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Review

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Traditional Chinese medicines and their active ingredients sensitize cancer cells to TRAIL-induced apoptosis

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Abstract: The rapidly developing resistance of cancers to chemotherapy agents and the severe cytotoxicity of such agents to normal cells are major stumbling blocks in current cancer treatments. Most current chemotherapy agents have significant cytotoxicity, which leads to devastating adverse effects and results in a substandard quality of life, including increased daily morbidity and premature mortality. The death receptor of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) can sidestep p53-dependent pathways to induce tumor cell apoptosis without damaging most normal cells. However, various cancer cells can develop resistance to TRAIL-induced apoptosis via different pathways. Therefore, it is critical to find an efficient TRAIL sensitizer to reverse the resistance of tumor cells to TRAIL, and to reinforce TRAIL's ability to induce tumor cell apoptosis. In recent years, traditional Chinese medicines and their active ingredients have shown great potential to trigger apoptotic cell death in TRAIL-resistant cancer cell lines. This review aims to collate information about Chinese medicines that can effectively reverse the resistance of tumor cells to TRAIL and enhance TRAIL's ability to induce apoptosis. We explore the therapeutic potential of TRAIL and provide new ideas for the development of TRAIL therapy and the generation of new anti-cancer drugs for human cancer treatment. This study involved an extensive review of studies obtained from literature searches of electronic databases such as Google Scholar and PubMed. "TRAIL sensitize" and "Chinese medicine" were the search keywords. We then isolated newly published studies on the mechanisms of TRAIL-induced apoptosis. The name of each plant was validated using certified databases such as The Plant List. This study indicates that TRAIL can be combined with different Chinese medicine components through intrinsic or extrinsic pathways to promote cancer cell apoptosis. It also demonstrates that the active ingredients of traditional Chinese medicines enhance the sensitivity of cancer cells to TRAIL-mediated apoptosis. This provides useful information regarding traditional Chinese medicine treatment, the development of TRAIL-based therapies, and the treatment of cancer.

Key words: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL); Cancer therapy; Chinese medicine; Apoptosis

1 Introduction

Apoptosis is a physiological pattern of cell death. It not only regulates tissue homeostasis, but also is a host mechanism that eliminates undesirable cell clones. For example, apoptosis is considered the final result of antitumor immune surveillance. Like other members of the tumor necrosis factor (TNF) family, TNF-related apoptosis-inducing ligand (TRAIL) is a

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homotrimer expressed as a type II transmembrane protein that can be proteolytically cleaved from the cell surface and released in a soluble form (Holland, 2013). TRAIL induces apoptosis through two well-known apoptotic pathways: an intrinsic pathway involving interaction with its receptors, and an extrinsic pathway (Falschlehner et al., 2007). TRAIL triggers extrinsic apoptotic pathways through its receptors death receptor 4 (DR4) and DR5. The trimerization of DR4 and DR5 occurs due to TRAIL linkage of TRAIL. By recruiting Fas-associated death domain (FADD) proteins, these multiple protein complexes are assembled into a death-inducing signaling complex (DISC) (Wang and El-Deiry, 2003) (Fig. 1). An adaptor protein is translocated to DISC, and its death domains (DDs) interact directly with the DD of the death receptors (Trivedi and Mishra, 2015). This protein complex assembly then begins to recruit caspase-8 and/or caspase-10, which belong to the starting caspases. These caspases, also known as apoptotic promoters, are activated automatically via proteolysis and subsequently transmit apoptotic signals to an effector caspase, such as caspase-3. Several cellular proteins dissociate from activated caspase-3 and constitute a signal cascade, leading to the biochemical and morphological features of apoptosis (Johnstone et al., 2008). In most cancer cell lines, in addition to the extrinsic pathway, researchers have found that activating an intrinsic apoptotic pathway via the mitochondrial pathway regulated by B-cell lymphoma 2 (Bcl-2) can enhance TRAIL-induced apoptotic signals (Gonzalvez and Ashkenazi, 2010) (Fig. 2). Bcl-2 homeodomain 3 interaction domain death agonist (Bid) is a substrate of caspase-8 (Li et al., 1998) that leads to an activated truncated Bid (tBid). Next, tBid interacts with Bcl-2 homologous antagonist/killer (Bak) and Bcl-2-associated X protein (Bax), which belong to the pro-apoptotic Bcl-2 family, leading to their oligomerization in the mitochondrial membrane. This causes a loss of mitochondrial membrane potential, directing the release of mitochondrial

