Mcl-1: A Gateway to TRAIL Sensitization

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Abstract

The proapoptotic cytokine tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) is being evaluated presently as a selective anticancer agent, but its limited effects against cancer cell lines has raised some concerns about its ultimate clinical utility. Here, we review recent findings that cancer cell sensitivity to TRAIL is greatly increased when the Bcl-2 family protein Mcl-1 is down-regulated by the Raf/vascular endothelial growth factor kinase inhibitor sorafenib, a Food and Drug Administration–approved cancer drug. Using the TRAIL-sorafenib combination as a tactic to more effectively kill cancer cells may provide an effective tool to attack a variety of human cancers that are largely presently untreatable. [Cancer Res 2008;68(7):2062–4]

Introduction

Development of cancer-specific therapy with little or no toxic side effects is one of the goals of molecular oncology and translational research. Targeting specific oncogenes encoding kinases or receptors using small-molecule inhibitors or recombinant antibodies has proven to be an excellent treatment paradigm for cancer patients (e.g., imatinib mesylate/Gleevec; trastuzumab/Herceptin). Targeted therapies have favorable side effects but are effective only in minor populations of patients with tumors harboring their specific oncogene targets (1). But tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) is different in its potential to target a broad host of tumors, which it normally does as part of the host immune system. Since its discovery, TRAIL has attracted great interest as a cancer cell–specific cytotoxic agent with virtually no off-target effects (2). TRAIL or antibodies that target the receptors of TRAIL are currently in clinical trials. However, many human tumor cell lines are resistant to TRAIL therapy, suggesting the potential that many patients will be nonresponders. Therefore, considerable effort is being directed at the identification of mechanisms to increase sensitivity to the tumor killing effects of TRAIL and to sensitize TRAIL-resistant cancers.

Determinants of the Resistance of a Cancer Cell to TRAIL Therapy

TRAIL is a type II transmembrane protein, and its extracellular carboxy terminal domain is proteolytically cleaved from the cell surface as a 19-kDa circulating molecule that binds one of two agonistic receptors, DR4 and DR5. TRAIL binding to DR4 or DR5 activates the apoptotic machinery of the target cell (3). After activating its death receptors, there are many potential defects in the apoptotic pathway that can block cell death triggered by TRAIL. Cells may have too little death receptor expression or may have high TRAIL decoy receptor expression to gain protection from the proapoptotic effects of TRAIL (4). Cells may lose apical caspase expression (5) or may harbor high expression of the initiator caspase activation inhibitor c-FLIP leading to TRAIL resistance (6). Cancer cells may inhibit intrinsic mitochondrial pathways leading to cell death by increasing antiapoptotic or decreasing proapoptotic molecules, including those of the Bcl-2 family (7). Such tumors are likely to resist TRAIL-mediated apoptosis if they require amplification of the death signal through the mitochondria. With such heterogeneity and multiple mechanisms that tumors use to evade TRAIL-mediated apoptosis, is it possible to identify a useful strategy to sensitize a wide-range of TRAIL-resistant cancers? Three recent papers revealing previously unknown relationships between TRAIL, Mcl-1, and sorafenib suggest that it may indeed be possible to use a single sensitizing agent when the apoptotic defect is harbored in the intrinsic apoptotic pathway.

TRAIL Resistance Conferred by Mcl-1 and Its Reversal by c-Myc

Our investigation into the relationship between TRAIL and Mcl-1 began with c-Myc (8). c-Myc is not only a potent cellular oncogene but also a potent apoptosis inducer. Previously, we identified that c-Myc expression is a major determinant of TRAIL sensitivity and can also sensitize cells to TRAIL (6). As an extension of this earlier study, we recently examined whether c-Myc overexpression in TRAIL-resistant Bax-deficient HCT116 human colon cancer cells could sensitize these cells through c-Myc. We hypothesized that c-Myc might sensitize Bax-deficient cells to TRAIL through effects on c-FLIP or possibly through effects on mitochondria that amplify signaling through the extrinsic apoptotic pathway. Myc did seem to be a potent sensitizer of the Bax-deficient human colon tumor cells to TRAIL, but we found that the underlying mechanism involved the intrinsic rather than extrinsic pathway (8). We also found that antiapoptotic molecules Mcl-1 and cIAP-2 were induced by TRAIL treatment in TRAIL-resistant Bax-null and other TRAIL-resistant cells in a nuclear factor-κB (NF-κB)–dependent manner (8). The induction of the antiapoptotic NF-κB targets was significantly diminished by c-Myc expression, and this correlated with TRAIL sensitization. We confirmed that Mcl-1 plays the predominant role for this mitochondrial form of TRAIL resistance.

