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Oriental Medicine

Theoretical development and mechanism research of evodiamine's antitumor effect

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Author contributions

Li-Ping Liu and Tian-Xiang Lei wrote the manuscript together. Li-Ping Liu, De-Liang Li and Feng Li jointly drew the pathway diagram. Li-Ping Liu and Ping-Xiang Li searched the literature together. Ke-Li Ge and Cheng-Shan Ma jointly issued the idea, the framework of the article, and repeated revisions. Ke-Li Ge gave the funding support. All authors read and approved the final manuscript.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

EVO, evodiamine; AIF, apoptosis inducing factor; Cyt-c, cytochrome-C; Bcl-2, B-cell lymphoma-2; BAX, BCL2-Associated X; MMP, mitochondrial membrane potential; PERK, protein kinase-like endoplasmic reticulum kinase; UPR, unfolded protein response; eIF2α, eukaryotic initiation factor 2α; MAPKs, mitogen-activated protein kinases; ERK1/2, cellular signal-regulated kinase 1/2; JNK, c-Jun n-terminal kinase; Top I, Topoisomerase I; HSP, heat shock protein; TRPV1, transient receptor potential vanilloid 1; CSC, cancer stem cells; VM, vasculogenic mimicry; CDKs, cyclin-dependent kinases; Ber, berberine; Caspase, cysteinyl aspartate-specific proteinase; END, 10-Nitro Evodiamine derivatives; UVEC, umbilical vein endothelial cell; ICG, indocyanine green.

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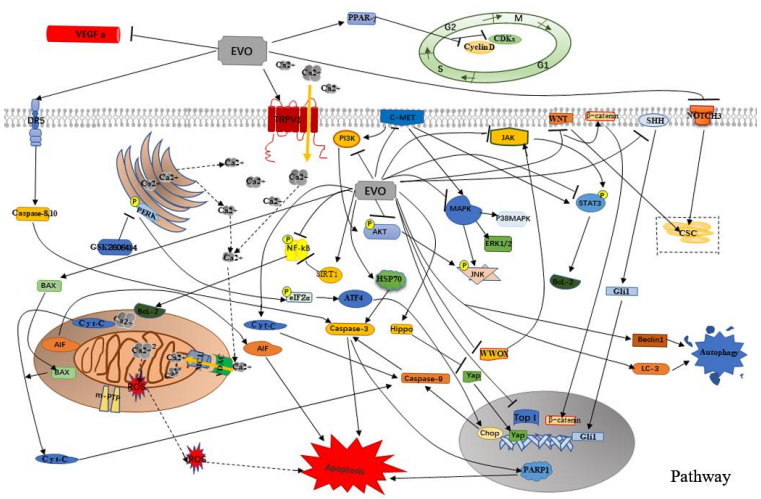
Abstract

Evodiamine (EVO), one of the bioactive components of traditional Chinese medicine, has been widely concerned for its anti-tumor properties in recent years. In this review, the anti-tumor mechanism of EVO was reviewed from the aspects of inducing tumor cell apoptosis (internal pathway, external pathway and other pathways), inducing autophagy, inhibiting tumor stem cells, inhibiting cell proliferation and migration, inhibiting peripheral vascular proliferation, and recent research progress of EVO anti-tumor drugs.

Keywords: evodiamine; anti-tumor; apoptosis; autophagy; cancer stem cells



Background

With the development of the social economy and the continuous improvement of average life expectancy, the incidence of cancer is increasing year by year and the trend of younger development. As a result, tumor-related research is also growing rapidly. In traditional Chinese medicine, cancer has been recorded: ancient book of Chinese medicine "*Difficult Classics 55 Difficult*" (8–23 C.E.) on the nature and characteristics of cancer: "the product, the five Tibetan born... The pain does not leave the body, the upper and lower parts end and start, and the left and right parts are poor". He believes that the accumulation of phlegm, blood stasis, Yin and turbidity caused by physical deficiency and cold is the essence of tumor.

