·Review·

TNF related apoptosis –inducing ligand and its receptors in ocular tumors

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Abstract

• Most of the ocular tumors have poor prognosis, and they remain a difficult problem in the area of ophthalmology. With the rapid development of molecular biology and immunologic techniques and the deep research on ocular tumor related genes, it becomes possible to diagnose and treat malignant tumors from the molecular level. The tumor necrosis factor related apoptosis-inducing ligand (TRAIL), a member of the tumor necrosis factor (TNF) super family, is a promising candidate, either alone or in combination with established cancer therapies, since it can initiate apoptosis through the activation of their death receptors. The ability of TRAIL to selectively induce apoptosis of transformed, virus-infected or tumor cells but not normal cells promotes the development of TRAIL-based cancer therapy. Here, we will review TRAIL and its receptors' structure, function, mechanism of action and application in ocular tumors therapy.

• KEYWORDS: tumor necrosis factor related apoptosis-inducing ligand; ocular tumors; apoptosis

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INTRODUCTION

Ocular tumor is one of the malignant diseases detrimental to human health. Operation, radiotherapy and chemotherapy are the major treatment means at present. These methods could help the patients to live longer, however, the problem on recurrence and distant metastasis still remains a big challenge. With the rapid development of molecular biology and immunologic techniques and the deep research on ocular tumor related genes, it becomes possible to diagnose and treat disease from the molecular level. Gene therapy, with a favorably therapeutic activity in patients with ocular tumor, is becoming an important part in biological therapy and will become a promising candidate for the treatment of ocular tumor. Tumor necrosis factor related apoptosis-inducing ligand (TRAIL), belonging to the tumor necrosis factor (TNF) superfamily, is the most promising candidate, either alone or in combination with established cancer therapies, since it can initiate apoptosis through the activation of their death receptors. It has five specific receptors which can be classified as three groups: The first group comprises DR4 and DR5, which are expressed widely in many normal cells and cancer cells. The combination of TRAIL with DR4 or DR5 can induce cell apoptosis. The second group includes DcR1 and DcR2. DcR1 is only expressed on normal cells but not cancer tissues, and DcR2 is found on fetal liver and adult testis tissue, both can competitively binds with TRAIL to avoid or inhibit TRAIL-induced cell damage on normal cells. The third group has only one member-osteoprotegerin (OPG). The combination of TRAIL and OPG will inhibit the TRAIL-induced cell apoptosis. The ability of TRAIL to selectively induce apoptosis of transformed, virus-infected or tumor cells but not normal cells promotes the development of TRAIL-based cancer therapy. Although there have been many excited achievements on the research of TRAIL's anti-tumor effects, the mechanism of TRAIL-induced cell apoptosis still remains unclear. The specificity of TRAIL-induced cancer cell apoptosis also becomes the points at issue. Here, we will review TRAIL and its receptors' structure, function, mechanism of action and application in ocular tumors therapy.

TRAIL AND ITS RECEPTORS

Structure and Function of TRAIL TRAIL, a member of the TNF superfamily which shares higher homology with Fas-L, was firstly cloned by Wiley *et al*[1] from the human cardiac muscle cDNA library in 1995. The same gene was

cloned by Pitti *et al* [2] in 1996, and was named as Apo-2L. TRAIL gene is located at chromosome band 3q26, 1769bp in length and includes 5 exons. Its cDNA full-length is 1042bp. TRAIL protein is a type Ⅱ transmembrane protein of which hydrophobic N terminal is in the cytoplasm and has no signal peptide, and hydrophilic C terminal is out of the cytoplasm. The molecular mass is 32.5kDa, and isoelectric point 7.63. The full-length has 281 amino acid residues, of which 241 are out of the cytoplasm, and the function sites are located between 114-281 amino acid residues. The amino acids located between 137-152, which near the cleavage site in N-terminal, can form an elongated "AA" ring by 12-16 amino acid residues. This ring has a central role both in the cytotoxic activities of TRAIL and its binding to the receptors. The chelation of Cys230, an unpaired Cys- residue located at 230, with Zn~(2+) form the typical chain-sandwich homotrimer structure. So Cys230 plays a decisive role in maintaining TRAIL's space structure and biological activities.

TRAIL can selectively induce apoptosis of transformed, virus infected or tumor cells, but has little effect on normal cells. There is expression of TRAIL on activated T cells, natural killer (NK) cells, monocytes and dendritic cells, which hints that TRAIL participates in modulating body's immune function and has an effect on host defense and immune homeostasis [3]. The latest studies on TRAIL indicated the potential therapeutic benefit of TRAIL in neutrophilic inflammation [4].

