Apoptosis is a metazoan process of controlled cell elimination that plays critical roles in embryonic development and adult tissue homeostasis. Apoptosis dysregulation contributes to several important diseases, including cancer. Two distinct yet interconnected signaling pathways control apoptosis by activating a core intracellular machinery of death proteases called caspases. The intrinsic apoptotic pathway engages caspases via members of the BCL-2 protein family and the mitochondria in reaction to severe cellular damage or stress. The extrinsic pathway activates caspases via cell-surface death receptors, which respond to cognate death ligands expressed on immune-effector cells. Tumor cells can acquire various apoptosis-evasion mechanisms; nevertheless, the transformed state of these cells makes them uniquely susceptible to apoptosis reactivation if resistance is circumvented. Molecular approaches to reengage the apoptotic pathways in cancer have been underway for over two decades. Gratifyingly, BCL-2 antagonists — which drive the intrinsic pathway — are beginning to bear clinical fruit. In contrast, clinical attempts to stimulate the extrinsic pathway with proapoptotic receptor agonists (PARAs) have been disappointing, despite compelling preclinical efficacy with this class of agents. Here, I discuss some of the possible reasons for this translational discrepancy and suggest strategies to overcome it with the next generation of PARAs.
Targeting the extrinsic apoptotic pathway in cancer: lessons learned and future directions

Avi Ashkenazi
Cancer Immunology, Genentech Inc., South San Francisco, California, USA

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From Coley’s toxins to PARAs
In 1894, Coley showed that cell extracts from gram-negative bacteria caused tumor shrinkage in patients. Nearly a century later, the discovery and cloning of TNF-α as a host factor induced by bacterial LPS made it possible for the first time to attempt to recapitulate Coley’s seminal observation with a single, molecularly defined agent. Although purified TNF-α was too toxic for systemic therapy, it was later approved in Europe for the treatment of sarcoma by isolated limb perfusion (I). Subsequently, Krammer and Nagata identified the death receptor Apo1/Fas (CD95), which helped decipher the extrinsic apoptotic pathway (2–5). However, attempts to activate CD95 for cancer therapy were again hampered by toxicity, in this case due to excessive apoptosis of hepatocytes (2, 3).

A third opportunity to test the concept that tumor cells could be killed deliberately through a specific biological pathway arose in the mid-1990s, when my laboratory at Genentech and Ray Goodwin’s laboratory at Immunex independently discovered another death ligand, called Apo2 ligand or TNF-related apoptosis-inducing ligand (Apo2L/TRAIL or TNFSF10) (6, 7). My team developed a recombinant soluble version of the human ligand comprising the extracellular domain of the endogenous protein and possessing a homotrimeric structure. In our 1999 JCI article (8), we reported that recombinant Apo2L/TRAIL induced apoptosis in a wide range of cancer cell lines while sparing various normal cell types. Moreover, the recombinant ligand exerted significant antitumor activity as a single agent and in combination with chemotherapy in a murine cancer xenograft model (8). The Immunex group reported similar results with a version of the ligand that was trimerized via a yeast-Gal4 leucine zipper (9). These findings were corroborated and expanded in numerous studies (10–12). X-ray crystallography later revealed that stabilization of the homotrimeric molecule by an internal zinc ion was crucial for its selective proapoptotic activity against malignant, but not normal, cells (13, 14).

The work with Apo2L/TRAIL and the identification of its cognate proapoptotic death receptors DR4 (TNFRSF10A) and DR5 (TNFRSF10B) (4, 15) prompted several groups, including my own, to develop agonistic anti-DR4 and anti-DR5 antibodies (16, 17). Compared with soluble Apo2L/TRAIL, these antibodies enable less frequent dosing; however, the agonistic activity of anti-DR4 and anti-DR5 antibodies in vivo is restricted by a requirement for binding to Fcγ receptors (18). Conversely, cross-linking of Apo2L/TRAIL — either directly or via anti-DR5 antibody — triggers apoptosis in tumor-associated endothelial cells, disrupting the tumor vasculature while sparing normal vessels (19, 20). Thus, optimizing geometry and stoichiometry of PARAs appears crucial for effective and selective apoptosis engagement.

The impressive efficacy of PARAs in preclinical cancer models provided a compelling rationale for testing these agents in the clinic. To date, about 30 phase I and/or phase II trials have been conducted to evaluate PARAs in various cancers, including non–small-cell lung cancer, colorectal cancer, pancreatic cancer, multiple myeloma, and non-Hodgkin’s lymphoma (http://www.clinicaltrials.gov). Importantly, unlike TNF-α and anti–CD95 agonist antibodies, Apo2L/TRAIL and DR4- or DR5-targeting agonist antibodies were relatively well tolerated. Disappointingly,
these PARAs failed to show significant efficacy either as monotherapies or in combination with conventional chemotherapies and/or certain biological agents. There were some rare, yet notably durable positive responses, for example, in a patient with chondrosarcoma (21).

