Selective TRAIL-Induced Apoptosis in Dysplastic Neoplasia of the Colon May Lead to New Neoadjuvant or Adjuvant Therapies

Commentary on Jalving et al., p. 4350

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In this issue of Clinical Cancer Research, Jalving et al. (1) shows that treatment with recombinant human tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL) triggers apoptosis in human dysplastic colorectal adenomas of the colon. These observations suggest the potential to develop neoadjuvant or adjuvant therapies for susceptibility syndromes and may in the future involve incorporation of TRAIL into strategies for chemoprevention. More evidence is required, however, both in animal models and in human subjects before initiation of therapeutic trials.

Cytokines, endogenous components of the immune system, that may enhance the immune surveillance against disease has been an attractive approach in the treatment of malignancies. In addition, these compounds may trigger apoptosis directly in a range of tumor cells. Successful use of cytokines in the treatment of cancers, however, has proven less successful than what could have been expected. The TNF super family of cytokines harbors members with the potential to kill cancer cells in vitro and in vivo. Inflammatory responses and sepsis, however, have proven to be dose limiting for clinical use of some family members (e.g., TNF-α; ref. 2) and severe liver toxicity for the Fas-L (3). These examples point to some severe dose-limiting toxicities that have made Fas-L and TNF less suitable for systemic anticancer therapy.

Another member of the TNF super family, constitutively expressed in most tissues, TRAIL has been shown to be well tolerated (4, 5). Indeed TRAIL belongs to a putative new class of cytokines harbors members with the potential to kill cancer cells in vitro and in vivo. Inflammatory responses and sepsis, however, have proven to be dose limiting for clinical use of some family members (e.g., TNF-α; ref. 2) and severe liver toxicity for the Fas-L (3). These examples point to some severe dose-limiting toxicities that have made Fas-L and TNF less suitable for systemic anticancer therapy.

TRAIL is poorly understood, but it suggests low toxicity in the treatment of malignancies. Both recombinant human TRAIL and agonistic monoclonal human antibodies to the death-inducing TRAIL receptors are currently under clinical evaluation (6–8).

The cell death signaling (i.e., the death receptors Fas, DR4, DR5, TNFR1, and DR3) members of the TNF family function as transmembrane signal transducers that contain a cytoplasmic death domain not present in other receptors of the TNF family, which respond to ligand binding (9). The death domain facilitates intracellular interaction with specific adaptor proteins (e.g., TNFR-associated death domain for TNFR1 and DR3 and the Fas-associated death domain for FAS, DR4, and DR5; refs. 10–12). These adaptor proteins contain specific sequences necessary for the binding of important proapoptotic effector proteins, such as caspase-8 and caspase-10, that following proximal recruitment to TNFR-associated death domain and Fas-associated death domain, undergo autoactivation through the cleavage from a pro-form to an enzymatically active form (Fig. 1; refs. 13–15). The complex between Fas-associated death domain/TNF-associated death domain, procaspase-8, and/or procaspase-10 is commonly referred to as the death-inducing signaling complex (16). Active caspase-8/caspase-10 triggers proteolysis and activation of apoptotic executioner caspase-3, caspase-6, and caspase-7. This pathway is commonly described as the extrinsic cell death pathway. In contrast, the intrinsic pathway involves signaling from the mitochondria through Bcl-2 family members (e.g., Bax and Bak that cause the release of cytochrome c, activation of caspase-9, and activation of the executioner caspases; i.e., caspase-3, caspase-6, and caspase-7; see Fig. 1; refs. 17, 18). Both the intrinsic and the extrinsic pathways can be activated following DNA damage triggered by chemotherapeutic compounds, a process commonly linked to the stabilization of the transcription factor p53. p53 regulates the expression of the proapoptotic proteins NOXA and Puma (19, 20), which prevent Bcl-2 from blocking Bax, a key protein that promotes the release of mitochondrial cytochrome c. The extrinsic pathway is stimulated by p53 through increased expression of the death receptors Fas and DR5 (21, 22). It should be mentioned that these processes are not merely or exclusively controlled by p53, but other factors may increase the expression of death receptors. The extrinsic and intrinsic pathway may crosstalk through the cleavage of the proapoptotic Bcl-2 family member Bid that promotes Bax and Bak activity (23, 24). For some cells, such crosstalk is essential for efficient triggering of apoptosis through the extrinsic pathway.