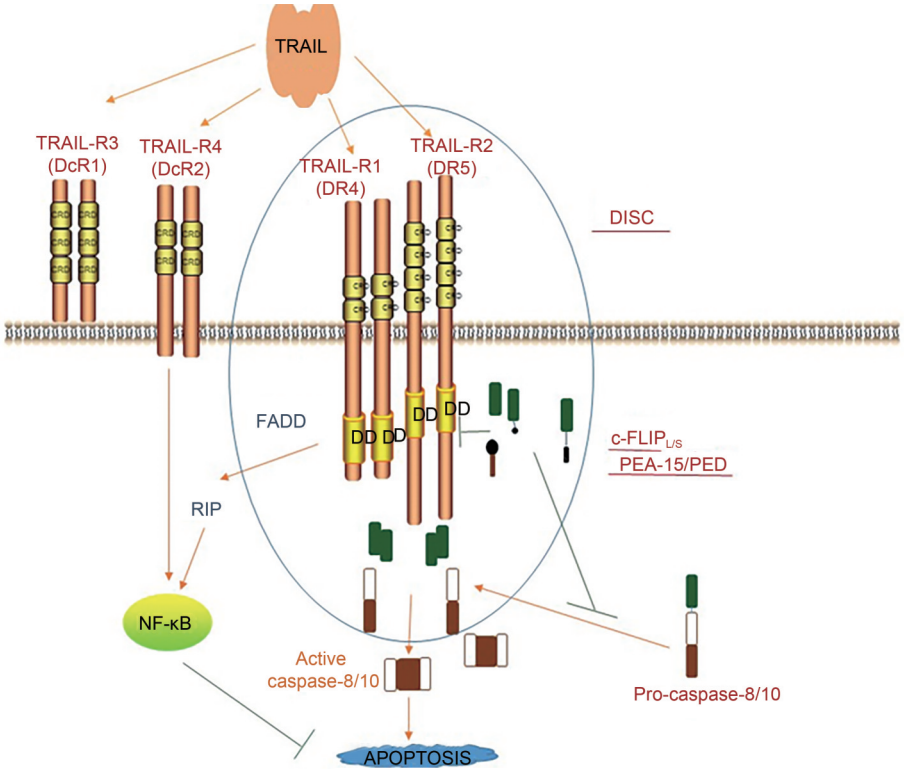

Fig. 1 Apoptosis induction at the level of TRAIL DISC. TRAIL binds to DR4 or DR5 to form a trimer of the receptor, thereby recruiting the receptor molecule FADD and pre-caspase-8 to jointly form DISC. The pre-caspase-8 is activated at the level of the DISC, which in turn activates downstream caspase-3, inducing apoptosis. Arrows denote activation, and blunt arrows indicate inhibition. c-FLIP: cellular FADD-like interleukin-1β (IL-1β)-converting enzyme (FLICE)-like inhibitory proteins; DISC: death induction signal complex; DR: death receptor; FADD: Fas-associated death domain (DD); NF-κB: nuclear factor-κB; TRAIL: tumor necrosis factor-related apoptosis-inducing ligand.

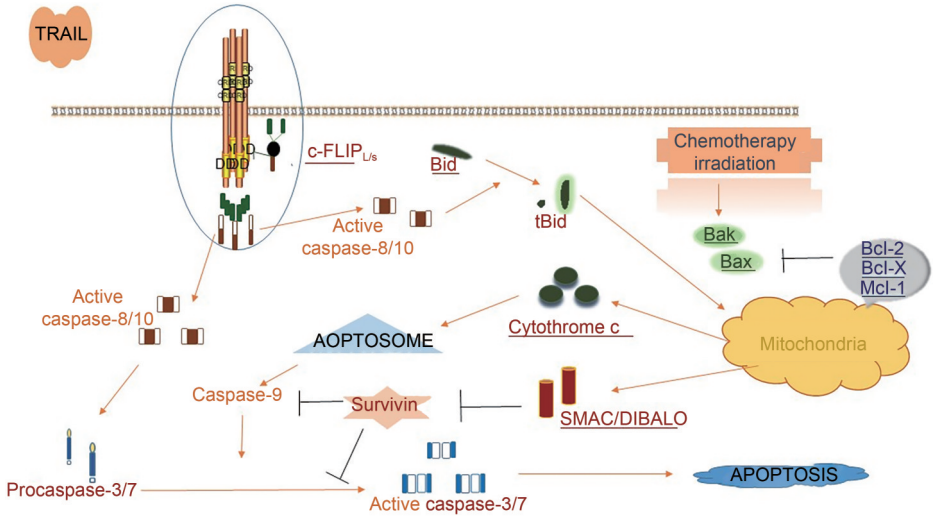

Fig. 2 TRAIL induces apoptosis by intracellular apoptosis (mitochondrial pathway). Overexpression of anti-apoptosis proteins (Bcl-2, Bcl-xL, Mcl-1), as well as dysregulation of pro-apoptosis proteins (Bax and Bak), can lead to hindrance of apoptosis. In addition, members of the apoptosis-inhibiting protein IAP family (like survivin) can inhibit the function of caspase-9 and caspase-3 directly and prevent the transmission of apoptosis signals. Arrows denote activation, and blunt arrows indicate inhibition. Bak: B-cell lymphoma 2 (Bcl-2) homologous antagonist/killer; Bax: Bcl-2-associated X protein; c-FLIP: cellular FADD-like interleukin-1β (IL-1β)-converting enzyme (FLICE)-like inhibitory proteins; DIABLO: direct inhibitor of apoptosis protein-binding protein with low pI; FADD: Fas-associated death domain (DD); IAP: inhibitor of apoptosis protein; Mcl-1: myeloid cell leukemia-1; SMAC: second mitochondrial-derived activator of caspase; tBid: truncated Bcl-2 homeodomain 3 interaction domain death agonist (Bid); TRAIL: tumor necrosis factor-related apoptosis-inducing ligand.

proteins, such as cytochrome *c* and second mitochondrial-derived activator of caspase/direct inhibitor of apoptosis protein-binding protein with low pI (SMAC/DIABLO), which participates in the pro-apoptotic process (de Miguel et al., 2016). Cytochrome *c* bonds to pepsin-9 and apoptotic protease activating factor 1 (APAF-1) to form apoptotic bodies, causing the activation of caspase-9. Therefore, in the apoptotic effectors of caspase-3, -6, and -7, the apoptotic signal reaches a vertex, leading to apoptosis and cell death (Almasan and Ashkenazi, 2003).

