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TRAIL and targeting cancer cells: between promises and obstacles

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Abstract

Targeting cancer cells is one of the challenges of current treatment strategies. TRAIL represents a promising therapeutic approach and over the past decades there was an increased interest in targeting TRAIL signaling to treat cancer. Indeed, TRAIL can specifically target cancer cells and exhibits very low cytotoxicity towards normal cells. However, rapidly accumulating experimental evidence has started to shed light on multiple factors which induce resistance against TRAIL in cancer cells. This resistance consists of various mechanisms including downregulation of death receptors and caspase-8 and overexpression of decoy receptors as well as antiapoptotic factors such as members of Bcl-2 family. Even if several studies focused on elucidating those resistance mechanisms, there still remain gray areas that need to be fully elucidated. Thus, therapeutic approaches could consist of targeting both resistance signaling pathways and TRAIL signaling to enhance TRAIL therapy efficiency.

Key words: Cancer, TRAIL, Targeted therapy, Apoptosis, TRAIL resistance, Clinical Trials.

Introduction

Cancer is a major health concern and one of leading causes of death worldwide. Current therapy consists mainly of surgical resection -when it is possible- in combination with radiation and chemotherapy. Data obtained through high-throughput technologies has considerably improved our understanding of complex and multifaceted nature of cancer. The advances in molecular biology to understand the molecular basis of tumor initiation and progression allowed developing strategies that directly target molecular mechanisms in cancer cells. Most current therapeutic strategies aim to induce apoptosis to inhibit excessive cell proliferation and to overcome resistance to apoptosis, one key hallmark in cancer (1). Despite progress in tumor treatment over the past decades, cancer is still a major health concern mainly due to intrinsic and acquired drug resistance and to the side effects of current therapeutic strategies. Furthermore, conventional anticancer drugs lack selectivity and target generally signaling pathways that are essential for the survival of normal cells too. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has emerged as one amongst the most extensively and deeply studied protein reportedly involved in selective targeting of cancer cells (2, 3).

TRAIL

TRAIL was first identified by Wiley et al. in 1995, however one year later, the same protein was described by Pitti et al. but called it Apo-2 ligand (Apo-2L) (4,5). TRAIL has attracted considerable attention because of its ability to selectively induce apoptosis in cancer cells while leaving normal cells intact (6-9).

Structurally, TRAIL possesses characteristics of type II membrane proteins. The C terminus of TRAIL is proteolytically cleaved into a soluble form. Both soluble form and full-length of TRAIL efficiently induce apoptosis in diverse cancer cells as evidenced by in-vitro analysis (10). Trail expression is reported in various cell types and human tissues, including ovary, liver, thymus, lung, small and large intestine, placenta and heart.

TRAIL is structurally made up of shorter cytosolic domain, a transmembrane and a large extracellular region and undergoes homotrimerization. Homotrimerized form binds to extracellular portion of three cysteine-rich receptors (10-12). TRAIL can interact with five distinct receptors having higher sequence based homology in their extracellular domains even if separate genes encode them. While two of these receptors, DR4 and DR5 can transduce apoptotic signals. Contrarily, two other receptors, membrane bound decoy receptors (DcR1, DcR2) do not transduce TRAIL-induced signals intracellularly. It is relevant to mention that DcR1 lacks cytosolically located domain, while functionally inactive truncated death domain (DD) is present in DcR2. The other receptor that can interact with TRAIL is osteoprotegerin (OPG) and plays a potentially regulatory role in TRAIL function but it shows very low affinity binding to it (13-15).

TRAIL-induced apoptosis

Apoptosis is a widely studied physiological process that control tissue homeostasis. Several diseases including cancer and neurodegenerative diseases show a defect in apoptosis program. Therefore, the understanding of the mechanisms that lead to these defects (excess or default of apoptosis) should provide a better compre-

hension of these diseases and thereby a better treatment (16).

Two major proapoptotic pathways have been described; intrinsically controlled mitochondrial pathway and extrinsic or death receptor pathway (17). The mitochondrial pathway is generally initiated by an intracellular stimuli such as endoplasmic reticulum (ER) stress or DNA damage which lead to mitochondrial membrane permeabilization and cytosolic accumulation of pro-apoptotic mitochondrial factors to the cytoplasm such cytochrome c and Smac (second mitochondria-derived activator of caspases). Once released, cytochrome c activates initiator caspase-9 while Smac acts as an antagonist of cIAPs (cellular inhibitor of apoptosis proteins), e.g. XIAP which prevents effector caspase activity (18, 19).

The extrinsic pathway is initiated by binding of death ligands as TNF-α, FasL or TRAIL to respective death receptors (20).

