Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) Pathway Signaling

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Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/Apo2L is a death ligand, a cytokine that activates apoptosis through cell surface death receptors. TRAIL is thought to be important in host tumor surveillance and metastasis suppression, and various therapeutic agonists that activate TRAIL receptors to induce tumor cell apoptosis are in clinical development. This review discusses recent findings about TRAIL pathway signaling and relates the signaling mechanisms to issues that need to be considered as we try to manipulate TRAIL signaling to treat cancer.

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FUNCTIONS OF TRAIL

The creation of TRAIL-deficient and TRAIL receptor-deficient mice has allowed examination of the physiological functions of TRAIL. Knockout mice are viable and fertile with no obvious developmental defects. The TRAIL pathway is involved in the regulation of innate immunity and in the homeostasis of memory T cells. From the cancer perspective, TRAIL signaling is important in T cell- and natural killer cell-mediated tumor surveillance and metastasis suppression. Although TRAIL receptor deficiency has no apparent effect on intestinal tumor development caused by p53 or APC loss, TRAIL-deficient animals have more hematological malignancies and are more susceptible to chemical carcinogens. TRAIL receptor-deficient animals are also less sensitive to damage caused by ionizing radiation. Together, these findings indicate that the TRAIL pathway has important roles in host anti-tumor defense and tumor suppression.

MECHANISM OF TRAIL PATHWAY SIGNALING

Upon DR4 or DR5 activation by binding TRAIL or the agonistic DR4 and DR5 antibodies, a protein complex called the death-inducing signaling complex (DISC) is formed (Fig. 1). In the DISC, the adaptor protein FADD is bound directly to the intracellular death domain (a protein interaction domain) of the receptors. FADD simultaneously binds to the intracellular death domain (a protein interaction domain) of the receptors and FADD simultaneously binds to the intracellular death domain (a protein interaction domain) of the receptors. This complex, in turn, leads to activation of effector caspases that digest cellular proteins to allow apoptosis to proceed.

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cause apoptotic cell death. The stoichiometry of these interactions is unclear. The TRAIL receptor functional unit is a trimer, but it is likely that multiple trimers must be activated together to create a functional DISC, and aggregation of death receptors in large groups is probably required for effective signaling. For the Fas/CD95 receptor, it has been proposed that FADD dimers link together receptor trimers. Similar interactions (with the added complexity that they may include both DR4 and DR5 receptors) likely occur for TRAIL signaling.

Like other death receptors (particularly Fas), two distinct kinds of apoptosis signaling have been described, and cells can be classified into one of two groups depending on how they activate death receptor-induced apoptosis. Type I cells activate large amounts of active caspase-8 in the DISC when death receptors are activated, whereas type II cells activate smaller amounts of caspase-8 directly and require amplification of the apoptotic signal through the mitochondrial apoptosis pathway to activate effector caspases. A second complex in the cytoplasm activates other signaling pathways, including JNK and NFκB.

Other signaling pathways leading to NFκB activation and activation of the ERK, JNK, and p38 MAP kinase pathways are induced by TRAIL receptors. Some of these signals (NFκB, JNK, and p38) are generated by a second cytoplasmic complex that forms after the DISC. The mechanism of ERK activation is currently unclear. The importance of these pathways is shown by studies in cells that are resistant to the apoptotic signaling. In such cells, TRAIL receptor stimulation can lead to increased tumor cell growth and survival through the activation of NFκB by the TRAIL receptors. Similar pro-tumorigenic effects of death receptor activation have also been noted for the Fas/CD95 receptor. Other pro-tumorigenic mechanisms of TRAIL receptor signaling have also been noted. For example, TRAIL stimulation of pro-inflammatory cytokines has been linked to increased metastasis in a pancreatic cancer model. An important conclusion arising from these studies is that, as we try to activate TRAIL receptor signaling for cancer therapy (see “TRAIL Signaling and Cancer Therapy”), we should try to minimize the ability of the TRAIL receptor agonists to activate these various pro-tumorigenic signals.