Although TRAIL is expressed in multifarious tissues (Daniels et al., 2005), it is expressed mainly in cells of the immune system, and plays a key role in inducing apoptosis of virally and oncogenically transformed cells (Hayakawa et al., 2004). TRAIL interacts with five receptors: TRAIL-R1 (DR4), TRAIL-R2 (DR5) (Pan et al., 1997), TRAIL-R3 (DcR1/TRID/LIT), TRAIL-R4 (DcR2/TRUNND), and osteoprotegerin (OPG) (Kwilas et al., 2016). DR4 and DR5 each contain a conserved DD motif and function in apoptotic signaling. TRAIL-induced apoptosis can be inhibited by the overexpression of these proteins, which act as "decoys." Although DcR2 has a truncated non-functional cytoplasmic DD, DcR1 lacks a cytoplasmic region. The physiological relevance of OPG as a TRAIL receptor is unclear (Wang and El-Deiry, 2003). Because DR4 and DR5 are expressed mainly in cancer cells, rather than in most untransformed cells, selective apoptosis can be triggered without significant tissue toxicity. This makes using other agonists or TRAIL for cancer treatment by DR4/DR5 a promising methodology (de Miguel et al., 2016).

As previously mentioned, Bcl-2 family proteins also play significant roles in apoptosis induced by TRAIL. A subset of these proteins routinely governs the intrinsic apoptotic pathway. In addition to the Bcl homologous domain 3 (BH-3) only proteins, there are four BHs (BH1−4) (Lemke et al., 2014). These include: the anti-apoptotic proteins Bcl-2, Bcl-xL, Bcl-B, Bcl-w, myeloid cell leukemia-1 (Mcl-1), and A1; the pro-apoptotic proteins Bax, Bak, and Bcl-2-related ovarian killer (Bok); and the BH-3 only proteins Bid, Bcl-2-interacting mediator of cell death (Bim), p53 up-regulated modulator of apoptosis (Puma), Bik, and Noxa (Yamaguchi et al., 2019). Structural studies have shown the importance of the interaction of these protein domains to anti-apoptotic and pro-apoptotic

effects. For example, anti-apoptotic activity is neutralized by pro-apoptotic proteins, thus breaking down the obstacle to apoptosis induction during cytotoxic stress. Similarly, increasing the activation of Bak and Bax further guides caspase activation and enhances the apoptosis process (Delbridge and Strasser, 2015). During cytotoxic stress, caspase-8 activation leads to Bid rupture and the formation of tBid, directs the oligomerization of Bak and Bax, and subsequently causes mitochondrial outer membrane permeability (MOMP), which is a marker of the intrinsic apoptotic pathway. Intermembrane space proteins such as cytochrome *c* are subsequently released. This triggers the activation of the caspase cascade, resulting in cell death (Kalkavan and Green, 2018). However, the existence of anti-apoptotic proteins inhibits apoptosis by maintaining mitochondrial membrane integrity against Bax and Bak activity (Seervi et al., 2018). Because Bcl-2 plays an important role in regulating apoptosis, TRAIL's regulation of the intrinsic apoptotic pathway is strictly dependent on cell type. TRAIL can induce wild-type cytochrome *c* release and apoptosis, which proves the importance of these two molecules in apoptosis. It takes place in $Bax^{-/-}$ or $Bak^{-/-}$ mouse embryonic fibroblasts, but not in double-knockout $Bax^{-/-}/Bak^{-/-}$ cells (Zhang and Fang, 2005). The impact of Bcl-2 in regulating apoptosis has been further proved by knockout studies of pro-apoptotic proteins, as has the mechanism by which the absence of Bcl-2 reverses the difficulty in inducing apoptosis (Huang and Sinicrope, 2008). The level of Bcl-2 anti-apoptosis protein can be inhibited by compound management, thereby eliminating the blocking of TRAIL-induced apoptosis (Kisim et al., 2012).

In summary, the resistance to apoptosis induced by TRAIL can be reduced via the following mechanisms: upregulation of death receptors (DR5 or DR4); downregulation of cell survival proteins such as Bcl-2, Bcl-xL, survivin, the X-linked inhibitor of apoptosis protein (XIAP), and Mcl-1, as well as cellular FADD-like interleukin-1β (IL-1β)-converting enzyme (FLICE)-like inhibitory proteins (c-FLIP, a caspase-8 inhibitor also known as I-FLICE); or upregulation of pro-apoptotic proteins and downregulation of TRAIL-binding antagonistic decoy receptors that lack the functional domains necessary for transduction of apoptosis signals (Gupta et al., 2013) (Fig. 3). In theory, TRAIL can be used as an inducing factor in many human cancers and is an ideal target for cancer treatments. However, there are many tumor cells that show insensitivity or

