Targeting TNF-related apoptosis-inducing ligand (TRAIL) receptor by natural products as a potential therapeutic approach for cancer therapy

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Abstract

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has been shown to selectively induce apoptotic cell death in various tumor cells by engaging its death-inducing receptors (TRAIL-R1 and TRAIL-R2). This property has led to the development of a number of TRAIL–receptor agonists such as the soluble recombinant TRAIL and agonistic antibodies, which have shown promising anticancer activity in preclinical studies. However, besides activating caspase-dependent apoptosis in several cancer cells, TRAIL may also activate nonapoptotic signal transduction pathways such as nuclear factor-kappa B, mitogen-activated protein kinases, AKT, and signal transducers and activators of transcription 3, which may contribute to TRAIL resistance that is being now frequently encountered in various cancers. TRAIL resistance can be overcome by the application of efficient TRAIL-sensitizing pharmacological agents. Natural compounds have shown a great potential in sensitizing cells to TRAIL treatment through suppression of distinct survival pathways. In this review, we have summarized both apoptotic and nonapoptotic pathways activated by TRAIL, as well as recent advances in developing TRAIL–receptor agonists for cancer therapy. We also briefly discuss combination therapies that have shown great potential in overcoming TRAIL resistance in various tumors.

Keywords: TRAIL, cancer, NF-κB, apoptosis, natural products

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Introduction: Discovery and structure of TRAIL

Generally, in normal tissues, a tight balance exists between self-renewal and cell death, and it aims to maintain the tissues' integrity. Once this balance is broken, cells might grow out of control and exhibit resistance to cell death. Uncontrolled growth and apoptosis resistance are two critical hallmarks for cancer initiation as well as progression[1]; therefore, therapies targeting these two important aspects might be ideal modalities for cancer treatment. Furthermore, in comparison to proliferation inhibition, which only stops tumor growth without removing cancer cells, apoptosis induction might be a more potent therapy because it is also able to completely eliminate the cancer cells that have accumulated diverse mutations over a period of time.

There are two major pathways involved in the process of apoptosis: the intrinsic and extrinsic. The intrinsic pathway depends on mitochondria[2]; it can eliminate damaged cells via sensing cell damage such as oxidative stress and DNA damage.[3] The tumor-suppressor protein p53 is critical in this pathway, as many intrinsic pathways are dependent on this molecule. Thus, p53 is considered as a potential target for cancer therapy. However, mutation or inactivation of p53 is commonly found in tumor cells, leading to the development of resistance to p53-dependent radio- and chemotherapy.[4] The extrinsic apoptosis pathway is dependent on death ligands binding to the death receptors (DRs). With ligand engagement to the transmembrane receptors, a death signal is transmitted from the outside to the inside of cells. The first cell death ligand used for anticancer treatment was tumor necrosis factor (TNF), which was discovered in 1975.[5] Although TNF showed apoptotic effect

in some cancer types, its major function was later found to be involved in the pro-inflammatory process. Subsequently, the DR FAS/APO-1 (CD95) was found to be another anticancer target since antibodies targeting this receptor were able to induce apoptosis in a wide range of cancer cells.[6,7] However, stimulation of CD95 also showed acute and lethal hepatic toxicity during its anticancer therapy.[8] A few years later, TNF-related apoptosis-inducing ligand (TRAIL) was identified based on its sequence homology to TNF and CD95L.[9,10] TRAIL has similar apoptotic effects as CD95L, but it does not affect normal cells,[11,12] which makes TRAIL a promising therapeutic for cancer therapy.

There are five types of TRAIL receptors. They are four-membrane receptors TRAIL-R1 (DR4), TRAIL-R2 (DR5), TRAIL-R3 (DcR1), and TRAIL-R4 (DcR1), and one soluble receptor called osteoprotegerin. Among them, TRAIL-R1 (DR4) and TRAIL-R2 (DR5) mediate the apoptosis pathway, and hence are termed DRs, while the others protect cells from apoptosis, and are called decoy receptors (DcRs). With ligand binding to the DR, TRAIL apoptotic signaling is initiated and further induces caspases or mitochondrial-dependent death. Various agents such as recombinant human soluble TRAIL and selective agonistic antibodies targeting TRAIL-R have been developed. Their robust anti-tumor activities have been demonstrated in a number of preclinical studies. However, subsequent clinical trials revealed only limited therapeutic benefit. This sobering performance might be due to the resistance to TRAIL therapy in most primary cancer cells, since major cell survival signaling cascades including nuclear factor-kappa B (NF-κB), mitogen-activated protein kinases (MAPKs), and phosphatidylinositol-3-kinases (PI3K/AKT) could also be activated by TRAIL. Therefore, besides the TRAIL apoptotic signaling pathway, this review also describes the nonapoptotic signaling pathways that can be induced upon TRAIL treatment. An update on the potential anticancer effects of TRAIL in both preclinical and clinical studies is also summarized in this review. The role of few selected, natural compounds that can sensitize tumor cells to TRAIL treatment has been also highlighted briefly.

