Fas (CD95/APO-1) is a 45-kDa type I transmembrane protein belonging to the tumor necrosis factor superfamily of receptors (1, 2). Fas was identified in 1989 as a cell death inducer of malignant human cancer and leukemia cell lines (3, 4). Fas contains a classic “death domain” within its cytosolic tail, typical of a branch of the tumor necrosis factor family implicated in apoptosis induction. Indeed, for many tumor cell lines, cross-linking Fas with agonistic anti-Fas antibodies leads to apoptosis (5, 6). Nonapoptotic roles for Fas have also been found, however, including stimulating proliferation of several types of normal cells under some conditions, including T cells, thymocytes (7), fibroblasts (8–10), and hepatocytes (11). This dual role of Fas complicates the biology of this tumor necrosis factor family receptor from the standpoint of predicting the role of Fas in tumor biology. An article in a previous issue of Clinical Cancer Research by Mitsiades et al. (12) illustrates the conundrum of Fas by showing a proliferative rather than apoptotic role for this “death receptor” in thyroid carcinoma cell lines and also providing some evidence that Fas expression is associated with more aggressive thyroid cancers.

The findings of Mitsiades et al. beg the question of how cross-linking Fas with an antibody (specifically CH-11) that is well known for its ability to induce apoptosis instead triggers proliferation of thyroid cancer cell lines. This is the first report to show that Fas can induce cell proliferation in thyroid cancer cells, but similar observations have been made previously for epithelial cancer, melanoma, myeloma cell lines, and primary chronic lymphocytic leukemia B cells (13–15). What makes these malignancies different from the many other tumor cell types where CH-11 anti-Fas antibody triggers apoptosis?

Previous work by Mitsiades et al. has shown that Fas stimulation failed to induce apoptosis of papillary thyroid carcinoma cell lines (16). In the current report, Mitsiades et al. (12) expand their analysis and show that Fas resistance does not seem to result from diminished Fas or Fas-associated death domain protein (FADD) expression. FADD is a unique cytosolic adapter protein that contains a death domain and a death effector domain (DED) and is an integral component of the death-inducing signaling complex formed upon activation of Fas (see Fig. 1). The death-inducing signaling complex is comprised of Fas, FADD, the DED-containing cell death proteases caspase-8 and/or caspase-10 (which bind the DED of FADD). Mitsiades et al. (12) used immunoblotting and immunohistochemistry methods to show the presence of Fas and FADD expression in thyroid cancer cell lines and tissue specimens. Furthermore, the investigators found that the death domain of Fas was free of mutations, unlike some malignancies (17, 18), excluding this as a possible explanation for the Fas-resistant phenotype. Despite apparent integrity of Fas, FADD, and procaspase-8 expression, these investigators previously showed that treatment of thyroid cancer cell lines with agonistic antibody does not induce recruitment of procaspase-8 to Fas (16), showing a defect in death-inducing signaling complex assembly.

An important clue to the mechanisms modulating Fas activity in thyroid cancer cell lines was uncovered when Mitsiades et al. found the protein synthesis inhibitor cycloheximide sensitized these cells to Fas-induced cell death, suggesting the presence of a short-lived inhibitory protein. One logical candidate for such an inhibitor is the Flice-like inhibitory protein (FLIP), a DED-containing antiapoptotic protein that associates with caspase-8 and caspase-10 at the death-inducing signaling complex and is known to have a short half-life (19–21). The flip gene encodes two principal proteins, a more abundant longer isoform (FLIP-L) that contains tandem DEDs resembling the prodomains of caspase-8 and caspase-10 followed by a caspase-like domain and a less prevalent shorter protein (FLIP-S) composed of only the DEDs. Although FLIP-S is exclusively a caspase inhibitor, the longer FLIP-L has a dual function as either a caspase inhibitor or activator, depending on a variety of issues including the ratio of FLIP-L to caspase-8 (22). The investigators show that agonistic anti-Fas antibody CH-11 induces FLIP-L association with Fas in thyroid cancers, using coimmunoprecipitation experiments. Consistent with an antiapoptotic role for FLIP in these thyroid cancer cell lines, FLIP antisense oligodeoxynucleotides sensitized to Fas-mediated apoptosis, thereby implicating FLIP-L in the Fas resistance mechanism. These findings are enlightening, given that FLIP-L can display either proapoptotic or antiapoptotic phenotypes, depending on the cellular context (22–24).

But, what about the proliferative actions of Fas in thyroid cancers? Does FLIP explain that phenomenon? It has been suggested that FLIP may provide the molecular switch allowing Fas to promote cell proliferation as opposed to apoptosis (reviewed by refs. 25, 26). For example, flip−/− murine T cells are defective in proliferation induced by stimulation through the T-cell antigen receptor complex (27, 28). Although FLIP antisense was interrogated for its effects on apoptosis, Mitsiades et al. were unable to explore whether experimentally reducing FLIP expression alters proliferation due to induction of apoptosis.

