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Review

Signaling and transcriptional control of Fas ligand gene expression

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Abstract

Fas ligand (FasL), a member of the tumor necrosis factor family, initiates apoptosis by binding to its surface receptor Fas. As a consequence, there is sequential activation of caspases and the release of cytochrome *c* from the mitochondria, with additional caspase activation followed by cellular degradation and death. Recent studies have shed important insight into the molecular mechanisms controlling FasL gene expression at the level of transcription. Nuclear factors such as nuclear factor in activated T cells, nuclear factor-kappa B, specificity protein-1, early growth response factor, interferon regulatory factor, c-Myc and the forkhead transcriptional regulator, alone or cooperatively, activate FasL expression. These factors are often coexpressed with FasL in pathophysiologic settings including human atherosclerotic lesions. Here, we review these important advances in our understanding of the signaling and transcriptional mechanisms controlling FasL gene expression.
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Keywords: Fas ligand; apoptosis; gene expression; transcription factor

Abbreviations: FasL, Fas ligand; SMC, smooth muscle cell; EMSA, electrophoretic mobility shift assay; DN, dominant-negative

Introduction

Fas ligand (FasL) was identified in 1993 as a type II transmembrane protein of 40 kDa belonging to the tumor necrosis factor (TNF) family. FasL is one of the major effectors of CD8+ cytotoxic T lymphocytes and natural killer (NK) cells.[1] The FasL system has been implicated in a number of pathogenic states. Soluble forms have been isolated from patients with large granular lymphocytic leukemia, NK cell lymphoma[1] and a number of nonlymphoid tumor cells.[2] Metalloproteinases are believed to be involved in the proteolytic cleavage of membrane-bound FasL, producing its soluble form,[3] which exists as a trimer.[4]

The intracellular and extracellular domains of FasL are located in the N- and C-terminal regions, respectively (see Figure 1 for FasL structure). FasL also consists of a single transmembrane domain and an oligomerization domain, which is required for self-assembly and appears to be well conserved in all TNF family ligands.[5] The receptor-binding domain is located at the very end of the C-terminus, and deletion of at least three amino acids from this region is sufficient to interfere with interactions with its receptor, Fas.[5] The proline-rich region in the cytoplasmic domain of FasL (amino acids 46–65) is responsible for sorting FasL to secretory lysosomes.[6] A putative casein kinase I (CKI) motif (-SSASS-) has been identified; however, its role in FasL signaling remains to be determined.[7] Additionally, three potential N-glycosylation sites have also been acknowledged.[5]

Binding of FasL with Fas triggers the formation of the death-inducing signaling complex (DISC) by recruiting an adaptor molecule FADD (Fas-associating protein with death domain) to the cytoplasmic tail of Fas (C-terminal region). The N-terminal region or death effector domains (DED) of FADD are critical for the recruitment of procaspase 8. Immediately after recruitment, procaspase 8 is proteolytically processed to its active large and small subunits. At this point, the death-receptor initiated pathway can diverge in different cell types. Type I cells (mitochondria independent, Bcl-2 insensitive) induce apoptosis through the death-receptor initiated pathway to activate procaspase 3.[8] In other cell types (type II), caspase 8 is inadequate to activate procaspase 3 and cleaves Bid instead (a cytoplasmic protein) to activate the mitochondrial pathway with the release of cytochrome *c*.[8] In type II cells, Fas-induced apoptosis can also be blocked by prosurvival factors such as Bcl-2. Upon release, cytochrome *c* is recruited to Apaf-1 (human homolog of *Caenorhabditis elegans* CED-4) followed by the formation of the apoptosome together with procaspase 9. This complex then triggers the activation of caspase 3 and the cleavage of a variety of substrates including DNA repair enzymes, structural proteins and endonucleases.[9] Although the idea of type I and type II cells has been widely accepted, Huang *et al.*[10] recently reported opposing data. There is some controversy regarding the function of the survival factor Bcl-2 on Fas-induced apoptosis. In this study, transgenic mice expressing Bcl-2 did not protect lymphocytes or hepatocytes from FasL/Fas-induced death. These results therefore imply identical FasL/Fas signaling in both type I and type II cells, and challenge the

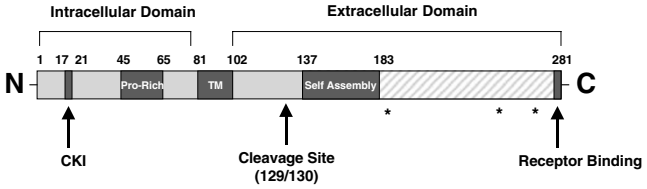

Figure 1 Structure of human FasL. FasL is synthesized as a 281-amino-acid protein. FasL contains a single transmembrane domain (TM), a proline-rich domain (Pro-Rich), a self-assembly domain and a putative casein kinase I (CKI) motif. Receptor binding occurs at the very end of the COOH-terminus. Cleavage of FasL occurs at site 129/130. * represents potential N-glycosylation sites (amino acids 184, 250 and 260, respectively). The hatched region denotes homology to other TNF family members

differential role of type I and type II cells in FasL-mediated death.