TRAIL-induced apoptotic signaling cascades

TRAIL interacts with five distinct receptors that are encoded by separate genes, but share high sequence homology in the extracellular domains. However, only DR4 and DR5, which contain an intracellular death domain, can produce apoptotic signals.[13] The apoptotic signaling pathway of TRAIL is triggered by trimerized TRAIL binding to DR4 and DR5, which enables the receptors to homotrimerize, thereby driving formation of the death-inducing signaling complex (DISC).[14] Upon ligand stimulation, DR4 and DR5 recruit Fas-associated death domain protein (FADD) through death domain interactions. FADD then recruits pro-caspase-8 and 10, and/or the cellular FLICE (caspase-8)-like inhibitory protein (c-FLIP) to the DISC (Figure 1). c-FLIP competes with caspase-8 for FADD binding in the DISC and inhibits the apoptosis signal.[15] Following recruitment, procaspase-8 comes into contact with the ubiquitin E3 ligase subunit (CUL3), which catalyzes polyubiquitylation of caspase-8 on its C-terminal region. Polyubiquitylated caspase-8 binds with the ubiquitin-binding protein p62, which promotes the translocation of caspase-8 from the DISC into intracellular ubiquitin-rich foci and subsequently leads to the cleavage and activation of caspase-8.[16] Activation of caspase-8 at the DISC transfers the apoptosis signal to executioners of apoptosis either directly via the extrinsic or indirectly via the intrinsic–mitochondrial pathway. In the extrinsic pathway, the DISC activates sufficient caspase-8 to stimulate the effector caspases 3, 6, and 7, and directly induce apoptosis. In the intrinsic–mitochondrial pathway, active caspase cleaves the BH3-interacting domain death agonist (Bid) to truncated Bid (tBid). tBid rapidly translocates to the mitochondria and drives permeabilization of the outer mitochondrial membrane by binding with Bax and Bak, releasing mitochondrial cytochrome c and mitochondria-derived activator of caspase (Smac).[17,18] This process can be blocked by overexpression of X-linked inhibitor of apoptosis protein (XIAP), B-cell lymphoma 2 (Bcl-2), and B-cell lymphoma-extra-large (Bcl-xL).[19,20] Once in the cytosol, cytochrome c conjugates with ATP and apoptotic peptidase-activating factor-1 (Apaf-1) to recruit the initiator caspase-9 into a signaling complex called the apoptosome. Activated caspase-9 then cleaves and activates the effector caspases-3, -6, and -7 to induce apoptosis.[18]

Effect of TRAIL on other cell survival signaling cascades

Although TRAIL exerts a remarkable effect in apoptosis induction, it has been also reported to activate anti-apoptotic pathways such as NF-κB, MAPKs (c-Jun NH2 terminal kinases [JNK], p38, and extracellular signal-regulated kinases [ERK]1/2), PI3K/AKT, and signal transducers and activators of transcription (STATs), which may repair TRAIL-induced apoptosis. The mechanism(s) underlying stimulation of these anti-apoptosis pathways are still not well understood. For example, Eugene et al. have indicated that, subsequent to assembly of the DISC, a secondary complex is formed that may stimulate the nonapoptotic signaling pathways. The secondary complex contains FADD, caspase-8, receptor-*interacting protein* (RIP1), TNF receptor associated factor-2 (TRAF2), and NF-κB essential modulator (NEMO). The association of the secondary complex might be dependent on formation of the primary complex, but also requires its dissociation. The specific localization of the TRAIL receptor complex may be another mechanism involved in the TRAIL-induced anti-apoptotic signaling events. Moreover, the TRAIL receptor localized in membrane lipid rafts activates apoptosis signaling, while the TRAIL receptor complex outside the rafts enables activation of nonapoptotic pathways. Other possible early molecular events for nonapoptotic pathways include the DISC inhibitor cFLIP and modification of TRAIL RIPs. We briefly discuss below few important cell survival pathways that can be activated upon exposure of tumor cells to TRAIL.

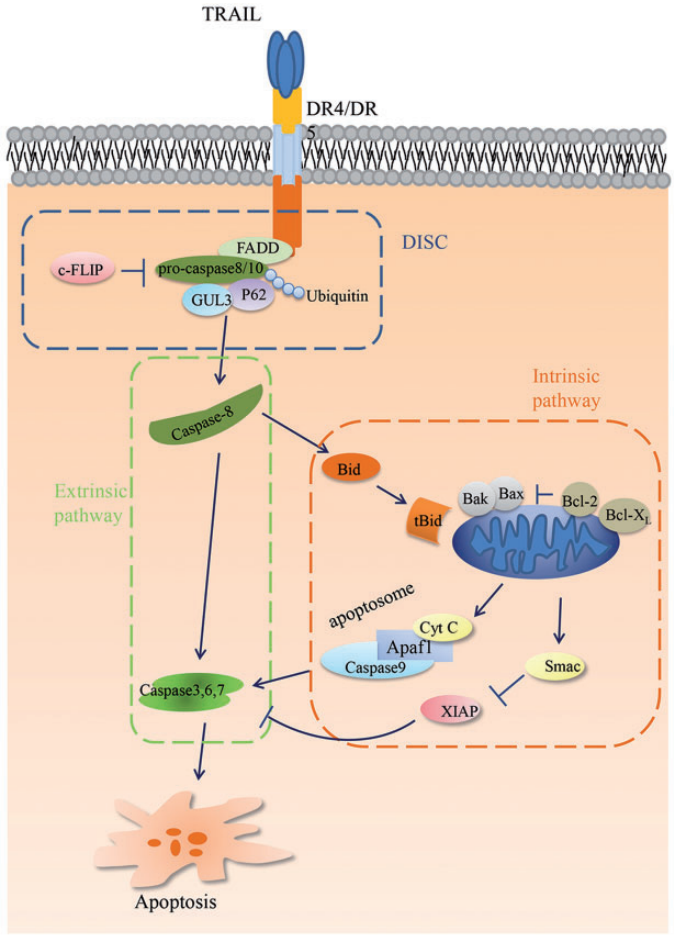

Figure 1 A schematic diagram of TRAIL-induced apoptotic signaling cascades. Binding of TRAIL to TRAIL death receptors results in recruitment of the FADD and caspase-8 to form DISC. DISC leads to the cleavage and activation of caspase-8, which can activate caspase effectors and the BH3-only protein Bid. In the extrinsic pathway, activation of caspase-8 is sufficient to activate downstream caspases-3, -6, and -7, leading to cell death. However, in the intrinsic pathway, cleavage of Bid into its truncated form (tBid) is essential to induce cell death. tBid can rapidly translocate to the mitochondria and drive (through Bax and Bak) permeabilization of the outer mitochondrial membrane, releasing mitochondrial cytochrome c and Smac. This process can be blocked by overexpression of Bcl-2 and Bcl-xL. Once in the cytosol, cytochrome c binds to the adaptor Apaf-1 to recruit the initiator caspase-9 into the apoptosome, which can activate caspase-9 and the effector caspases. Release of Smac augments apoptosis by antagonizing the inhibitory effect of XIAP on the various effector caspases. (A color version of this figure is available in the online journal.)