The biological function of TRAIL and sensitivity towards TRAIL is poorly understood. Apo2L/TRAIL mRNA is expressed in many human tissues, as is mRNA encoding for DR4 and
Thus, there must exist a mechanism that under physiologic conditions protects cells from Apo2L/TRAIL. Resistance to TRAIL can be overcome, at least in part, by coadministration of conventional chemotherapeutics together with TRAIL, presumably, because of DNA damage mediated engagement of the intrinsic pathway and activation of p53. Sensitivity to TRAIL does not always correlate well with the cell surface expression of the death-inducing TRAIL receptors; thus, regulation of downstream events from receptor signaling must be important. It has been suggested that down-regulation of the short form of FLIP, a molecule that inhibits the activation of caspase-8, might be of importance to TRAIL resistance (25, 26). Down-regulation of FLIP has been shown to correlate with enforced expression of c-Myc, a deregulated oncogene in several
different malignancies (27). Furthermore, increased nuclear factor-κB signaling has been shown to protect from TRAIL-induced death. Why normal cells do not show high sensitivity towards TRAIL, however, is still largely unclear. TRAIL-binding receptors lacking a death domain (DcR1 and DcR2) may function as decoy receptors (4).

Recombinant human TRAIL and monoclonal antibodies that specifically facilitate trimerization of the TRAIL death receptors (HGS-ETR 1 towards DR4 and HGS-ETR 2 towards DR5) are currently undergoing phase Ib and phase II clinical evaluations, respectively (refs. 6–8; Table 1). The advantage with the antibodies compared with recombinant TRAIL may be a longer plasma half-life, higher specific binding affinity, and subsequently less reactivity towards decoy receptors that may otherwise ameliorate the death receptor engagement. On the other hand, this could lead to an altered toxicity profile compared with recombinant TRAIL (4). Recombinant soluble TRAIL does not discriminate between DR4 and DR5 (or the decoy receptors) and has been shown to have little to no effect on normal cells in preclinical experiments (5, 28, 29). These agents constitute promising new anticancer therapies that specifically trigger apoptosis in cancer cells. The HGS-ETR antibodies show few dose-limiting toxicities, and an maximum tolerated dose was not reached during phase I. Treatment with HGS-ETR 1 at 10 mg/kg body weight to patients every 28 days with a range of solid tumors and non-Hodgkin’s lymphoma during phase I resulted in stabilized disease as best response (7, 8). HGS-ETR1 is currently in phase II clinical development as a single agent in patients with non–small cell lung cancer and colorectal cancer (for a review of the HGS-ETR antibodies, see ref. 29).