In addition to proliferation, the investigators observed that Fas induces signal transduction events, such as mitogen-activated protein/extracellular signal-regulated kinase kinase and extracellular signal-regulated kinase phosphorylation and nuclear factor-κB (NF-κB) and activator protein 1 activation in thyroid cancer cells. In this regard, it has been shown previously that FLIP can induce activation of extracellular signal-regulated kinase (ERK) 1 and 2 in breast cancer cell lines (29, 30). Furthermore, this work suggests that FLIP may be a candidate for regulating cell cycle progression and ERK activity in thyroid cancer through its dual role of Fas mediation.
kinases (apparently by binding the upstream kinase Raf-1), at least when overexpressed (29), raising the possibility of FLIP involvement in this Fas-induced signaling event observed in thyroid cancers. FLIP also reportedly activates NF-κB when overexpressed in HEK293 cells, and the two DED domains are sufficient for the activation (30). The role of FLIP in NF-κB activation, however, is complicated. When FLIP-L is overexpressed in Jurkat or HEK293 cells, and those cells are then stimulated with Fas ligand, NF-κB activation is suppressed (31, 32). Moreover, reducing FLIP using small interfering RNA also enhances Fas ligand–induced apoptosis (31). Thus, high levels of FLIP-L can also block Fas-induced apoptosis, presumably by competing for recruitment of procaspase-8 into the death-inducing signaling complex (3). The scenarios of (1), (2), and (3), therefore, result when FLIP-L is present at low, medium, or high levels, respectively. It is largely unknown how, under certain conditions, FLIP can inhibit NF-κB activation.

Fig. 1. Model depicting the dual functionality of components of the Fas/CD95 signaling complex. Fas stimulation leads to formation of the death-inducing signaling complex (DISC; dashed box). Within the death-inducing signaling complex, Fas recruits FADD via death domain (DD)–DD interactions, and FADD in turn recruits either procaspase-8 or FLIP via DED-DED interactions. If FLIP is absent at the death-inducing signaling complex, caspase-8: caspase-8 homodimers form (1), leading to caspase-8 activation via a process whereby the DED-containing prodomains are removed by proteolytic cleavage and the resulting p20/p10 heterotrimer is released into the cytosol to activate downstream caspases and induce apoptosis (left). Alternatively, if FLIP is present at the DISC, caspase-8:FLIP heterodimers form, activating caspase-8 via a mechanism whereby the DED-containing prodomains remain attached and also resulting in cleavage of FLIP-L to yield a p43 cleavage product (2). The caspase-8:FLIP heterodimer complex is capable of initiating cell proliferation by recruiting and activating downstream signaling proteins, such as TRAF, RIP, and Raf, resulting in NF-κB and extracellular signal-regulated kinase (ERK) activation (right). However, high levels of FLIP-L can also block Fas-induced apoptosis, presumably by competing for recruitment of procaspase-8 into the death-inducing signaling complex (3). The scenarios of (1), (2), and (3), therefore, result when FLIP-L is present at low, medium, or high levels, respectively. It is largely unknown how, under certain conditions, FLIP can inhibit NF-κB activation.

One possible explanation for this dual phenotype can be envisioned if FLIP-L participates in a multiprotein complex involving three or more proteins that engender NF-κB activation, such that the proper stoichiometry of the individual subunits is achieved only if the right amount of FLIP protein is present relative to the other components. In that case, either too much or too little FLIP-L would result in diminished signaling. Hints of a candidate complex that might link FLIP-L to proliferation are found in observations from knock-out mice that ablation of the genes encoding FADD, caspase-8, and FLIP phenocopies each other, and that all three of these proteins are required for T-cell proliferation, and all three of these proteins interact (reviewed by ref. 19). Thus, like FLIP-L, other components of the death-inducing signaling complex (i.e., FADD and caspase-8) lead surprising dual lives in both apoptosis regulation and signal transduction, apparently inducing apoptosis in some cellular contexts and proliferation in others.

Precisely how do FADD, caspase-8, and FLIP-L all collaborate in inducing NF-κB and other signaling events that promote cell proliferation? Many details remain unclear, but data collected from several prior studies suggest a model in which FADD recruits both caspase-8 and FLIP-L (presumably as a heterodimer), resulting in activation of caspase-8 and proteolytic cleavage of both FLIP and caspase-8. The cleavage of caspase-8 in
complex with FLIP-L occurs between the large and small catalytic domains, but unlike conditions where caspase-8 activation leads to apoptosis, the DED-containing prodomain remains connected to the large catalytic subunit; thus, caspase-8 stays associated with FLIP and FADD, presumably via DED to DED interactions. FLIP-L is also cleaved by caspase-8 to produce a 43-kDa DED-containing fragment. Next, cleaved FLIP (probably heterodimerized with caspase-8) seems to be recognized by several signaling proteins, either directly or indirectly through interactions with components of a multi-protein complex, including TRAF-families adapters (TRAF1, TRAF2, and possibly TRAF3), RIP, and maybe Raf-1 (24, 29, 34, 35). The result is activation of NF-κB and other downstream signaling pathways that promote cell proliferation (Fig. 1). Studies in which the p43-cleaved FLIP protein was expressed by gene transfer in cells suggest that its ability to induce NF-κB is caspase independent (35), implying that once FLIP-L is cleaved, caspases are no longer required.

Thus, the multiprotein signaling complex that contains p43-cleaved FLIP may define a new target for cancer therapies aimed at suppressing cell proliferation. As for apoptosis-inducing therapies, the challenge for devising future therapies based on exploiting Fas or other apoptosis-inducing members of the tumor necrosis factor family is to delineate the circumstances under which FADD, caspase-8, and FLIP functions as killers versus growth inducers. Answering this question may be critical for selecting the right patients for clinical trials, including testing of tumor vaccines that seek to induce tumor-recognizing cytolytic T cells that often use Fas ligand to effect their killing of tumor targets (36).

References
The FLIP-Side of Fas Signaling
Marc L. Hyer, Temesgen Samuel and John C. Reed


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