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Review

Broad targeting of triptolide to resistance and sensitization for cancer therapy

Zhen-yan Hou[a,b], Xiao-pei Tong[a,b], Yong-bo Peng[c], Bi-kui Zhang[a,b,*], Miao Yan[a,b,*]

[a] Department of Pharmacy, The Second Xiangya Hospital, Central South University, Changsha 410011, China
[b] Institute of Clinical Pharmacy, Central South University, Changsha 410011, China
[c] Molecular Science and Biomedicine Laboratory, College of Life Sciences, State Key Laboratory for Chemo/Bio-Sensing and Chemometrics, Hunan University, Changsha 410082, China

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ABSTRACT

Cancer cell resistance to current anticancer therapeutics as well as the side effects are still obstacles to successful cancer therapy. Hence, the development of novel anticancer agents or therapeutics is of vital significance, and especially rational adjuvant therapies containing low-cost natural products with multiple targets have attracted great interests. Triptolide, the main biocomponent of *Tripterygium wilfordii* Hook F, is restricted in clinical applications mainly due to its severe systemic toxicities, although it has shown strong antitumor activities in preclinical studies. Mounting evidence suggests that triptolide at low doses as an adjuvant therapeutic agent circumvents resistance to current anticancer therapies, enhances the anticancer effectiveness, and relieves toxicities of both triptolide and anticancer therapies. Furthermore, several unique antitumor targets of triptolide make it superior to other therapeutics. The molecular mechanisms of triptolide-induced anti-resistance and sensitization effects include changes in ATP-binding cassette transporters, induction of apoptosis pathways, increase in tumor suppressors and decrease in oncogenic factors, and interactions with the RNA polymerase II complex; targeting cancer stem cells and tumor-microenvironment-mediated resistance are also involved. Besides, some synthetic derivatives and novel delivery systems of triptolide are also developed to enhance the water-solubility and reduce the toxicity, which will also be discussed.

1. Introduction

Although the cancer death rate was declined by approximately 1.5% annually from 2005 to 2014, cancer is still the second cause of death, accounting for 23% of deaths in the United States in 2014 according to the US Final Mortality Data [1]. Among the causes behind cancer treatment failure, resistance of malignant cells to current cancer therapeutics ranks the top, leading to tumor metastasis and relapse. Both intrinsic resistance resulting from genetic characteristics of cancer cells and acquired resistance from exposure to cancer therapies make malignancies refractory and challenging [2,3]. In addition, severe side effects and high costs of current cancer therapies hamper their clinical applications [4–6]. Thus, the development of novel anticancer drugs or therapeutics is urgently needed.

As the phenotype of malignant cell resistance to cancer therapies is a comprehensive result of multiple complex cellular process, targeting

Abbreviations: ABC, ATP-binding cassette; AKT, V-Akt murine thymoma viral oncogene homolog; ALDH1, aldehyde dehydrogenase 1; AR, androgen receptor; Bad, Bcl-2-associated death promoter; Bak, Bcl-2 homologous antagonist killer; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma; Bcl-xL, Bcl-extra large; Bcl-xs, Bcl-extra short; BCRP, breast cancer resistance protein; Bid, BH3 interacting domain death agonist; Bik, Bcl-2-interacting killer; BNIP3, Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3; CAF, cancer-associated fibroblast; CAIX, carbonic anhydrase IX; cFLIP, cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein; ChIP, chromatin immunoprecipitation; CI, Combination Index; cIAP1/2, cellular IAP1/2; CML, chronic myelogenous leukemia; CSC, cancer stem cell; DISC, death-inducing signaling complex; DR, death receptor; DRI, Dose Reduction Index; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; EpCAM, epithelial cell adhesion molecule; GLUT, glucose transporter; GSK3β, glycogen synthase kinase-3β; HIF-1, hypoxia inducible factor-1; HSF1, heat shock factor 1; HSP, heat shock protein; IAP, inhibitor of apoptosis protein; JNK, *c-JUN* N-terminal kinase; MAPK, mitogen-activated protein kinase; Mcl-1, myeloid leukemia cell differentiation protein; MDM2, murine double minute 2; MDR, multidrug resistance; MKP, MAPK phosphatase; MRP1, multidrug resistance protein 1; NF-κB, nuclear factor-kappaB; pAkt, phosphor-Akt; PARP, poly ADP-ribose polymerase; P-gp, P-glycoprotein; PI3K-PTEN, phosphatidylinositol 3-kinase-phosphatase and tensin homolog; PKC, phosphorylated at Ser^9 by protein kinase C; pp2A, protein phosphatase 2A; REST, repressor element-1 silencing transcription factor; SAR, structure-activity relationship; TAM, tumor-associated macrophage; TFIIH, transcription factor IIH; TKI, tyrosine kinase inhibitor; TM, tumor-microenvironment; TRAIL/Apo-2L, TNF-related apoptosis-inducing ligand; tRXRα, the N-terminal truncated form of the nuclear retinoid X receptor-α; TWHF, *Tripterygium wilfordii* Hook F; VEGF, vascular endothelial growth factor; XIAP, X-linked IAP; XPB, xeroderma pigmentosum group B protein