Lessons learned and future strategies

What might account for the discrepant preclinical and clinical results with PARAs? One plausible explanation is that tumors encountered in the clinic have a higher threshold for reactivation of the extrinsic pathway than do those in preclinical models. This potentiality could be addressed by implementing several strategies (Figure 1): (a) augmenting PARA potency a step beyond the first generation of agents while ensuring a favorable therapeutic index; (b) stratifying patients and optimizing dosing based on predictive and pharmacodynamic diagnostic biomarkers; and (c) combining PARAs with other targeted agents to achieve synthetic lethality against tumors. Approaches to augment potency include the presentation of two Apo2L/TRAIL trimers on Fc fusion platforms (22) or multiple trimers on liposomal membranes (P. Nair and A. Ashkenazi, unpublished observations), or a combined treatment with Apo2L/TRAIL and a suitable DR5 antibody (20). Biomarkers that may help predict responsiveness to PARAs include membranous expression of DR4 and DR5 on malignant and endothelial cells within tumors; O-glycosylation enzymes involved in post-translational modification of DR4 and DR5 in the Golgi apparatus — a modification that augments ligand-induced receptor clustering (23, 24); Fcγ receptor polymorphism (18), which may impact the affinity and hence efficacy of agonistic antibodies; expression of E-cadherin, which facilitates ligand activation of DR4 and DR5 by dynamically coupling these receptors to the actin cytoskeleton in epithelial cancer cells (25); and ubiquitin E3 ligases involved in potentiating or curtailing caspase-8 activation in epithelial cancer cells (26, 27), among other components and modulators of the extrinsic pathway. Pharmacodynamic biomarkers may include cleaved caspase-8 and caspase-3 or other readouts for caspase activation and apoptosis. Potential synthetic lethal strategies include combinations with BCL-2 antagonists, IAP antagonists, proteasome inhibitors, agents targeting aberrant signaling cascades such as the

Figure 1. Apoptotic signaling pathways engaged by PARAs that target the death receptors DR4 and DR5. There are several potential strategies to improve the clinical efficacy of these PARAs. One strategy would be to augment potency by increasing the oligomeric state of Apo2L/TRAIL or the affinity of agonistic anti-DR4 or anti-DR5 antibodies for Fcγ receptors (blue asterisks). Another strategy to improve efficacy would be the implementation of predictive and pharmacodynamic diagnostic biomarkers that might help predict or determine whether a patient’s cancer is sensitive (green asterisks) or resistant (red asterisks) to PARA treatment. A third approach would be to improve synthetic lethality against cancer cells by combining PARAs with pharmacological agents that target various other intracellular signaling components or modulators of the apoptotic pathways (black asterisks). BAX/BAK, BCL-2-associated X protein/BCL-2 antagonist killer 1; BCL-2/X, B cell lymphoma-2/extra long; BID, BH3-interacting domain death agonist; cFLIP, cellular FLICE-inhibitory protein; CUL3, cullin 3; DISC, death-inducing signaling complex; FADD, Fas-associated death domain; FUT3/6, fucosyltransferase 3/6; GALNT14/3, polypeptide N-acetylgalactosaminyltransferase 14/3; SMAC, second mitochondria–derived activator of caspases; TRAF2, TNF receptor–associated factor 2; XIAP, X-linked inhibitor of apoptosis.
Conclusions

An attractive feature of apoptosis reactivation is the potential to cause tumor regression rather than just stasis. On the other hand, discriminating between malignant and healthy cells is crucial to avoid untoward side effects. Attempts to translate Coley’s seminal findings by directly engaging the extrinsic apoptotic pathway were hampered by toxicities associated with TNF-α or anti-CD95 antibodies. We now have progressed beyond these safety hurdles with DR4- and DR5-targeted PARAs, although clinical efficacy with these agents has yet to be achieved. This creates a unique opportunity to overcome tumor resistance by (a) developing second-generation PARAs with enhanced potency while maintaining a therapeutic index; (b) implementing diagnostic biomarker approaches; and (c) investigating more advanced combinatorial strategies. Albert Einstein said: “failure is success in progress.” I hope that researchers in academia and industry will find this article helpful in their quest to harness the extrinsic pathway for medical benefit.

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Address correspondence to: Avi Ashkenazi, Cancer Immunology, Genentech Inc., 1 DNA Way, South San Francisco, California 94080, USA. Phone: 650.225.1853; E-mail: aa@gene.com.