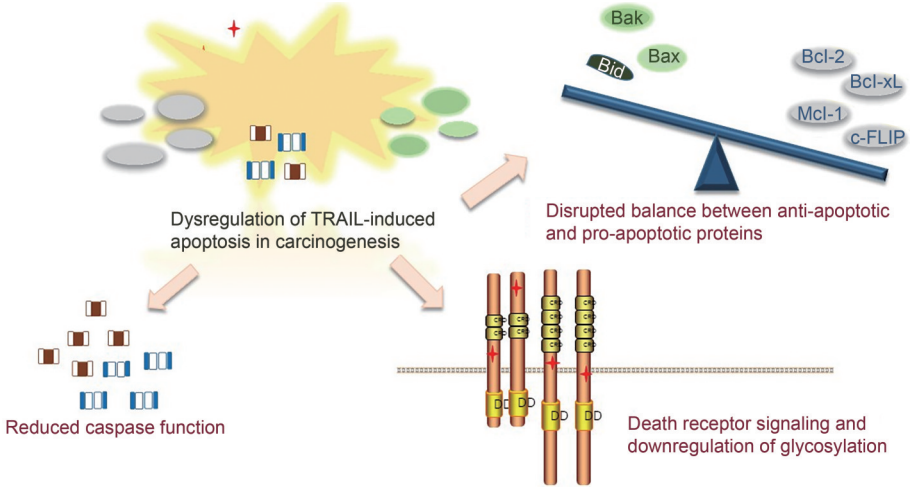

Fig. 3 TRAIL-induced apoptosis is out of balance during carcinogenesis. The mechanisms that inhibit the execution of apoptosis include: (1) destruction of the balance between anti-apoptotic and pro-apoptotic proteins, causing apoptosis imbalance in affected cells due to lower expression of pro-apoptotic proteins (such as Bid, Bak, and Bax) or overexpression of anti-apoptotic proteins (such as Bcl-xL, Bcl-2, c-FLIP, and Mcl-1); (2) inhibition of apoptosis by reducing caspase function; (3) impaired death receptor signal transduction attributed to a decrease in glycosylation, leading to escape from extrinsic apoptotic pathways and lower receptor surface expression. Bak: B-cell lymphoma 2 (Bcl-2) homologous antagonist/killer; Bax: Bcl-2-associated X protein; Bid: Bcl-2 homeodomain 3 interaction domain death agonist; c-FLIP: cellular FADD-like interleukin-1β (IL-1β)-converting enzyme (FLICE)-like inhibitory proteins; FADD: Fas-associated death domain (DD); Mcl-1: myeloid cell leukemia-1; TRAIL: tumor necrosis factor-related apoptosis-inducing ligand.

even resistance to it. This has become a major problem for scientists. The clinical effects of TRAIL, when applied individually, are often unsatisfactory (Kretz et al., 2018).

Therefore, it is very important to find effective TRAIL sensitizers to reverse the resistance of tumor cells to TRAIL and enhance the ability of TRAIL to induce tumor cell apoptosis. The combination of some traditional Chinese medicines (TCMs) and TRAIL is a promising way to reverse acquired TRAIL resistance, and also a very promising treatment option. Many studies have demonstrated that in cancer treatment, the combination of Chinese herbal medicine with chemotherapy, radiation therapy, or targeted therapy can be used to enhance the efficacy and reduce the side effects and complications caused by the therapies (Wang et al., 2018). The compounds of TCM have shown characteristics of low toxicity and high efficiency, and have multiple targets. Furthermore, their corresponding anti-tumor sensitizing effects have attracted increasing attention, rendering TCM an important source of new anti-tumor drugs (Singh et al., 2014). In this review, we summarize the role of TCMs and their ingredients capable of actively sensitizing TRAIL to induce cancer cell apoptosis.

2 Combining TRAIL with traditional Chinese medicine to promote tumor cell apoptosis

2.1 Compounds used in Chinese medicine induce apoptosis by upregulating DR5 in cancer

Auriculasin (AC), sourced for example from roots of *Flemingia macrophylla* (Willd.) Merr., is found in various food ingredients as a prenylated isoflavone (Wang et al., 2013). AC can lead to cellular apoptosis and thus reduce tumor growth by targeting reactive oxygen species (ROS)-mediated caspase-independent pathways and inhibiting phosphoinositide-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling (Cho et al., 2018). Prostate cancer is the most common cancer globally, and an important cause of cancer-induced death in males. Co-treatment with TRAIL and non-cytotoxic concentrations of AC can increase the apoptosis of RC-58T/h/SA#4 cells, which have observable resistance to TRAIL. AC can also effectively reduce the expression of apoptosis-related proteins and AC-TRAIL-induced cell death by pretreatment with a chimeric blocking antibody against DR5. This indicates that non-toxic concentrations of AC make TRAIL-resistant primary prostate cancer cells sensitive to TRAIL-mediated apoptosis by upregulating downstream signaling pathways and DR5 (Cho et al., 2019).