Physiological and Pathological Roles of FasL-Mediated Apoptosis

Regulation of the immune response

The FasL–Fas-mediated death pathway plays a major role in immune function, particularly in activation-induced cell death (AICD). AICD is an essential mechanism required to maintain cellular homeostasis in multicellular organisms. AICD functions to limit the excess proliferation of activated lymphocytes in the periphery after the elimination of antigen. It is also required for the elimination and inactivation of autoreactive thymocytes by negative selection within the thymus. In T cells and T-cell hybridomas, AICD arises through upregulation of FasL and Fas expression. T-cell receptor (TCR) triggering or stimulation by *Staphylococcus* enterotoxin B superantigen in T cells induces FasL expression to promote apoptosis by AICD.[11] FasL and Fas are both upregulated in T-cell hybridomas,[12] and antagonists, including soluble Fas and antibodies directed to FasL, have been demonstrated to inhibit AICD.[13]

The role of FasL–Fas in AICD is further demonstrated by the development of lymphoproliferative disorders in mouse mutants. Mutants *gld/gld* and *lpr/lpr* are defective in the genes encoding FasL and Fas, respectively. They are also defective in AICD. Mature T cells from *gld* and *lpr* mice have defects in antigen-stimulated suicide, mediated by the FasL/Fas-dependent pathway.[14] In confirmation, activated T-cell hybridomas also do not undergo cell death in the presence of a Fas neutralizing antibody. B-cell homeostasis also appears to be regulated by FasL–Fas interactions. Observations in *gld* and *lpr* mice have demonstrated an accumulation of B cells and elevated levels of autoantibodies.[15] FasL is not expressed on the surface of resting or activated B cells.[16] B cells, however, can express its receptor, Fas. Deletion of B cells by FasL on CD4+ T cells has been demonstrated,[17] and a transgenic mouse line expressing Fas only in T cells was created using *lpr* mice.[18] These mice did not accumulate T cells but instead produced elevated levels of autoantibodies. These results suggest that FasL-expressing T cells can kill Fas-expressing activated B cells.

T-cell-mediated cytotoxicity is also an important factor in targeting and eliminating potentially harmful cells by apoptosis. Cytotoxic lymphocytes (CTL) comprise mainly CD8+ cytotoxic T cells and NK cells, and function to kill target cells (virus-infected and malignant cells) by two mechanisms. One pathway of CTL death occurs by calcium-dependent exocytosis of cytolytic granules from CTL. Cytotoxic granules contain proteins that are required for the destruction of the target cell including perforin and granzymes (serine proteases) (reviewed in Smyth *et al*.[19]). These proteins are secreted toward the target cell where they can penetrate the cytoplasm and nucleus of a cell, initiating cytosolic and nuclear apoptotic changes. The mechanism of granzyme death is not completely understood. Granzyme A and B processes however, do initiate DNA fragmentation in the target cell but require perforin for activity.[20,21] The most potent factor, granzyme B, has been shown to activate cdc2 (a G_2 cell cycle kinase), procaspase 3[21] and the cytoplasmic protein Bid (involved in the mitochondrial apoptotic pathway).[22] These actions are sufficient to induce cell death.

An additional mechanism of CTL cytotoxicity has been proposed based on the notion that effector T cells from perforin knockout mice are still capable of inducing cell lysis and DNA fragmentation.[23] This perforin/granzyme-independent pathway is thought to be because of FasL-mediated death. FasL is expressed in some CTL,[24] and CTL hybridomas that lyse Fas^+ but not Fas^- cells suggest a role for FasL–Fas death in this process.[25] Consistent with these observations and in a granule-independent manner, activated T cells from *gld* mice do not lyse Fas^+ target cells.[26]

Recently, it was demonstrated that Fas engagement induced disseminated endothelial cell apoptosis *in vivo*.[27] This study provides important immunopathological implications. Injection of anti-Fas monoclonal antibody (mAb) into mice produced an increase in endothelial cell apoptosis and vascular damage in a number of organs. Interestingly, when allogeneic lymphocytes from wild type, *gld*- or *lpr*–deficient mice were transferred to SCID recipient mice, no lesions were formed from FasL-deficient *gld* cells. On the contrary, wild-type and Fas-deficient *lpr* recipients displayed vascular lesions and endothelial cell apoptosis at levels similar to those observed with anti-Fas mAb.[27] These results suggested that FasL-expressing activated T lymphocytes interact with Fas-expressing endothelial cells during nonallogeneic immune responses.[27] Such responses may include infectious pathogens and tumors.

