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Editorial

Taming TRAIL: the winding path to a novel form of cancer therapy

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Cancer therapeutics have for many decades primarily and relatively nonspecifically targeted the proliferative capability of cancer cells, because it was assumed that neoplastic transformation is mainly triggered by cellular changes that cause normal cells to proliferate in an uncontrolled fashion. This is still achieved by either irradiating tumors or exposing them to chemotherapeutic drugs that target various intracellular pathways. A major problem with this strategy is that these modalities cannot completely eradicate cancer cells in most cases (indeed cancer cells often become resistant to treatment), and they have serious undesirable side effects such as severe immunosuppression, death of proliferating cells in the gastrointestinal tract, or hair loss due to the effects of the chemotherapeutic agents.

It is only in the last 20 years that we have come to recognize the significance of another process that regulates cellular homeostasis: apoptosis. It is now widely accepted that in order for cancer to develop, tumor cells must find a way of avoiding targeted elimination. Indeed, it is likely that most tumors have evolved mechanisms of apoptosis resistance having undergone a rigorous selection in the hostile tumor microenvironment. Many attempts to target tumor cells for destruction, therefore, have the goal of apoptosis induction or of finding mechanisms to overcome apoptosis resistance that the tumor cells have developed. One of the earliest projects to selectively target tumor cells for destruction through induction of apoptosis was initiated in the late 1980s. Two groups independently isolated monoclonal antibodies that slowed the growth of tumor cells by inducing apoptosis.[1,2] Both of these antibodies were found to bind to the same cell surface structure that was later identified as the death receptor CD95 (APO-1/Fas). Early experiments suggested that the ligand to CD95 could be used to induce apoptosis in tumors cells *in vivo*.[2] Unfortunately, subsequent experiments with a mouse CD95-specific agonistic antibody, Jo2, and with the CD95 ligand (CD95L) demonstrated major complications for the clinical use of CD95 ligation. Mice injected with Jo2 died due to massive apoptosis induction in the liver,[3] reminiscent of the effects of tumor necrosis factor α (TNF). CD95L turned out to be a member of a family of death ligands that also includes TNF, and its therapeutic use encountered similar limitations. Another member of this family, TNF-related apoptosis inducing ligand (TRAIL) or APO-2L, was found to have a remarkable property: it killed many types of tumor cells without resulting in the systemic toxicity observed with either TNF or CD95L treatment.[4] Two of the four membrane-bound receptors to which TRAIL could bind were similar in structure to CD95 and were called TRAIL receptor 1 (DR4) and TRAIL receptor 2 (DR5/TRICK). Similar to CD95L, binding of TRAIL to its death receptors induces recruitment of a complex of proteins (forming the death-inducing signaling complex (DISC)) that initiates apoptosis through activation of the caspase cascade of cysteine proteases. It was found that the majority of TRAIL-induced apoptosis was mediated through DR5, which was therefore viewed as the more potent receptor.[5]

TRAIL-deficient mice are more susceptible to experimental and spontaneous tumor metastasis than mice with TRAIL,[6] suggesting that TRAIL is part of a surveillance system that eliminates developing tumors, raising hopes that TRAIL could be used as a novel anticancer reagent. For reasons that are not completely clear, TRAIL preferentially kills tumor cells without inducing apoptosis in normal cells. Interestingly, a large number of compounds have been reported that, when coadministered with TRAIL, further sensitize tumor cells to TRAIL-induced apoptosis without compromising the apoptosis resistance barrier of untransformed cells.[7] One novel class of antitumor reagents is a group of compounds that target histone deacetylases, which are enzymes that modify chromatin in a way that allows certain genes to be transcriptionally activated, often breaking the apoptosis resistance of tumor cells.[8] The activity of HDAC inhibitors (HDAC-I) to sensitize human tumor cells to TRAIL-induced apoptosis has recently been demonstrated for a large number of human tumor types.[9–14] Furthermore, a number of standard chemotherapeutic drugs also have this sensitizing activity, giving rise to the hope that the efficiency of TRAIL can be further increased by combining its use with low doses of standard chemotherapy.