Clematis flammula L. (CFI) is an herb native to North Africa and Southern Europe, and is widely used in TCM. CFI is used by the Chinese to treat rheumatism. In recent reports, its component, boehmenan, has been confirmed to induce lung cancer cell apoptosis by regulating epidermal growth factor (EGF)-dependent pathways (Pan et al., 2016). *Clematis* hederagenin saponin (CHS), another compound extracted from CFI, can also induce apoptosis through the mitochondrial pathway in breast cancer cells (Cheng et al., 2018). In another experiment, HCT116 cells of colon cancer were treated by a combination of CFI and TRAIL. Compared to cells treated with CFI or TRAIL alone, it was found that CFI synergizes with TRAIL by downregulating the expression of cell survival proteins involved in apoptosis. The results showed that CF1 sensitizes TRAIL-resistant cells to apoptosis by activating DR5-expressing mitogen-activated protein kinase (MAPK), SP1, and CCAAT/enhancer-binding protein homologous protein (CHOP) (Guesmi et al., 2019).

Luteolin is an important family member of the flavonoids and is highly distributed in vegetables, fruits, tea, olive oil, honeycomb propolis, and red wine (Mittra et al., 2000). It exists in the form of glycoside in Chinese herbal medicines such as *Lonicera japonica* Thunb, *Perilla frutescens* (L.) Britton, and *Dendranthema morifolium* (Ramat.) Tzvelev (Hempel et al., 1999). Luteolin is effective against oxidation, inflammation, and cancer, and has an ability to reverse multidrug resistance (MDR) in many types of cancer cells (Ou et al., 2013). It can also weaken the cytotoxicity caused by various chemotherapeutic drugs (Lin et al., 2008). Luteolin can directly induce apoptosis by activating c-Jun NH_2-terminal kinase (JNK), thereby inhibiting TNF-α-mediated nuclear factor-κB (NF-κB) p65 to nuclear (Cai et al., 2011). It has been proven that luteloin can inhibit gastric cancer (GC) progression by suppressing Notch1 signaling and reversing epithelial-mesenchymal transition (EMT) (Zang et al., 2017). Furthermore, it has been demonstrated that luteolin, as a flavonoid, can cross the blood–brain barrier (Youdim et al., 2004). In hepatocellular carcinoma (HCC) cells,

treatment with TRAIL or luteolin alone causes little or no cell death, while co-treatment with TRAIL and an increasing concentration of luteolin can significantly reduce cell viability as well as increase apoptotic bodies. Luteolin induces autophagy and sensitizes cells to TRAIL-induced apoptosis, and its treatments significantly upregulate microtubule-associated protein light chain 3 (LC3)-II and downregulate p62 expression, which is consistent with DR5 upregulation. These outcomes suggest that luteolin sensitizes TRAIL-induced apoptosis by upregulating DR5 expression (Nazim and Park, 2019).

It has been reported that tetrandine (TET), a natural compound isolated from *Stephania tetrandra* S. Moore, has been used to treat inflammation and hypertension in China for hundreds of years. It has shown a variety of pharmacological activities, including immunosuppressive, antihypertensive, and antitumor activities (Wang et al., 2004). In recent years, TET's novel antitumor effects have been extensively studied. Impressively, TET affects various biological activities of cancer cells, including inhibition of invasion, migration, proliferation, and angiogenesis. It induces autophagy and apoptosis, and also enhances radiation sensitivity and MDR (Liu T et al., 2016). TET derivative H1 exerts anti-MDR activity by inhibiting the activation of Erk1/2, Akt1/2, and an intrinsic apoptotic pathway (Wei et al., 2011). In prostate cancer, TET treatment leads to increased levels of DR5 and DR4 proteins. To clarify the functions of DR5 and DR4 in sensitizing TET to TRAIL in LNCaP cells, researchers used a lentiviral short hairpin RNA (shRNA) construct to knock down TET and TRAIL. Based on western blot analysis results, DK cells (lentivirus shRNA-transfected LNCaP cells infected simultaneously with DR4 and DR5) showed a significant reduction in the levels of DR4/DR5 protein, but TET treatment failed to affect their protein levels (20 μmol/L for 24 h). Next, researchers figured out the concentration-dependent effect of TET on DR4/DR5 protein levels in DK cells and LNCaP shRNA control (scrl)-control cells. In LNCaP scrl-control cells, they discovered that TET treatment led to a time-dependent increase in the levels of DR4 and DR5 mRNA, yet this effect was not seen in DK cells. Therefore, DR4 and DR5 are necessary for inducing PCa cells to sensitize TRAIL by TET (Shishodia et al., 2018).

Ginsenoside is the main active ingredient from *Panax ginseng* C.A. Meyer. The protopanaxadiol-type ginsenosides include CK, CMc, CMc1, CO, CY, Rb1, Rb2, Rc, Rd, F2, Rg3, and Rh2. CK is regarded as the major ginsenoside metabolite in blood and urine (Hasegawa et al., 1997). It is reported that CK enhances the apoptosis induced by γ rays by generating ROS in human lung cancer cells and destroying mitochondrial membranes (Chae et al., 2009). CK can also induce apoptosis and autophagy by activating JNK and producing ROS in human colon cancer cells (Kim et al., 2013). TRAIL can also induce apoptosis in colon cancer cells. In an experiment examining the effect of CK on the expression of TRAIL DRs, the results showed that DR5 and DcR2 were upregulated in a dose-dependent manner. Additionally, CK treatment can increase cell surface expression and DR5 transcription, which coincides with protein changes. To further substantiate these findings, the expression of DR5 can be silenced by applying two specific small interfering RNAs (siRNAs). The silencing of DR5 reduced the percentage of cell apoptosis induced by CK, as indicated by flow cytometry analysis. This evidence indicates that DR5 plays a decisive role in enhancing the influence of CK on TRAIL-induced apoptosis (Chen et al., 2016).

