The Fas signalling pathway and its role in the pathogenesis of cancer
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Tumor cells frequently exhibit de novo expression of Fas ligand (FasL/CD95L). Coupled with resistance to Fas-mediated apoptosis, FasL expression enables many cancers to deliver a pre-emptive strike or ‘counterattack’ against the immune system. New studies also indicate that FasL expression on tumor cells could confer a double advantage to these cells by stimulating their own proliferation. However, pro-inflammatory effects of FasL have also been observed. New findings are beginning to reconcile the paradoxical effects of FasL, with the clinical significance of the Fas counterattack only beginning to emerge.

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Abbreviations
DD death domain
DISC death-inducing signalling complex
FADD Fas-associated death domain
FasL Fas ligand
mFasL membrane FasL
NK natural killer (cells)
TIL tumor-infiltrating lymphocyte
TNF tumor necrosis factor

Introduction
During carcinogenesis, tumors develop multiple mechanisms to subvert the immune system and suppress the anti-tumor immune response. Upregulation of Fas ligand (FasL/CD95L) expression appears to represent one such mechanism. FasL is a transmembrane protein belonging to the tumor necrosis factor (TNF) superfamily that can trigger apoptotic cell death following ligation to its receptor, Fas (CD95/APO-1). FasL was originally discovered on cells of the lymphoid/myeloid lineage, including activated T cells and natural killer (NK) cells, where it plays an important role in immune homeostasis and T cell- and NK cell-mediated toxicity [1]. FasL has since been found to be expressed in sites such as the eye [2] and testis [3], where it contributes to immune privilege by inducing apoptosis of infiltrating proinflammatory immunocytes. The discovery of FasL expression by a variety of tumor cells raised the possibility that FasL could mediate immune privilege in human tumors by inducing apoptosis of anti-tumor lymphocytes. Moreover, new data suggest that tumor-expressed FasL may also stimulate proliferation of tumor cells. However, tissue transplantation studies have recently identified a pro-inflammatory activity for FasL, questioning the ability of FasL to mediate tumor immune privilege. This review summarizes our current knowledge about the role of this pleiotropic molecule in tumorigenesis and in the evasion of the immune response.

Fas-mediated apoptosis
Higher organisms have developed several mechanisms to ensure the rapid and selective elimination of unwanted cells, one of which involves the interaction of cell surface Fas with its cognate ligand, FasL (Figure 1). Structurally, Fas is a transmembrane cell surface receptor containing three cysteine-rich extracellular domains at the amino-terminus, which are responsible for ligand binding, and an intracytoplasmic death domain (DD) of about 80 amino acids that is essential for transducing the apoptotic signal [4]. Binding of FasL to Fas causes a higher-order aggregation of the receptor molecules [5*] and recruitment of the adaptor molecule Fas-associated death domain (FADD) via DD–DD interactions. FADD also has another domain called the death effector domain, which in turn recruits pro-caspase-8 (FLICE) and/or pro-caspase-10 to the receptor. The resulting multimeric protein complex is called the death-inducing signalling complex (DISC), and forms within seconds of receptor engagement [4].

At the DISC, pro-caspase-8 (and/or -10) is activated. Caspases are cysteine proteases that cleave their substrates at aspartic acid residues [6]. They are synthesized as relatively inactivezymogens called pro-caspases. Initiator caspases (e.g. caspases-8 and -9) are the first to be activated in response to a pro-apoptotic stimulus and initiate a cascade of increasing caspase activity by cleaving and activating effector caspases (e.g. caspases-3, -6, and -7). The effector caspases, in turn, selectively cleave a restricted set of target proteins and are responsible for the demise of the cell.

Effective formation of the DISC is required for Fas-mediated apoptosis. Cells in which caspase-8 is activated...
Apoptosis signalling via the Fas receptor. Binding of FasL to Fas induces the recruitment of FADD and pro-caspase-8 to the cytoplasmic tail of Fas, and the formation of the DISC. At the DISC, caspase-8 is activated. In type I cells, sufficient caspase-8 is generated to activate pro-caspase-3 directly. However, in type II cells, activation of pro-caspase-3 occurs indirectly through cleavage and activation of Bid. Truncated Bid (tBid) triggers the release of pro-apoptotic molecules from the intermembrane space of mitochondria. Released cytochrome c (cyto c) clusters with Apaf-1 and pro-caspase-9 in the presence of dATP to activate caspase-9. Activated caspase-9 cleaves and activates caspase-3, triggering a caspase cascade, which ultimately results in the death of the cell.