Traditional Chinese medicine has a long history in tumor treatment and has played an important role in the development of anti-cancer drugs in modern medicine [1]. Wuzhuyu (*Fructus Euodiae*) has the functions of dispersing cold and stopping pain, warming and stopping vomiting, helping Yang and stopping diarrhea. According to the ancient book of Chinese medicine "*Compendium of Materia Medica*" (1552–1578), "*Fructus Euodiae*, heat energy disperses heat temperature, bitter heat energy dryness can strengthen"; "*Shennong's Classic of Materia Medica*" (25–220 C.E.) also describes: "Warming the middle and lower Qi, pain relief, dehumidifying blood bi, driving wind evil, open Cou rationale, cough against cold and heat", from the performance and efficacy of *Fructus Euodiae* anti-tumor mechanism is described. Evodiamine (EVO) is the main alkaloid in EVO. EVO also has many pharmacological effects, including anti-inflammatory, analgesic and blood pressure lowering, etc. In addition, experimental studies have found that EVO also has some preventive and therapeutic effects on lung cancer, gastric cancer, cervical cancer and other malignant tumors [2–5]. EVO has a wide range of anti-tumor mechanisms, including inducing apoptosis of tumor cells, inducing programmed death of tumor cells, inhibiting proliferation/migration/invasion of tumor cells, and inhibiting proliferation of tumor stem cells. In this review, the mechanism of action and drug use of EVO against tumors in recent years were reviewed.

EVO induces apoptosis of tumor cells

Induce tumor cell apoptosis through internal pathway

Uncontrolled apoptosis is an important cause of tumorigenesis, and apoptosis mainly occurs through two main pathways, endogenous and exogenous, and the two pathways have different initial signals [6]. It has been shown that EVO can induce tumor cell apoptosis through the above two pathways.

Imbalance of oxidative stress and calcium homeostasis. Yang et al. showed that EVO can lead to a rapid increase in intracellular ROS and induce apoptosis of human melanoma cells A375-S2 [7]. Jiang et al. have found that EVO can exhibit oxidase-like catalytic activity and has a significant inhibitory effect on oral squamous cell carcinoma [8]. Liu et al. found in an experiment that blocking er calcium channels significantly reduced EVO-induced intracellular Ca^{2+} elevation, suggesting that EVO can induce Ca^{2+} mediated internal apoptosis pathway [9]. Another study also showed that intracellular Ca^{2+} chelating agents significantly improved EVO induced cytotoxicity, confirming that EVO induces cell death through Ca^{2+} overload [10].

Mitochondrial damage. Mitochondria, as the key part of apoptosis regulation, contain apoptosis inducing factor (AIF), cytochrome-C (Cyt-c) and mitochondrial permeability transition pore play key roles in cell apoptosis. It activates cysteinyl aspartate-specific proteinase (Caspase) containing cysteine by releasing apoptotic enzyme activator, which leads to endogenous apoptosis. As early as 2006, Lee et al. found that EVO induced apoptosis of leukemia U937 cells was mediated by AIF [11]. Fang et al. showed that EVO had cytotoxic effects on multiple myeloma U266 and RPMI8226 cells [12]. The expressions of Caspase-3, Caspase-9, BAX and cytoplasmic Cyt-C were significantly increased and the expressions of Bcl-2 and Mito-Cyt-C were decreased in U266 and RPMI8226 cells treated with EVO at different concentrations for 24h, while the expressions of Caspase-8 were not significantly changed. These results indicated that EVO induced apoptosis of U266 and RPMI8226 cells through mitochondrial induction pathway. Liu et al. confirmed that EVO can induce the increased expression of caspase-3, caspase-9 and Poly ADP-ribose Polymerase 1 in human melanoma A-375 cells [13]. Mitochondrial membrane potential (MMP) dissipated in a time-dependent manner, suggesting that EVO induced apoptosis of A-375 cells through the Mitochondrial pathway.