FasL has been thought to play an important role in sites of immune privilege (such as the eye and testis).[28] Certain locations in the body are excluded from immune surveillance, as they cannot tolerate the damaging effects of inflammation. The eye and testis are immune privilege sites that have developed a protective mechanism against such damaging immune responses. Both the eye and testis constitutively express FasL.[29] For some time, it was believed that FasL expression resulted in the death of invading Fas^+ cells within immune privilege sites. Evidence for such theories was provided by mutant mice models. Eyes of *gld* mice do not express functional FasL, and when infected with virus, the

eyes were destroyed by inflammation.[29] Corneal allografts from *gld* mice were also rejected.[30] These results provided evidence that FasL expression in the eye was responsible for the successful corneal transplants observed in human patients.

Additional studies provided further confirmation on the role of FasL in immune privilege. Islets of Langerhans allograft rejection was prevented with myoblasts engineered to express FasL in mice.[31] Moreover, testis grafts from mice expressing FasL survived when transplanted into allogeneic animals.[32] On the contrary, grafts derived from mutant *gld* mice were rejected.[32] Some groups also demonstrated that cancer cells became resistant to Fas-mediated apoptosis.[33] The idea of cancer as a region of immune privilege was also recognized. There was evidence that tumor cells may have used FasL as a mechanism of immune evasion . Some tumor cells express FasL constitutively,[34] and FasL from these cancer cells may have led to apoptosis of infiltrating lymphocytes. This had been demonstrated by a number of groups.[35,36]

FasL has been thought to confer immune privilege, however recent data implies that this may not be the case. Restifo[37] demonstrated that deficiency of FasL or Fas had no effect on the pathology of the eye, in an autoimmune uveitis model.[37] Additional conflicting data were observed from a study conducted by Allison *et al*.[38] In this study, fetal pancreas grafts from transgenic mice, expressing FasL on their islets β cells, were transplanted under the kidney capsule of allogeneic mice. FasL expression failed to protect the grafts from rejection. Furthermore, granulocytic infiltration was observed in the pancreata of the transgenic mice.[38] These results suggest that FasL expression may have a proinflammatory role and may not protect organ allografts from rejection.[38] Over time several others have demonstrated FasL expression to cause rejection of transplantations with extreme inflammation.[39] Similar observations were also seen in experimental tumor systems.[39]

The alleged role of FasL in immune privilege may have been because of false-positive data, based on controversial monoclonal antibodies used in experimental conditions. Additional controversy regarding the use of Fas antibodies has been raised.[10] Huang *et al.*[10] questioned the validity of Fas antibodies and how accurately these antibodies reflected the physiological mechanisms of Fas-induced apoptosis. Most data on sensitivity to Fas have been derived from studies using anti-Fas mAbs. To resolve these significant issues, a number of experiments were performed to elucidate the effects of Fas inducers on different cell lines. Huang *et al.*[10] demonstrated that only membrane-bound and multimerized (aggregated) FasL induced apoptosis reliably. This was also observed by Janin *et al.*[27] The capacity of soluble FasL to trigger apoptosis depended on the degree of soluble FasL multimerization.[27] It was also observed that antibodies to Fas did not dependably mimic FasL.[10] They proposed that anti-Fas mAb alone did not sufficiently support receptor crosslinking, particularly in type II cells. Type II cells became highly sensitive to crosslinked anti-Fas mAb.[10] Additionally, under certain conditions, mAbs to Fas could antagonize Fas-induced cell death. Therefore, studies using anti-Fas mAbs may not provide reliable data, since they may not accurately imitate the physiological functions of FasL. Thus, the role of FasL in immune privilege, tumor counterattack and inflammation needs to be considered with caution.

FasL-induced death in vascular disease

Apoptosis is not limited to an immune response. Programmed cell death has also been observed during vascular development and interestingly within the arterial wall in atherosclerosis, hypertension and restenosis.[40,41] Regression of the thickened arterial wall early in these pathologies by apoptosis could reduce the neointima.[42] Both FasL and Fas are expressed in the normal and diseased vessel walls.[43] Sata *et al*.[44] demonstrated FasL-induced cell death by an adenovirus encoding FasL (adeno-FasL). Adeno-FasL induced apoptosis in Fas^+ vascular smooth muscle cells (SMCs) in a paracrine manner and inhibited neointima formation in rats. Local delivery of adeno-FasL to proliferating vascular SMCs after balloon injury in rats also induced apoptosis.[44] In addition, a flow-restricted ligation model of injury, performed by Sata and Walsh[45] in FasL-defective *gld* mice, displayed greater neointima and enhanced leukocyte infiltration compared to wild type.[45] These results suggest that the FasL–Fas pathway can function to restrict inflammation and intimal hyperplasia during vascular remodeling.

