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Activation of systemic antitumor immunity via TRAIL-induced apoptosis

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TNF-related apoptosis-inducing ligand (TRAIL) continues to be intently studied as a cancer therapeutic because of its selective tumoricial activity. We have been interesting in evaluating the ability of TRAIL to induce systemic antitumor immunity through the generation of apoptotic tumor cells. Recent observations suggest that localized administration of TRAIL in combination with CpG ODN generates a systemic antitumor immune response to eliminate the primary tumor and distant metastases.

For many years cell death was studied with far less interest than other basic cellular processes such as proliferation and differentiation. As it was thought that cell death was simply a degenerative phenomenon produced by injury, the idea that cell death would also occur as a genetically controlled event in healthy animals did not gain wide acceptance. It is now clear that failure or suppression of apoptosis likely contributes to the initial development of cancer and to the appearance of tumor cells resistant to cytotoxic therapy. Over the years, a variety of drugs have been developed to kill tumor cells by inhibiting some component of key biomolecular pathways that contribute to uncontrolled tumor growth. Many of these drugs have limited specificity for tumor cells, so the potential for drug-induced death of normal cells is increased. In 1995, Wiley et al. described the selective tumoricidal activity of a (then) newly identified member of the TNF superfamily—TNF-related apoptosis-inducing ligand (TRAIL).[1] Since that initial paper, there have been numerous reports examining the direct tumoricidal activity of TRAIL (alone or in combination with a wide variety of drugs). Most of these studies have used recombinant TRAIL, but there has been considerable interest in using agonistic monoclonal antibodies (mAbs) specific for the death-inducing TRAIL receptors, TRAIL-R1 and -R2. These reagents (both recombinant TRAIL and agonistic mAbs) are therapeutically appealing because they can be administered systemically to (at least on theoretical grounds) directly kill tumor cells while leaving normal, non-transformed cells unscathed.

As an alternative to the systemic administration of a TRAIL receptor agonist, we were the first to describe the in vitro and in vivo tumoricidal activity of a recombinant adenovirus encoding the *TRAIL*cDNA (Ad-TRAIL).[2,3] Our initial studies were designed to only examine tumor cell death as induced by Ad-TRAIL (alone or in combination with a drug to increase Ad infectivity and/or tumor responsiveness to TRAIL), but our recent studies have examined the impact of Ad-TRAIL-induced tumor cell death on the subsequent induction of systemic antitumor immunity. Our hypothesis was that TRAIL-induced tumor cell death would generate apoptotic tumor cells that would be recognized by the immune system to generate a systemic antitumor response, in turn killing residual tumor cells at the primary site along with any distant metastases (**Fig. 1**). Using a murine model of renal cell carcinoma, we demonstrated that the local administration of Ad-TRAIL and CpG oligonucleotides (which are thought to increase the immunogenicity of apoptotic tumor cells)[4] increased tumor regression and prolonged animal survival.[5,6] In addition to directly decreasing the primary tumor burden, Ad-TRAIL/CpG therapy induced systemic antitumor immunity that decreased the metastatic tumor burden at distant sites. Furthermore, mice that went on to clear the primary tumor after Ad-TRAIL/CpG treatment were also able to resist a second challenge with tumor cells. It remains to be determined just how much tumor cell death must occur as a result of Ad-TRAIL/CpG treatment to stimulate a systemic anticancer immune response. These preclinical results suggesting the therapeutic potential of Ad-TRAIL provided the necessary "proof-of-concept" data to justify the initiation of a Phase I clinical trial in men with prostate cancer. Our preliminary results from that trial show that the intra-prostatic injection of Ad-TRAIL is well-tolerated in patients, and produce no adverse side effects.[7] In addition, there was evidence of apoptotic death (as measured with TUNEL staining of DNA fragmentation), suggesting that TRAIL expressed from the transferred transgene was functional. Unfortunately, this Phase I trial was not designed to test therapeutic efficacy.