at the DISC, leading to the rapid activation of caspase-3 and cell death, are known as type I cells. In some cells, however, DISC formation following Fas stimulation is strongly reduced. In these cells, known as type II cells, mitochondria play an essential role as signal amplifiers (Figure 1) [7]. This mitochondrial or ‘intrinsic’ apoptosis pathway is activated by caspase-8-mediated cleavage of the Bcl-2 family member Bid. Truncated Bid translocates to the mitochondria, where it can induce both the oligomerization of pro-apoptotic Bax and/or Bak in the membrane and the release of pro-apoptotic molecules, including cytochrome c, from the mitochondrial intermembrane space. Cytochrome c can then associate with the scaffolding protein Apaf-1, dATP and pro-caspase-9 to form a high-molecular mass complex called the apoptosome. Within the apoptosome, pro-caspase-9 is activated. Caspase-9 then activates caspase-3, resulting in cell death.
The Fas counterattack and tumor immune privilege

The demonstration of FasL expression at sites of immune privilege identified an important role for FasL in the interaction between non-lymphoid tissues and the immune system. Tumor expression of FasL was first demonstrated in the colon carcinoma cell line SW620, where it could induce apoptosis of Fas-sensitive lymphoid cells in vitro [8], raising the possibility that FasL may also mediate immune privilege in human tumors. FasL expression has since been reported on numerous tumors of varying origin, including colon [9], gastric [10] and lung [11] carcinoma, and astrocytoma [12]. FasL expressed by these cells is functional, as demonstrated by the ability of the tumor cells to kill Fas-sensitive target cells when co-cultured in vitro. Furthermore, apoptosis of tumor-infiltrating lymphocytes (TILs) has been detected in situ within FasL-expressing human tumors [9,10,13]. For instance, in esophageal carcinoma, a quantitative study demonstrated that the number of TILs was reduced concomitantly with increased TIL apoptosis within FasL-expressing areas of the tumor [13].

Numerous animal studies provide further evidence demonstrating the ability of tumor-expressed FasL to downregulate anti-tumor immune responses. Subcutaneous injection of FasL-expressing murine melanoma cells into Fas-deficient lpr mutant mice resulted in delayed tumor growth compared with that in wild-type mice [14]. Delayed tumor growth was proposed to be a result of the insensitivity of the T cells to FasL expressed on the tumor cells, enabling them to mount an effective anti-tumor response. Moreover, immunosuppression was demonstrated directly in vivo in allogeneic mice injected with FasL-transfected colon carcinoma cells. Alloantibodies were absent, whereas allospecific cytotoxic T cells were abolished and helper T cell activity was reduced [15]. The ability of FasL to promote tumor immune escape in the face of an active specific immune response was demonstrated recently using mice transgenic for the rat HER-2/neu oncogene (NEU-TG) [16,17]. These mice develop spontaneous breast tumors after a first pregnancy. Some rNEU-TG mice were found to develop late ‘escape’ tumors despite the presence of an rNEU-specific immune response induced by immunotherapy. Characterization of these ‘escape’ tumors revealed that they continued to express rNEU but had acquired constitutive FasL expression, which was associated with apoptosis of infiltrating T lymphocytes in situ.

Expression of FasL by tumors implies that cancer cells are themselves resistant to Fas-mediated apoptosis. Indeed, resistance to apoptosis is believed to be one of the hallmarks of cancer [18]. Most cancer cells are relatively resistant to apoptosis mediated through Fas, with molecular defects being identified at several levels of the apoptotic signalling pathway (Figure 2) [19]. A common mechanism employed by cells to decrease sensitivity to Fas-mediated apoptosis is to regulate cell surface expression of Fas [20,21]. Alternatively, cells may secrete an antagonistic ‘decoy’ receptor [22]. The Fas signal can also be inhibited at the level of the DISC via increased expression of cFLIP (FLICE-inhibitory protein), which can inhibit interaction of caspase-8 and -10 with the DISC [23], or reduced expression of FADD [24] or caspase-8 [25]. Loss of Fas signalling as a consequence of Bel-2, Bel-xL or IAP (inhibitor-of-apoptosis) protein expression can also occur, favoring tumor survival [26]. Thus, because of their insensitivity to Fas-mediated apoptosis, tumor cells can express FasL without undergoing apoptosis [27].

Resistance to Fas-mediated apoptosis protects tumor cells not only from tumor-expressed FasL but also from FasL expressed as a cytotoxic mediator by infiltrating anti-tumor T cells and NK cells [28]. Because activated lymphocytes express Fas and are sensitive to Fas-mediated apoptosis, expression of FasL by apoptosis-resistant tumor cells represents a powerful asset to malignancy, enabling tumor cells to take the offensive and ‘counterattack’ the immune response (Figure 3).