Triptolide is a diterpenoid compound isolated from the Chinese herbal medicine *Tripterygium wilfordii* Hook F., which can effectively induce apoptosis and autophagy in a variety of cancer types, including neuroblastoma (Antonoff et al., 2009; Krosch et al., 2013), pancreatic cancer (Mujumdar et al., 2010), and colon cancer (Dudeja et al., 2009). It can also sensitize a variety of solid tumor cells to TRAIL-induced cell death (Clawson et al., 2010). Research indicates that triptolide can sensitize various cancer cells to apoptosis induced by the TRAIL-induced death receptor pathway. Triptolide treatment can result in p53 accumulation and inhibit the Akt/Hdm2 signaling pathway, which upregulates DR5 expression and makes prostate cancer cells significantly more sensitive to TRAIL-mediated apoptosis (Hu and Sun, 2012). In pancreatic cancer, it increases the death receptor DR5 levels and the pre-survival c-FLIP is decreased, thus promoting the activation of caspase-8. This combination further results in cell death (Chen ZY et al., 2014). In vivo treatment of mice bearing renal cell carcinoma (RCC) in situ showed that TRAIL induced increases in

apoptosis and death of human RCC cells in the presence of triptolide. Treatment of Renca mice with Minnelide and anti-DR5 monoclonal antibody (mAb) was able to significantly reduce the tumor burden and prolong survival time (Brincks et al., 2015).

Andrographolide (Andro) is a diterpene lactone isolated from the traditional herbal medicine *Andrographis paniculata* (Burm. F.) Nees. It is known that the compound exerts anti-inflammatory activity primarily by inhibiting the nuclear transcription factor NF-κB (Xia et al., 2004). According to a research report, Andro is able to induce apoptosis of human cancer cells via the DR-mediated apoptosis pathway. Through the p53-dependent transcriptional upregulation of DR4 to achieve this sensitization, this process is mediated by multiple consecutive events, including the production of ROS, the activation of JNK, and p53 stabilization and phosphorylation (Zhou et al., 2008). In bladder cancer, co-therapy by TRAIL and Andro can inhibit the growth of T24 cells, reduce proliferation and colony formation, inhibit migration, and promote caspase-mediated apoptosis. Andro's sensitization is achieved by upregulating DR4 and DR5 in a p53-dependent manner. Additionally, Andro is able to inactivate the NF-κB signaling pathway by downregulating p65/RelA, which further contributes to TRAIL-mediated cytotoxicity (Deng et al., 2019). Moreover, Andro increases p53 expression in prostate cancer cells, and also leads to ROS production in cells. At low toxic concentrations, Andro preferentially increases the sensitivity of PCa cells to TRAIL-induced apoptosis, and the regulatory mechanism is related to the increase in DR4 (Wei et al., 2018).

2.2 Compounds used in Chinese medicine sensitize TRAIL-induced apoptosis through intrinsic pathways in cancer

Curcumin (Cur), found in the rhizomes of TCM *Zingiber officinale* Roscoe, is a highly pleiotropic molecule (Devassy et al., 2015). Cur has shown strong antioxidative, anti-inflammatory, antiangiogenic, anticancer, and pro-apoptosis properties (Aggarwal et al., 2003). In nanoparticles (TRAIL-Cur-NPs), TRAIL is used as both a therapeutic agent and an active targeting ligand. Cur can upregulate DR4 and DR5 to enhance the apoptosis-inducing effect of TRAIL (Yang et al., 2017). Extensive research has shown that Cur has chemopreventive properties, mainly because it has the ability to prevent the cancer cell cycle when used independently or in combination with radiation therapy or chemotherapy. Cur also induces cancer cell apoptosis by regulating a variety of important cellular signaling pathways, including JNK, NF-κB, Janus kinase/signal transducers and activators of transcription (JAK/STAT), PI3K/Akt, Notch-1, and TRAIL. Cur is known to induce apoptosis of cancer cells and activate cell death signals, and inhibit tumor progression without affecting normal cells (Chen J et al., 2014). It has been shown to sensitize TRAIL-resistant cells to apoptosis. Treatment with Cur can produce intracellular ROS and inhibit Akt activity, thereby increasing the quantity of DR5 after activating NF-κB (Deeb et al., 2004). Cur inhibits NF-κB by inhibiting the pro-survival Akt signaling pathway, thereby making human prostate cancer cells sensitive to TRAIL-induced apoptosis (Deeb et al., 2007). Research also shows that Cur sensitizes breast cancer cells to TRAIL-induced apoptosis by regulating apoptosis-related proteins. Data suggest that Cur-induced Mcl-1 in MCF-7 cells may reduce the Cur-induced sensitization to TRAIL, while Cur downregulates Mcl-1 in SK-BR-3 and T47D cells, which may help sensitize cancer cells to TRAIL (Park et al., 2013).

