Clearing the TRAIL for Cancer Therapy

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The death receptor ligand TRAIL has shown remarkable promise as an anticancer agent. However, TRAIL signaling also activates NF-κB, which induces the antiapoptotic regulators Mcl-1 and cIAP2, thus compromising its efficacy. In this issue of *Cancer Cell*, El-Deiry and colleagues explore pathways that disrupt TRAIL-induced survival signaling and show that the Myc oncoprotein and the Raf kinase inhibitor Sorafenib sensitize otherwise TRAIL-resistant colon cancer cells by effectively reducing NF-κB-mediated transcription of *Mcl-1*. These findings suggest that combining TRAIL with agents that disrupt NF-κB regulation or binding or those that directly destabilize or disable Mcl-1 will have therapeutic benefit.

In designing the *ideal* therapy for cancer treatment, three criteria are highest on the list. First, the therapy has to be effective in killing cancer cells and prevent them from returning. Second, the treatment needs to be selective for the cancer cell and avoid off-target effects. Indeed, the untoward side effects and poor tolerance of conventional chemotherapeutic agents are common pitfalls in the clinic. Finally, the ability to predict efficacy based on biomarkers permits informed treatment, saving both time and suffering. In this issue of *Cancer Cell*, the studies of Ricci et al. tackle all three of these criteria, and importantly, they do so in the setting of chemorefractory disease, which ultimately kills the patient.

TRAIL (*T*NF-α-*r*elated *a*poptosis-*i*nducing *l*igand) has attracted a lot of interest as a cancer therapeutic agent. TRAIL is trimeric and binds to and induces trimerization of the death receptors DR4 or DR5, which recruits the *d*eath-*i*nducing *s*ignaling *c*omplex (DISC) to the "death domains" present in the cytoplasmic region of these receptors. Formation of the DISC induces transcleavage and activation of the initiator caspase, caspase 8. At this point, the signal bifurcates with one route (the extrinsic pathway) leading to caspase 8-mediated activation of caspase 3, while the other channels through caspase 8 cleavage of Bid, activating the intrinsic cell death pathway and amplifying caspase 3 activation (Figure 1A) (Takeda et al., 2007). Cells have been broadly delineated as type I, in which the extrinsic pathway is sufficient to trigger cell death, and type II, which require amplification through the mitochondrion for apoptosis. In most tumor cells the latter is operational. The finding that TRAIL selectively kills cancer cells quickly spawned clinical trials with TRAIL that showed promise, yet resistance to TRAIL is now recognized as a common and unfortunate outcome. Thus, the critical questions are how one can discriminate TRAIL-resistant and -sensitive tumors, what the underlying mechanisms of resistance are, and whether resistance can be reversed.

Previous forays into these questions have demonstrated that Myc is a critical arbiter of TRAIL sensitivity. At one level Myc represses the expression of FLIP (Figure 1B), a dedicated inhibitor of the DISC that prevents caspase 8 activation, by interfering with Miz-1-dependent transcription (Ricci et al., 2004). In addition, Myc induces the transcription of *DR5* (Wang et al., 2004) (Figure 1B). Now the studies of Ricci et al. (2007) have revealed more complexity to the mix. As a model for type II, TRAIL-resistant cells, the authors utilized HCT116 human colon carcinoma cells lacking the proapoptotic protein Bax, which together with Bak is essential for all forms of apoptosis. Loss of *Bax* cripples TRAIL-induced death, yet Myc sensitizes these cells to TRAIL, which is rather surprising given that Myc's effects on the intrinsic pathway have been ascribed to Bax in preference to Bak (Nilsson and Cleveland, 2003). Thus, in this setting one would predict that Myc must somehow provoke a type II to type I switch, but this is not the case either, as inhibition of caspase 9 or caspase 8 has equal effects on cell death, and there is little, if any, effect on the DISC.

Insights into how Myc might liberate TRAIL-induced death sans Bax came from experiments demonstrating that TRAIL induces profound increases in Mcl-1 and cIAP2 in $Bax^{-/-}$, Bcl-X_L-overexpressing-$Bax^{+/-}$, or caspase 8/9 inhibitor-treated $Bax^{+/-}$ colon cancer cells. Further, in $Bax^{-/-}$ cells, TRAIL also provokes the disappearance of Bak, effectively creating a *Bax*/*Bak*-deficient cell that should be resistant to all forms of death (Wei et al., 2001), which obviously is not the case. Curiously, Myc does *not* restore Bak expression in Bax-deficient TRAIL-treated cells; thus, Myc sensitization seems to break all the rules for Bax/Bak engagement—so what gives?

