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Review Article

Anti-tumor Effects of Triptonide Derived from Chinese Herb Tripterygium Wilfordii Hook F

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Abstract: Tripterygium wilfordii Hook F (TwHF), a traditional Chinese herb, has been used to treat autoimmune and inflammatory diseases for centuries. In the past decades, anti-tumor effects of two key ingredients of TwHF, triptolide and celastrol, have been reported in numerous research studies and review articles. However, the importance of triptonide (another important ingredient of TwHF) in cancer therapy has not been recognized until recent years. In the article, we review recent advances of triptonide as an antitumor agent with a focus on its biological activities, action mechanisms and clinical development.

Keywords: Triptonide, anti-tumor, cancer, Tripterygium wilfordii Hook F, mechanisms.

Introduction

Tripterygium wilfordii Hook F. (TWHF), also known as Thunder God Vine or Lei gong teng, is a traditional Chinese herb that has been widely used to treat autoimmune and inflammatory diseases for centuries. Over years, hundreds of natural compounds have been identified from TWHF [1, 2]. Among those natural compounds, triptolide and celastrol have been extensively studied for decades, and their therapeutics have been investigated in rheumatoid arthritis, systemic lupus erythematosus, neurodegenerative diseases and tumors [3-9]. In contrast, studies of triptonide, another important natural compound isolated from TWHF, were only reported in recent years. Triptonide have been shown to inhibit cell growth of multiple cancer types including prostate cancer, pancreatic cancer, melanoma, myeloid leukemia, lymphoma, gastric cancer, lung cancer, nasopharyngeal carcinoma, prostate cancer, breast cancer, osteosarcoma and etc [10-20]. Here, we review and discuss the potential therapeutics of triptonide for tumors with a focus on its action mechanisms (**Figure 1**).

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Molecular Mechanisms of triptonide as an anti-cancer agent

By targeting Epithelial to Mesenchymal transition (EMT)

EMT is a process by which epithelial cells lose their cell apical-basal polarity and adherent tight junctions, and acquire migratory and invasive mesenchymal cell properties. Physiologic EMT is a crucial process during embryonic development and adult tissue regeneration [21, 22], while abnormal pathological EMT plays an important role in tumor progression [23].

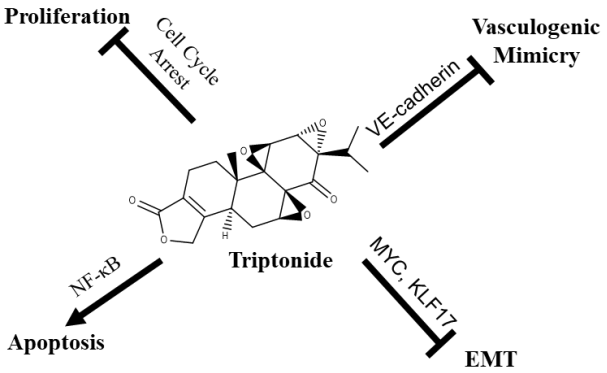

Figure 1. Mechanisms of triptonide as an anti-cancer agent

Triple-negative breast cancer (TNBC) is characterized by lacking expression of estrogen receptor, progesterone receptor and human epidermal

growth factor receptor 2, and currently no targeted therapies are available for this type of cancer [24]. Very recently, Gao et al. has reported that triptonide inhibits human TNBC cell growth *in vitro* and *in vivo* in xenograft mammary tumor model [11]. MYC and ITGA6 genes are positive regulators of EMT and KLF17 gene is a negative regulator of EMT. The authors further showed that triptonide treatment decreases expression of MYC and ITGA6 genes while increases expression KLF17 gene, which are well in line with EMT inhibition by triptonide treatment in TNBC cells. However, SNAI1, an important transcript factor in EMT, is upregulated in triptonide treated TNBC cells, which appears to be paradoxical. To address this question, the authors generated triptonide-resistant HCC1806 cells which show markedly higher expression of SNAI1 compared with the parental cells, and knockdown of SNAI1 in the triptonide-resistant HCC1806 cells restores the cell sensitivity to triptonide, suggesting that a SNAI1 feedback mechanism may lead to acquired resistance to triptonide and targeting SANI1 pathway overcomes the acquired resistance.

Tumor-associated fibroblasts, which display different gene expression profiles and functions from normal fibroblasts, provide microenvironment to support cancer progression, and targeting cancer-associated fibroblasts to destroy cancer microenvironment is a therapeutic strategy to treat cancer [25, 26]. In 2017, Wang and colleagues reported that triptonide effectively inhibits the colony formation-, migration-, and invasion-promoting capacities of gastric cancer-associated fibroblasts through regulating microRNA-301a and microRNA-149 expression. This microRNA expression re-establishment increases the tissue inhibitor of metalloproteinase 2 and decreases IL-6 production in gastric cancer-associated fibroblasts, resulting in EMT prevention in gastric cancer cells [19]. Therefore, triptonide holds promise as anti-tumor agent to treat gastric cancer by targeting cancer-associated fibroblasts.

