Review Article

Targets and molecular mechanisms of triptolide in cancer therapy

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Abstract: Triptolide (TPL/TL) is a natural drug with novel anticancer effects. Preclinical studies indicated that TPL inhibits cell proliferation, induces cell apoptosis, inhibits tumor metastasis and enhances the effect of other therapeutic methods in various cancer cell lines. Multiple molecules and signaling pathways, such as caspases, heat-shock proteins, NF-κB, and deoxyribonucleic acid (DNA) repair-associated factors, are associated with the anti-cancer effect. TPL also improves chemoradiosensitivity in cancer therapy. Phase I trials indicate the potential clinical value of TPL use. However, further trials with larger sample sizes are needed to confirm these results.

Keywords: Triptolide (TPL/TL); neoplasms; antineoplastic; cancer

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Introduction

Triptolide (TPL/TL), which is also called tripterygium wilfordii lactone alcohol or tripterygium wilfordii lactone, is extracted from tripterygium wilfordii celastraceae plants. TPL contains three epoxy groups of diterpene lactone compounds, and it has anti-tumor, anti-inflammatory and immunosuppressive activities. TPL exerts proapoptotic and anti-proliferative effects on tumor cell lines *in vitro*, and reduces tumor size or restricts tumor growth *in vivo*. Furthermore, TPL sensitizes tumor cells to other therapeutic methods, such as chemoradiation. The synergistic effects of TPL with other chemotherapeutic agents showed efficacy in preclinical animal models (1). This review summarizes the targets and molecular mechanisms of TPL in cancer therapy.

TPL inhibits tumor cell proliferation

Disorder of cell cycle regulation plays a significant role in the occurrence and development of tumors. The completion of the cell cycle is under the control of the serine/threonine kinase cyclin dependent kinase (CDK) family. The cell cycle can be completed when CDKs are activated. Periodic proteins (cyclins) positively regulate the cell cycle, and inhibitors of CDKs reverse regulation. Cell proliferation is out of control when CDK inhibitors are inhibited or cyclins are overexpressed, which leads to the occurrence of tumors. p21 is an inhibitor of CDKs. The expression of p21 inhibits the formation of all cyclin-CDK complexes. Inhibiting the expression of cell cycle proteins and CDK2 phosphorylation leads to cell cycle arrest, which inhibits cell proliferation. TPL up-regulates p27 and p21 expression, but down-regulation of CDC25A and cyclinA expression leads to S phase arrest (2). TPL inhibits the proliferation of colon cancer cells via inhibition of messenger ribonucleic acid (mRNA) expression, which affects cyclin expression. CyclinB promotes the G2-M transition. CyclinA and CyclinD are the regulatory proteins of G1-S. TPL down-regulates the expression of cyclinA, cyclinB, cyclinC, and cyclinD, which causes cell cycle arrest and inhibits cell proliferation (3).

TPL induces tumor cell apoptosis

TPL activates signaling pathways of caspases

There are two signaling pathways of apoptosis. One pathway

is the death receptor signaling pathway, which combines cell membrane receptors with ligands and leads to the activation of apoptosis precursors to induce cell apoptosis. Another pathway is the signaling pathway of mitochondria, which induces apoptosis factors, damages mitochondrial structure and function, promotes apoptosis molecules from mitochondria, releases cytochrome into the cytoplasm, and affects a series of biological characteristics, resulting in cell apoptosis. TPL-induced apoptosis occurs by the release of cytochrome c and activation of caspase-3, and it has been found to be associated with the down-regulation of Bcl-2 expression and the up-regulation of Bax expression (4). TPL induces apoptosis by regulating apoptosis-related protein expression, such as the regulation of caspase-3, caspase-9, poly adenosine diphosphate ribose polymerase (PARP), and Bcl-2 activation (5). TPL up-regulates caspase-3/9 expression and down-regulates Bcl-2 expression without changing Bax levels (6). TPL also down-regulates Mcl-1 mRNA and protein levels. Furthermore, TPL reduces Mcl-1 protein, which correlates with caspase activation and induces apoptosis. TPL inhibits Mcl-1 synthesis at the mRNA transcription level, and the underlying molecular mechanism is associated with inhibition of RNA polymerase II carboxyl-terminal domain (CTD) phosphorylation (7,8). TPL down-regulates the expression of the anti-apoptotic proteins Bcl-2 and MDR1 at the gene and protein level, and it increases the expression of pro-apoptotic proteins, such as Fas and Bax (9). TPL induces endoplasmic reticulum (ER) stress via the PERK/eIF2α pathway. TPL induces extracellular signal-regulated kinase (ERK) activation via the regulation of the expression of the Bcl-2 protein family, but it is not involved in the down-regulation of Bcl-xL expression (10).

