR expression and the ability to induce angiogenesis in Y79 cells at different concentrations of Fisetin. Quercetin (Que) is a flavonoid with antitumor

activity and is widely found in plant-based herbs. Song *et al*[59] found that Que inhibited the proliferation of RB cells and also inhibited the VEGFR expression in the cells, blocking the angiogenesis-promoting effect of RB cells, and observed that Que inhibited RB cell-induced angiogenesis. The effect of Que on RB cell-induced angiogenesis was also observed to be dose-dependent. In addition, Wang *et al*[60] found that methyl lotusine from lotus seed germ could also significantly inhibit RB cell-induced microangiogenesis. These experimental studies indicate that plant activities with anti-RB angiogenic effects are widely available, and the results of the experiments provide a basis for further drug development and show that active ingredients derived from plants have great potential in the therapeutic field of anti-RB angiogenesis.

Additional results of anti-RB studies using active ingredients such as Neferine and Sinomenine[61-62] showed that both can produce clear anti-RB cell invasion through different pathways. The above studies indicate that there are a large number of active ingredients in plants that can inhibit tumor metastasis, and their study can help to develop drugs or adjuvant classes of drugs that inhibit tumor metastasis, solve the most difficult problems in tumor treatment, and improve patient survival.

TRIGGER RETINOBLASTOMA CELL CYCLE ARREST

The cell cycle promotes the replication of genetic material and cell division[63] and has an important role in the normal growth and development of cells and in maintaining the homeostasis of the organism, a process that is precisely regulated by a variety of genes[64]. The cell cycle is divided into interphase and metaphase, the longer of which is interphase, consisting of G0 phase in the resting state, G1 phase in preparation for DNA synthesis, S phase for DNA synthesis, and G2 phase at the end of DNA synthesis in preparation for mitosis, and the shorter of which is M phase, when cells undergo division. The transition between different periods in the cell cycle depends on the joint coordination of cyclins, cyclin dependent kinases (CDKs), and checkpoint pathways in the cell cycle, and abnormal activation of these cyclins and checkpoint pathways will lead to abnormal cell cycle progression[65], causing them to exhibit a higher proliferation rate than normal cells[66].

In recent national and international literature, it has been shown that inhibitory treatment of genes involved in the cell cycle is a promising anticancer strategy and has become an attractive therapeutic target in cancer therapy[67-68], and data show that there is a significant upregulation of cell cycle pathway factors in RB cells[69], and there are also a large number of plant-derived active molecules applied in cell cycle studies of RB cells that anti-tumor effects by causing blockage of RB cells at different periods. The blocking effect of plant-derived active molecules on RB cells in the current study can be roughly divided into G0/G1, S and G2/M phase arrest.

G0/G1 Phase Arrest G0/G1 phase is the preparatory phase of cellular DNA synthesis, in which mitogenic stimulus signals first induce the synthesis of cyclin D[70], and the synthesized Cyclin D1 mutates the cyclin-dependent kinases 4 (CDK4) and cyclin dependent kinases 6 (CDK6), and forms Cyclin D1/CDK4 and Cyclin D1/CDK6 complexes, which in turn activate transcription of a large number of genes related to cell cycle progression and proliferation, facilitating the transition from G1 to S phase[71-72], and the inhibition of cycle-regulated proteins in this phase prevents cells from transitioning to S phase for DNA replication, which in turn produces antitumor effects. Studies have shown that various plant extracts have the ability to induce G0/G1 phase arrest in RB cells. The results of Zhao *et al*'s[73] study showed that treatment of vincristine-resistant RB SO-RB50 cells (SO-RB50/VCR cells) with Matrine alkaloids, an alkaloid found in the legume family, resulted in a significant inhibition of SO-RB50/VCR cell proliferation, and cell cycle analysis revealed that SO-RB50/VCR cells were blocked in G0/G1 phase, with the strongest inhibition in high concentrations of Matrine, and analysis of the relevant proteins in the cells revealed that the G0/G1 phase arrest in SO-RB50/VCR cells was associated with the inhibition of Cyclin D1 protein expression in the cells by Matrine.