Structure and Function of TRAIL´s Receptor Five specific receptors were found so far, with genes located at chromosome band 8q21 (Figure 1). These receptors can be classified into three classes.

Class 1 Death receptors (DR), which include DR4 and DR5, also known as TRAIL-R1 and R2, contain a functional death domain (DD) in their cytoplasmic region which is capable of activating the extrinsic pathway for apoptosis after TRAIL-induced receptor trimerization. They can activate both the caspase and nuclear factor-kappa B (NF-kB). When the death receptors (DR4 /DR5) are over expressed, they can also directly induce cell apoptosis independent of the corresponding ligands. DR4 is a type Ⅰ transmembrane protein, and consists of 455 amino acids. The signal peptide contains 13 amino acids (1-13) at N-terminus out of the cytoplasm. Between the amino acids 108-206, there were two Cys-rich repeating motifs, which are the binding sites of TRAIL. The transmembrane domain is located at 227-245 amino acids. In the cytoplasm, there is a death domain comprised of 70 amino acids, which has a highly homologous to TNFR, FasL and other DDs. The DD in the cytoplasm can be activated upon ligation with

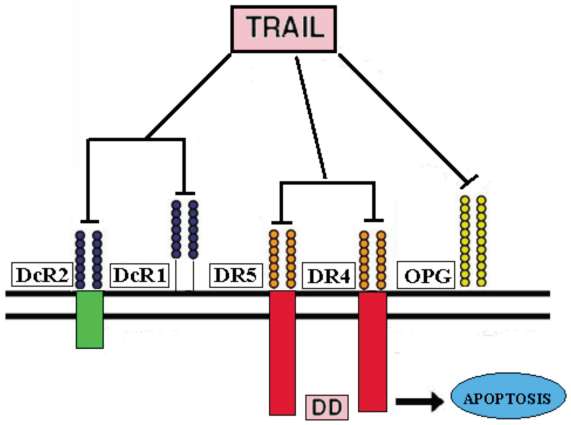

Figure 1 TRAIL-TRAIL receptor system

TRAIL, and then the apoptosis message will be sent into the cytoplasm, caspase system will be activated, and finally induces the cell apoptosis. This is why it is so called as DD [5]. DR5 is also a type Ⅰ transmembrane protein [6], which consists of 411 amino acids，and has structural and functional similarities to DR4. The signal peptide contains 15 amino acids (1-15) out of the cytoplasm. Two Cys-rich repeating motifs, which are the binding sites of TRAIL, are located between amino acids 84-79. Amino acids 184-206 are the transmembrane domain. The region after transmembrane domain is a DD comprised of 70 amino acids. TRAIL-inducing apoptosis is related to the expression of DR4 and DR5 on surface of tumor cells. The homology of the corresponding region, out of the cytoplasm and in the DD, between DR4 and DR5 is 66% and 64%, respectively. In humans, TRAIL interaction with either DR4 or DR5 triggers an apoptotic signal by recruitment of the adapter molecule Fas-associated DD protein to the receptor cytoplasmic DD.

Class 2 Decoy receptors (DcR), which include DcR1 and DcR2, also known as TRAIL-R3 and R4, are lack of the functional DD in the cytoplasm, and can activate NF-kB but not caspase. They cannot induce but inhibit cell apoptosis, and this is why they are so called as DcR. DcR1 is a type Ⅰ transmembrane protein, and consists of 259 amino acids. Amino acids 1-236 are out of the cytoplasm with an N terminal. Amino acids 237-259 are the transmembrane domain, lack of intracellular region. Of which the amino acids 1-23 maybe the signal peptide, amino acids 52-150 have two Cys-rich repeating motifs, and amino acid 5 has glycosylated repeating motifs, and 15 amino acids as an unit, are followed. This motif can covalently binds to the carbonhydrate chain of glycosylphosphatidylinositol (GPI) and form a protein immobilized on the cell surface. The extracellular domain of DcR1 is 69% and 52% homologous to DR4 and DR5, respectively. Because of the absence of DD, it can only bind to TRAIL, but cannot induce cell

apoptosis. DcR2 is also a type Ⅰ transmembrane protein, and consists of 386 amino acids. The amino acid sequencing is 58%-70% homologous to the three receptors stated above. The intracellular domain is comprised of 155 amino acids, only one third of the whole DD [7]. So it is also lack of the ability of inducing apoptosis.