Magnolol is a biologically active compound extracted from *Magnolia officinalis* Rehder. It has many pharmacological effects, such as anti-depressive, antioxidant, anti-inflammatory, and anti-tumor properties (Chuang et al., 2011). Evidence suggests that magnolol may be applied as a therapeutic agent for a variety of cancers, such as breast, lung, prostate, and colon cancers. For lung cancer, magnolol or polyphenol mixture (PM) treatment increased the expression of the pro-apoptotic protein Bax significantly, while it inhibited the expression of the anti-apoptotic protein Bcl-2. Moreover, in A549 and H1299 cells, magnolol and PM treatments significantly increased capsase-3, as well as the levels of PARP cleavage protein and caspase-3 cleavage (Liu YT et al., 2016). Honokiol is also a natural biochenolic compound extracted from the *Magnolia* plant. It can activate caspase-3, -8, and -9 through TRAIL treatment, whereas its upregulation of DR5 does not participate in the increase of TRAIL sensitivity. In addition, honokiol reduces survivin and c-FLIP to increase TRAIL sensitivity (Woo et al., 2019).

Bakuchiol, a pre-cured phenolic monomer isolated from the seeds of the pine cell *Cullen corylifolium* (L.) Medik, is commonly used in traditional folk medicine

in India and China as a nephrogenic agent to relieve diarrhea, osteoporosis, and asthma (Lim et al., 2009; Chen et al., 2010; Xu et al., 2014). A previous study has shown that bakuchiol can activate JNK and then transfer Bax to the mitochondria of rat liver fibroblasts to induce caspase-3-dependent apoptosis (Park et al., 2005). Also, bakuchiol exerts an anti-tumor effect in lung cancer cells through activating caspase-9/3 and p53, arresting cells in S-phase, upregulating Bax, and downregulating Bcl-2 through apoptosis, which is related to ROS (Chen et al., 2010). Further research may prove that bakuchiol can enhance the anti-tumor activity of TRAIL. In colon cancer, experiments show that only HCT116 cells are sensitive to TRAIL. Even at concentrations as high as 50 or 100 ng/mL, HT-29 and HCT116 cancer cells are resistant to TRAIL (data not shown). However, when bakuchiol was combined with TRAIL, it enhanced TRAIL-induced cytotoxicity in TRAIL-resistant HT-29 cells and TRAIL-sensitive HCT116 cells. The experimental results indicated that bakuchiol reduced the expression of Bcl-2, survivin, XIAP, and c-FLIP via the overexpression of DR4 and DR5 to overcome TRAIL resistance (Park et al., 2016).

Shikonin is a crude ingredient from the roots of *Lithospermum erythrorhizon* Siebold & Zucc. that has for centuries been widely used in China to treat many diseases (Huang et al., 2014; Wang et al., 2019). Shikonin has been demonstrated to have antiproliferative ability (Gong and Li, 2011; Gorrini et al., 2013). It can enhance cytotoxicity and reverse drug resistance to chemotherapy (Huang, 2007; Lee et al., 2015; He et al., 2016), and has the ability to inhibit the invasion and migration of cancer cells and kill certain cancer cells, while causing less cytotoxicity to normal cells (Goncharenko-Khaider et al., 2010; Li et al., 2014; He et al., 2016). In cholangiocarcinoma cells, ROS-triggered JNK contributes to the TRAIL-induced antiproliferative activity of shikonin and induces apoptosis (Zhou et al., 2017). In the same way, the AKT, JNK, and signal transducer and activator of transcription 3 (STAT3) pathways are engaged in shikonin-mediated sensitization of TRAIL in A549 cells. More importantly, cotreatment with shikonin and TRAIL can inhibit the expression of anti-apoptotic proteins such as Bcl-2, Bcl-xL, Mcl-1, XIAP, and c-FLIP. This can enhance Bid expression, but has no effect on Bax expression (Guo et al., 2018). Recently, a study also found that in HepG2 cells, acetylshikonin, a shikonin derivative, was able to combine with TRAIL to activate the caspase signaling pathway. Furthermore, Bax promotes the apoptosis induced by TRAIL and acetylshikonin (Hong et al., 2019).

Irigenin is an isoflavonoid (Jung et al., 2002) isolated from the rhizomes of *Iris domestica* (L.) Goldblatt & Mabb. (Wozniak et al., 2010). *I. domestica* (L.) Goldblatt & Mabb. has long been used as an antidote, expectorant, antiphlogistic, analgesic, and antipyretic agent, and is a TCM (Zhang et al., 2016). Irigenin has been found to have an antiproliferative effect on cancer cells (Morrissey et al., 2004). A recent study showed that irigenin can sensitize GC cells to TRAIL-regulated cell death. When irigenin was combined with TRAIL, the expression of Bax, FADD, and DR5 was greatly enhanced, while the expression of survivin, c-FLIP, and Bcl-2 was strongly reduced. The results show that irigenin sensitizes GC cells to TRAIL-induced apoptosis by increasing pro-apoptotic proteins and reducing anti-apoptotic proteins (Xu et al., 2018).