Yu *et al*[24] conducted an anti-RB study on curcumin extracted from the ginger family and found that curcumin significantly inhibited the expression of Cyclin D3 and CDK2 and CDK6 proteins in Y79 cells, as well as upregulated the expression of CDK inhibitor proteins p21 and p27, causing Y79 cells to undergo G1 phase arrest and reducing the proliferation viability of the cells. In addition, puerarin extracted from *Pueraria lobata* has the same effect of inducing G0/G1 phase arrest in RB cells, and Yang *et al*[22] used different concentrations of puerarin to act on Y79 cells and SO-RB50 cells, respectively, and found that the application of puerarin inhibited p-MEK and p-ERK protein expression in both RB cells, causing Cyclin D1, Cyclin B1 and CDK2 transcription and expression decreased, the percentage of cells in S phase decreased, and the percentage of cells in G0/G1 and G2/M phases increased, producing the effect of double phase arrest in G0/G1 and G2/M phases.

S Phase Arrests Cyclin E plays an important role in the transition from G1 to S phase of the cell cycle, a period of rapid DNA replication. In the late G1 phase cyclin E expression is elevated and forms a complex with CDK2, which regulates cell entry into S phase while activating phosphorylation of E2 factor (E2F) protein, which further promotes cyclin E transcription after E2F activation. High levels of Cyclin E induce increased expression of S phase

specific thymidine kinase and cell cycle protein A and other proteins, which promote cell entry into S phase and initiate DNA replication[74]. S-phase is also the target of cell cycle blocking effect of some natural drugs on RB cells. Zhu *et al*[75] found that nootkatone, a natural compound isolated from plants, can cause cell cycle S phase arrest in a dose-dependent manner. Chen *et al*[45] studied 7-methoxyhepatocarpine extracted from Brassicaceae plant Huangpi and found that the addition of 7-methoxyheptaxanthine to Y79 cells could also induce S phase arrest in Y79 cells, and assay analysis revealed a drug concentration-dependent decrease in the expression of the cycle-related proteins Cyclin A, Cyclin D1 and Cyclin E in the cells, resulting in a significant increase in the percentage of Y79 cells in S phase.

G2/M Phase Arrest G2/M phase is a critical period for cell mitosis, during which relevant factors in cells examine and repair the DNA damage present after DNA synthesis and induce cells to undergo mitosis. Cyclin B1 and CDK1 are key regulators during this period. Since CDK1 is dependent on the formation of Cyclin B1-CDK1 complex to exhibit kinase activity, the activity of CDK1 is largely influenced by Cyclin B1 protein levels. In the cell cycle Cyclin B1 first appears in S phase and gradually accumulates in G2 and M phases[76], and in G2 phase Cyclin B1 levels increase and form a complex with CDK1, which can regulate the expression of DNA damage repair and mitosis-related genes[77-78], and finally complete cell division. The G2/M phase is an important target for many plant active ingredients to exert their antitumor effects. Corosolic acid, an active ingredient from the plant Zea mays, was used in an antitumor study of Y79 cells by Wang *et al*[79]. They found a significant increase in the number of Y79 cells in the G2/M phase, and analysis of cellular protein assays revealed that corosolic acid exerted a G2/M phase blockade on Y79 cells through its dose-dependent upregulation of p53 and p21WAF1 protein expression in Y79 cells, as well as inhibition of cyclin B1, Cdc25B and Aurora B protein activity, producing a G2/M phase blockade in Y79 cells. Artesunate is a natural active substance extracted from the plant Artemisia annua, which is widely used in the treatment of malaria, and in recent years, it was found that this active substance also has anti-tumor activity. Tang[80] used artesunate to treat RB WERI-RB1 and SO-RB50 cells and found that artesunate could concentration-dependently inhibit the cycle-related proteins CDK1, Cyclin B and Cdc25 in the cells. Cyclin B and Cdc25 expression, causing both cells to be blocked in G2/M phase. Also in the anti-RB studies with Polyphyllin I and methyl eugenol[81-82] showed to have the effect of inducing G2/M phase arrest in RB cells and inhibited the proliferation of RB cells.

In summary, natural active drugs of plant origin have a significant cell cycle arresting effect on RB cells, and the period of action of the drugs with different components varies, and the cell cycle arrest lead to a decrease in survival and an increase in apoptosis of RB cells. After making this class of drugs act on RB cells, the mechanism of inducing cell cycle arrest can be studied, which can provide more options for RB cell treatment.