To explain it all, the authors turned to the inhibitory effect of Myc on TRAIL-induced cIAP2 and Mcl-1 expression. Knockdown experiments demonstrated that Mcl-1 is really the relevant target for TRAIL sensitization, and here they demonstrate that Myc suppresses *Mcl-1* transcription and that Myc is constitutively bound to the *Mcl-1* promoter. Mechanistically how this all occurs is not clear,

and one would predict that the Myc-Miz-1 axis plays a role in this response. Nonetheless, insights came from experiments implicating NF-κB in the Mcl-1 response to TRAIL—here Myc binding somehow blocks the activity of NF-κB when bound to its recognition elements in the *Mcl-1* promoter. Sorting out how all this occurs, not to mention that the Myc/TRAIL combo is lethal in a cell that is effectively Bax/Bak null, are areas of important investigation.

Obviously, one does not want to overexpress Myc as a therapeutic strategy, yet the ability of Myc to short circuit NF-κB in the TRAIL-to-Mcl-1 pathway suggested other avenues for intervention. El-Deiry and colleagues tested the interesting hypothesis that signaling through the Ras pathway might represent an Achilles' heel one could exploit, and here the authors tested the effects of the Raf kinase inhibitor Sorafenib (BAY43-9006), which is in several clinical trials for malignancies with Raf activation. Sorafenib was designed to target Raf-1, B-Raf, and activated, mutant B-Raf (a hallmark of melanomas and other tumor types) and does so with nanomolar IC_{50}s, yet it also inhibits several tyrosine kinase receptors and was known to downregulate Mcl-1 expression to induce apoptosis (Rahmani et al., 2005; Wilhelm et al., 2006; Yu et al., 2005). Mcl-1 is a short-lived protein and is rapidly degraded by the proteasome in the response to several apoptotic triggers, yet here Ricci et al. demonstrate that Sorafenib sensitizes Bax-deficient HCT116 colon cancer cells, as well as other TRAIL-resistant tumor cells, to TRAIL by rather blocking *Mcl-1* transcription. How this happens is also a bit fuzzy, as Sorafenib blocks binding of NF-κB to the *Mcl-1* promoter without effecting its translocation to the nucleus (Figure 1C). Again, the devil is in the details, and sorting out how this really happens is an important "to-do." Experiments that evaluate the potential effects of Sorafenib on NF-κB phosphorylation and/or acetylation, which are important for its transactivation functions, should be high on the list.

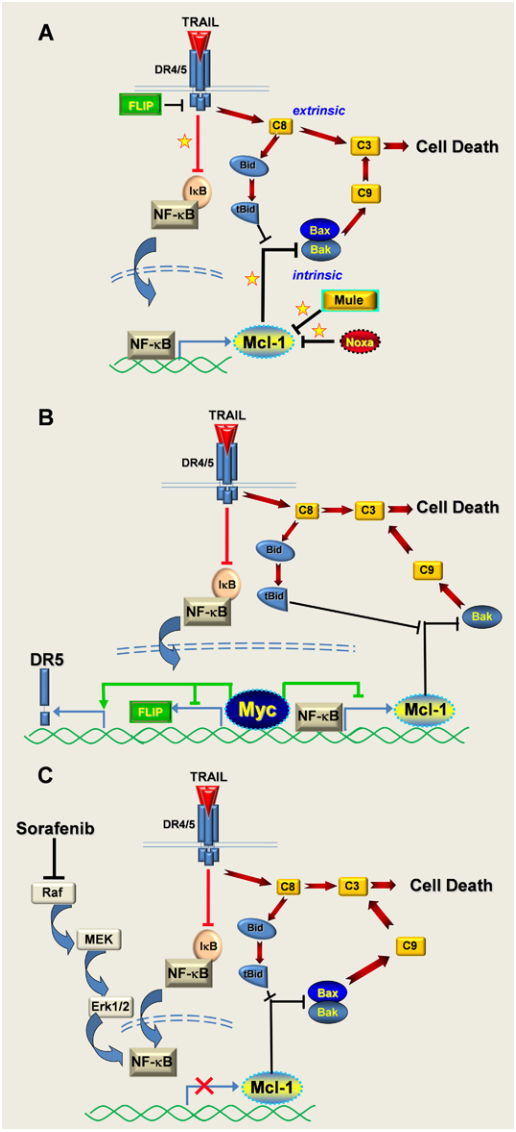

Figure 1. Myc and the Raf Kinase Inhibitor Sorafenib Disable TRAIL-Directed Survival Pathways by Targeting the Intrinsic Apoptotic Machinery

(A) The binding of trimerized TRAIL ligand (red) to its cognate death receptors DR4 or DR5 simultaneously propagates a proapoptotic signal through caspase 8 (C8) and a prosurvival signal by activating NF-κB. In turn, NF-κB directly induces the transcription of *Mcl-1*, accounting for TRAIL resistance. When the intrinsic cell death machinery is intact, the proapoptotic signal dominates, and cell death results. Additional points where one could sensitize the pathway are marked with stars. Caspases-3, -8 and -9 are abbreviated C3, C8, and C9, respectively.