Accumulating evidence suggests that, in addition to local defense, Fas/FasL may also play an important role in
tumor progression and in the establishment of metastasis. Numerous studies have shown that disease progression is associated with progressively increased expression of FasL [10,29,30,31]. FasL expression was also found to be higher in metastatic tumors than in primary ones. In breast and cervical tumors, high FasL expression was significantly associated with lymph node metastases [30,32], whereas stronger FasL expression was found in liver metastases of colon cancer relative to the primary tumor [33]. Together, these studies suggest that FasL expression favours the establishment of tumor metastases, perhaps contributing to the survival of the tumor cells in immunologically hostile sites or at sites where the indigenous cells are sensitive to Fas-mediated apoptosis.

Additional benefits for the tumor
Apoptosis of infiltrating anti-tumor T cells might not be the only advantage associated with FasL expression. Recent studies have shown that FasL can also suppress T cell activation [34] and inhibit immunoglobulin production in B cells [35]. In addition, expression of FasL by tumor cells correlates with loss of CD3ζ chain expression by TILs [36,37]. The CD3ζ chain is essential for T cell receptor signalling, transducing activation signals from the antigen-binding T cell receptor to the T cell nucleus. Thus, FasL expressed on tumors might contribute to the functional abnormalities frequently found in lymphocytes isolated from the tumor microenvironment. Furthermore, FasL expressed by tumor cells can increase proliferation of tumor cells (reviewed in [38]). Recent studies have shown that Fas can transduce activation and proliferation signals, although the mechanisms by which this occurs are poorly understood. Given that many tumors co-express Fas and FasL, tumor-expressed FasL might act in an autocrine or juxtacrine manner to promote tumor growth and so confer a double advantage to these cells — both protecting the tumor cells from the immune system and stimulating their own proliferation.

Pro-inflammatory and anti-tumor properties of FasL
Because of evidence that FasL contributes to immune privilege in tumors and some tissues, it was hoped that FasL could be exploited to confer immune privilege on organ transplants. Although some studies demonstrated an immunoprotective effect [39,40], many others revealed that genetically engineered overexpression of FasL in allografts of tissues [41] or tumor cells [42] could target these cells for rapid destruction by neutrophils, questioning the ability of FasL to mediate immune privilege.

Investigations into the apparent discrepancy among results revealed that membrane FasL (mFasL) possesses a pro-inflammatory activity, which is opposed by proteolytically shed FasL [43]. Further studies revealed that mFasL can trigger the production of pro-inflammatory cytokines by monocytes and macrophages [44,45]. A high level of mFasL, such as that found in overexpression systems, may thus trigger excessive apoptosis of macrophages present in the allograft microenvironment and the production of pro-inflammatory cytokines or chemokines, given that the apoptotic demise of these cells precedes neutrophil infiltration [44]. Indeed, all studies demonstrating inflammation and neutrophil recruitment involve forced ectopic overexpression of mFasL (reviewed in [46]). Tumor cells could therefore avoid the inflammatory response associated with strong expression of mFasL by expressing both mFasL and soluble FasL [47]. However, whether naturally expressed FasL possesses this potent pro-inflammatory activity remains to be determined. Also, anti-inflammatory cytokines present in the...
tumor microenvironment might favour an immunosuppressive role of FasL [48], suggesting that other factors may determine whether FasL mediates immune privilege or inflammation. Importantly, significant neutrophil recruitment has not been observed in FasL-expressing human tumors in vivo [49]. However, the precise molecular conditions under which FasL promotes immune privilege or evokes a pro-inflammatory response remain to be defined.

Conclusions

The importance of the Fas counterattack as a mechanism of tumor immune evasion is only beginning to emerge, and represents an exciting new avenue of research in tumor immunology. Understanding the basis of the Fas counterattack could be important for the development of effective tumor-specific therapies. For example, reinstating sensitivity to Fas-mediated apoptosis is likely to have an enormous impact on the disease. Alternatively, rendering cytotoxic T lymphocytes insensitive to Fas-mediated apoptosis might offer a potential therapeutic intervention. Thus, disarming the Fas counterattack is a conceptually appealing goal for tumor immunotherapy, which may ultimately result in improved anti-cancer therapies.

Acknowledgements

The authors would like to thank their colleagues, in particular Aileen Ryan and Snead Lane, for their help with our research. The authors acknowledge financial support from the Wellcome Trust, Enterprise Ireland, Science Foundation Ireland, the Irish Higher Educational Authority and the Irish Health Research Board.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


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tumor development upregulation of Fasl was accompanied by reduced microvesicles are associated with T cell apoptosis and suppression of CD3, Fasl expression in cells. Thus, in addition to TIL apoptosis, tumor-expressed Fasl might also contribute to the functional abnormalities seen in patients with advanced malignancies.