ER stress. Protein kinase-like endoplasmic reticulum kinase (PERK) is a sensor of ER stress. Stressed or misfolded proteins accumulate to a certain extent, it may induce the unfolded protein response (UPR), which may further initiate apoptosis of the stressed protein. When there are a lot of unfolded proteins in the cell, GRP78, which is closely bound to the PERK, dissociates and further binds to the unfolded protein, thus revealing the PERK. The mutual aggregation with PERK increases the phosphorylation of the protein. The phosphorylation of eukaryotic initiation factor 2α (eIF2α) was also catalyzed. Studies showed that EVO inhibited the activity of human ovarian cancer cells A2780, A2780CP, ES-2 and SKOV-3, and phosphorylated eIF2α (P-EIF2α) and phosphorylated PERK (P-perk) were detected in EVO treated cells [14]. These results suggested that EVO induced tumor cell apoptosis may be related to er stress. Wu et al. found that EVO significantly increased P-PERK at Thr980 when applied to human renal cell carcinoma cell A498, and PERK inhibitor GSK2606414 significantly inhibited EVO-induced apoptosis [15].

Induce tumor cell apoptosis by extracellular signal

Death receptor family cells all contain death domains (Fas/CD95, DR5, DR4, DR3 and TNFRl). Apoptosis is induced by the formation of death inducing signal complex. Zhang et al. showed that EVO can increase the expression level of DR5 in HUMAN hepatocellular carcinoma cells Huh7 and reduce the resistance of tumor cells to TRAIL, thus inducing apoptosis [16]. Khan et al. found that EVO can increase the expression of death receptors DR4 and DR5 in glioblastoma U87 cell line [17]. The apoptotic rate of tumor cells increased after synergistic action with TRAIL. Mohan et al. showed that EVO can induce high expression of DR5 in human lung cancer CELLS A549 and H1299, and promote cell apoptosis together with endogenous pathways such as CytC [18].

Induce apoptosis of tumor cells through other signaling pathways

PI3K signaling pathway. Dysregulation of PI3K signaling pathway plays a significant role in the occurrence and progression of tumors. Abnormally activated PI3K signaling pathway can promote normal cell variation, tumor cell migration and metastasis, etc. AKT is an important downstream protein kinase of the PI3K signaling pathway. Yang et al. found that EVO can down-regulate the expression of p-Akt and Bcl-2 proteins in hepatocellular carcinoma HepG2 cells, leading to apoptosis of hepatocellular carcinoma cells [19]. Qidi et al. also showed that EVO can significantly antagonize the activity of SMALL cell lung cancer H1688 and H446 cells, effectively down-regulate the activity of PI3K/AKT signal transduction pathway in SMALL cell lung cancer H1688 and H446 cells, and reduce the phosphorylation level of AKT protein to induce apoptosis [20].

Mitogen-activated protein kinase (MAPK) signaling pathway. MAPKs mainly include P38MAPK, extra Cellular signal-regulated kinase 1/2 (ERK 1/2) and c-Jun n-terminal kinase (JNK) three kinases, each of which has a pathway that is closely associated with uncontrolled cell growth. Du et al. proved that in human breast cancer cell MDA-MB-231, ERK inhibitor PD98059 or P38 MAPK inhibitor SB203580 combined with EVO can enhance cell apoptosis [21]. Wu et al. also found that EVO can increase the phosphorylation of JNK protein and induce apoptosis of human glioma cells U87 and C6 [22].

NF-κB signaling pathway. NF-κB signaling pathway can inhibit apoptosis, and long-term activation of NF-κB signaling pathway can lead to loss of inhibition of cell growth, leading to the development of tumor. Bcl-2 is an anti-apoptotic protein located downstream of NF-κB signaling pathway. Activation of NF-κB-P65 upregulates the expression of bcl-2 protein, thereby controlling apoptosis. Li et al. found that EVO combined with verofinil (PLX4032) significantly down-regulated the protein levels of P-Akt, P-NF-κB-p65 and Bcl-2 in melanoma A375 cells, thereby inducing apoptosis of melanoma cells [23]. Sui et al. found that EVO can inhibit the growth of HUMAN colon cancer oxaliplatin resistant HCT-116/L-OHP cells [24]. EVO induces apoptosis mainly by inhibiting phosphorylation of NF-κB pathway, especially p50/ P65. Hwang et al. concluded that EVO can exert a variety of tumor suppressive effects by blocking PI3K/Akt, MAPK, C-Met and NF-κB signaling pathways, leading to apoptosis of PROSTATE cancer

cells PC-3 and DU145 [25].

