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Fas function and tumor progression: Use it and lose it

Recent studies have provided evidence that Fas and FasL interactions are important in the control of malignant disease and that changes in the level of Fas expression can determine immune escape and therapeutic responses.

Imbalanced rates of apoptosis have been proposed to create a platform that is necessary and sufficient for tumor formation (Green and Evan, 2002). The host environment can influence cancer outgrowth by altering tumor gene expression, resulting in tumor proliferation and suppression of the endogenous apoptotic program. Fas and Fas ligand (FasL) are an interacting, extracellular proapoptotic receptor/ligand pair (reviewed in Nagata, 1999). Trimerization of membrane bound Fas with FasL causes recruitment of the FADD adaptor protein and procaspase-8, the key initiator caspase in the death receptor pathway. Procaspase-8 is activated by induced proximity and further activates downstream caspases and initiates cleavage of critical apoptotic substrates. Active caspase-8 also engages the intrinsic mitochondrial pathway of apoptosis through the cleavage of Bid, which translocates to the mitochondria and promotes release of cytochrome *c*.

The role of Fas-induced apoptosis in the maintenance of immune homeostasis is well established (Nagata, 1999). More recently, Fas-induced apoptosis has been implicated in the control of tumor progression and chemotherapeutic drug-induced death. Functional Fas is highly expressed on a variety of nonmalignant tissues, while Fas loss-of-function commonly accompanies the malignant phenotype. Multiple molecular mechanisms underlie Fas loss-of-function in cancer including downregulation of transmembrane Fas by promoter methylation (reviewed in Owen-Schaub et al., 2000), transcriptional repression (Ivanov et al., 2001), histone acetylation (Maecker et al., 2000), and alternative mRNA splicing to produce soluble Fas protein lacking a transmembrane anchor (reviewed in Owen-Schaub et al., 2000). Overexpression of the degenerate caspase homolog c-FLIP (Bullani et al., 2001) and inactivating Fas mutations (reviewed in Owen-Schaub et al., 2000) have also been shown to contribute to Fas loss-of-function in nonhematopoietic cancers. In several cancer types, Fas loss-of-function has been shown to track with an aggressive disease presentation and decreased patient survival. In experimental animal models (reviewed in Owen-Schaub et al., 2000), disruption of Fas has been shown to result in enhanced tumor development while Fas restoration has been shown to delay primary tumor outgrowth. The acquired ability to spread and metastasize represents the most intractable feature of cancer. Recent studies have implicated Fas and FasL interactions in the control of distant metastases (Owen-Schaub et al., 1998) as well as in the development of chemotherapeutic resistance in some cell types (reviewed in Johnstone et al., 2002). These observations suggest that Fas is a frequent target for inactivation during oncogenesis and that Fas-induced apoptosis plays a crucial role in the biology and response of malignant disease.

Both transmembrane and cleaved FasL can induce Fas clustering and initiate apoptotic cell death (Nagata, 1999). Although some nonhematopoietic tissues (retinal pigment epithelial cells and lung epithelial cells, for example) display FasL, expression is most prominent in bone marrow-derived immune cells including activated lymphocytes, neutrophils, natural killer (NK) cells, and macrophages. Conceivably, $FasL^+$ immune effectors could inhibit Fas^+ tumor survival by direct

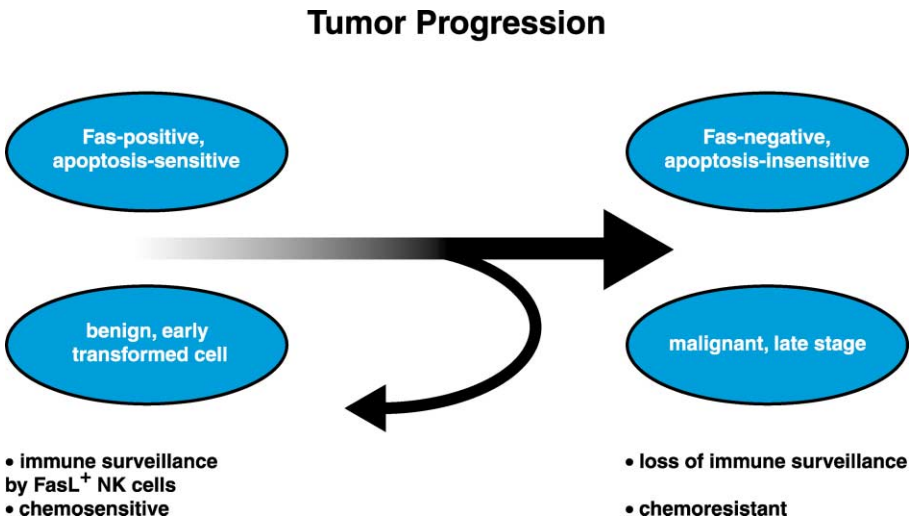

Figure 1. Model for Fas loss-of-function in tumor progression

This model is supported by the findings of Maecker et al. (2002) that Fas is important for NK cell-mediated immune surveillance and chemosensitivity.