Mechanism and Reversal of TRAIL Resistance by the Multikinase Inhibitor Sorafenib

Our observations initially led us to investigate the ability of TRAIL to activate Raf signaling because Mcl-1 (and cIAP-2) were

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both identified to be cAMP-responsive element binding protein (CREB) targets, and CREB signaling is downstream of Raf. This led us to discover that the Raf/vascular endothelial growth factor kinase inhibitor, sorafenib, could inhibit Mcl-1 transcriptional induction by TRAIL, and that Mcl-1 induction was mediated by NF-κB. TRAIL-induced NF-κB–mediated transcriptional activation of Mcl-1, and NF-κB binding to the Mcl-1 promoter could be blocked by sorafenib. Sorafenib, however, did not alter the nuclear localization of NF-κB after TRAIL treatment, but it did decrease the DNA-binding ability of NF-κB. Therefore, sorafenib seems to inhibit the DNA-binding capacity of NF-κB after translocation into nucleus through unclear mechanisms. One possible mechanism for sorafenib action on the DNA-binding function of NF-κB is potentially through changes in the phosphorylation status of NF-κB subunits in the nucleus. Alternatively, a recent report showed that oncogenic BRAF increased β-Trcp, which functions as the substrate recognition subunit of the E3 ligase for IκB and potentiates NF-κB activity. Sorafenib can mediate decreased IκB kinase activity through this pathway (9). There is also the possibility that sorafenib directly regulates other transcription factors involved in Mcl-1 regulation (Fig. 1). Furthermore, recent reports have shown that sorafenib can down-regulate Mcl-1 through translational or posttranslational mechanisms (10).

Sorafenib Sensitizes TRAIL-Resistant Leukemia Cells through Mcl-1 Down-regulation

Two additional studies recently found that Mcl-1 plays a significant role in mediating TRAIL-resistance and that sorafenib can be used to inhibit Mcl-1 action. Meng et al. (11) reported that Mcl-1 confers TRAIL resistance by serving as a buffer for Bak, Bim, and Puma but not tBid and showed that down-regulation of Mcl-1 by sorafenib sensitizes resistant cells to TRAIL-induced death in TRAIL-resistant leukemia cell lines and acute myelogenous leukemia (AML) patient specimens. Although they did not examine Mcl-1 levels after TRAIL treatment, Meng et al. (11) did observe an increase in Bak activation with the combination of TRAIL plus sorafenib. Rosato et al. (12) examined human malignant hematopoeitic cells and showed down-regulation of Mcl-1 by sorafenib sensitized cell lines and AML patient samples to TRAIL. They found that sorafenib down-regulated Mcl-1 and also Bcl-xL, and that Mcl-1 preferentially inhibits Bak activation when compared with Bax.

Mcl-1 is an antiapoptotic Bcl-2 family protein that can bind to BH3-only proteins, such as Bim, Bid, Puma, and Bak, thereby, when at sufficiently high levels, functions as a reservoir for those proapoptotic proteins (13). Regulation of Mcl-1 protein level can be a critical barrier for preventing unwanted cell death. Conversely, overexpression of Mcl-1 can desensitize cells to a cell death signal,