Induce apoptosis of tumor cells by other target proteins

DNA topoisomerase. DNA topoisomerase, a ribozyme, is a target of many anticancer drugs. Among them, Topoisomerase I (Top I) is an important target of antitumor drugs. Chan et al. proved that EVO can act as an inhibitor of Top I, leading to DNA replication and transcription disorders by stabilizing the process of DNA strand breaking and reconnection [26]. Pan et al. studied the effects of EVO on the anti-proliferative activity of human leukemia cells K562, THP-1, CCRF-CEM and CCRF-CEM/C1, as well as the inhibitory mechanism of Topo I and Topo II [27]. Their results showed that EVO is a dual catalytic inhibitor of Topo I and Topo II with IC50 of 60.74μmmol/L and 78.81μmmol/L, respectively.

Heat shock protein. The Heat shock protein (HSP) system plays an important role in preserving the expression and function of many oncoproteins. Hyun et al. showed that EVO reduced the diversity, volume and weight of lung tumors in both in vivo and in vitro models [28]. EVO disrupts the HSP system by binding n-terminal ATP and can be used as an effective HSP70-targeted anticancer drug.

Transient receptor potential vanilloid 1. Transient receptor potential vanilloid 1 (TRPV1) is a new type of ion channel, which plays a key role in cell function and signaling pathway transmission. Liu et al. showed that EVO induces ROS-dependent cytotoxicity through the TRPV1/Ca^{2+} pathway, which then leads to apoptosis of BGC-823 gastric cancer cells, and the overexpression of exogenous TRPV1 can enhance EVO-induced cytotoxicity [10].

EVO induces autophagy in tumor cells

Autophagy refers to the process of phagocytosis, degradation and reuse of unnecessary or damaged cellular structures, and is an adaptive response to stress. Loss of autophagy destroys double-stranded DNA and activates proto-oncogenes, leading to the occurrence of new cancers. Activation of autophagy mechanism can induce anti-tumor inflammatory response and control tumor metastasis. Recent studies have shown that a variety of Chinese herbal medicines can play an anti-tumor role by activating autophagy [29]. Beclin-1 is an important autophagy protein, and its loss can promote the generation of tumors. In addition, many tumor suppressor genes, such as P53 and PTEN, have been proved to control tumor growth and migration by regulating intracellular autophagy. At present, the number of intracellular autophagosomes and LC3-II can be used as important indicators of autophagy activity. Wang et al. observed that EVO up-regulated the expression of LC3-II and Beclin-1 while inducing apoptosis of colon cancer cell SW480 [30]. Electron microscopy showed that the number of autophagosomes in SW480 cells was correlated with EVO concentration. Lv et al. also found that EVO could control the growth of HCT-116 cells, increase the production of ROS, activate AMPK signaling pathway and cause autophagy [31]. In addition, the study showed that EVO-induced autophagy and apoptosis can complement each other.

EVO inhibits tumor stem cells

Cancer stem cells (CSC) are self-renewing cells in the tumor cell population, which may play an important role in the occurrence, progression, metastasis and recurrence of tumors [32]. By analyzing the expression of 84 CSC-related genes and 24 CSC genes in two colon cancer cell lines CSC, Dr. Astrologer Kim et al. found that EVO inhibits WNT and NOTCH genes to control key CSC signaling pathways [33]. Su et al. suggest that EVO is a novel NOTCH3 methylation stimulator that inhibits CSC self-renewal through the Hippo signaling pathway and significantly inhibits lung cancer in vivo and out of the astrologer [34]. Zhao et al. also found that EVO affects Hippo-YAP signal transduction in vitro and exerts anti-hepatocellular carcinoma activity [35].