TPL inhibits heat shock protein (HSP) expression

The family of HSPs, including HSP27, HSP70, and HSP90, is a group of stress proteins. These small-molecule chaperones participate in the regulation of apoptosis, and they are involved in carcinogenesis. Evidence shows that HSP27 plays an important role in drug resistance, and it is highly expressed in drug-resistant cancers, including colon cancer, gastric cancer, ovarian cancer, and lymphoma. Significant elevation of HSP27 expression in aggressive androgen-insensitive cell lines and malignant pancreatic cancer cell lines predicts a more aggressive cell type and poorer clinical outcome. These studies suggest that HSP27 should be considered as a potential target for chemotherapy-resistant carcinoma. TPL down-regulates HSP27 and HSP70 expression (11). TPL inhibits the promoter of the HSP70 gene, which down-regulates heat shock gene expression, and induces more sensitive cells to a stress-induced cell death, which may be closely related to cancer therapy (12). TPL down-regulates HSP70 expression in pancreatic cancer. The mechanism of HSP70 expression down-regulation is that it changes the acetylglucosamine modification of Sp1 to cause inactivation, which leads to cell death (13).

TPL induces cell apoptosis through the NF-κB pathway

NF-κB is cell nuclear factor that is involved in transcription regulation in the process of inflammation, stress, cell growth and proliferation. NF-κB promotes cell proliferation, inhibits cell apoptosis, and plays an important role in the process of tumor development. NF-κB is a heterologous dimer composed of p50 and p65. TPL inhibits the transactivation effect of the p65 subunit of NF-κB to inhibit NF-κB activity and promote cell apoptosis. TPL inhibits growth via apoptosis induction in THP-1 cells. The mechanism of apoptosis is through caspase activation and NF-κB inhibition (14). TPL indirectly affects NF-κB and suppresses NF-κB signaling mainly through the AKT/GSK3β/mTOR pathway (15). TPL induces apoptosis in ovarian cancer through the suppression of NF-κB expression to reduce human epidermalgrowth factor receptor-2 (HER2), which is downstream of the PI3K/Akt-signaling pathway (16). TPL inhibits complex I of the mitochondrial respiratory chain (MRC) in SKOV3 platinum-resistant human ovarian cancer cells. TPL down-regulates reactive oxygen species (ROS), inhibits nuclear factor NF-κB activation and decreases the anti-apoptotic proteins Bcl-2 and X-linked inhibitor of apoptosis protein (XIAP) (17). The induction of apoptosis by TPL in undifferentiated thyroid cancer cells is not through the p53 pathway but via the NF-κB pathway. Therefore, TPL may become a drug used in the treatment of p53-mutation-type thyroid cancers (18).

TPL enhances apoptosis that is induced through other mechanisms

Deoxyribonucleic acid (DNA) rupture is the most important threat for cellular genome stability. Blockade or incomplete DNA repair can lead to chromosome replacement, misses or breaks (19). TPL induced DNA damage and suppressed DNA repair-associated gene expression (mRNA) in A375.S2 cells, which may underlie the TPL-mediated inhibition of

cell proliferation (20). TPL modulates apoptosis in MCF-7 cells via the induction of liposomal membrane permeability, up-regulation of the expression of pro-apoptotic proteins, and the inhibition of cell proliferation in a time- and dose-dependent manner (21).

TPL inhibits tumor metastasis

The metastasis of solid tumors is the main cause of patient death. Vascular endothelial growth factor (VEGF) is an important angiogenesis factor that induces endothelial cell proliferation and migration and promotes angiogenesis. VEGF plays an important role in tumor growth and metastasis. VEGF combined with its receptors releases numerous growth factors and cytokines, enhances the permeability of blood vessels, causes plasma protein extravasation, and provides the substrate for the growth of tumor cells. VEGF also stimulates the proliferation and migration of vascular endothelial cells and promotes angiogenesis formation. TPL can be a promising antiangiogenic agent. The potent antiangiogenic action of TPL occurs via inhibition of angiogenic pathways mediated by Tie2 and vascular endothelial growth factor receptor (VEGFR)-2 (22). TPL inhibits human pancreatic cancer cell proliferation in a concentration- and time-dependent manner and down-regulates VEGF expression *in vitro*. Furthermore, medium from TPL-treated PANC-1 cells inhibits the proliferation, tube formation, and migration of Human Umbilical Vein Endothelial Cells (HUVECs). Analysis of CD31 expression, which is a marker of tumor angiogenesis, further showed that TPL down-regulated blood vessel formation in previous experimental models (23).