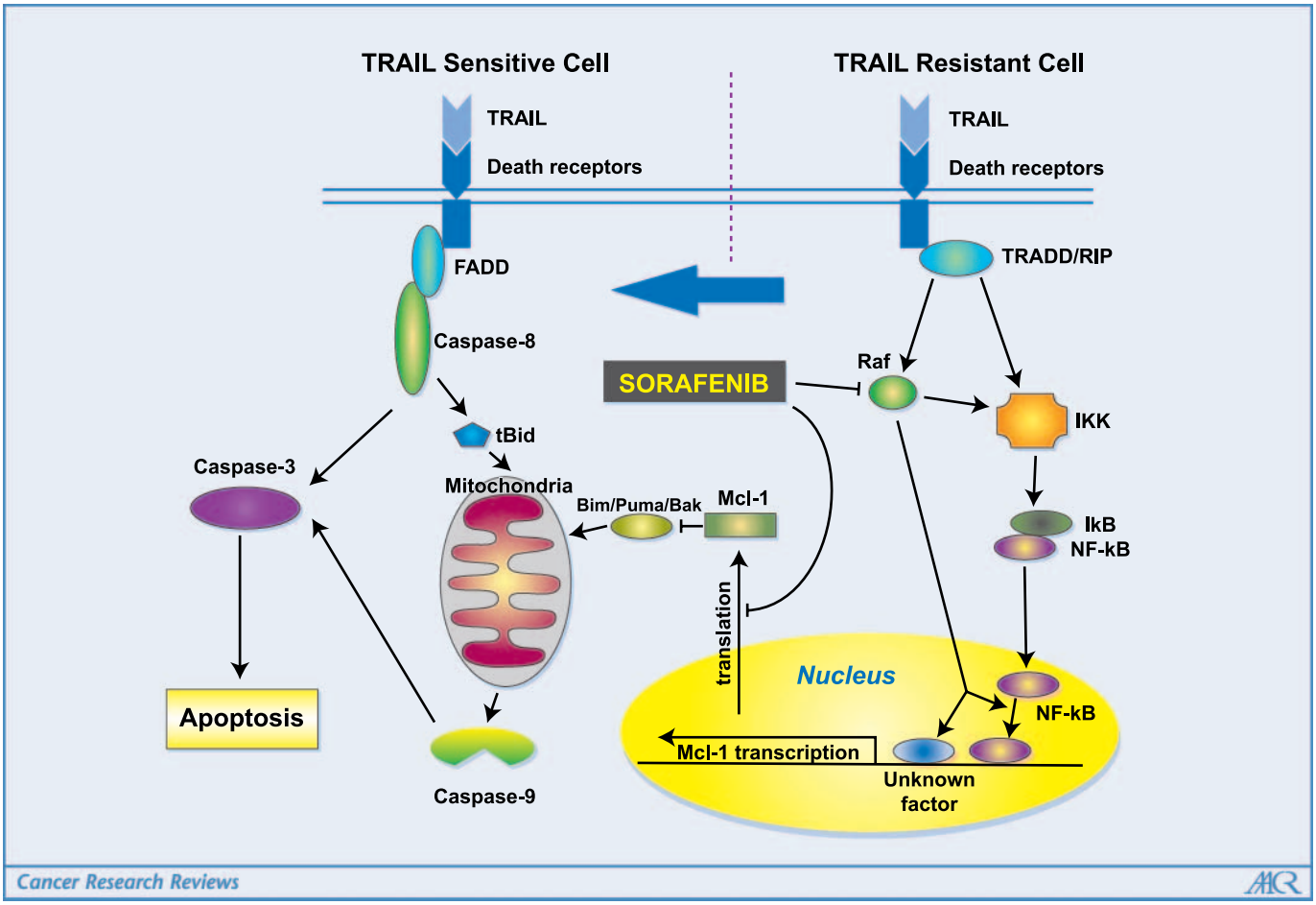

Figure 1. Pathways mediating sensitization to TRAIL by down-regulation of Mcl-1. In TRAIL-resistant cells, TRAIL induces NF-κB activity and Mcl-1 transcription to block BH-3 only protein function. Treatment using sorafenib blocks NF-κB activity by transcriptional inhibition of Mcl-1. Sorafenib also inhibits translational expression of Mcl-1. Released BH-3–only proteins shift TRAIL-resistant cells toward sensitivity and apoptotic death.

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especially in the context of preventing the death of an unwanted (i.e., transformed) cell (14). The importance of Mcl-1 in mediating TRAIL-resistance was shown previously by studies showing that down-regulation of Mcl-1 by siRNA enhanced TRAIL-mediated cell death (15, 16), and a microRNA, mir-29b, was reported to endogenously regulate Mcl-1 expression, and its enforced expression made cancer cells sensitive to TRAIL (17). However, a practical method to down-regulate Mcl-1 expression in cancer cells was not described until the recent reports (8, 11, 12).

Derivation of a Rationale for a Potentially Effective Therapy in the Clinic

Sorafenib, which was suggested as a potent TRAIL sensitizer in the three recent papers described here (8, 11, 12), is approved for use in treatment of renal cancers and is currently undergoing investigation in >30 clinical trials against a wide range of human cancers. Sorafenib has been shown to affect survival in hepatocellular carcinoma and was approved as therapy for this disease by the Food and Drug Administration in late 2007. It is therefore possible that the TRAIL plus sorafenib combination therapy may be effective at least for treatment of some TRAIL-resistant cancers. In the rare instances where the resistance to TRAIL relies on defects in the extrinsic pathway, such as absence of death receptor expression or down-regulation of caspase 8, the TRAIL plus sorafenib combination is unlikely to be helpful. Down-regulation of FLIP (6) or death receptor *O*-glycosylation have emerged as mechanisms of TRAIL sensitization (18), and if effective ways to modulate these molecules are found, then overcoming extrinsic pathway defects might be possible to some extent. For a wide-range of human cancers possessing intrinsic pathway defects that confer TRAIL resistance, sorafenib now seems to be a promising sensitizing agent for use in combination with TRAIL or proapoptotic TRAIL receptor agonists.

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