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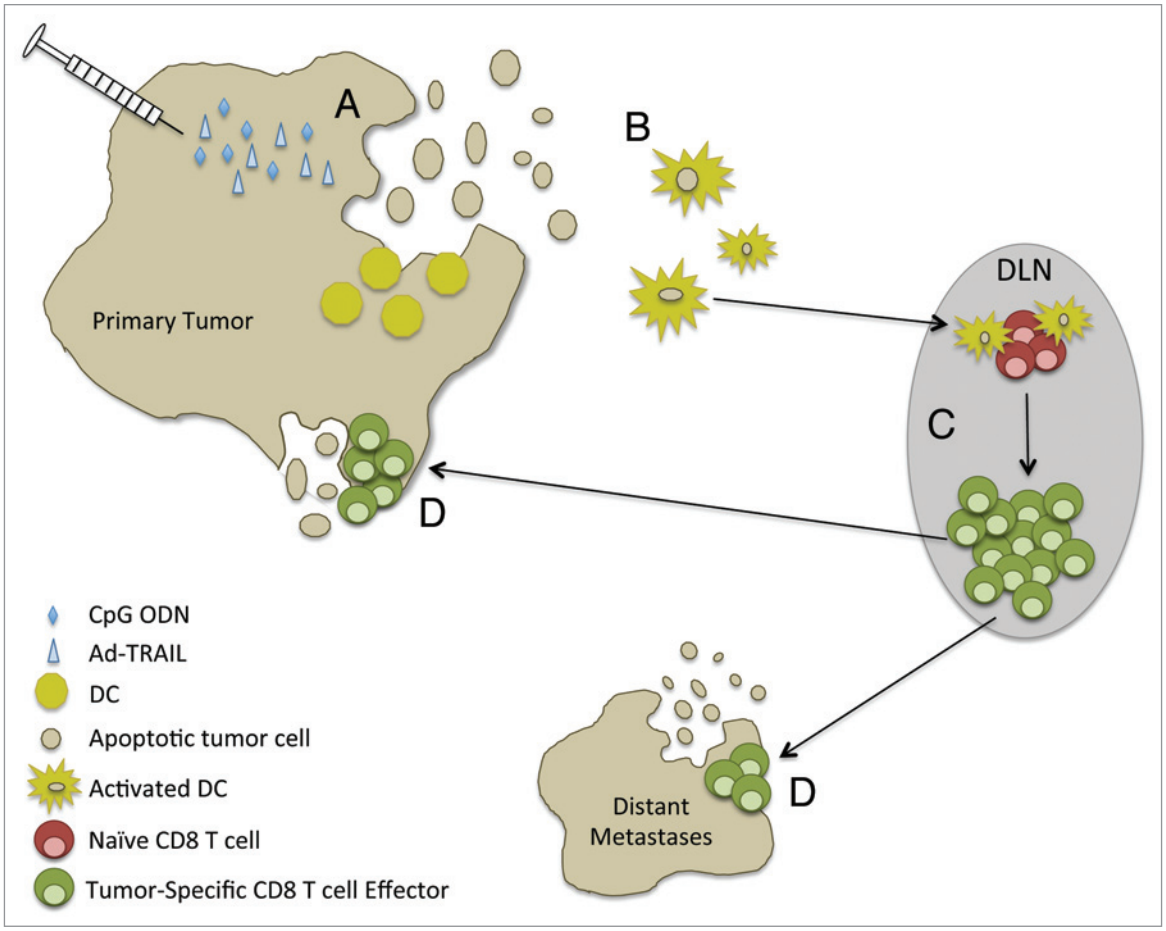

Figure 1. Ad-TRAIL/CpG oligonucleotides immunotherapy leads to a robust anticancer immune response and tumor clearance. (**A**) Intratumoral injection of Ad-TRAIL/CpG leads to TRAIL-induced tumor cell death and CpG-induced activation of dendritic cells (DCs). (**B**) Activated DCs take up apoptotic tumor cell debris and migrate to the draining lymph node, where (**C**) they cross-present tumor antigens to naïve $CD8^+$ T cells. This leads to the activation and expansion of tumor-specific $CD8^+$ T-cell effectors, which (**D**) then migrate to the primary tumor as well as to distant metastases to mediate further tumor cell killing and systemic antitumor immunity.

As with most preclinical studies, our model was optimized in that we were using a tumor cell line that was susceptible to adenovirus infection and TRAIL-induced apoptosis. The success of Ad-TRAIL, as with any adenoviral vector, is dependent on coxsackie adenovirus receptor (CAR) expression on target cells. Despite being widely expressed on most normal epithelial cells, CAR levels can be variable on different tumor cell lines of the same histological origin. Thus, genetically modifying the adenoviral vector to target tumor-specific proteins or some other cell surface receptor (besides CAR) has been one approach for increasing adenoviral infectivity. It has also been well-documented that many of the tumor cell lines used experimentally vary in their susceptibility to TRAIL-mediated killing. Thus, it is not that surprisingly that there have been hundreds of papers published describing the ability of a plethora of drugs to sensitize tumor cells to TRAIL. Many of these drugs target a component of a molecular pathway that protects the tumor cells from apoptosis in general, and not specifically death receptor (extrinsic)-mediated apoptosis. Interestingly, several histone deacetylase inhibitors (HDACis) have been reported to increase tumor cell sensitivity to both adenoviral infection[8] and TRAIL-induced cell death.[9] The notion of using HDACis to treat cancer largely stems from the fact that HDACis maintain chromatin in a hyperacetylated state, leading to the expression of normally repressed genes that result in growth arrest, terminal differentiation, and/or death of the tumor cell.[10] Combination therapy may be the best approach for increasing the responsiveness of tumor cells to adenoviral infection and TRAIL cytotoxicity, notably with the sensitizing drug being administered systemically prior to localized Ad-TRAIL delivery. One important aspect of such combination therapy that is frequently overlooked is the potential for the sensitizing drug to increase the susceptibility of normal, non-transformed cells to TRAIL-induced apoptosis. As the development of TRAIL-based cancer therapies continues, future work must take into consideration the potential for unexpected side-effects when a second drug is used to increase TRAIL sensitivity.

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