Class 3 Osteoprotegerin (OPG) was identified as the fifth TRAIL receptor by Emery in 1998[8]. It is a secreted soluble glycoprotein, homologous to secreted TNF, and is significant in bone turnover *via* its role as a decoy receptor for the receptor activator of NF-kB ligand (RANKL). RANKL activates NF-kB through its membrane-bound receptor, receptor activator of NF-kB, leading to osteoclast-mediated bone resorption [9]. It has been thought that TRAIL may play a role in bone homeostasis, but TRAIL knockout mice demonstrate a normal skeletal phenotype. The binding site has some overlap with that of DR5, but the affinity is much weaker than that of DcR1 and DR5. It is a special receptor of TRAIL. When binding to TRAIL, it can inhibit TRAIL-induced cell apoptosis and protect the normal human epithelial cell from TRAIL-induced cell apoptosis. OPG's action may work through the competitive inhibition for DD [10]. In turn, TRAIL can obstruct the inhibitory effect of OPG on bone resorption osteoclasts. From what we know OPG and TRAIL are in a state of being coordinate [11].

MECHANISM OF TRAIL –INDUCED CANCER CELL APOPTOSIS

Pathways Two pathways of TRAIL-induced cell apoptosis have already been generally accepted [12,13], which are mitochondria-dependent and -independent pathways. The apoptosis signal transduction pathway is activated through the specific binding of TRAIL and death receptor (DR4/DR5) on the target cell surface [14]. Ligand-receptor trimer is formed when the receptor binds to the DD of Fas-associated protein with death domain (FADD) in the C terminal through its DD in the cytoplasmic region. FADD binds to procaspase-8 through its death effector domain (DED) in the N terminal and forms the DR4/DR5/FADD/ procaspase-8 death-inducing signaling complex (DISC), which promotes the cleavage of procaspase-8 and brings about the active caspase-8 [15]. There are two pathways to transmit apoptosis signal after the activation of caspase-8 (Figure 2) [16], i.e. type Ⅰ cells are through mitochondria-independent pathway (extrinsic pathway), in which is the active caspase-8 directly activates downstream effector-caspase-3, caspase-6 and caspase-7, and induces apoptosis; type II cells are through mitochondria-dependent pathway (intrinsic pathway), in which the active caspase-8 promotes the cleavage of Bid, activates truncated Bid (tBid) which is

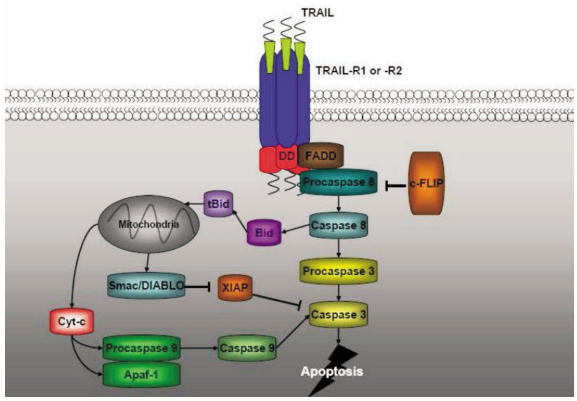

Figure 2 Schematic representation of TRAIL –R1/–R2 apoptotic signaling pathway (Cited from: Holoch PA, Griffith TS.TNF-related apoptosis-inducing ligand (TRAIL): a new path to anti-cancer therapies. *Eur J Pharmacol*2009;625(1-3):63-72)

located on the formed mitochondria membrane. Then the mitochondial transmembrane potentials decrease or destroy, and cytochrome C (cytC), pro-death protein Smac /Diablo are released by mitochondria, then apoptosome is formed by the binding of cyt C, Apaf-1,procaspase-9 and dATP. The dimerization of apoptosome triggers the activation of procaspase-9, then the active caspase-9 activates downstream effector, and finally induces cell apoptosis. But some studies suggest that in many cancer cells only one of the two death-inducing TRAIL receptors is functional, and most cells exhibit TRAIL resistance [17]. So, there are ways to re-sensitize TRAIL-resistant tumors to TRAIL either by a combination of TRAIL with chemotherapeutics or irradiation, or avoid decoy receptor-mediated neutralization of TRAIL.

Except for the pathways stated above, TRAIL can also activate other apoptosis-inducing signal pathways or factors after binding to the receptor, such as AKT pathway, NF-kB, protein kinase C (PKC), mitogen-activated protein kinases (MAPK), *etc.* These activated pathways or factors can regulate TRAIL's apoptosis-inducing activity.