* Corresponding authors at: Department of Pharmacy, The Second Xiangya Hospital, Institute of Clinical Pharmacy, Central South University, Changsha, 410011, China.
E-mail addresses: 505995@csu.edu.cn (B.-k. Zhang), yanmiao@csu.edu.cn (M. Yan).

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only one contributor to resistance is rarely effective to combat all kinds of mechanisms behind the cellular resistance [7], especially when specific mechanisms exist [8]. Nowadays, natural products have attracted much attention of researchers because of their multi-target characteristics and their ability to bind to specific cellular targets [9]. Particularly, combining natural products with traditional cancer therapeutics to reverse resistance and sensitize cancer cells are under consideration, in order to enhance cell-killing mechanisms as well as decrease the likelihood of toxicity by decreasing the drug or radiation doses [10].

Triptolide, the main biocomponent of traditional Chinese herb *Tripterygium wilfordii* Hook F (TWHF, thunder god vine, or Lei Gong Teng), has shown strong antitumor activities as well as severe systemic toxicities [11]. Several preclinical screening studies indicate that this low-cost natural product is more effective than other anticancer agents, showing strong anticancer activities at nanomolar concentrations even in highly resistant malignant cells [12–14]. The action mechanism of triptolide is distinct from that of most conventional anticancer agents [14], making triptolide an promising sensitizer to circumvent drug resistance. Considering the dose-dependent toxicity of triptolide, combination of low-dose triptolide with other anticancer therapeutics is a good choice to improve the antitumor activities and reduce the toxicity of every single drug [15], but the action mechanisms remain unclear. Furthermore, with regard to the water-insolubility and high cytotoxicity of triptolide [16], a number of triptolide derivatives and novel triptolide-loaded delivery systems, especially nanostructured carriers, have been developed, which aim to enhance the anticancer activities and water-solubilities as well as mitigate the toxicities [17,18].

This review summarizes the broad targets of triptolide to resistance and sensitization for cancer therapy. The progress in the development of triptolide derivatives and novel delivery systems will also be discussed to further study the anti-resistance and sensitization activity of triptolide as well as promoting its clinical applications.

2. Isolation, structure, and bioactivities of triptolide, and types of triptolide-targeted cancer therapy

Triptolide was first isolated and structurally elucidated by S. Morris Kupchan et al in 1972 [19]. They fractionated the ethanol extract of TWHF, conducted multistep chromatography, and obtained 0.001% triptolide, which showed significant antileukemic and anti-KB cell activities [19]. The structure of triptolide is shown in Fig. 4, which was determined by S. Morris Kupchan et al by X-ray crystallographic analysis, Hamilton's R-factor ratio test, and the measurement of intensity difference in Friedel pairs of reflections [19]. Triptolide is the first recognized deterpenoid triepoxides.

Nowadays, multiple bioactivities of triptolide have been elucidated, such as the anti-cancer, anti-inflammatory, anti-fertility, immunosuppressive and neuroprotective activities [11,20–22]. As a sensitizer of anticancer therapies, several evidences have shown that triptolide can target resistance to conventional chemotherapies, radiotherapies, targeted therapies, immunotherapies, hormaonotherapies, and cytokines. The mechanisms behind its broad effects will be discussed (Fig. 3).