In this issue of Clinical Cancer Research, Jalving et al. (1) showed that treatment with recombinant TRAIL might have implications for the prevention and management of colorectal cancer, a disease affecting ~158,000 people globally and annually. Colorectal cancer is the leading cause of cancer death and may develop progressively through histologically distinct lesions, spanning from dysplastic aberrant crypt foci, early to late adenoma and carcinoma (30). Each lesion in colorectal tumorigenesis is associated with specific genetic changes in tumor suppressors and oncogenes. In the case of hereditary disease, preventive measures equals frequent colonoscopy and resection of visible polyps in the colon as well as prophylactic colectomy in familial adenomatous polyposis. To date, it has not been fully clear when TRAIL sensitivity occurs during tumorigenesis of the colon. Indeed, a number of colon cancer cell lines show sensitivity to TRAIL (Table 1), and one previous study suggested that TRAIL sensitivity is triggered during the adenoma-to-carcinoma transition of colorectal carcinogenesis (31). In this study, four colorectal adenoma cell lines were shown to be less sensitive to TRAIL (0.25-1 µg/mL), although there were variations in sensitivity between some of the carcinoma cell lines. Another study in which colonic tissues were explanted from a resected colon, containing a large number of small polyps, of a familial adenomatous polyposis patient and treated with TRAIL ex vivo suggested that TRAIL by itself triggered a very modest apoptotic response over baseline values (32). This was in stark contrast, however, to a readily detected apoptotic response by the combination of CPT-11 and TRAIL. Jalving et al. show a clear sensitivity in two adenoma cell lines to 0.1 µg/mL of TRAIL and a number (n = 38, from a total of five patients) of resected dysplastic adenomas (1 µg/mL of TRAIL), but importantly, not in resected colonic tissue with normal morphology (n = 15). Although different TRAIL preparations can differ in killing potency, that does not seem to be a reasonable explanation for the difference between the studies. Rather, Jalving et al. may suggest from their experiments with resected adenomas that the adenoma cell line used by Hague et al. may be derived from adenomas with a low-grade dysplasia, not sensitive to TRAIL. Furthermore, in determining TRAIL sensitivity, ample data exist on sensitive tumor cell lines and xenograft models thereof, whereas normal cells are less frequently tested in parallel. Therefore, the data of Jalving et al. set a good example in the use of an internal control to monitor the effect of TRAIL on normal cells. Colonic explants with no dysplasia showed no signs of cell death after TRAIL treatment, whereas high-grade dysplastic lesions from the same patient reminiscent of (pre) neoplasia showed cell death after treatment with TRAIL. Although this was only shown at one time point (5 hours), another study showed that colonic explants from a familial adenomatous polyposis patient could respond to TRAIL treatment (0.1 µg/mL) after 30 hours (32). Thus, it is possible that the response to TRAIL in adenomas with low grade of dysplasia has a slower onset of apoptosis.

### Table 1. TRAIL receptor – activating agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Class</th>
<th>Phase of development (Institution)</th>
<th>Target</th>
<th>LOEC* and EC50</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>APO2L/TRAIL</td>
<td>Cytokine</td>
<td>Phase I (Amgen/Genentech)</td>
<td>TRAIL receptors; death inducing (i.e., DR4 and DR5) and decoy receptors (i.e., DcR1 and DcR2)</td>
<td>10 and 35 ng/ml (Colo205) 10 and 50 ng/ml (SW480)</td>
<td>(35–37)</td>
</tr>
<tr>
<td>HGS-ETR1</td>
<td>Monoclonal antibody</td>
<td>Phase Ib (Human Genome Sciences)</td>
<td>Specific for DR4</td>
<td>100 and 500 ng/ml (SW480) 500 and 1000 ng/ml (Colo205)</td>
<td>(38)</td>
</tr>
<tr>
<td>HGS-ETR2</td>
<td>Monoclonal antibody</td>
<td>Phase II (Human Genome Sciences)</td>
<td>Specific for DR5</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NOTE: NA refers to no published literature on colon cancer cells or cell lines.
Abbreviations: LOEC, lowest effective concentration; EC50, 50% effect concentration.
*Extrapolation made over different cell death/cell growth end points for a number of known TRAIL-sensitive colon cancer cell lines. LOEC and EC50 are approximative.
The data of Jalving et al. may, however, have implications for TRAIL in the treatment and management of colorectal cancer. The investigators suggest that TRAIL treatment may serve as adjuvant treatment for patients undergoing resection of polyps. Considering the limitation of resecting visible polyps and the toxicity involved with standardized adjuvant treatment, TRAIL death receptor agonist or recombinant TRAIL could be a useful strategy to eliminate residual neoplastic cells. The data also raise issues concerning when TRAIL treatment could be an effective strategy. In general, clinical research has largely been focused on defining new molecular targets that are specific for cancer cells and in developing lead pharmaceutical compounds. Few diagnostic tools have been developed to reach a clinical standard to discover when a particular molecular target is best used for the treatment of malignancies (i.e., when a particular treatment strategy might be useful to ablate a tumor mass). As an example, there are no standardized methods to analyze p53 mutations in tumor tissue (biopsies or resected tissue) in the clinic, although there are ample preclinical and clinical data that suggest that the p53 gene status could have a significant effect on the outcome of treatment with chemotherapy or radiotherapy. The lack of understanding of the basic biology with regard to what makes cells sensitive to TRAIL may be more hampering in comparison to the ample p53 data available, but clearly, with increased molecular specificity of therapy comes a demand for better diagnostic tools and biomarkers. Not all colon cancer cell lines are susceptible to TRAIL, and combination treatment with more toxic modalities might need to be used. Thus, to design better treatments, a more thorough understanding for TRAIL signaling in the colon is required. Meanwhile, the data of Jalving et al. hopefully suggest that TRAIL could be in the forefront battling colorectal cancer in the early 21st century.