NF-κB

NF-κB is a transcription factor that is involved in inflammation and cell survival. The NF-κB family has five members: p65, Rel B, cRel, p50, and p52. In the TRAIL/TRAIL-R system, NEMO/IKKγ in the secondary complex recruits IKKα/β, which phosphorylates IκBα and induces its ubiquitination and degradation. Degradation of IκBα activates NF-κB, and allows its nuclear translocation. NF-κB then binds to the DNA and induces transcription of anti-apoptotic genes such as *Bcl-xl*, *Mcl-1*, *cFLIP*, and *cIAPs*. Inhibition of NF-κB by using either an IκB dominant negative version or selective chemical factor was found to enhance TRAIL-induced apoptosis in several preclinical tumor models such as leukemia, neuroblastoma, pancreatic cancer, and non-small cell lung carcinoma (NSCLC).[21–25] Interestingly, a pro-apoptotic effect was also reported in TRAIL-induced NF-κB activation. For example, deficiency of cRel resulted in resistance to TRAIL treatment in glioma cell lines,[26] and a similar anti-apoptotic effect was also observed in human β islets cells.[27] The mechanism(s) for the pro-apoptotic role of NF-κB are still elusive. However, it was found that NF-κB can help recruitment of FADD and caspase 8, and facilitate DISC formation. Few other evidences further indicates that a pro-apoptotic role of NF-κB might be related to the relative amount of RelA and cRel in activated NF-κB, as cRel upregulation was found to enhance the expression levels of TRAIL1 and TRAIL2 receptors,[28] while Rel A overexpression had opposite effects.[29] Besides above described apoptosis-related roles, NF-κB activation was also reported to be involved in the TRAIL-enhanced invasion of apoptosis-resistant pancreatic ductal adenocarcinoma cells.[30]

MAPKs

The MAPKs are kinases that control different cellular processes such as immunoregulation, inflammation, cell growth, cell differentiation, and cell death. This family consists of six members: the ERK1/2, ERK3/4, ERK5, ERK7/8, JNK1/2/3, and the p38-MAPK. Among them, TRAIL can significantly activate JNK, p38, and ERK1/2 in diverse

tumor cell lines. For example, TRAIL-induced JNK activation requires both RIP and TRAF2 in the secondary complex,[31–33] and JNK might be activated through the TRAF2–MEKK1–MKK4 signaling pathway.[32] Different mechanisms are involved in JNK-mediated apoptosis induced by TRAIL. Bim is a pro-apoptotic Bcl-2 family member, which can mediate lysosome permeabilization and induce cell death through activating Bax. It was found that TRAIL can enhance Fas-induced cell death through activating JNK and its downstream substrate Bim in isolated murine hepatocytes[34]; and the pharmacological JNK inhibitor SP600125 can attenuate TRAIL-induced lysosomal permeabilization and cell death in cholangiocarcinoma.[35] Stimulation of autophagic cell death might be another mechanism contributing to JNK-mediated cell death, because Beclin 1 (which is an important autophagy regulator), could be phosphorylated by TRAIL-induced JNK activation.[36] In addition, JNK was found to have dual activity as inhibition of JNK-enhanced TRAIL-induced apoptosis in hepatocellular carcinoma cells,[37] and this dual effect might be due to the magnitude of signal transduction and the isoforms involved. For example, prolonged JNK activation or long isoforms of JNK such as JNK1α2 and JNK1β2 induce cell apoptosis, while transient activation or short isoforms (JNK1α2 and JNK1β2) prevent apoptosis.[38,39] Therefore, JNK may act as a pro- and/or anti-apoptotic molecule in different cell types and experimental systems.

TRAIL-induced p38 activation is RIP1 and TRAF2 dependent,[31] and it has been reported that TRAIL-induced p38 activation through upstream kinases such as TGF-β activated kinase-1 (TAK1) and MKK4/MKK6.[40] The roles of p38 in TRAIL-induced apoptosis are also controversial. In Hela cells, p38 activation was found to be responsible for TRAIL-induced apoptosis, because specific p38 kinase inhibitor SB203580 prevented apoptosis.[41] TRAIL-induced reactive oxygen species (ROS) production may also contribute to p38 activation. Pretreatment with antioxidants such as glutathione attenuated p38 kinase activation as well as TRAIL-induced apoptosis. Meanwhile, TRAIL-induced p38 activation has also shown an anti-apoptotic effect. Son et al. reported that TRAIL can induce p38 activation in prostate cancer cells, and activated p38 can further upregulate the expression of *Mcl-1* gene, which can suppress the intrinsic apoptosis pathway by inhibiting mitochondrial membrane permeabilization.[40] In addition, p38 inhibition sensitized breast carcinoma cells to TRAIL treatment.[42] However, in some other tumor cells such as the human colorectal cancer cell line DLD1, p38 did not play a major role in TRAIL-mediated apoptosis.[43] For example, although p38 was activated in TRAIL-sensitive DLD1 cells but not in TRAIL-resistant DLD1 cells, p38 inhibition did not block TRAIL-mediated cell death. Therefore, the role of p38 in TRAIL-induced apoptosis might also be cell-type dependent.