It has also been proposed that since vascular SMCs express Fas and inflammatory cells express FasL, FasL–Fas-mediated apoptotic cell death may contribute to atherosclerotic plaque instability.[43] SMCs are the principal cellular components of atherosclerotic plaques capable of producing the collagen required to maintain tensile strength. It has been proposed that vascular SMC death within vulnerable regions of atherosclerotic plaques may lead to destabilization and plaque rupture.[46] Several lines of evidence suggest a positive role for the FasL/Fas death pathway in atherosclerotic plaques. Firstly, expression of Fas has been found in both inflammatory and vascular SMCs.[43] Fas^+ vascular SMCs are located prominently within the intima of plaques[43] and Fas has also been demonstrated to colocalize with TUNEL-positive vascular SMCs in regions consisting of CD3+ T-cells and CD68+ macrophages.[47] Geng *et al.*[48] also demonstrated positive staining for FasL in 34 out of 34 carotid plaques and the majority of this staining was localized with intimal vascular SMCs. Recently, it was also concluded that human macrophages induce apoptosis of vascular SMCs derived from carotid plaque.[49] Macrophage-induced SMC apoptosis was inhibited by a neutralizing antibody to FasL or Fas–Fc fusion protein, suggesting that this process may promote plaque rupture.[49] In a more clinical setting, the role of FasL-mediated death in myocardial infarction was examined by Shimizu *et al.*[50] In this study, plasma of acute myocardial infarction (AMI) or stable/unstable angina pectoris (AP) patients were measured for soluble FasL (sFasL). Shimizu *et al.*[50] demonstrated that patients with AMI and unstable AP have elevated levels of sFasL, indicating a role for the FasL–Fas system in vascular disease.

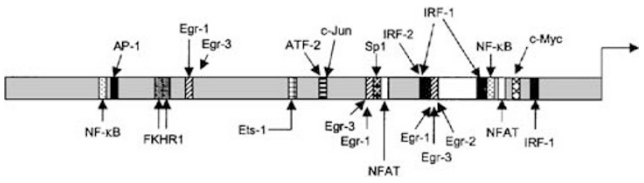

Figure 2 Transcriptional regulation of Fas ligand. Nuclear factors activating transcription of the FasL gene are illustrated in this schematic. Boxes represent *cis*-regulatory elements located in the proximal FasL promoter known to regulate FasL gene expression with indicated transcription factors. References are provided in the text.

Transcriptional Regulation of Fas Ligand

FasL gene expression is controlled by distinct protein–DNA interactions at the FasL promoter. Transcriptional control of FasL gene expression has previously been the focus of a review.[51] Transcriptional regulation of FasL since then, however, has significantly developed and a number of different factors have been identified to regulate FasL gene expression. Examples of these include transcription factors such as specificity protein-1 (Sp1), Ets-1 (homolog of viral *ets*), interferon regulatory factor-1 (IFN-1) and inducible cAmp early repressor (ICER) (Figure 2; see Table 1 for coordinates). Here we will discuss in some detail the transcriptional signaling machinery involved in FasL gene expression, including mechanisms of stress and cytokine-induced expression. Elucidation of precise mechanisms underlying FasL gene expression may provide useful molecular insights on the disease states associated with FasL.

Nuclear factor in activated T cells (NFAT), a member of the enhancer binding protein family, is critical for the expression of many cytokine genes[52] and is involved in the regulation of TCR-mediated FasL expression.[53] Two NFAT sites have been identified through DNase I footprinting studies from nuclear extracts of activated T cells. Both sites are shown to be of importance, although mutational studies have demonstrated the distal NFAT binding site to have a more significant role.[53] Human T-cell leukemia virus type I (HTLV-1)-infected T cells constitutively express FasL. HTLV-1 induces transcription of FasL through the viral transactivator, tax.[54] The previously identified consensus NFAT binding site[53] is required for tax activation, and the NFAT motif is essential for activation of the FasL promoter by TCR signals.