(B) When the intrinsic pathway is compromised, for example, by inactivating *Bax* (shown here) or by mutations that affect other apoptotic regulators found in cancer, cells become resistant to TRAIL. TRAIL-resistant cells can be resensitized by Myc, which induces *DR5* expression, and which represses the transcription of both *FLIP* and *Mcl-1*.

(C) The Raf-to-Erk pathway also converges on NF-κB activation, and the Raf kinase inhibitor Sorafenib blocks NF-κB binding to the *Mcl-1* promoter, by as yet unknown mechanisms. This releases the intrinsic pathway, and cell death ensues.

Since a major conclusion of Ricci et al. (2007) is that the "TRAIL" leads to Mcl-1, its mechanism of induction warrants closer inspection. Indeed, the response to TRAIL is actually quite complex, with Mcl-1 protein in Bax-deficient colon cancer cells initially declining and then rebounding to markedly increased levels. Fluctuations are also apparent in $Bax^{+/-}$ cells treated with TRAIL (i.e., this pathway is intact). All of these gyrations precede the modest elevations in *Mcl-1* transcripts. Therefore, TRAIL-mediated control of Mcl-1 turnover or translation may also be in the mix, and the roles of additional regulators of Mcl-1 protein such as the E3 ubiquitin ligase Mule and Noxa, which disrupts Mcl-1 binding to Bak (Willis and Adams, 2005) should be interrogated in this system. Further, Mule and Noxa may represent missing pieces to the "disappearing Bak" puzzle and could provide additional targets for combination therapy with TRAIL. Finally, other regulators could also contribute. For example, the authors show that BH3-only protein Puma is also repressed by TRAIL in $Bax^{+/-}$ colon cancer cells, which may also be relevant.

So, does this new combination of TRAIL and Sorafenib represent a step toward the *ideal* cancer treatment? Certainly, TRAIL is an effective, cancer-cell-selective agent,

and thus identifying regulators, such as Myc, that mediate sensitivity is critical to its success. Indeed, sensitizing cancer cells to TRAIL using combination therapy has been successfully tried before, for example, using DNA-damaging agents. But the elegant approach of targeting the resistance pathway (upregulation of Mcl-1) using the new targeted therapy Sorafenib is an appealing advance and opens avenues for combining TRAIL with small molecules or peptides that act directly on apoptotic sensitizers and effectors (such as stabilized BH3-peptides targeting Mcl-1 and/or Bak) or other drugs that destabilize Mcl-1. However, a word of caution—sensitizing TRAIL-resistant cancer cells may also sensitize normal cells to this "death" ligand. Thus, one needs to choose the "TRAIL" wisely...

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Drugging the Bad "AKT-TOR" to Overcome TKI-Resistant Lung Cancer

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EGFR kinase inhibitors constitute an important class of lung cancer treatments. While they produce dramatic responses in a subset of patients—primarily those with activating EGFR mutations—remissions are typically limited to several months due to acquired drug resistance, frequently associated with the secondary T790M mutation in EGFR. In this issue of *Cancer Cell*, Li et al. report that an irreversible EGFR kinase inhibitor, HKI-272, had limited activity in a mouse lung cancer model driven by an EGFR mutant harboring T790M and an activating mutation. However, combining HKI-272 with rapamycin promoted rapid tumor regression, suggesting a therapeutic strategy to overcome drug resistance.

Lung cancer remains the leading cause of cancer deaths worldwide. While the disease is largely refractory to conventional chemotherapy, the recent emergence of selective tyrosine kinase inhibitors (TKIs) that elicit dramatic clinical responses in a subset of treated patients represents a highly promising therapeutic development. Specifically, about 10%–20% of chemotherapy-refractory non-small-cell lung cancers (NSCLCs), which constitute the vast majority of lung cancer cases, exhibit clinical responses to gefitinib (Iressa) and erlotinib (Tarceva), small-molecule inhibitors of the epidermal growth factor receptor (EGFR) kinase (Sequist et al., 2007). Moreover, such responses have been well correlated with the presence of somatic activating EGFR kinase domain mutations in tumors and have been linked to an overall survival benefit (Sequist et al., 2007).

However, the excitement around this therapeutic advancement has been somewhat tempered by the fact that clinical responses to EGFR TKIs are of limited duration—typically, 6–9 months, due to acquired drug resistance. Paralleling the clinical experience with the BCR-ABL kinase inhibi-