On the other hand, ERK1/2 activation has been mainly implicated in cell survival and proliferation. The activation of ERK1/2 by TRAIL has been reported in a number of cell types,[44,45] and the mechanism may be Mst1 (mammalian sterile 20-like kinase 1) dependent, as a caspase-3-generated 36 kDa form of Mst1 was found to activate ERK1/2.[46] ERK1/2 protects cells from TRAIL-mediated apoptosis. Smac/direct IAP binding protein with low pI (DIABLO) release from mitochondria is an important pathway mediating TRAIL-induced apoptosis. In melanoma cells, it was shown that release of Smac/DIABLO was downregulated by EKR1/2 activation, thus attenuating TRAIL-induced apoptosis.[45] Inhibition of ERK1/2 sensitized cells to TRAIL-induced apoptosis in breast cancer cells and HT-29 colon cancer cells, and further indicates that ERK1/2 is a critical proliferation mediator.[47] In NSCLC, which lack caspase-8, TRAIL caused an increase in proliferation, and the induced proliferation was mediated by ERK1/2, as ERK inhibition attenuated the TRAIL-induced proliferation.[48] A similar role of ERK1/2 was also observed in TRAIL-resistant human glioma cells, in which TRAIL-induced ERK1/2 increased cell proliferation via increasing cell cycle progression and inhibiting c-FLIP(L) (the long form of the caspase 8 inhibitor).[49]

PI3K/AKT

Akt is a PI3K-activated protein kinase, which is mainly involved in regulating cellular functions such as cell growth, apoptosis, and survival.[50] TRAIL-induced Akt activation has been demonstrated in various cancer types. In the TRAIL-sensitive prostate cancer cell line DU145, TRAIL stimulated Akt activation via Rous sarcoma oncogene cellular homolog (Src) and c-Cbl, and suppression of Akt enhanced the TRAIL-induced apoptosis.[51] Akt activation may also contribute to development of TRAIL resistance, as inhibition of TRAIL-induced Akt phosphorylation sensitized the TRAIL-resistant NSCLC cells for TRAIL treatment.[52] A similar phenomenon was also observed in TRAIL-resistant ovarian and breast cancer cell lines.[53]

STAT3

STAT3 is a cytoplasmic transcription factor involved in cell proliferation, apoptosis, angiogenesis, and immune response. With the ligands (cytokines or growth factors such as epidermal growth factor [EGF]) binding to the receptors, monomeric STAT3 are phosphorylated by the receptor-associated tyrosine kinases such as JAK and Src, and then form dimers to migrate into the nucleus and activate gene transcription. In 2012, Azijli et al.[52] found that TRAIL can enhance cell migration and invasion through activating the Src-STAT3 pathway in the TRAIL-resistant NSCLC cells. Inhibition of Src or STAT3 by either a chemical inhibitor or shRNA-attenuated TRAIL-induced migration and invasion. Activation of Src and STAT3 is mediated through RIP1 kinase. Silencing of RIP kinase suppressed TRAIL-induced Src and STAT3 phosphorylation as well as TRAIL-induced migration and invasion. TRAIL-R2 might mediate TRAIL-induced activation of Src and STAT3, as DHER (D269H/E195R, a selective TRAIL variant against TRAIL2) significantly enhanced cell migration and invasion. Src activation may also contribute to the apoptosis resistance, as it was found to impair DR/caspase 8-dependent apoptosis by phosphorylating caspase 8 at tyrosine 380.[54] Src also can induce an autocrine or paracrine loop of TGF-α–EGFR activation.[55] Figure 2 summarizes the various cell survival pathways activated upon exposure of tumor cells to TRAIL.

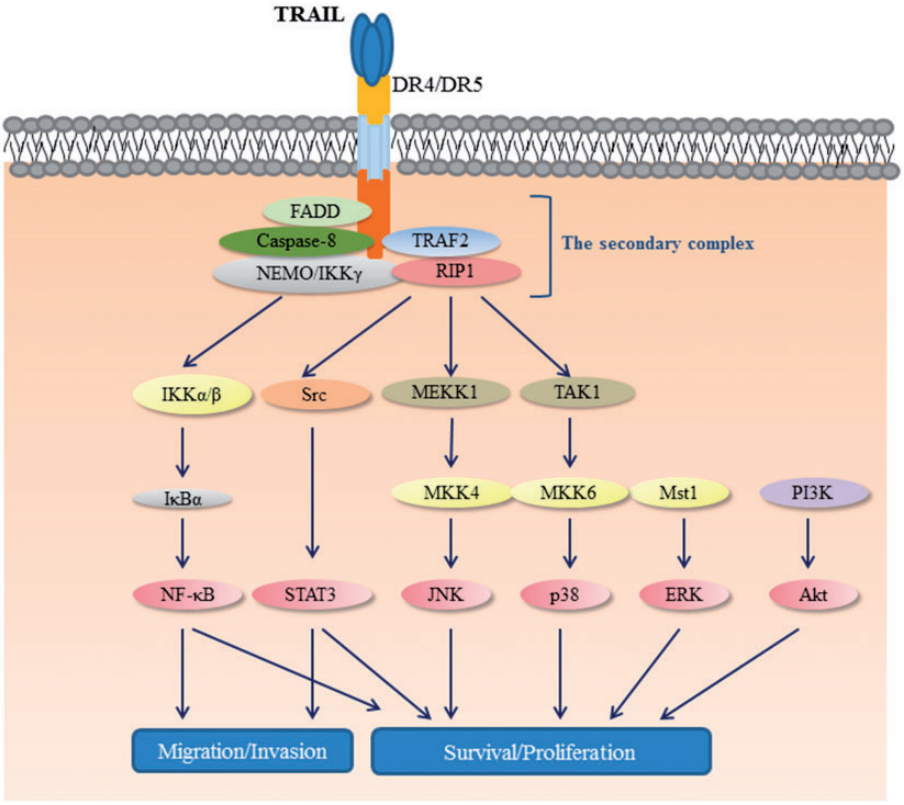

Figure 2 A schematic overview of prosurvival signals elicited by the activation of TRAIL receptors. With agonists binding to TRAIL-R1/R2, a secondary complex can be formed after receptor activation, leading to the activation of various signaling pathways that are involved in induction of nonapoptotic responses as indicated. (A color version of this figure is available in the online journal.)