Nuclear factor-kappa B (NF-κB) is a ubiquitous transcription factor involved in the expression of many genes including cytokines, growth factors and cell adhesion molecules.[55] The inducible form of NF-κB is a heterodimer of NF-κB1 and Rel A. Inactive NF-κB is found in the cytoplasm in a complex with an inhibitory protein I-κB. Once activated, NF-κB is released from I-κB, translocates to the nucleus and activates target genes.[56] Two NF-κB sites have been identified in the mouse promoter of FasL (FasL-κB1 and FasL-κB2).[57] Both sites were found to bind NF-κB; however, only the FasL-κB1 site was able to activate gene expression.[57] NF-κB-dependent upregulation of FasL has also been demonstrated in apoptosis of etoposide- and teniposide-treated Jurkat T-cells.[58] Moreover, the FasL promoter was responsive to DNA damage and coexpression with p65 (Rel A) or Fos/Jun. Mutations in NF-κB and activator protein-1 (AP-1) binding sites eliminated these responses, indicating a crucial role for both NF-κB and AP-1 in FasL expression and apoptosis.[58] Like NFAT, NF-κB also plays a role in T-cell activation-induced FasL expression.[59] Inhibition of NF-κB activity in T-cell hybridomas reduced FasL expression and apoptosis upon

Table 1 Transcriptional regulators of FasL gene expression

Transcription factor	Strand	Response element	Coordinates	Reference
NFAT	+ve	5′-GGAAA-3′	−137/−133	53
NFAT	+ve	5′-GGAAA-3′	−270/−272	53,54
NF-κB	−ve	5′-GGGGACTTTCT-3′	−1086/−1076	58
NF-κB[a]	+ve	5′-AGGTGTTTCCC-3′	−138/−128	57
NF-κB[a]	+ve	5′-TGGTCTTTTCCC-3′	−440/−429	57
AP-1	+ve	5′-TTAGTCAG-3′	−1050/−1043	58
Sp1	+ve	5′-GGGCGG-3′	−280/−275	60,62
Ets-1	+ve	5′-GGAA-3′	−366/−363	61
Egr-1, Egr-3, Egr-2	+ve	5′-GTGGGTGT-3′	−215/−208	63
Egr-1, Egr-3	+ve	5′-GTGGGCGG-3′	−282/−275	65,66
Egr-1, Egr-3	+ve	5′-GTGGGTGT-3′	−784/−777	65
IRF-1, IRF-2	+ve	5′-AAGTGA-3′	−221/−216	69
IRF-1	+ve	5′-GAGAAGAAGTAAAACCGTTTG-3′	−49/−29	70
IRF-1	+ve	5′-AGAGAAAGAGAAAGACAGAGG-3′	−174/−154	70
c-Myc	−ve	3′-ATTCTCT-5′	−127/−121	76
FKHRL1	+ve	5′-TAAATAAATA-3′	−897/−888	78
FKHRL1	+ve	5′-TAAATAAATA-3′	−885/−876	78
FKHRL1	+ve	5′-TAAGTAAATA-3′	−889/−880	78
ATF-2/c-*jun*	+ve	5′-TTGGGTAGCACAGCGA-3′	−335/−320	81

Nuclear factors involved in the transcriptional regulation of FasL are listed. Coordinates and sequences corresponding to transcription factor *cis*-elements (relative to transcriptional start site) in human FasL proximal promoter are noted
[a]Mouse FasL promoter sequence

TCR stimulation. Coexpression of p65 (Rel A) on the FasL promoter induced FasL activity. In contrast, coexpression of I-κB dramatically reduced inducible FasL promoter activity.[59] Unlike inducible FasL expression by DNA-damaging agents, AP-1 was not required for activation-induced FasL gene expression.[59]

A broadly expressed zinc-finger transcription factor, Sp1, is involved in the transcriptional regulation of many genes and can influence gene expression by promoter interactions through architectural support and by chemical modification. Sp1 has also been identified to be important in the regulation of FasL gene expression and apoptosis. We recently demonstrated that inducible FasL gene expression in SMC involves the zinc-finger transcription factor Sp1, which in turn is regulated by the atypical protein kinase C-zeta (PKCζ).[60] Sp1 activated the FasL promoter via a distinct recognition element, and inducible FasL promoter activation was abrogated by expression of the dominant-negative (DN) mutant form of Sp1. Inducible FasL transcription and apoptosis were also blocked using DN-PKCζ. We also showed that Ets-1 positively activates FasL gene expression in SMC.[61] Furthermore, Ets-1 activation of the FasL promoter involved a distinct recognition element and cooperative interactions with Sp1.[61] Using immunohistochemical staining, we found that Ets-1, Sp1, PKCζ and FasL were expressed in smooth muscle-actin$^+$ TUNEL$^+$ SMCs of human carotid atherosclerotic plaques.[60] The Sp1 element in the FasL promoter overlaps with an NFAT binding motif. This region (5′-GGGCGGAAA-3′) is critical for FasL promoter activity in IL-2 treated T cells.[62] Mutation of the Sp1 and/or NFAT sites reduced FasL promoter activity.[62] In contrast, mutation at the early growth response factor (Egr) site had no effect on FasL transcription.[62]