TPL enhances the effect of chemotherapy

TPL sensitizes several cancer cell lines to chemotherapy *in vivo* and *in vitro*. TPL highlights the synergistic anti-tumor effect in cells in combination with many cytotoxic drugs. The synergistic anti-tumor effect of TPL and cisplatin or 5-FU down-regulates cancer cell viability in liver cancer cell lines *in vitro* and in nude mice and induces higher levels of apoptosis compared to single treatments. Furthermore, cells treated with TPL plus cisplatin or 5-FU exhibit a marked production of intracellular ROS and caspase-3 activity, down-regulate Bcl-2 expression and up-regulate Bax expression (24). TPL sensitizes cells to carboplatin activity. Previous studies showed that combined-agent-treated groups almost stopped growing, and tumor weights *in vivo* were much lighter than with single-drug treatment (25). TPL in combination with sorafenib is superior to single drug treatment in inducing apoptosis and down-regulating viability via decreasing NF-κB activity. Tumor growth inhibition rates in combined-agent-treated groups in a nude mouse model are increased compared to single drug treatment (26). TPL combined with oxaliplatin (OXA) effectively inhibits proliferation in the colon cancer cell line SW480 and induces cell apoptosis. The mechanism partly involves the inhibition the expression of target genes in the cell cycle and nuclear translocation of β-catenin. Moreover, combined-agent-treated groups in a nude mouse model significantly suppressed tumor growth (27). TPL in combination with temozolomide (TMZ) significantly up-regulates the percentage of apoptotic cells in glioma-initiating cells via up-regulation of NF-κB transcriptional activity and increased expression of downstream genes (28).

The combination of TPL with non-cytotoxic drugs has synergistic effects in numerous types of cancer cells. The main mechanism of the TPL-enhancing apoptosis effect of dexamethasone is that TPL affects the PI3k/Akt/NF-kB pathway, mitogen-activated protein kinase (MAPK) signaling pathway, and Bcl-2 expression (29). The synergistic antitumor effect of TPL and iron deficiency anemia (IDA) in acute myelocytic leukemia (AML) cells is due to induction of ROS and the inhibition of the Nrf2 and hypoxia inducible factor (HIF)-1α pathways (30). The synergistic antitumor effect of TPL and aspirin in cervical cancer involves a reduction in cyclin E expression, up-regulation of Bax and P21 expression, the inhibition of cell proliferation and induction of cell apoptosis (31).

TPL enhances the effect of radiotherapy

TPL improves radiosensitivity and has synergistic antitumor effects *in vitro* and *in vivo*. Cell survival in combined-agent-treated groups in pancreatic cancer cells is significantly suppressed, and the percentage of apoptotic cells is significantly up-regulated compared to single treatment. Moreover, tumor growth in a nude mouse model is significantly suppressed in combined-agent-treated groups. Immunohistochemistry and terminal-deoxynucleotidyl transferase mediated nick end labeling (TUNEL) of caspase-3 cleavage in tumor tissues showed similar evidence (32). TPL in combination with radiation has synergistic anti-tumor effects on the expression of XIAP and Mcl-1 proteins in oral cancer cells especially. Tumor size and volume of combined-agent-treated groups in a

nude mouse model were decreased significantly compared to single treatment groups. Caspase-3 expression was up-regulated and XIAP protein expression was decreased in this model (33).

Clinical trials of TPL for the treatment of solid tumors

The activation of anti-apoptotic effectors, such as NF-κB, can cause the resistance of cancer cells to cytotoxic therapy. Therefore, compounds that inhibit NF-κB stimulation could overcome chemotherapy resistance. One phase I and pharmacological study of F60008 (a semi-synthetic derivate of TPL) was performed in patients with advanced solid tumors. Twenty patients were enrolled, and they received a total of 35 cycles. For one cycle, F60008 was given intravenously as a weekly infusion for 2 weeks every 3 weeks. The most frequent hematological side effect was mild grade 1-2 anemia. Non-hematological toxicities included constipation, fatigue, vomiting, diarrhea and nausea, which were all grades 1-2. Two lethal events were observed in which an up-regulated caspase-3 activity and overt apoptosis in neutrophils and monocytes were observed. Pharmacokinetic studies showed high inter-individual variability and demonstrated that F60008 was a far-from-optimal derivative of TPL (34). There are few clinical trials of TPL in solid tumors, and further clinical studies and trails are warranted to investigate clinical applications.

Conclusions

TPL is widely used for the treatment of autoimmune and inflammatory diseases. The anti-tumor activity of TPL *in vitro* and in various tumor-bearing animal models has been investigated for years, and many findings showed that TPL is a promising agent in anti-tumor therapy. However, clinical evidence is very limited as far as we know. TPL has been approved for Phase I clinical trials for the treatment of prostate cancer, but the anti-tumor effect and mechanism of TPL need to be further elucidated. We hope and believe that TPL would provide more benefit in the treatment of malignant tumors in the future as a prospective anti-tumor drug candidate.

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