By targeting tumor cell apoptosis signaling

Apoptosis evasion is also a hallmark of cancer, which allows cancer cells to live longer with more time for mutation accumulation for cancer progression, therefore inducing cancer cell apoptosis is a common mechanism for anti-cancer therapy [27]. Several studies have reported that triptonide promotes apoptosis in gastric cancer, prostate cancer, acute myeloid leukemia and osteosarcoma [12-15].

In contrast to the study that triptonide inhibits gastric cancer growth by indirectly targeting the gastric cancer-associated fibroblasts [19], Xiang et al discovered that triptonide can also directly targets gastric cancer cells to inhibit their migration, invasion and proliferation *in vitro* and tumor growth and metastasis in xenograft mice without obvious toxicity [14]. Their molecular mechanistic studies have demonstrated that triptonide dramatically reduced Notch1 protein levels in gastric cancer via the ubiquitin-proteasome pathway, which leads to inhibition of the downstream nuclear factor-kappa B (NF-κB) activation to induce apoptosis of gastric cancer cells. In summary, triptonide may exert its functions to suppress gastric cancer progression via two ways collectively: indirectly targeting gastric cancer-associated fibroblasts to inhibit EMT and directly targeting gastric cancer cells via NF-κB signaling for apoptosis.

Endoplasmic reticulum (ER) stress caused by reactive oxygen species (ROS) can induce unfolded protein response (UPR) to prevent cancer cell apoptosis [28]. Upon sensing ER stress, glucose regulated protein 78 (GRP78) activates UPR process resulting in upregulation of PKR-like ER kinase (PERK). PERK oligomerizes and phosphorylates itself and the eukaryotic initiation factor-2α, resulting in an increased translation of activating transcription factor 4 (ATF4) to alleviate ER stress [29]. Transient PERK activation is protective; however, chronic ER stress and sustained PERK activation eventually results in apoptosis with up-regulation of characteristically pro-apoptotic proteins including C/EBP homologous transcription factor protein (CHOP) and ER-associated caspase 12 [30]. Zheng et al. reported that triptonide inhibits osteosarcoma cell growth through the oxidative driven ER stress-mediated apoptosis. In the study, triptonide treatment increases intracellular ROS level leading to ER stress, upregulate PERK, GRP78, ATF4 and CHOP in in the human osteosarcoma cells indicating that triptonide induces oxidative driven ER stress-mediated apoptosis. This result is further supported by the experiments that siRNA knockdown of CHOP and ER stress responsive pharmacological inhibitor azoramide can abolish apoptotic effect of triptonide [13].

The hedgehog (Hh) pathway abnormal activation plays a critical role in cancer and inhibition of Hh signaling to induce apoptosis has been used to treat many types of cancer including leukemia [31-34]. Acute myeloid leukemia (AML) harboring internal tandem duplication of FMS-like tyrosine kinase 3 (FLT3) is highly aggressive with poor prognosis. Xu et al. demonstrated that triptonide can efficiently inhibit FLT3-driven AML *in vitro* and *in vivo* [12]. They found that triptonide targeted Hedgehog signaling by inhibiting its critical effectors such as GLI2, c-Myc and FLT3, resulting in apoptosis of FLT3-driven AML cells [12].

The tyrosine kinase Lyn is implicated in initiation

and malignant progression of many types of cancer including prostate, glioblastoma, colon, breast cancer and lymphoma, and Lyn has recently become an anticancer drug target [35-39]. Yang and colleagues showed that triptonide potently inhibits the proliferation of human B-lymphoma Raji and T-lymphoma Jurkat cells *in vitro*, nearly completely inhibits the lymphoma growth in a xenografted mouse model without apparent side effects[16]. Further study indicates that triptonide promoted apoptosis through activation of PARP and caspase 3 as well as reduction of BCL2 level in the lymphoma cells[16].