3. Molecular mechanisms of triptolide-induced anti-resistance and sensitization in cancer therapy

3.1. Alternation in drug efflux transporters

Among the categories of resistance in cancer therapy, multidrug resistance (MDR) is the main cause of therapy failure [23], which protects cancer cells not only against the used chemotherapies, but also against a series of structurally and functionally unrelated agents [2]. Since multiple ATP-binding cassette (ABC) transporters can export several anticancer drugs by hydrolyzing ATP, thus lowering the intracellular drug concentrations and the subsequent anticancer effects, inhibition of the efflux transporters may circumvent MDR, especially P-glycoprotein (P-gp, MDR1 or ABCB1), multidrug resistance protein 1 (MRP1 or ABCC1) and breast cancer resistance protein (BCRP or ABCG2), which are closely associated with MDR [24].

To date, several studies have investigated the role of triptolide in regulating the efflux transporters to reverse MDR. Wang et al. reported that pre-administration of low dose triptolide increased the rat plasma concentration of sorafenib, a tyrosine kinase inhibitor (TKI) as well as a substrate of P-gp, and that may be related to the inhibition of P-gp by triptolide [25]. But this explanation is just a speculation without experimental validation. Another study indicated that low nanomolar dose of triptolide showed strong anticancer effects on KB-7D cells overexpressing MRP and KB-tax cells overexpressing P-gp by down-regulating MRP and P-gp expressions. The combination of triptolide and 5-fluorouracil showed strong antitumor activities against cancer-resistant xenografts *in vivo* [26]. However, KB-7D cells also overexpressed topoisomerase II. Hence, whether decreasing efflux transporter expressions correlated with the anti-resistance results was unclear. Besides, the subset of MRP was not elucidated, making the study results confusing. Guo et al. reported that triptolide overcame MDR and reduced P-gp expression in DU145/ADM cells [27], and Li et al. proved that triptolide reversed the adriamycin resistance in K562/A02 cells via decreasing P-gp expression and increasing intracellular adriamycin accumulation [28]. Despite this many studies, none of them show either the escape from triptolide-mediated anti-resistance effects after overexpressing the transporters, or a direct correlation between a transporter expression and the phenotype of MDR, and other studies indicated that triptolide did not inhibit the P-gp drug efflux function [29,30]. Further, some cancers do not overexpress the efflux pump gene [31]. Therefore, whether alternation in drug efflux transporters correlates with the anti-resistance effects of triptolide is insufficient in evidence and needs further validation.

3.2. Induction of apoptosis pathways

Since most current anticancer therapies primarily act by activating the cell death pathways, particularly the apoptosis pathways, which are the final goals of anticancer therapies [6], apoptosis resistance is a major hallmark of anticancer therapies. There are 2 types of apoptosis pathways. One is the intrinsic pathway leading to the increased mitochondrial permeability, mitochondrial release of cytochrome *c*, and subsequent caspase activations. The other one is the extrinsic pathway mediated by the binding of ligands to their death receptors, formation of the death-inducing signaling complex (DISC), subsequent activation of caspases, and final induction of the mitochondrial pathway [10]. In both pathways, several anti-apoptotic proteins and pro-apoptotic signals are involved, such as B-cell lymphoma (Bcl-2) family proteins, inhibitor of apoptosis protein (IAP) family proteins and so on, which are attractive targets for anticancer resistance.

Considering the dose-dependent toxicities of triptolide and other cancer therapies as well as the multi-target characteristics of triptolide, researchers have investigated the combination of low-dose triptolide and current approved therapeutics to induce the pro-apoptotic signals and inhibit the anti-apoptotic proteins in malignant cells without affecting the normal cells, hoping to result in the anti-resistance and sensitization effects. Table 1 summarizes the studies related to triptolide mediated anti-resistance and sensitization activities. It can be concluded that triptolide sensitizes most currently available therapies to apoptosis, including conventional chemotherapy, targeted therapy, radiotherapy and cytokine-therapy, even in highly-resistant pancreatic and leukemia cancer cells, without significant adverse effects, as demonstrated by xenograft mouse models. The Combination Index (CI) and Dose Reduction Index (DRI) indicate their synergistic effects. These anti-resistance and sensitization effects are triggered via either the intrinsic or the extrinsic apoptosis pathways, during which expressions of