Anticancer effects of TRAIL

There is strong experimental evidence that the TRAIL pathway has an important role in the regulation of tumor initiation and development. As detailed below, TRAIL may contribute to host immune surveillance against tumors. Moreover, dysfunction of TRAIL-Rs through mutation or decreased expression may promote tumor progression and confer intrinsic resistance to TRAIL-induced apoptosis. TRAIL is expressed by effector lymphocytes, which are well known to contribute to host immune surveillance against primary tumor development and metastasis. TRAIL, along with perforin 1 and CD95L, is constitutively expressed on murine natural killer (NK) cells in the liver, but not NK T cells or ordinary T cells, and is responsible for spontaneous cytotoxicity against TRAIL-sensitive tumor cells *in vitro* and *in vivo*.[56,57] Both the mouse and human Apo2L/TRAIL promoters are regulated by interferon-gamma (IFN-γ),[58,59] and Apo2L/TRAIL expression and its contribution to preventing liver metastases depend on IFN-γ signaling.[60] Furthermore, the IFN-γ-induced expression of TRAIL might change the tumor microenvironment to enable enhanced antigen presentation and tissue infiltration. The TRAIL-sensitive tumor cells interaction with TRAIL-expressing tumor infiltrating immune cells might be involved in tumor resistance and metastasis.[61]

In addition to Apo2L/TRAIL's contribution to immune surveillance, Apo2L/TRAIL appears to play an important role in suppressing tumor progression and determining chemosensitivity. Both neutralization of TRAIL by monoclonal antibody and TRAIL knockout mice promoted tumor development in mice and supported a direct role for NK cells expressing TRAIL in the suppression of tumor metastasis, while no metastasis occurred with the TRAIL-resistant cells.[60,62] A more recent study shows that syngeneic renal cell carcinomas grow faster and shows increased metastasis to the liver in Apo2L/TRAIL knockout mice as compared with wild-type controls.[63] Furthermore, metastasis to lymph nodes was significantly enhanced in TRAIL-R-deficient mice, which indicates that TRAIL-R2 is a metastasis suppressor in the mouse multistage model of squamous cell carcinoma.[64] Wang and El-Deiry[65] showed that silencing of TRAIL-R2/DR5 *in vivo* promotes tumor growth and renders tumor cells resistant to the chemotherapeutic agent 5-fluorouracil. These findings provide the evidence for the physiological function of TRAIL as a tumor suppressor.

Ongoing clinical trials with TRAIL alone and in combination with other pharmacological agents

Since TRAIL was observed to have promising anticancer effects in preclinical research, agonists targeting the TRAIL receptor have been developed and have already undergone Phase I and Phase II clinical trials. There are

two categories of clinically tested TRAIL receptor agonists (TRAs): recombinant forms of TRAIL and agonistic antibodies specific for TRAIL-R1 or TRAIL-R2. Recombinant forms of TRAIL have a stronger apoptotic effect compared to agonistic antibodies because they can target and trigger both apoptosis receptors (TRAIL-R1 and TRAIL-R2). However, since it has no selectivity, it also increases their chance to bind the DcRs such as TRAIL-R3 and TRAIL-R4, and thus attenuates its apoptotic activity. Recombinant forms of TRAIL were also found to be cleared by the body within hours, so repeated administration is required for systemic application.[66]

Considering the less selectivity of the recombinant forms of TRAIL, agonistic antibodies specific for TRAIL-R1 or TRAIL-R2 might be more effective for cancer treatment. The antibodies can selectively bind to specific apoptotic TRAIL-Rs, therefore they would not bind to nondeath-inducing TRAIL-Rs and activate the survival pathway. In addition, the half-life of agonistic antibodies is in the range of several days to weeks, which allows a more stable concentration within tissues and avoids the need for continuous application.[66]

Recombinant forms of TRAIL

Dulanermin, which contains the TNF homology domain within the extracellular domain of human soluble TRAIL, is the only recombinant TRAIL developed for clinical application.[11] In several phase I clinical trials, dulanermin has proved to be a safe and well-tolerated drug in the treatment of different tumors such as colorectal cancer, lung cancer, and lymphoma, even when combined with other chemotherapies (Table 1).[67–101] The antitumor activity of dulanermin has also been shown in patients for whom partial or complete clinical responses were observed. To further study the specific antitumor activity of dulanermin, Phase II b clinical trials (randomized control trials, RCTs) were performed in nonsmall cell lung cancer[71] and non-Hodgkin's lymphomas[73] (Table 1). However, although these two trials further confirm the tolerability of dulanermin in cancer treatment, no significant anticancer activities were observed.

Agonistic antibodies

Mapatumumab is the only agonistic TRAIL-R1 specific antibody that has entered clinical trials (Table 1). Its safety and broad tolerability have been revealed in both Phase I and Phase II trials. In the Phase I trials, partial responses were observed in advanced cancers when used in combination with chemotherapy. Although Phase IIa and IIb trials have been conducted in cancers such as colorectal and lung cancers, an objective response was only observed in patients with lymphoma undergoing a Phase II a trial, while no anticancer activity was observed in other trials. There are several agonistic TRAIL-R2 specific antibodies such as conatumumab, lexatumumab, tigatuzumab, drozitumab, and LBY-135 (Table 1). So far, different clinical trials have been carried out either alone or in combination with chemotherapy. Some positive trends were observed in Phase I and Phase II a trials, while no significant anticancer activity was achieved in Phase II b trials (Table 1). These TRAs although have been found to be well tolerated, but they exhibited only minimal therapeutic activity in these clinical trials. Therefore, future work might be to focus on strategies that could achieve a significant anticancer effect with these pharmacological modulators.