Berberine, a natural alkaloid, is isolated from many medicinal herbs, such as the Chinese herbs *Berberis vulgaris* L., *Berberis aquifolium* Pursh, and *Coptis chinensis* Franch. (Eom et al., 2008). Studies have shown that berberine targets multiple pathways to induce apoptosis in cultured human colon cancer cells (Murthy et al., 2012; Guamán Ortiz et al., 2014). TRAIL enhances berberine-mediated p38 activation and counteraction (Refaat et al., 2015). In the murine 4T1 breast cancer model, the combined treatment of DR5 and berberine can effectively inhibit primary tumor growth and metastasis (Refaat et al., 2013). Also, DR5 participates in the apoptosis caused by the TRAIL-berberine combination (Ke et al., 2018). Furthermore, berberine can enhance TRAIL-induced apoptosis in human renal cancer cells through ROS-mediated downregulation of Mcl-1 and c-FLIP proteins (Lee et al., 2011).

3 Conclusions

This review briefly summarizes the apoptotic effects of activated TRAIL when combined with different TCM components through intrinsic or extrinsic pathways (Table 1), and also reveals its physiological role in cancer. Various reports have shown that different

Table 1 List of Chinese medicine ingredients that enhance tumor cell sensitivity to TRAIL

Compound	Source of Chinese herbal medicine(s)	Tumor cell type	Mechanism of action
Auriculasin	*Flemingia macrophylla* (Willd.) Merr.	Prostate cancer	DR5↑
Boehmenan, *Clematis* hederagenin saponin	*Clematis flammula* L.	Colon cancer	DR5↑
Luteolin	*Lonicera japonica* Thunb, *Perilla frutescens* (L.) Britton, *Dendranthema morifolium* (Ramat.) Tzvelev	Hepatocellular carcinoma	DR5↑
Tetrandine	*Stephania tetrandra* S. Moore	Prostate cancer	DR4↑, DR5↑
Ginsenoside	*Panax ginseng* C.A. Meyer	Colon cancer	DR5↑
Triptolide	*Tripterygium wilfordii* Hook F.	Pancreatic cancer	DR5↑, c-FLIP↓
Andrographolide	*Andrographis paniculata* (Burm. F.) Nees	Prostate cancer	DR4 ↑
Curcumin	*Zingiber officinale* Roscoe	Breast cancer	Mcl-1↓
Magnolol	*Magnolia officinalis* Rehder	Lung cancer	Bax↑, Bcl-2↓
Bakuchiol	*Cullen corylifolium* (L.) Medik	Colon cancer	Bcl-2↓, survivin↓, XIAP↓, c-FLIP↓
Shikonin	*Lithospermum erythrorhizon* Siebold & Zucc.	Lung cancer	Bcl-2↓, Bcl-xL↓, Mcl-1↓, XIAP↓, c-FLIP↓
Irigenin	*Iris domestica* (L.) Goldblatt & Mabb.	Gastric cancer	Bax↑, Bcl-2↓, survivin↓, c-FLIP↓
Berberine	*Berberis vulgaris* L., *Berberis aquifolium* Pursh, *Coptis chinensis* Franch.	Renal cancer	Mcl-1↓, c-FLIP↓

Bcl-2: B-cell lymphoma 2, an anti-apoptotic protein; Bax: Bcl-2-associated X protein, a pro-apoptotic protein; c-FLIP: cellular FADD-like interleukin-1β (IL-1β)-converting enzyme (FLICE)-like inhibitory protein; DR4: death receptor 4, a receptor of TRAIL (TRAIL-R1); DR5: a receptor of TRAIL (TRAIL-R2); FADD: Fas-associated death domain (DD); Mcl-1: an anti-apoptotic protein; survivin, a member of the inhibitor of apoptosis protein (IAP) family with caspase activation inhibition functions; TRAIL: tumor necrosis factor-related apoptosis-inducing ligand; XIAP: X-linked IAP protein.

compounds derived from Chinese herbal medicines can make tumor cells sensitive to TRAIL by directly activating intrinsic apoptotic pathways or regulating multiple non-apoptotic pathways to upregulate DR.

Nevertheless, most of the studies were performed in preclinical mouse models or cell lines of cancer. Therefore, more clinical evidence is needed to confirm whether these Chinese herbal medicine derivatives can also synergize with TRAIL in cancer patients. Some other challenges remain. For instance, TRAIL can selectively induce apoptosis of tumor cells or transformed cells, but the mechanism of insensitivity to TRAIL in normal cells is still not completely clear. The molecular mechanisms that cause TRAIL resistance in tumor cells have also not been elucidated. Thus, more work is needed to address the mechanism of TRAIL resistance, which could lead to the development of new treatments to restore TRAIL sensitivity. In addition, TRAIL is known to induce tumor metastasis and activate survival pathways (Trauzold et al., 2001; Hartwig et al., 2017), although the detailed functions of the TRAIL pathway in drug resistance and tumor metastasis are not yet fully understood.

In recent years, the influence of TRAIL on cancer treatment has attracted increasing attention. With advancements in understanding of the mechanism of TRAIL and the elucidation of TRAIL-sensitive biomarkers, new TRAIL sensitizers will be discovered. This progress will be useful for the development of therapies based on TRAIL and new types of anticancer drugs to treat human cancers.

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Author contributions

Ming HONG, Wulin WEN, and Jiawei WANG conducted this review. Bingyu SUN, Yongqiang LIU, Danhua HE, and Jinke LI contributed in the writing and editing of the manuscript. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Bingyu SUN, Yongqiang LIU, Danhua HE, Jinke LI, Jiawei WANG, Wulin WEN, and Ming HONG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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