DISC formation leading to effective signaling was originally thought to occur randomly at the cell surface. However, it is now apparent that localization of these signaling complexes in particular regions of the membrane may be critical for the efficiency of signaling and may even lead to different cellular responses being mediated by the same signaling proteins. For example, the interaction among caspase-8, FADD, and receptors may occur on lipid rafts. Lipid rafts are specialized cholesterol-rich domains in the cell membrane that promote preferential concentration of particular signaling proteins to provide distinct signaling activities. Signaling from lipid rafts is often different from that achieved by the same signaling proteins in different subcellular locations. For example, lipid rafts differentially affect NFκB and ERK signaling by the death receptor TNFR1. Recent work indicates that redistribution of DR4 and DR5 into rafts may explain the anti-tumor effects of other agents, suggesting that lipid raft association of TRAIL receptors may be an important aspect of their regulation. In addition, COX2 inhibition sensitizes to TRAIL-induced apoptosis by promoting DR5 clustering in cholesterol- and ceramide-rich caveolae.

Death receptors also signal from other sites in the cell in addition to the plasma membrane. For example, some signaling by TNFR1 and Fas death receptors occurs within vesicles in the cytoplasm after receptor endocytosis. We know much less about the role of spatial organization in controlling TRAIL pathway signaling. However, given the marked similarities in signaling mechanisms between Fas/CD95 and DR4 and DR5 and the evidence that formation of cytoplasmic complexes after TRAIL receptor stimulation is important for activation of the non-apoptotic pathways, it
seems likely that similar spatial regulation will also be found to be important for TRAIL signaling.

INHIBITION OF TRAIL SIGNALING

There are several ways to inhibit the TRAIL pathway. Many of these mechanisms are found in tumor cells and contribute to decreased sensitivity to TRAIL-induced apoptosis. These mechanisms could therefore limit the efficacy of TRAIL receptor-targeted therapeutics and inhibit host tumor surveillance and metastasis suppression. As mentioned in “Mechanism of TRAIL Pathway Signaling,” two non-signaling receptors (DcR1 and DcR2) are expressed in mammalian cells along with DR4 and DR5. It was thought that these receptors, which lack all or most of the intracellular death domain, act as simple decoys by competing with DR4 and DR5 for ligand binding. However, recent work suggests that DcR1 and DcR2 inhibit signaling by different mechanisms; DcR1 works by titrating away the ligand, whereas DcR2-mediated inhibition involves the formation of heterologous complexes with DR5. In some cases, increased expression of these decoy receptors has been correlated with TRAIL resistance in tumor cells.

DR4 and DR5 map to chromosome 8p21–22, a site of frequent allelic loss in tumors. This led to the suggestion that, as potential tumor suppressors, TRAIL receptors may also harbor somatic mutations. Several studies identified somatic mutations in gastric, non-Hodgkin’s lymphoma, non-small cell lung, and breast cancers. Mutations were fairly common, arising in 7 of 57 breast cancers (12%; 20% small cell lung, and breast cancers. Mutations were almost always DR5 and affect the intracellular domain of the receptor; i.e., the region that binds FADD. Despite the small numbers of patients studied, some mutations have been found repeatedly in different tumor types and among different patients with the same disease, suggesting that DR4/DR5 mutations may have important functional effects among patients with cancer.

Other mechanisms that inhibit TRAIL signaling include increased expression of inhibitors such as FLIP. FLIP is a homolog of caspase-8 with mutations in the catalytic domain that prevent its activation as a protease. FLIP expression is controlled by other important oncogenic pathways. For example, Myc regulation of TRAIL sensitivity may be largely mediated through Myc’s ability to repress FLIP expression. When recruited to the activated receptor complex, FLIP can inhibit activation of caspase-8 and thus block downstream apoptosis signaling. However, the reality is actually more complicated because FLIP comes in two isoforms, and low levels of the longer isoform may actually stimulate caspase-8 activation or after its substrate specificity when death receptors are activated. Other, more general apoptosis inhibitors (e.g., IAP proteins that interfere with caspase activation), can also inhibit TRAIL signaling and may limit the effectiveness of tumor cell killing by TRAIL when over-expressed.