Mechanism of evasion of apoptosis in normal cells A distinctive feature for TRAIL is that it can selectively induce apoptosis of transformed, virus-infected or tumor cells, but not normal cells. The mechanism we have already found are as follows[18]:1) The protection of DcR: DcR1 and DcR2 are widely expressed in normal tissues, but hardly expressed in cancer tissues. DcR can competitively bind to TRAIL rather than DD, and this is why normal cells can successfully escape from TRAIL's killing. Pan *et al* [19] has confirmed that ectopic expression of DcR1 can protect mammalian cells from TRAIL-induced apoptosis; 2) The protection of FLIP (FADD-like ICE inhibitory protein): FLIP is the inhibitor of active caspases, and has the similar sequence

with caspase-8. It can block the apoptosis pathway through competitive inhibition for binding proteins with caspase-8. Kim *et al* [21] has discovered that the cell lines insensitive to TRAIL has a low expression of DR4, but high expression of FILP; 3) The protection of inhibitor of apoptosis (IAP) family: The family members are widely expressed [21], which include caspase-IAP1, caspase-IAP2, X chromosome associated IAP (XIAP) and auxin. They are the hidden inhibitors of caspases, and can block TRAIL-induced apoptosis. Their effects take place through binding to caspases, such as caspase-9, caspase-3 and caspase-7, suppress their activation or weaken their activities. Hörnle *et al* [22]found that the majority of melanoma cell lines are resistant against treatment with death ligand TRAIL, mainly due to incomplete processing of caspase-3 into catalytically inactive p21 by binding of the anti-apoptotic protein XIAP.

TRAIL AND OCULAR TUMOR THERAPY

At present, the treatment of malignant ocular tumors is in a comprehensive way, and operation is the main treatment mean, and chemoreduction, laser photocoagulation, thermotherapy, cryotherapy, episcleral plaque radiotherapy and external radiation are the adjuvant means. With the deep research on TRAIL's clinical application and the establishment of the experimental model of combination application of TRAIL with radiotherapy and chemotherapy on multiple tumor cell lines, a new method will develop to the biological therapy of ocular tumor.

Choroidal Malignant Melanoma Choroidal malignant melanoma (CMM) is one of the most common primary malignant ocular tumors in adults which constitutes 85% of the uveal malignant tumors. It has a great harm on human health, which can cause blindness and even death because of the distal metastasis [23]. It is well known that melanoma is a highly aggressive malignant tumor with an exceptional ability to develop resistance and no curative therapy is available for patients with distant metastatic disease, so successful treatment of melanoma is still challenging. There haven't been researches simply on the biological therapy of CMM by now, but the studies on malignant melanoma have already existed for many years and have got many achievements. The latest studies showed the regression of early-stage melanoma and the curative effect mediated by IFN-2a were related with the infiltration of CD4+ T lymphocytes, and the action of CD4+ T lymphocytes on killing melanoma cells was exerted by TRAIL-induced cell apoptosis. TRAIL can induce apoptosis of 2/3 melanoma cells when cultured *in vitro*, with no effect on normal tissues [24]. When the effect of recombinant human TRAIL (rhTRAIL) on the growth and apoptosis of melanoma cell line A-375 was investigated, Chen *et al* [25] found that rhTRAIL can significantly inhibit the growth of A-375 cells, and the inhibitory rate is dose-dependent. Many melanoma cell lines have been found insensitive to TRAIL now, which may be associated with the expression of DR and abnormality in many signal molecules, such as caspase, Bcl-2, XIAP [20,26,27]. When combined with other drugs, the sensitivity of melanoma cells to TRAIL may be significantly increased. Liu *et al* [28] found that 2-deoxy-D-glucose (2-DG) does not have cytotoxicity on melanoma cells, but enhances TRAIL-induced apoptosis in cultured melanoma cells and fresh melanoma isolates. It sensitizes human melanoma cells to TRAIL-induced apoptosis by up-regulation of DR5. Qin's [29] results are in concord with that of Liu's with improvement. He found that 2-DG enhances surface levels for both DR4 and DR5 expression, and mannose pre-treatment reduces the enhanced cytotoxicity by combination treatments. All the results indicated that the notorious death resistance of melanoma can be overcome by interfering with glucose metabolism in combination with TRAIL. Except for the glucose metabolism studied for melanoma, there were also studies targeting other metabolic pathways, for example, glutamine metabolism. Qin *et al* [30] also discovered that glutamine depletion can sensitize melanoma to TRAIL-induced cytotoxicity. Karasic *et al* [31] found that cyclolignan picropodophyllin (PPP), a specific inhibitor of insulin-like growth factor-1 receptor (IGF-1R) kinase, combined with TRAIL can substantially increases cytotoxicity by apoptosis for WM793 and WM9 cells. The mechanism is as follows. PPP inhibits the activity of IGF-1R kinase, the highly active IGF-1R signaling pathway and the downstream activation of PI3K-AKT and MAPK pathways are inhibited, which induces apoptosis in melanoma cells in coordination with TRAIL. Hörnle *et al*[22] discovered that cisplatin can sensitize TRAIL-resistant melanoma cells to TRAIL *via* cleavage of XIAP. Moreover, the combination of TRAIL with ionizing radiation in several *in vitro* settings as well as *in vivo* models resulted in highly increased rates of cell killing and long-term tumor control [32]. Zhou *et al* [33] indicated that radiation-inducible human TRAIL gene that therapy may be a novel treatment for radioresistant uveal melanoma. They found adenovirus Ad-ET (an adenovirus containing EGR1/ TRAIL) combined with radiation therapy significantly inhibits the growth of radioresistant cell line-SP6.5 and OM431. With the further knowing in the mechanism of chemo- and radio-resistance of TRAIL and synergism with other drugs, TRAIL is expected to be a new agent to treat CMM.