EVO inhibits tumor cell proliferation and migration

Tumor cell proliferation maintains the vitality of tumor cell population, and tumor cell migration causes tumor cell movement through signal transduction, invasion of surrounding tissues and access to blood vessels, and then spread to distant tissues. Tumor proliferation and apoptosis signaling pathways are basically consistent, such as MAPK signaling pathway, PI3K signaling pathway, NF-κB signaling pathway, etc. In addition, Notch pathway, SHH/GLI1 signaling pathway, Wnt/β-catenin pathway and JAK/STAT pathway are also involved in the Xia et al. found that EVO can target non-small cell lung cancer by inhibiting gamma-secretase and thus Notch receptor activation (e.g., Notch3) [36]. Li et al. showed that EVO significantly inhibited A549 cell proliferation and decreased the activity of AKT-κB and SHH/GLI1 signaling pathways [37]. Yang et al. found that EVO inhibits cell growth of human osteosarcoma cells in a dose-dependent manner by inhibiting the Wnt/β-catenin signaling pathway [38]. Hu et al. believed that EVO plays an anti-tumor role by inhibiting β-catenin activity, which is mainly realized by WW domain-containing oxidoreductase pathway [39]. Professor Yang Jie mentioned in 2012 that EVO is an inhibitor of STAT3 signaling pathway, which can inhibit STAT3 phosphorylation in HepG2 cells of liver cancer and block the STAT3 signaling pathway [40]. Zhao L C et al. showed that the antitumor activity of EVO on HUMAN colon cancer HCT-116 cells can be exerted by inhibiting the activation of JAK2/STAT3 signaling pathway [41]. Li et al. also proposed that EVO inhibits the proliferation of HUMAN colon cancer HCT-116 cells by inhibiting sirT1-mediated activation of NF-κB/P65 signaling pathway, and inhibits its vitro migration by reducing the expression of MMP-9 [42]. EVO can eliminate the activation of c-Met/Src/STAT3 signaling axis, and has a strong inhibitory effect on the survival, proliferation and angiogenesis of PROSTATE cancer PC-3 and DU145 cells [25]. In addition, studies at home and abroad have also shown that EVO can inhibit the proliferation of mouse lymphoma, human osteosarcoma HOS and U2OS cells, epithelial ovarian cancer cells A2780/WT and A2780/PTXR, lung A549 cells [28, 43– 46]. Inhibit the growth and apoptosis of HCT-116/L-OHP cells from colorectal cancer, inhibit the proliferation of BGC-823 cells and improve their sensitivity to radiotherapy, and inhibit the proliferation of HUMAN pancreatic cancer cells PAN-1 and SW1990 [47–49]. Inhibit the activity of cytotoxic thyroid cancer cells TPC-1 and SW1736 [50].

EVO inhibits peritumoral angiogenesis

Tumor cells rely on the metabolic conditions provided by tumor blood vessels for continuous proliferation and growth, and utilize the structural defects of high permeability of blood vessels in tumor tissues for metastasis. Inhibition of peritumor vascular hyperplasia and alteration of tumor microenvironment is an indispensable part of tumor therapy. Vasculogenic mimicry (VM) is a newly discovered tumor angiogenesis model, which is prevalent in various highly invasive tumors. The process of tumor induction of angiogenesis in vitro is similar to the formation of human umbilical vein endothelial cells (UVECs) tubes. An early study showed that EVO can directly inhibit the formation of HUVECs tubes in vitro, showing angiogenic mimicry, while EVO inhibits cell migration in vitro [51]. EVO also inhibited tumor VM formation in subcutaneous xenograft models. Shi et al. found that EVO inhibited tumor growth, various biomarkers of angiogenesis (such as CD31, CD34, β-catenin) and vascular endothelial growth factor VEGFa in hepatocellular carcinoma SMMC-7721 xenotransplantation model [52].

EVO blocks the cell cycle of tumor cells

The whole process that a cell goes through from the completion of one division to the end of the next is called the cell cycle. The cell cycle consists of early DNA synthesis (G1 phase), S phase, late DNA synthesis (G2 phase), and M phase. Abnormal operation of cell cycle is the core link of malignant growth of tumor cells. Cyclin, cyclin-dependent kinases (CDKs) and other factors all affect normal cell cycle operation. Sun et al. found that EVO can inhibit cyclin D1 expression and block cell cycle of leukemia K562 cells through peroxisome proliferators-Activated receptor γ signaling pathway [53]. The proportion of G2/M phase cells was significantly increased. Chen H et al. found that with the extension of EVO action time and the increase of drug concentration, the cell ratio of G2/M phase in pancreatic cancer SW1990 cells gradually increased [54]. Zhao et al. found that EVO can cause bel-7402 arrest in G2/M phase and inhibit tumor growth [35]. Ge et al. also demonstrated that EVO delayed cell cycle progression in a dose-dependent manner by inhibiting cyclin and CDKs expression [55].