TRAIL-induced apoptosis is initiated upon binding of trimerized TRAIL to DR4 and/or DR5 leading to receptor clustering and the subsequent recruitment of Fas-associated death domain (FADD). This latter recruits pro-caspase-8 and/or pro-caspase-10 forming the death-inducing signaling complex (DISC) and leads to its activation. Activated DISC leads to proteolytic cleavage of the executioner caspases-3, -6 and -7 leading to apoptosis (21-23). The extrinsic apoptotic pathway can cross activate the mitochondrial pathway through caspase-8-dependent cleavage and activation of Bid (24).

Characteristically, tumor cells are sub-divided into two categories including type 1 or type 2 cells. In type 1 cells, DISC activation significantly and stably induces caspase-8 activation. In this case, caspase-8 activation directly and fully activates caspase-3 which results in apoptotic cell death. Surprisingly, DISC-induced caspase-3 activation was not sufficient to trigger apoptosis in type 2 cells therefore mitochondrially amplified apoptotic signal via tBid is required to induce apoptosis (25).

TRAIL-induced necroptosis

Confluence of information highlighted that death receptors activation by TNF, FasL and TRAIL may also lead to necroptosis induction (26-30). Necroptosis is a caspase-independent mechanism that depends on the formation of a complex called necrosome. The necrosome is a nano-machinery consisting of RIP3 and RIP1 and assembles either in absence of caspase-8 or when its activity is inhibited. This complex recruits and phosphorylates the pseudokinase MLKL that is required for necroptosis induction (31, 32). However there are still gray areas by which mechanism MLKL leads to necroptosis induction. Nevertheless, recent studies suggest that MLKL positioning at plasma membrane might be contributory in pore formation leading to membrane permeabilization (33, 34).

Resistance to TRAIL-induced apoptosis

Even if TRAIL induces apoptosis selectively in cancer cells, not all tumor cells are responsive to TRAIL treatment. Indeed, numerous cancer cells show intrinsic or acquired resistance to TRAIL-induced apoptosis. In this paragraph, we are going to give an overview of different mechanisms that render cancer cells resistant to TRAIL.

Overexpression of DcRs and OPG

It has previously been experimentally verified that DcRs do not transduce signals intracellularly and their overexpression induced resistance against TRAIL-induced apoptosis. In a study, Bouralexis et al. demonstrated that DcR2 overexpressing cancer cells acquired resistance to TRAIL (35). However, blockade of DcR2 restored sensitivity to TRAIL (35). In another study, it has been shown that DcR1 upregulation also induced resistance against TRAIL in cancer cells (36). In addition, high expression levels of DcRs in mucosal T cells render them resistant to apoptosis (37).

Several lines of evidence demonstrated contributory role of OPG in development of resistance to TRAIL-induced apoptosis. Indeed, in OPG-expressing tumors OPG allows overcoming antitumor immune surveillance exerted by TRAIL by inhibiting its ability to induce apoptosis in cancer cells. Furthermore, *in vitro* studies showed that exogenous OPG causes resistance to TRAIL and this was reverted by the removal of OPG. Finally, advanced colorectal cancer patients exhibit increased levels of OPG in serum (38).

Dysregulation of DR4/DR5

Several studies demonstrated that downregulation of DR4/DR5 is instrumental in development of resistance against TRAIL-based therapeutics as these receptors are the only ones that possess death domain and therefore able to transmit apoptotic signal. Indeed, low expression of DRs at the surface of cancer cells causes resistance to apoptosis preventing further caspases-8 activation (39, 40). Furthermore, intriguingly, constitutive endocytosis of DR4 and DR5 significantly reduced cell surface expression of death receptors (41). Furthermore, low levels of DRs correlated notably with increased multidrug resistance (MDR) (42). Another study showed that mutation in or near the ligand binding domain of DR4 considerably impaired TRAIL interaction with DR4 (43). In addition it has been reported that aberrant methylation of DR4 promoters contributes to TRAIL resistance (44).

Upregulation of anti-apoptotic factors

Such as in the mitochondrial pathway, upregulation of anti-apoptotic factors leads to resistance to TRAIL-induced apoptosis. Thus, overexpression of factors such as members of the anti-apoptotic Bcl-2 family contributes to TRAIL resistance. For instance, Mcl-1 was found to play a crucial role in determining the sensitivity of glioblastoma to TRAIL (45). Also, a decrease in Bid content was associated with inhibition of TRAIL-induced caspase-8 activation and thereby TRAIL resistance (46). Furthermore, the Bcl-2 nineteen kilodalton interacting protein (BNIP3) was reportedly involved in developing resistance against TRAIL. Nuclear BNIP3 binds to DR5 promoter to transcriptionally downregulate DR5 expression (47). c-FLIP has also been studied extensively because of its involvement in TRAIL resistance. Mechanistically it has been noted to inhibit formation of functionally active caspase-8 (48). In addi-

tion, high levels of X-linked inhibitor of apoptosis protein (XIAP) also contribute to TRAIL resistance (49). Mutations in pro-apoptotic protein Bax have been described to induce resistance to TRAIL-induced apoptosis in human colon carcinoma cells (50).