Mittelstadt and Ashwell[63] identified a sequence on the FasL promoter that binds Egr transcription factor family members termed the FasL regulatory element (FLRE). Electrophoretic mobility shift assays (EMSA) identified two activation-induced nuclear protein complexes, Egr-1 and Egr-3 to bind this region.[63] The transcription factor Egr-1 is implicated in growth, differentiation and apoptosis.[64] The function of Egr-3 on the other hand remains unknown. Transient overexpression of Egr-3 increased FasL promoter activity in a cyclosporin A-insensitive manner. In contrast Egr-1 had no effect.[63] Li-Weber *et al*.[65], through DNase I footprinting, identified an additional two Egr binding sites on the FasL promoter. Both Egr-1 and Egr-3 were found to form nucleoprotein complexes at these identified sites.[65] Moreover, NFAT was also a component of the inducible-binding complexes formed. In the same study, Egr-1, Egr-3 and NFAT displayed cooperative and synergistic activation of the FasL promoter mediated by the three Egr/NFAT regulatory elements.[65] Egr-2 also showed a positive regulatory role in FasL transcription.[66] Mittelstadt and Ashwell[66] demonstrated that both Egr-3 and Egr-2 induced FasL-promoter-dependent reporter activity in T-cell hybridomas and HeLa cells. Egr-3 and Egr-2 also upregulated endogenous FasL mRNA.[66]

Regulation of FasL by NFAT via Egr factors has been established by Rengarajan *et al*.[67] Through Northern, EMSA and promoter-dependent studies using cells of NFAT-deficient mice, Egr2 and Egr3 were shown to be regulated by NFAT proteins.[67] Primary lymph node cells from mice lacking NFATp, NFAT4, Egr2 and Egr3 were also used to assess direct transactivation of the FasL promoter by Egr2 and Egr3, under the regulatory control of NFAT. Interestingly, expression of NFAT together with a minimal FasL promoter construct (containing the FLRE) demonstrated significant induction of the reporter vector. The fact that coexpression of Egr3 together with the minimal FasL reporter vector bearing a mutation in FLRE demonstrated near extinction of FasL-promoter upregulation further supports the notion that NFAT controls the regulation of Egr2 and/or Egr3 to regulate FasL transcriptional activity.[67] In addition to these studies, Yang *et al*.[68] recently established the synergistic activation of the FasL promoter by coexpression of Egr2 or Egr3 and the human immunodeficiency virus (HIV) transactivator, Tat. Mutations in FLRE no longer supported this superinduced activation.[68] This study also established that Egr2 and Egr3 physically interacted with Tat. Interaction with Egr3 was still supported by an amino-acid substitution in Tat that blocked its transactivation activity; however, this mutation failed to enhance Egr-dependent regulation of the FasL promoter.[68]

TCR-inducible FasL expression is under the direct influence of the interferon transcription factor family. Deletion and mutagenesis studies identified a 12 bp sequence in the FasL promoter containing a putative interferon regulatory factor (IRF) binding site.[69] EMSA demonstrated the formation of DNA-binding complexes to contain IRF-1 and IRF-2. Overexpression of either IRF-1 or IRF-2 resulted in FasL promoter activation, although the activation observed was more significant by IRF-1 overexpression.[69] IRF-1 and IRF-2 overexpression also lead to an increase in endogenous FasL mRNA levels in heterologous nonlymphoid cells. Kirchhoff *et al*.[70] identified two positive IRF-dependent domains in the FasL promoter. EMSA demonstrated IRF-1 binding to both sites, where IRF-1 overexpression induced FasL promoter activity. Interestingly, Kirchhoff *et al*.[70] demonstrated that both sites are important in TCR/CD3-mediated FasL induction, and that viral IRF of human herpesvirus 8 (HHV8) abolish IRF-1-mediated, and abrogate TCR/CD3-mediated FasL induction. Thus, inhibition of FasL-dependent T-cell function may contribute to the immune escape of HHV8.[70]

The transcription factor c-Myc dimerizes with Max to form an active transcriptional complex involved in cell cycle progression, neoplasia and cell death. Little is known regarding mechanisms of c-Myc-mediated apoptosis. c-Myc has been shown to promote apoptosis in fibroblasts.[71] Studies have demonstrated the involvement of c-Myc in AICD of T cells through the use of antisense oligonucleotides targeting c-Myc[72] and dominant-negative mutant forms of c-Myc or Max.[73] Hueber *et al*.[74] revealed that c-Myc-induced apoptosis requires functional FasL and Fas, and these findings were confirmed by Brunner *et al*.[75] Brunner *et al*.[75] demonstrated that T-cell activation-induced expression of FasL is regulated by c-Myc. c-Myc has been illustrated to interact directly with the FasL promoter. A 'noncanonical' binding site has been identified (3′-ATTCTCT-5′) for c-Myc–Max heterodimers,[76] and c-Myc activation of the FasL promoter was abolished

upon mutation of this binding element.[76] Transforming growth factor-beta (TGFβ) also downregulates FasL transcriptional activity via c-Myc. TGFβ1 has been shown to block c-Myc-induced FasL mRNA and subsequent activation of apoptosis in T cells.[77] This provides a possible mechanism for AICD downregulation that may allow for clonal expansion during an immune response.[77]