cytotoxicity and/or release of proinflammatory cytokines leading to increased cellular infiltration. Tumor immune escape has been implicated in disease progression following Fas loss-of-function in several experimental models (reviewed in Owen-Schaub et al., 2000). Recently, an intriguing study (Screpanti et al., 2001) implicated NK cells in the rejection of tumors through the death receptor pathway. These results raise the exciting possibility that immune surveillance resulting in NK cell-mediated tumor rejection could be accomplished through the upregulation of functional, transmembrane-anchored Fas in advanced tumors where Fas loss-of-function has occurred (Figure 1). Evidence for this exciting possibility is presented in studies by Maecker et al. (2002) in this issue of *Cancer Cell*.

Using mouse embryo fibroblasts (MEF cells), these investigators document that early-passage MEF cells containing wild-type p53 express high levels of Fas (equivalent to that observed in early transformed cells or benign tumors). On the other hand, p53 null MEF cells express low levels of Fas, consistent with previous reports that wild-type p53 can transcriptionally activate Fas and increase export of this protein to the surface (reviewed in Owen-Schaub et al., 2000). Late-passage, wild-type p53-containing MEF cells downregulate Fas expression to undetectable levels, paralleling findings observed in highly malignant cells in tumor progression models (Figure 1). Late-passage MEF can be induced to express high levels of functional Fas following infection with a retroviral vector containing Fas or treatment with the histone deacetylase inhibitor, Trichostatin A (TSA), to relieve Fas repression via an acetylation-dependent mechanism (Maecker et al., 2000). Using this model system, Maecker et al. (2002) has now provided direct evidence that changes in Fas levels can determine immune escape of transformed cells and influence responses to chemotherapeutic agents in vivo. In initial tumorigenecity studies, early-passage MEF (expressing high levels of functional Fas) were shown to be weakly tumorigenic, whereas late-passage MEF (expressing low levels of functional Fas) were shown to be highly tumorigenic in SCID animals (containing NK cells). When high levels of Fas were induced on late-passage MEF by retroviral Fas or treatment with TSA, local tumor growth was potently inhibited compared to late-passage, Fas-deficient MEF containing vector alone, a receptor control, or a dominant-negative Fas. These studies identify Fas as an important death receptor restricting local tumor outgrowth; however, they do not examine potential underlying effector mechanisms. In the next set of experiments, early- and late-passage MEF (expressing high and low Fas levels, respectively) were injected into SCID beige mice (lacking NK cells), and tumorigenecity was examined. In the absence of NK cells, early- and late-passage MEF formed local tumors at equivalent rates, implying a role for NK cells as the underlying effector mechanism restricting Fas^+ tumor growth in SCID mice. While encouraging as a potential therapeutic approach, it remained to be determined whether Fas upregulation could inhibit growth of established tumors in a more clinically relevant model. To this end, the investigators injected late-passage, Fas-deficient MEF in the subcutis of SCID animals and waited for 10 days to allow tumor establishment. After such time, tumors were treated with TSA or TSA in combination with the chemotherapeutic drug etoposide and the effects on tumor growth measured. In this model, TSA alone had little effect on growth, suggesting that Fas restoration in established tumors may be ineffective as a single agent. However, when TSA was used in combination with etoposide, tumor growth was potently inhibited. The effects of the combination treatment were NK cell dependent and independent of MHC class I changes in the tumor. These findings raise the exciting possibility that Fas upregulation in combination with other agents such as radiation and chemotherapy may be particularly effective in the treatment of established tumors. The strict dependence on NK cells for the elimination of "early" Fas^+ MEF tumors in TSA alone treatment groups and "late" Fas^+ MEF tumors in the TSA- and etoposide-treated group is intriguing and argues that increasing Fas levels may be a useful strategy to enhance immune-mediated clearance of tumors and reverse chemoresistance in some tumor types. It will be especially interesting to determine whether such therapeutic approaches are effective in the treatment of spontaneous, syngeneic tumors and to characterize the potential involvement of additional $FasL^+$ immune effectors (lymphocytes, neutrophils, macrophages) in immunocompetent animals. Understanding how apoptosis is silenced in the malignant cell and how apoptotic pathways can be restored is tantamount to designing more effective cancer therapies and preventing tumor progression.

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