Natural compounds that can sensitize tumor cells to TRAIL

Numerous natural compounds have shown great potential to enhance TRAIL-induced apoptosis through modulation of diverse nonapoptotic pathways such as NF-κB, STAT3, PI3K/AKT, MAPKs, and p53, which can be considered as a part of emerging treatments for unresponsive cancer (Table 2). For example, suppression of TRAIL-induced NF-κB activation is considered to be an important method to sensitize cancer cells to TRAIL by using natural compounds. Numerous natural compounds such as wogonin (derived from the popular Chinese herb Huang-Qin), sulforaphane (derived from enriched broccoli sprout extracts), and melittin (major component of bee venom) sensitize resistant malignant cells to TRAIL-induced apoptosis through the modulation of NF-κB signaling pathway.[102–104] Upregulation of TRAIL receptors through NF-κB is also mediated by other natural compounds such as the ethanolic extract of Brazilian green propolis (EEP) and curcumin (a substance found in turmeric).[105,106] However, kurarinone (a natural bioactive lavandulyl flavonoid),[107] resveratrol (a type of natural phenol),[108] artesunate (a derivative of the natural product artemisinin),[109] and combertastatin A-4 (isolated from the bark of *combretum caffrum*)[110] can also sensitize melanomas to TRAIL through abrogating TRAIL-induced NF-κB activation and modulation of expression of anti-apoptotic genes such as *cFLIP*, *XIAP*, and *Bcl-xL*.

Since suppression of the STAT3 pathway is linked to overcoming TRAIL resistance of tumor cells, numerous natural compounds have been investigated to determine whether they sensitize cancer cells via STAT3-dependent mechanism(s). Most of these natural compounds such as chrysin (a major constituent of Thai propolis), 6BIO (a derivative of indirubin), and bufadienolide (a major class of biologically active compounds isolated from ChanSu) can overcome TRAIL resistance of cancer cells through *Mcl-1* downregulation by inhibiting STAT3 phosphorylation.[111–113] Another natural agent, resveratrol, was found to increase sensitivity of melanomas to exogenous TRAIL through suppressed expression of cFLIP and Bcl-xL proteins and decreased STAT3 and NF-κB activation.[108] Upregulation of DRs through the suppression of STAT3 activation by parthenolide (a sesquiterpene lactone found in European fever few)[114] and luteolin (3′,4′,5,7-tetrahydroxyflavone, found in many pants)[115] has also been found to be involved in enhancing the sensitivity of tumor cells to TRAIL.

Cellular resistance to TRAIL could also be developed through phosphorylation (activation) of the PI3K/AKT pathway. Eupatolide, the sesquiterpene lactone isolated from the medicinal plant *Inula Britannica*, could augment

Table 1 Results of recombinant TRAIL or agonistic antibodies targeting TRAIL-R in clinical trials

Agents	Phase	Cancer type	Efficacy/*n*	References
Dulanermin	I	Advanced cancers	2PR/71	Herbst et al.[67]
	I	Colorectal	13PR/23, 6PR/27, NA/30	Wainberg et al.[68] Kasubhai et al.[69] Yee et al.[70]
	I	Lung	(1CR+13 PR)/24	Soria et al.[139]
	I	Lymphoma	(2CR + 1PR)/7	Yee et al.[72]
	II (RCT)	Lung	No cancer activity[a]/213	Soria et al.[71]
	II (RCT)	Lymphoma	No cancer activity[a]/48	Belada et al.[73]
Mapatumamab	I	Advanced cancers	No response/49, No response/41, 12PR/49, 5PR/27	Tolcher et al.[74] Hotte et al.[75] Mom et al.[76] Leong et al.[77]
	I/II	Lymphoma	(2CR + 1PR)/40	Younes et al.[78]
	II	Colorectal	No response/38	Trarbach et al.[79]
	II	Lung	No response/32	Greco et al.[80]
	II (RCT)	Multiple myeloma	No cancer activity[a]/104	Belch et al.[81]
	II (RCT)	Lung	No cancer activity[a]/109	von Pawel et al.[82]
Conatumumab	I	Advanced cancers	1PR/37, No response/18, No response/9	Herbst et al.[140] Doi et al.[83] Chawla et al.[84]
	I	Soft tissue sarcoma	No response/6	Demetri et al.[85]
	I	Lung	(1CR + 3PR)/12	Paz-Ares et al.[141]
	I	Colorectal	5PR/12	Saltz et al.[87]
	I	Pancreatic	4PR/13	Kindler et al.[88]
	II (RCT)	Soft tissue sarcoma	No anticancer activity[a]/128	Demetri et al.[85]
	II (RCT)	Lung	No anticancer activity[a]/172	Paz-Ares et al.[86]
	II (RCT)	Pancreatic	No anticancer activity[a]/83	Kindler et al.[89]
	II (RCT)	Colorectal	No anticancer activity[a]/103, No anticancer activity[a]/190	Cohn et al.[90] Fuchs et al.[91]
Lexatumumab	I	Advanced cancers	No response/37, No response/31, PR/41	Plummer et al.[92] Wakelee et al.[93] Sikic et al.[94]
	I	Pediatric cancers	No response/24	Merchant et al.[95]
Tigatuzumab	I	Carcinoma–lymphoma	No response/17	Forero-Torres et al.[96]
	II	Pancreatic	8PR/61	Forero-Torres et al.[97]
	II (RCT)	Lung	No anticancer activity[a]/97	Reck et al.[98]
Drozitumab	I	Colorectal	2PR/9	Rocha Lima et al.[99]
	I	Advanced cancers	No response/50	Camidge et al.[100]
LBY-135	I/II	Advanced cancers	2PR/73	Sharma et al.[101]

Note: TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; n, number of patients enrolled; CR, complete response; PR, partial response; NA, data about responses (efficacy) were not reported; RCT, randomized-controlled trials.
[a]Anticancer activity was considered when the addition of the recombinant TRAIL or agonistic antibodies demonstrated statistically significant activity as compared to the standard therapy.