REGULATE RETINOBLASTOMA RESISTANCE

In the treatment of tumors, the presence of cellular drug resistance is one of the main reasons for the failure of chemotherapy in human malignancies[83]. Drug resistance to chemotherapy may be related to the resistance of the tumor itself to the drug or may develop gradually during the course of chemotherapy. Cellular resistance is associated with increased activity of several resistance-related proteins, such as glutathione-S-transferase π (GSTπ), p-glycoprotein (P-gp), lung resistance protein (LRP) and multidrug resistance protein 1 (MRP1), the expression of which is elevated, can increase the detoxification capacity or decrease the sensitivity of tumor cells to chemotherapeutic agents[84], which in turn can lead to treatment failure. The literature shows that multidrug resistance in the treatment of RB is likewise one of the main challenges facing chemotherapy and is closely related to the prognosis of RB[85]. Shukla *et al*[86] studied induced drug-resistant Y79 cells and found that the expression of P-gp protein and MRP1 protein was significantly higher in drug-resistant cells compared to normal Y79 cells, but the expression of LRP protein did not differ between the two. However, Krishnakumar *et al*[87] found the same expression of P-gp and LRP proteins in unchemothered RB tissues, indicating that multidrug resistance proteins are inherently present in RB cells and that changes in the activity of some resistance proteins under drug induction may be an important reason for RB treatment failure, and therefore the study of RB resistance will help to improve Therefore, the study of RB resistance will help to improve the therapeutic effect of patients. Cui[88] used resveratrol, a plant-derived natural polyphenol, to study multidrug resistance-related factors in RB SO-RB50 cells and found that Resveratrol could inhibit both mRNA and protein expression of resistance-related genes MDR-1, MRP1, COX-2, and GST-π in SO-RB50 cells, resulting in a decrease in cellular resistance. Li[89] used ginsenoside Rg3 to study primary drug resistance in HXO-RB44 cells and showed that the use of ginsenoside Rg3 reduced MRP protein expression in HXO-RB44 cells, and ginsenoside Rg3 with vincristine (VCR) or homoharringtonine (HTT). When combined, the inhibitory effect of both on HXO-RB44 was enhanced, suggesting that inhibition of MRP protein expression inhibits primary drug resistance in HXO-RB44 cells and improves cellular sensitivity to chemotherapeutic agents. Piperlongumine (PLGM) is an alkaloid extracted from the fruit of the plant wicker, which is a biologically active substance

that inhibits many types of cancer. Wang *et al*[90] used PLGM to treat two drug-resistant RB cell lines, HXO-RB44/VCR and SO-Rb50/CBP, and showed that the addition of PLGM to drug-resistant RB cells increased drug sensitivity and apoptosis were increased, and the expression of P-gp, MDR1, MRP1, Top-II, GST-π, Survivin, Bcl-2, CDK1, ABCB1, and ABCG1 proteins were inhibited while downregulating the activity of PI3K/AKT signaling pathway in the cells, suggesting that PLGM may enable the inhibition of PI3K/AKT signaling pathway through reversal of drug resistance in RB cells through inhibition of PI3K/AKT signaling pathway. The above study confirmed that the active molecules extracted from plants have the effect of reversing the drug resistance of RB cells, which can effectively improve the chemotherapy sensitivity of RB cells and bring new hope to patients.

OUTLOOK

Due to their long history of use, species richness and wide range of sources, plant-based drugs have been the focus of attention for disease treatment. In previous basic studies against RB, we found that many natural active ingredients of plant origin have shown good antitumor effects, especially in reversing RB drug resistance with advantages that are difficult to be matched by other drugs, and their research has broad application prospects. Although their direct targets of action on RB cells, mechanisms of anticancer effects, and monomeric drug components of antitumor activity need further research, there are also many shortcomings such as *in vivo* experiments have not been fully carried out, less research on the mechanism of action of reversing resistance, and lack of data on clinical applications. However, it is believed that with the progress of technology and in-depth research, all these problems will be solved gradually, and more and better drugs will be available, so that the treatment of RB will no longer be a problem.

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