By targeting cell cycle arrest to inhibit tumor cell proliferation

Cell proliferation occurs through the four phases of cell cycle, and each phase is finely coordinated by cell-cycle regulators to ensure genomic integrity [40]. However, gene mutations along with defects in cell cycle checkpoints may lead to uncontrolled cell proliferation and eventually cancer. Therefore, it has become a clinical strategy to inhibit cancer cell proliferation via the cell-cycle arrest by inhibitors that specifically targeting key proteins during cell-cycle progression [41]. In mammalian cells, CDK3 and p38 are required for the cell cycle G0/G1-S transition and p38 activation causes cell cycle arrest at G2 phase [42-44]. Zhang et al. showed that triptonide can activate p38 and inhibits CDK3 expression in pancreatic cancer cells, which induce cell cycle arrest by hitting the multiple phases of cell cycle, namely cancer cell cycle arrest at G2/M phase and G0/G1 [16]. In a prostate cancer study, Dong et al. has demonstrated that triptonide effectively inhibits the proliferation of human prostate cancer cells *in vitro* and prostate cancer growth *in vivo* at least partially through the cancer cell G2/M phase of cell cycle arrest [15].

By targeting cancer vasculogenic mimicry

Different from angiogenesis, vasculogenic mimicry is the formation of microvascular channels without endothelial cells by aggressive, metastatic and genetically deregulated tumor cells, and it is associated with poor survival in cancer patients [45, 46]. Therefore, tumor vasculogenic mimicry is a target for anti-cancer drug discovery. Han et al. showed that triptonide effectively inhibited pancreatic cancer cell-formed capillary-like structures *in vitro* and blood vessels *in vivo* [18]. VE-cadherin is a master gene involved in vasculogenic mimicry, and chemokine C-X-C motif ligand 1/2 (CXCL1/2) secretion at metastatic sites can promote the establishment of a metastatic niche [47]. Triptolide was shown to effectively blocked pancreatic cancer cell migration, invasion, and vasculogenic mimicry by inhibition of VE-cadherin and CXCL2 gene expression[18]. Most importantly, triptonide can achieve this outcome at concentration as low as 2.5nM, which is over 400-fold potent in comparison to all the other reported small molecules [48, 49].

By targeting other mechanisms

Abnormal activation of Hedgehog pathway is involved in lung cancer progression and metastasis [50-52], and recent studies have shown that Sonic Hedgehog pathway plays an important role in regulating the self-renewal of cancer stem cells (CSCs) which have been believed to be responsible for tumor initiation, growth, and recurrence [53-57]. Zhang et al. examined triptonide anti-lung cancer effect and found that triptonide at very low concentrations of 5-10nM remarkedly suppressed cell proliferation and colony formation of lung cancer cells [58]. In addition, they showed that triptonide effectively disrupted the lung cancer cell sphere formation and reduced the stemness and tumorigenicity *in vitro*. In a xenograft mouse model, administration of triptonide significantly inhibited the lung tumor growth with low toxicity. Further molecular mechanistic studies revealed that triptonide inhibits the Sonic Hedgehog signaling by repressing Gli1 gene promoter activity, resulting in downregulation of Gli1, an important hedgehog signaling effector. In addition, the authors showed that triptonide inhibited self-renewal of lung cancer CSCs by blocking the Shh signaling pathway, a potential way to eradicate CSCs in lung cancer [58].

Recently, Tan et al. reported that triptonide robustly suppressed melanoma cell tumorigenicity, migration, and invasion, and markedly reduced tumor growth and melanoma lung metastasis in tumor-bearing mice with low toxicity [10]. In addition, they revealed that triptonide increased tumor suppressors Salvador homolog-1 and large tumor suppressor 1 (LATS1) expression, to activate the tumor-suppressive Hippo pathway. The activated Hippo pathway leads to degradation of oncogenic YAP via the lysosomal pathway, and reduction of tumorigenic microphthalmia-associated transcription factor in melanoma cells [10]. These findings suggest that triptonide may be a novel therapy to treat metastatic melanoma.

Conclusion

Triptonide is a key ingredient of TWHF, and its anti-tumor effects have not been recognized till recent years. Triptonide inhibits cell growth of multiple tumor types including prostate cancer, pancreatic cancer, melanoma, myeloid leukemia, lymphoma, gastric

cancer, lung cancer, nasopharyngeal carcinoma, prostate cancer, breast cancer, osteosarcoma and etc [10-20]. Triptonide exerts it anti-tumor effects via various mechanisms including EMT, apoptosis signaling, cell cycle arrest, cancer vasculogenic mimicry and so on (Figure 1). Though triptonide has been shown to be an effective therapeutic agent to multiple tumors *in vitro* and *in vivo*, to date, no human clinical trials have been conducted for triptonide to treat tumor. However, in comparison to triptolide, triptonide displays the markedly lower toxicity and higher potency *in vivo*, suggesting that triptonide may hold a promise as an effective anti-tumor agent in clinic [59].

Conflicts of interest

The author declares that there are no conflicts of interest.

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