With all of this promise, why is TRAIL not already being used in the clinic? A major reason likely lies in the complexity of the interaction of TRAIL with its five receptors, of which only two are death receptors. As a result of the detrimental *in vivo* experiences with CD95L and TNF, studies had to confirm that TRAIL does not adversely effect normal tissues under any circumstances. A promising initial demonstration that TRAIL caused regression of established tumors in mice[15] was followed by reports suggesting that TRAIL could kill normal liver and brain cells.[16,17] These findings prompted careful analysis of the preparations of TRAIL that were administered, further investigation into the structure of TRAIL itself, and the development of novel antibodies against TRAIL receptors. The culmination of these investigations demonstrated that

certain TRAIL preparations indeed seemed to be safe when used in a specific way (reviewed in Kelley and Ashkenazi[4]).

After this necessary delay to further characterize the potential toxicity of TRAIL therapy, two pharmaceutical companies have now begun to conduct clinical trials with reagents that target TRAIL receptors. Genentech/Amgen are testing a preparation of the TRAIL ligand, whereas Human Genome Sciences developed two humanized anti-TRAIL receptor antibodies, HGS-EGR1 and HGS-EGR2, which target DR4 and DR5, respectively. There are, however, many open questions as to the activity and specificity of these reagents, and a direct comparison of these agents has not been performed.

In this issue of Cell Death and Differentiation, MacFarlane *et al.*[18] compared the activities and specificities of a number of TRAIL receptor-specific agents side by side, including the compounds currently being tested in clinical trials. Not only did the authors test the specific activities of these agents, they also compared their activities in combination with HDAC-Is. As a tumor cell model, they chose a cancer type known to be notoriously resistant to the apoptosis including activity of TRAIL – chronic lymphocytic leukemia (CLL). In contrast to many other studies, the current study tested mostly primary tumor material from patients who had undergone conventional chemotherapy. In agreement with previous studies by the same group, these tumor cells could be substantially sensitized to TRAIL-induced apoptosis by cotreating them with HDAC-Is, which seemed to increase the efficiency of TRAIL-induced DISC formation.[19] Interestingly, it was found that, in contrast to expectations, CLL cells did not die through DR5 but mainly through DR4, and the relative ratio of expression of the two TRAIL-binding death receptors alone was not sufficient to predict apoptosis sensitivity to any of the reagents.[18] The study demonstrated that it may even be possible to treat forms of cancer that are highly resistant to the effects of TRAIL when treatment is combined with HDAC-Is. Nonetheless, for each cancer type, it may be necessary to determine the susceptibility to different TRAIL receptor-targeting agents.

There are still many open questions that need to be addressed before TRAIL can be safely and effectively used to treat various forms of human cancer: Why do different preparations of TRAIL induce apoptosis through different TRAIL receptors? What determines which TRAIL receptor mediates apoptosis in different tumors? Is there a difference in these effects between tumor cell lines and primary tumors? What protects normal cells from TRAIL-induced apoptosis, and what does it take to compromise this barrier? What are the mechanisms that render tumor cells sensitive to TRAIL when they are exposed to various anticancer agents?

As a result of the study by MacFarlane *et al.*[18], it seems that not only the combination of TRAIL with more traditional cancer therapeutics may be required to effectively combat cancer with TRAIL, but that even combinatorial administration of different TRAIL receptor-specific reagents could be beneficial. Since both receptor-specific antibodies[20] and TRAIL muteins[5] are now available, this seems a feasible strategy. As there is now a beginning to an end of the long and winding road to develop TRAIL into a novel anticancer agent, we may know relatively soon whether TRAIL is the holy grail of cancer therapy that neither TNFα nor CD95L could be.

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