Survival factors such as nerve growth factor (NGF) and insulin-like growth factor 1 (IGF1) trigger a cascade of events leading to the activation of the phosphatidylinositol 3-kinase (PI3K)–Akt pathway. Akt directly inhibits members of the apoptotic machinery including BAD and caspase 9. The forkhead transcriptional regulator-1 (FKHRL1) is a substrate of Akt phosphorylation.[78] The release of survival factors leads to the phosphorylation of FKHRL1, rendering it inactive as a transcription factor. Dephosphorylation of FKHRL1 contributes to FasL upregulation and apoptosis.[78] Brunet *et al*.[78] identified three putative overlapping response elements to FKHRL1 in the FasL promoter. Only two of these response elements were found to bind FKHRL1.[78] Recently, Suhara *et al*.[79] demonstrated that serum deprivation and treatment of vascular SMC with wortmannin (PI3K inhibitor) ablated Akt signaling and led to the upregulation of FasL. Akt suppression also induced c-Jun N-terminal kinase (JNK) and DN mutants of c-Jun inhibited FasL promoter activity.[79] Induction of FasL by FKHRL1 was dependent on c-Jun activation.[79] Suhara *et al*.[79] established a positive feedback loop mechanism where FasL participates in and promotes apoptosis under conditions of cellular stress.

Stress-Induced FasL Transcription

Environmental stress stimuli such as cytotoxic stress and DNA-damaging agents can trigger responses that control cellular processes including repair, cell cycle arrest and programmed cell death. The induction of FasL gene expression in T lymphocytes in response to environmental stress has been shown to be dependent on JNK activation.[80] c-Jun translocates to the nucleus following phosphorylation by JNK. Within the nucleus it binds to c-Fos forming a complex, AP-1. The MEKK1 (JNK kinase kinase)-regulated response element was identified by Faris *et al*.[80] on the FasL promoter. Mutation of this response element greatly reduced MEKK1-mediated FasL promoter activation. EMSA demonstrated specific binding by an AP-1 heterodimer consisting of activating transcription factor 2 (ATF-2) and c-Jun. Transfection of c-*Jun* and ATF-2 mutants (lacking JNK phosphorylation sites) decreased transcriptional activation of FasL. Faris *et al*.[80] thus demonstrated that MEKK1, and transcription factors regulated by the JNK pathway play a role in committing lymphocytes to undergo apoptosis via FasL transcription in a stress-responsive manner.

JNK to FasL signaling pathways also play important roles in the induction of neuronal cell death in response to various stresses.[81] Treatment with truncated MEKK1 (MEKK1Δ) or NGF withdrawal leads to an increase in FasL transcriptional activity. The p38 inhibitor SB202190 blocked FasL induction and c-Jun phosphorylation.[81] SB20358 activation induced FasL expression, and overexpression of mitogen-activated protein kinase (MAPK) kinase 3b (activator of p38 MAPK) led to an increase in FasL promoter activity and an increase in transcript in T cells. In addition, Kasibhatla *et al*.[58] demonstrated that etoposide, teniposide and ultraviolet-induced T-cell apoptosis occurs through the activation of FasL. They also demonstrated that these stimuli activated the JNK pathway. These responses were abrogated by mutations in AP-1 and NF-κB, indicating a role for AP-1 and NF-κB in stress-induced apoptosis.[58]

Cytokine-Induced FasL Gene Expression

The role of IL-2 in apoptosis is not completely established. In fact, IL-2 rescues activated T cells from apoptosis by inducing antiapoptotic genes including Bcl-2.[82] Other studies, however, suggest that IL-2 primes T cells to TCR-mediated programmed cell death.[83] These findings are confirmed by the observation that IL-2-deficient mice exhibit an increase in lymphocyte production, uncontrolled T-cell activation and autoimmunity.[84] More recently, Haux *et al*.[85] revealed that NK cells exposed to IL-2 over 3 days became apoptotic and released soluble FasL, indicating the involvement of FasL/Fas in the downregulation of IL-2-activated human NK cells.[85] IL-2 also increased transcription and surface expression of FasL,[86] and Xiao *et al*.[62] further demonstrated this by functional FasL promoter studies. It was found that IL-2-induced FasL-promoter-dependent expression was mediated via the Sp1 and NFAT binding motifs.[62]