TRAIL SIGNALING AND CANCER THERAPY

Notwithstanding these caveats about the pro-tumorigenic effects of TRAIL pathway activation, most of the current interest in TRAIL pathway as it relates to cancer focuses on plans to directly activate TRAIL receptors as a way to kill tumor cells. These ideas arise from early indications that there was preferential TRAIL receptor-dependent killing of tumor cells compared with normal cells, with the result that TRAIL and agonistic antibodies that activate DR4 or DR5 have become attractive candidates as cancer therapeutics. The first phase I trials of TRAIL receptor agonistic antibodies were reported at the 2004 ASCO meeting. It is encouraging that these agents do not display systemic toxicity in humans and several clinical trials are ongoing at multiple sites worldwide. In preclinical testing, these agents can be highly effective in reducing tumor growth as single agents. For example, in an orthotopic lung cancer model, treatment with recombinant TRAIL caused 60% reduction in tumor growth and HGS-ETR1 (mapatumumab), a human DR4 monoclonal antibody has been shown to induce cell death and tumor regression in multiple tumor types, including lung, colon, and renal tumors. It is unclear whether recombinant TRAIL or the agonistic antibodies will be more effective drugs. TRAIL requires careful manufacture to correctly coordinate an essential zinc atom that is required for proper structure and has a shorter half-life than the antibodies; therefore, one might believe that the antibodies would be more effective than the ligand. However, because the antibodies activate either DR4 or DR5 but do not affect the other receptor, whereas TRAIL activates both DR4 and DR5, optimal clinical use of these agents may require selection based on the specific characteristics of the tumor. For example, a patient whose tumor has a mutant version of DR5 but wild-type DR4 may obtain no benefit from treatment with anti-DR5 but might benefit from anti-DR4 or TRAIL. Similarly, at least in some tumor types, efficient apoptosis signaling may occur through only one of the receptors even when the other receptor is present and not mutated.

Conventional chemotherapy agents and ionizing radiation can synergize with TRAIL receptor agonists. For example, paclitaxel, other tubulin-targeted drugs, radiation, and DNA-damaging chemotherapy synergize with TRAIL. Thus, it is hoped that combining TRAIL or agonistic TRAIL receptor antibodies with chemotherapy or radiation may be more effective than either treatment alone. For example, in the lung cancer study described above, combining TRAIL with taxol plus carboplatin led to a 97% reduction in tumor growth. It may also be beneficial to combine TRAIL with agonistic antibodies that activate T cells.

TRAIL receptor signaling may also be an important determinant of the therapeutic response with other agents (i.e., when exogenous TRAIL or the agonistic antibodies are not part of the treatment). Direct evidence for such an effect comes from the finding that inducible silencing of DR5 leads to acquired resistance to 5-fluorouracil. In addition, increased expression of DcR2 can regulate sensitivity to DNA damaging chemotherapy. Anti-tumor effects of chemother-
apy and synergy between TRAIL and other chemotherapy drugs or radiation may be mediated by alterations in the subcellular location\textsuperscript{52,53} or increased expression of the TRAIL receptors.\textsuperscript{59} However, the simple view frequently found in the literature that other agents increase TRAIL receptors and that this is the source of the synergy may be incorrect.\textsuperscript{66} More complicated synergy mechanisms may, in fact, apply. Thus, in addition to the direct use of TRAIL receptor agonists as anti-cancer therapeutics, alterations in TRAIL receptor signaling may affect response to other therapies.

**SUMMARY**

Although the TRAIL pathway may seem like a rather simple apoptosis signaling pathway (the receptors bind the ligand and then directly activate caspases), it is, in fact, quite complicated. We have two receptors that may not function equivalently and that can induce both pro-apoptotic and pro-survival/growth signaling. Somatic mutations in tumor cells can affect one or other of the TRAIL receptors while leaving the other untouched. We have two membrane-bound decoy receptors that work in subtly different ways and numerous other inhibitors such as FLIP, which themselves may have different effects in different contexts. Spatial regulation of signaling complex formation (in different regions of the cell membrane, such as lipid rafts and inside the cell) can differentially regulate the signaling that is obtained. All of these effects may be influenced by other treatments such as DNA damaging chemotherapy and ionizing radiation. It is inherently attractive to manipulate these pathways in cancer treatment because we are trying to boost signaling through pathways that are probably an important aspect of normal host defenses against tumor development and metastasis. Moreover, a number of different agents (antibodies and the recombinant ligand) are now being tested in the clinic to do just this. By better understanding how the signaling works and making sense of the complexity, we will hopefully be able to maximize the benefit to be obtained from these agents.

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