Orbital Lymphoma Orbital lymphoma is common in space-occupying lesion of the orbit, accounting for 10% of the orbital malignant tumor [34]. TRAIL showed promising

therapeutic activity against solid tumors and lymphomas in numerous phase Ⅰ and Ⅱ clinical trials. Wissink *et al*[35] found that the combination of irradiation and TRAIL synergistically induces apoptosis in malignant lymphatic cell lines, and the combination can strongly enhance the efficacy of tumor therapy with irradiation in the radiotherapy-tolerant lymphatic cells with Bcl-2 overexpression. The studies from Unnithan *et al*[36] provided a proof of *in vivo* experiment of the synergistic effect of the combination of TRAIL and radiotherapy. They evaluated the TRAIL levels in 17 patients treated with radiation for Hodgkin's and non-Hodgkin's lymphoma, and found that the TRAIL expression was heightened after radiotherapy. At present, the mechanism of the synergistic effect of TRAIL and radiation in inducing apoptosis still remains unclear. The generally accepted theory is the activation of caspase. Chinnaiyan *et al* [37] found that the synergistic effect is p53-dependent and may be the result of radiation-induced up-regulation of the TRAIL-receptor DR5. Also there are many studies on the treatment of lymphoma with a combination of TRAIL and chemotherapeutic agents. Georgakis *et al* [38] found that TRAIL can enhance doxorubicin- or bortezomib-induced cell death. But the mechanism of the synergistic effect is also not very clear now. There are also clinical trials on TRAIL's receptors. Mapatumumab, a fully human agonistic monoclonal antibody to DR4 showed promising clinical activity in patients with follicular lymphoma [39]. Tigatuzumab, an agonistic humanized monoclonal antibody targeting DR5, was also well tolerated and the MTD was not reached [40]. Radiotherapy and chemotherapy are still the main treatment means of orbital lymphoma, and the combination of TRAIL can improve the curative effect.

Rhabdomyosarcoma Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children, and constitutes 10% of the malignant solid tumors in children. Nearly 35-40% rhabdomyosarcoma occurs in the head and neck, including orbital tissue. It is insensitive to chemotherapeutics and has a high malignancy, so the prognosis still remains poor. TRAIL is a hopeful candidate for new strategies in chemotherapy. Xu *et al* [41] found that TRAIL together with cisplatin has synergistic effect on RMS cells. And the synergistic effect may be related with the down regulation of mitochondrial membrane potential in RMS cells and expression of cFLIPmRNA. And the results in Komdeur's experiment revealed that the combination of TRAIL and doxorubicin has synergistic effect on inducing apoptosis of RMS cells [42]. Also the effects of TRAIL and melphalan (Mel) in the Mel-resistant rhabdomyosarcoma cell line TE-673 were investigated by Klüttermann *et al* [43], and the results demonstrated that TRAIL can sensitize Mel -resistant tumor cells to melphalan *via* a caspase-2 and -3-dependent mechanism From what we know the TRAIL combined with chemotherapy may be a new candidate in the treatment of RMS.

In conclusion, the ability of TRAIL to selectively induce apoptosis of tumor cells, but not normal cells and its synergistic effect when combined with radiotherapy and chemotherapy promotes the development of TRAIL-based cancer therapy. But the mechanism of drug-resistance and synergistic effect are still not very clear. The specificity of TRAIL-induced cancer cell apoptosis also becomes the points at issue. Moreover, the researches of TRAIL's application in ocular tumor are still in the beginning stage. So more experiments and clinical trials are needed to lay the foundations for TRAIL's application in ocular tumor.

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