Dysregulation of caspase-8

As described previously, caspase-8 activation is a key element in TRAIL signaling as upon its activation, it induces caspases cascade leading to apoptosis. Then, loss or just a lower level expression of caspase-8 cause sTRAIL resistance as shown in several studies (51-53).

Signaling pathways involved in TRAIL resistance

Several studies reported a critical role for NF-κB in TRAIL resistance in various cancer cells. Indeed, NF-κB is a transcription factor that positively regulates several genes implicated in TRAIL resistance including Mcl-1, c-FLIP and XIAP. Studies showed that inhibition of NF-κB restored cell sensitivity to TRAIL (54-56). However, certain hints have emerged emphasizing on apoptosis promoting role of NF-κB in TRAIL-treated cancer cells (57). Overexpression of Akt, PKC and MAPK also contribute in development of resistance against TRAIL (46, 58, 59). Recently, another study revealed that Src and CXCR4 overexpressing breast cancer cells were resistant to TRAIL (60).

Preclinical Studies

Leucine zipper-tagged TRAIL has recently been tested for efficacy in xenografted mice and results showed that it was pharmacokinetically safe in cynomolgus monkeys without abnormalities in drug exposed animal models (61). Albumin-cross-linked polyethylene glycol (PEG) hydrogel loaded with TRAIL protein considerably inhibited tumor growth in mice xenografted with pancreatic cancer MIA PaCa-2 cells (62). Recombinant CD19-Ligand × Soluble TRAIL (CD19L-sTRAIL) combined with low dose total body irradiation was highly efective against radio-resistant advanced stage $CD19^{+}$ murine lymphoblastic leukemia with lymphomatous features in CD22ΔE12xBCR-ABL double transgenic mice (63).

Clinical Trials

It is essential to mention that TRAIL has attracted most interest in translational oncology because of its ability to selectively kill cancer cells. Tremendous breakthroughs have been made in translating the laboratory findings to clinically effective therapeutics and accordingly, first human recombinant TRAIL was generated by Genentech. In this section we discuss most recent advancements in TRAIL based therapeutics.

TAS266, a novel agonistic tetravalent Nanobody(®) has been designed to specifically target DR5 receptor. In a Phase I study, significant but reversible hepatotoxicity was noted in TAS266 treated patients with advanced solid tumors (64). Circularly permuted TRAIL (CPT), a recombinant mutant of TRAIL was tested for clinical efficacy in an open-label phase II trial. Results revealed that CPT was well tolerated with no dose-limiting toxicities in thalidomide resistant multiple myeloma patients (65). Mapatumumab, a fully human agonist monoclonal antibody directed against DR4 did not show any clinical efficacy when administered combinatorially with carboplatin and paclitaxel in advanced non-small-cell lung cancer patients (66). Tigatuzumab (CS-1008), a humanized monoclonal antibody directed against DR5 in combination with gemcitabine was well tolerated in chemotherapy-naive patients (67).

Conclusion

Most current therapeutic approaches in cancer treatment aim to overcome two key hallmarks of cancer, i.e., uncontrolled proliferation and resistance to apoptosis. Understanding the mechanisms that underlie resistance and developing strategies to overcome it is crucial for effective treatment.

TRAIL represents a promising therapeutic approach as it specifically targets cancer cells with very low cytotoxicity towards normal cells (68, 69, 70). However, some issues have been raised using TRAIL in cancer treatment due to intrinsic and acquired resistance to TRAIL-induced apoptosis. Indeed, many therapeutic strategies have been developed to sensitize TRAIL resistant cancer cells (71). There are direct evidences related to efficient delivery of TRAIL gene and proteins to target sites using nanotechnologically assisted methods (72). Thus, many recombinant variants of TRAIL and agonistic antibodies against its receptors have been used in clinical trials. However, the clinical efficacy was not very promising mainly due to resistance to TRAIL-induced apoptosis. Even if the signaling pathways involved in TRAIL resistance have been widely investigated, there are still gray areas to clarify probably due to the type of tumor and to heterogeneity of tumors which furthermore might differ from a patient to another.

Nevertheless, this approach remains a promising therapeutic strategy. The challenge, then, is to overcome this resistance may be using combined treatment to target resistance signaling pathways to render cancer cells sensitive to TRAIL treatment.

Other articles in this theme issue include references (73-84).

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