Indeed, in addition to death receptor agonists as single-agent therapy, several combination therapies with TRAIL are being explored in preclinical settings and clinical trials. Combinations with currently used chemotherapeutics (e.g., gemcitabine, 5-fluorouracil, and CPT-11) that engage the intrinsic pathway through p53 (Fig. 1) to amplify TRAIL-mediated killing of resistant clones are being exploited. In addition, activation of p53 and up-regulation of the DR5 receptor have been shown to overcome resistance to TRAIL in at least some cell types. Proteasomal inhibitors (i.e., Velcade), currently undergoing clinical evaluation for treatment of myeloma, have been shown to overcome TRAIL resistance in several cancer cell lines. Thus, it seems that prososomal degradation of TRAIL’s death-inducing receptors and inhibition of nuclear factor-κB or other downstream component might limit the efficacy of TRAIL treatment alone. Thus, Velcade might prove to be useful for colorectal cancers that show resistance to TRAIL. A recent article, however, suggests that inhibition of the proteasome may require prior knowledge about the presence of antiapoptotic or proapoptotic proteins present in the particular tumor to be treated (33). Intriguingly, Sohn et al. showed that the proteasome actually is required for the initiation of death receptor–induced apoptosis. The proteasome removes antiapoptotic proteins in early phases after activation of the death receptors, whereas at later stages, proapoptotic proteins are being removed (33).

The data presented by Jalving et al. may warrant clinical trials for neoadjuvant and adjuvant therapy with TRAIL. Before such plans can be realized, however, careful investigation in animal models may be helpful. To date, no studies have addressed whether TRAIL can be used as described by Jalving et al. in an animal tumor model. For example, studies using TRAIL in APCmin mice or MSI12-deficient mice could serve as a model that (to some extent) recapitulate the human hereditary conditions, whereas chemical carcinogenesis models may better mimic sporadic colorectal cancers. DR5 knockout mice could be bred into these colon tumor–prone mice to better establish the role of TRAIL signaling in their suppression in vivo (34). Furthermore, although unlikely to be reduced, studies on the serum levels of TRAIL in patients with familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer may show any deficiency in endogenous TRAIL that may allow TRAIL-sensitive cancers to develop and may suggest that TRAIL could be given to reduce the risk of developing colorectal cancer. It remains to be determined whether pharmacokinetics or safety related to the long-term administration of TRAIL or TRAIL agonists would allow such approaches in humans.

Further chemopreventive measures could involve TRAIL given together with cyclooxygenase-2 inhibitors that have been shown to have effects on colonic polyps in familial adenomatous polyposis. Different mouse models could here provide a useful tool that may address dose-related issues like systemic toxicity in relation to tumor response. In addition, because chemopreventive studies can take years or even decades to establish the most appropriate regimen, dosage, and duration of therapy to affect a particular disease, animal studies may greatly accelerate the process.

In conclusion, although many questions remain, the data published in this issue of Clinical Cancer Research by Jalving et al. may motivate more thought and effort directed at these questions in different models. This may ultimately shed more light on the usefulness of TRAIL in the treatment and management of colonic neoplasms at various stages of their evolution.

References
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