TRAIL-induced apoptosis in human breast cancer cells by downregulating c-FLIP expression through the inhibition of AKT phosphorylation.[116] Besides this, there are various other natural compounds reported to activate TRAIL-induced apoptosis through inhibition of the PI3K/AKT pathway, such as sanguinarine (a benzophenanthridine alkaloid derived from the root of *Sanguinaria Canadensis*),[117] artesunate,[109] and luteolin.[115]

Several natural compounds can significantly increase the expression of ERK1/2, which further induces the expression of DRs. Gossypol (a polyphenol derived from cotton seed oil), curcumin (a natural compound derived from *curcuma longa*), apigenin (4′,5,7-trihydroxyflavone found in many plants), and butein (active component of the stems of *Rhus verniciflua* Stokes) induce DRs directly through activation of ERK1/2.[106,118–120] Azadirone (a limonoid tetranortriterpene), γ-tocotrienol (an unsaturated vitamin E present predominantly in palm oil), and nimbolide (a terpenoid lactone derived from *azadirachta indica*)[121–123] induce DRs through activation of ERK1/2 mediated by activation of p53 pathways. Zerumbone (a component of Asian ginger) has been shown to upregulate DR expression through

Table 2 A list of selected natural compounds that can sensitize tumor cells to TRAIL

Compound	Tumor type(s)	Mechanism(s) of action	References	Compound	Tumor type(s)	Mechanism(s) of action	References
NF-κB dependent				ERK dependent			
Wogonin	Leukemia	NF-κB ↓	Fas et al.[102]	Zerumbone	Human colorectal cancer	ERK/P38↑-DR4/5↑	Yodkeeree et al.[124]
Sulforaphane	Human prostate cancer	NF-κB ↓	Labsch et al.[103]	Azadirone	Human cancer cells	ERK↑-P53↑-DR4/5↑	Gupta et al.[122]
Melittin	Hepatocellular carcinoma	NF-κB↓	Wang et al.[104]	γ-tocotrienol	Human cancer cells	ERK↑-P53↑-DR4/5↑	Kannappan et al.[123]
Curcumin	Human bladder cancer	NF-κB↓-DR5↑	Hussain et al.[106]	Curcumin	Human breast cancer	ERK↑-DR5↑-Mcl-1↓	Hussain et al.[106]
Kurarinone	Human cervical carcinoma	NF-κB↓-cFLIP↓	Seo et al.[107]	Apigenin	Hepatocellular carcinoma	ERK↑-DR5↑	Kim et al.[120]
Resveratrol	Melanoma	NF-κB↓/STAT3↓-cFILP↓	Ivanov et al.[108]	Butein	Hepatocellular carcinoma	ERK↑-DR5↑	Moon et al.[118]
Artesunate	Human cervical carcinoma	NF-κB↓/PI3K↓-Bcl-XL↓	Thanaketpaisarn et al.[109]				
Combretastatin A-4	Human colorectal cancer	NF-κB↓-XIAP↓	Zhang et al.[110]				
STAT3 mediated				JNK mediated			
Chrysin	Human lung adenocarcinoma	STAT3↓-Mcl-1↓	Lirdprapamongkol et al.[111]	SVT	Human cancer cells	ROS↑-JNK↑-DR4/5↑	Park et al.[127]
6BIO	Human breast cancer	STAT3↓- Mcl-1↓/cFLIP↓	Braig et al.[112]	Tricetin	Hepatocellular carcinoma	ROS↑-JNK↑-DR4/5↑	Hsu et al.[129]
Bufadienolide	Human breast cancer	STAT3↓-Mcl-1↓	Dong et al.[113]	Capsazepine	Human colorectal cancer	ROS↑-JNK↑ -DR4/5↑	Sung et al.[128]
Parthenolide	Hepatocellular carcinoma	STAT3↓-DR4/5↑	Carlisi et al.[114]	Ursolic acid	Human cancer cells	ROS↑-JNK↑-DR4/5↑	Prasad et al.[126]
Luteolin	Human renal carcinoma	STAT3↓/AKT↓-DR4/5↑	Ou et al.[115]	Cordycepin	Hepatocellular carcinoma	JNK↓-Bcl2↓	Lee et al.[125]
PI3K/AKT dependent				p53 dependent			
Artesunate	Human cervical carcinoma	NF-κB↓/PI3K↓-Bcl-XL↓	Thanaketpaisarn et al.[109]	Triptolide	Human prostate cancer	p53↑-DR5↑	Xiaowen et al.[134]
Sanguinarine	Human gastric adenocarcinoma	PI3K↓/AKT↓-Bid↓	Choi et al.[117]	α-TOS	Human cancer cells	p53↑-DR4/5↑	Tomasetti et al.[135]
Eupatolide	Human breast cancer	AKT↓-cFLIP↓	Lee et al.[116]	Andrographolide	Hepatocellular carcinoma	P53↑-DR4↑	Zhou et al.[136]
Luteolin	Human renal carcinoma	STAT3↓/AKT↓- -DR4/DR5↑	Ou et al.[115]	6-DHGD	Human hepatoblastoma	ROS/p53↑-DR5↑	Chen et al.[137]
p38 mediated				Nimbolide	Human colon cancer	ERK/p53↑-DR4/5↑-Bax↓	Gupta et al.[121]
Zerumbone	Human colorectal cancer	ERK/P38↑-DR4/5↑	Yodkeeree et al.[124]	γ-tocotrienol	Human cancer cells	ERK/P53↑-DR4/5↑	Kannappan et al.[123]
Diosgenin	Human colorectal cancer	P38↑-DR5↑	Lepage et al.[131]	Lupulone	Human colorectal cancer	P53/P38↑-DR4/5↑	Lamy et al.[130]
CAPE	Hepatocellular carcinoma	P38↑-DR4/5↑	Kim et al.[132]	Damnacanthal	Human cancer cells	P53/P38↑-DR5↑	Lin et al.[138]
Lupulone	Human colorectal cancer	P3↑8-P53↑-Mcl-1↓	Lamy et al.[130]				