Recent progress of EVO antitumor drugs

Derivatives and new dosage forms

Zhao et al. designed a series of EVO derivatives and evaluated the cytotoxicity of three human tumor cell lines (BEL-7402, A549 and BGC-823) and normal human liver cell L-02 [56]. Among them, nitrate derivatives 11A and 11B showed moderate activity, while furan-based derivatives 13A-C, 14A and 14B showed good activity. 13C showed good cytotoxic selectivity between tumor cells and normal liver cells. Fan et al. designed and synthesized 15 EVO derivatives with inhibitory effect on liver cancer cell lines [57]. Among them, compound 8 had a good effect in vitro, and had a good inhibitory effect on Topo I. By inducing G2/M block and apoptosis, it could significantly reduce the metastasis and invasion of HCC cells. In vivo studies showed that compound 8 significantly reduced tumor volume and weight (TGI=40.53%). Qiu et al. combined EVO with HP-β-CD to improve drug solubility by preparing EVO/ HYDROXY propyl-β -cyclodextrin (EVO/HP-β-CD) [58]. Cell viability evaluation showed that EVO and EVO/HP-β-CD had 8.516 and 0.977μM semi-inhibitory concentrations on HepG2 cells, respectively. Analysis of Caspase-3 enzyme activity and Annexin V/PI double staining showed that EVO/HP-β-CD had better anti-tumor activity than EVO, which was more likely to induce apoptosis of HepG2 cells. Jiang et al. used two derivatives of EVO: The antitumor activity of 10-Nitro Evodiamine derivatives (END) and 10-Amino Evodiamine derivatives on small cell lung cancer cell line H1688 was detected [59]. The results showed that END had better antitumor effect in vitro. Wei et al. have integrated EVO and Indocyanine Green (ICG) into nanoliposomes, which not only functions as anti-tumor chemotherapy agents, but also can be used as a contrast agent for positron emission tomography/computed tomography imaging [60]. EVO/ICG nanoliposomes also showed peroxidase-like catalytic activity, which significantly inhibited the proliferation of cancer cells and eventually induced apoptosis of tumor cells by penetrating into tumors and converting endogenous H2O2 into cytotoxic reactive oxygen species.

EVO combination

At present, mainstream research believes that the combination of Chinese and Western medicine has the effect of enhancing efficacy and reducing toxicity. As an extract of traditional Chinese medicine, EVO can effectively increase the anti-tumor efficacy when combined with a variety of anticancer drugs. The combination of EVO and Bortezomib can enhance the anticancer effect of multiple myeloma MM cells [12]. The combination of EVO and Erlotinib significantly increased the apoptosis rate of NSCLC cells, and the combination efficiency was higher than that of single treatment [61]. Combined treatment with EVO and berberine (Ber) significantly reduced the level of Mir-429, a malignant tumor marker, in colorectal cancer [62]. When EVO is co-applied with chloroquine, it has a more significant inhibitory effect on the cell activity and tumor angiogenesis of hepatocellular carcinoma HepG2 [63].

Conclusion and future perspective

EVO can induce the apoptosis of tumor cells through the Caspase pathway and non-caspase-dependent pathways through the exogenous death receptor pathway, classical pathways such as endogenous mitochondria and endoplasmic reticulum, as well as a variety of signaling pathways. TRPV1, Top I and HSP70 are all precise target proteins discovered in recent years. Intracellular Ca^{2+} levels and ROS levels are altered, leading to cell death. EVO can also act on tumor stem cells and inhibit tumor cell proliferation. Furthermore, EVO can also block tumor cell cycle, inhibit tumor cell angiogenesis, reduce their migration, and induce autophagy. Significantly, the synergistic and attenuating effect of the new model is an important basis for its potential use as a clinical antitumor drug.

To sum up, EVO has obvious control effect on the tumor, in recent years in inhibiting tumor cell proliferation, blocking the cell cycle regulation of signaling pathways of antitumor mechanism has also been a certain degree of research, especially for the regulation of tumor cell signaling pathways, will provide a wider range of tumor targeting therapy research and application of space.

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