Ayroldi *et al*.[87] also demonstrated that transcription of FasL/Fas is controlled by IL-2 production and that CD2 stimulation rescued T-cell hybridomas from AICD through a reduction in IL-2. Ayroldi *et al*.[87] also showed negative regulation of the FasL/Fas system with the involvement of interleukin-6 (IL-6). IL-6 was found to inhibit anti-CD3-induced apoptosis in a T-cell hybridoma line, thus demonstrating a protective effect. IL-6 did not inhibit IL-2 production, suggesting IL-2-independent mechanisms of FasL/Fas expression.[87] A significant decrease was also observed in the anti-CD3-induced expression of FasL and IL-6 rescued resting T cells from apoptosis, by activating Bcl-2 expression.[88]

TGFβ, a cytokine that regulates cell growth, adhesion and differentiation,[89] has contradictory actions and its role in apoptosis is not clear. There are a number of conflicting studies that demonstrate TGFβ to have a negative[90] and a positive role in apoptosis.[77] Recently, Genestier *et al*.[77] demonstrated negative regulation of apoptosis by TGFβ1 through the transcriptional regulation of FasL. They showed that TGFβ1 inhibited c-Myc expression in T-cell hybridomas, and a chimeric molecule consisting of c-Myc and the estrogen receptor's steroid binding domain blocked FasL and AICD stimulated by TGFβ1.[77] Schlapbach *et al*.[91] showed that TGFβ induced Flice-inhibitory protein (c-FLIP) in resting and activated microglia, and they demonstrated that the presence of FLIP strongly interfered with FasL-induced activation of caspase 8 and caspase 3, preventing apoptosis.[91] On the contrary, TGFβ is required for programmed cell death in the developing mouse limb,[92] where levels of apoptosis were reduced in the interdigital spaces of the developing limbs of Tgf-beta2$^{-/-}$ Tgf-beta3$^{-/-}$ double

knockouts. Arsura *et al*.[93] also demonstrated a positive role in TGFβ-induced apoptosis. They showed that TGFβ-induced programmed cell death is preceded by a reduction in c-Myc expression, which is associated with a decrease in NF-κB expression.[93]

Negative Regulation of FasL Transcription

Repression of FasL expression has been demonstrated by a number of factors including retinoic acid, nitric oxide, vitamin D_3 and the transcriptional repressor ICER. Treatment of T-cell hybridomas with retinoic acid and glucocorticoids inhibits FasL upregulation and subsequent apoptosis.[94,95] The ability of retinoic acid to inhibit AICD was enhanced by over-expression of the retinoid X receptor (RXR).[96] The inhibition of apoptosis was blocked by the dominant-negative mutant form of RXR.[96] Yang *et al*.[96] demonstrated efficient inhibition of FasL upregulation and T-cell apoptosis by retinoids binding to their respective receptors.

Recently, Lee *et al*.[95] illustrated the repression of FasL by retinoic acid, mediated via an NFAT binding element. EMSA determined a reduction in the ability of NFAT to bind DNA following retinoic acid treatment and revealed that retinoic acid blocked the translocation of NFAT from the cytosol to the nucleus.[95] In a similar manner, nitric oxide also inhibited FasL expression and apoptosis by interfering with the ability of AP-1 to induce FasL expression.[97] Moreover, vitamin D_3 inhibited activation-induced apoptosis and FasL gene expression. This repression was shown to be mediated by a noncanonical c-Myc binding element.[98] In addition, the transcriptional repressor ICER has also been identified as a downregulator of FasL expression in T lymphocytes. Bodor *et al*.[99] recently demonstrated the transcriptional repression of activated human FasL promoter by ICER, and the involvement of the proximal NFAT binding element. The study illustrated the formation of a ternary complex between ICER and the DNA-binding domain of NFAT via the proximal NFAT element.[99] Increased expression of ICER also correlated with a decrease in FasL expression in both T and NK cells.[99] In support of these observations, Bodor *et al*.[99] illustrated that a proximal NFAT binding site participates in the downregulation of the FasL promoter by ICER. Thus, FasL is likely to be controlled by complex interactions via positive and negative regulatory factors.

Conclusion

In recent years, a distinct pattern of FasL gene expression has emerged, involving transcription factor interactions with distinct promoter elements, protein–protein combinatorial interactions and phosphorylation. These molecular events integrate signals from outside the cell to changes in FasL gene expression, altering cell phenotype and triggering cell death. Future work should provide a more complete picture of the signaling, transcriptional and post-transcriptional regulation of this mediator of cell death.

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