Note: NF-κB, nuclear factor-kappa B; ERK, extracellular signal-regulated kinases; PI3K, phosphatidylinositol-3-kinases; Brazilian EEP, Brazilian green propolis; 6BIO, 6-bromo-indirubin-3′-oxime; CAPE, caffeic acid phenethyl ester; SVT, snake venom toxin; α-TOS, alpha-tocopheryl succinate; 6-DHGD, 6-dehydrogingerdione; ↓, downregulated; ↑, upregulated.

induction of ERK and P38 activation.[124] These studies indicate that ERK-dependent upregulation of TRAIL receptor DR4/5 can form the basis of an important strategy method to sensitize tumor cells to TRAIL.

Since inhibition of JNK can enhance TRAIL-induced apoptosis,[37] numerous natural compounds have been investigated to determine whether they sensitize tumor cells to TRAIL via a JNK-dependent mechanism(s). One of these natural compounds, cordycepin, an active component of the caterpillar fungus *cordyceps militaris*, increases sensitivity of human hepatocellular carcinoma Hep3B cells to TRAIL-mediated apoptosis directly by inactivating the JNK signaling pathway.[125] However, other natural compounds such as SVT (snake venom toxin from *vipera lebetina turanica*), ursolic acid (a pentacyclin triterpene), capsazepine (the active ingredient of chilli pepper), and tricetin (a flavonoid derivative found in *Myrtaceae* pollen and *Eucalyptus* Honey) induce DRs mediated by JNK1/2 activation through production of ROS.[126–129]

DRs can also be upregulated by diverse natural compounds through the activation of p38 signaling cascade. Another natural agent, lupulone, a β-acid largely present in hops (*Humulus lupulus* l), can significantly enhance the expression of p38, which plays a major role in the activation of p53 and the TRAIL-DR apoptotic pathway in SW620 human colon cancer-derived metastatic cells.[130] Zerumbone (a sesquiterpene from the edible plant *Zingiber zerumbet Smith)*, diosgenin (obtained from fenugreek), and caffeic acid phenethyl ester (CAPE; a phenolic compound derived from honeybee propolis) have also been shown to upregulate DR expression through induction of p38 activation.[124,131,132] Our group has also recently reported that emodin, a naturally occurring anthraquinone present in the roots and barks of numerous plants, and an active ingredient of various Chinese medicinal herbs can downregulate the expression of various cell survival proteins, and induce the cell surface expression of both TRAIL receptors, DR 4 as well as 5 in hepatocellular carcinoma cells. In addition, emodin increased the expression of C/EBP homologous protein (CHOP) in a time-dependent manner.[133] Knockdown of CHOP by small interfering RNA (siRNA) decreased the induction of emodin-induced DR5 expression and apoptosis. Emodin-induced induction of DR5 was mediated through the generation of ROS, as N-acetylcysteine blocked the induction of DR5 and the induction of apoptosis.

A critical factor for the TRAIL resistance of p53-mutant cell lines is the limited upregulation of the expression of DR4 and DR5 by mutant p53. Numerous natural compounds such as triptolide (isolated from the Chinese herb *Tripterygium wilfordii Hook*), alpha-tocopheryl succinate (α-TOS, an analogue of vitamin E), and andrographolide (a diterpenoid lactone isolated from a traditional herbal medicine *Andrographis paniculata*) upregulate the expression of DRs directly through the induction of p53.[134–136] It has also been reported that ROS participates in the induction of DRs by 6-dehydrogingerdione (a compound isolated from the rhizomes of *Zingiber officinale*) mediated through expression of p53.[137] However, nimbolide, azadirone, and γ-yocotrienol induce DR expression through p53 expression that is mediated by an ERK-p53 mechanism(s).[121–123] Similarly, lupulone and damnacanthal (isolated from *Morinda citrifolia*) can upregulate DRs through induction of both p38–p53 mediated mechanism(s).[130,138] These studies indicate that p53 plays an important role in induction of DRs by natural compounds, which can significantly sensitize tumor cells to TRAIL therapy. Table 2 summarizes the list of various natural compounds that can significantly summarize tumor cells to TRAIL.

Conclusions

This review briefly summarizes both the apoptotic and non-apoptotic pathways that can be activated upon TRAIL treatment as well as its physiological role in cancer. However, the molecular mechanism(s) contributing to TRAIL resistance in tumor cells still remain to be elucidated. Experimental preclinical as well as clinical evidences show that both TRAIL antibody and TRAIL used in combination with chemotherapeutics have a significant potential for anticancer treatment 139–141. From various reports, it is also clear that various pharmacological agents derived from natural sources can sensitize tumor cells to TRAIL through direct activation of intrinsic apoptotic pathway or modulation of diverse nonapoptotic pathways to upregulate DRs. However, as most of these studies have been conducted in cell lines or in preclinical mouse models of cancer; hence, additional clinical evidences are required to confirm whether these natural agents may also have synergistic therapeutic effects with TRAIL in cancer patients. Furthermore, since several recombinant TRAIL antibodies and agonistic antibodies against DRs are being used in the clinic, the combination of natural agents with these antibodies may greatly revolutionize cancer treatment. Therefore, in the coming years, we hope that such studies will be conducted and yield promising results.

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