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REVIEW

ATF3, a Hub of the Cellular Adaptive-Response Network, in the Pathogenesis of Diseases: Is Modulation of Inflammation a Unifying Component?

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Activating transcription factor 3 (ATF3) gene encodes a member of the ATF family of transcription factors and is induced by various stress signals. All members of this family share the basic region-leucine zipper (bZip) DNA binding motif and bind to the consensus sequence TGACGTCA in vitro. Previous reviews and an Internet source have covered the following topics: the nomenclature of ATF proteins, the history of their discovery, the potential interplays between ATFs and other bZip proteins, ATF3-interacting proteins, ATF3 target genes, and the emerging roles of ATF3 in cancer and immunity (see footnote 1). In this review, we present evidence and clues that prompted us to put forth the idea that ATF3 functions as a "hub" of the cellular adaptive-response network. We will then focus on the roles of ATF3 in modulating inflammatory response. Inflammation is increasingly recognized to play an important role for the development of many diseases. Putting this in the context of the hub idea, we propose that modulation of inflammation by ATF3 is a unifying theme for the potential involvement of ATF3 in various diseases.

Key words: Activating transcription factor 3 (ATF3); Adaptive-response network; Inflammatory response

ACTIVATING TRANSCRIPTION FACTOR 3 (ATF3) AS A HUB OF THE CELLULAR ADAPTIVE-RESPONSE NETWORK

ATF3 as a Hub

Overwhelming evidence indicates that ATF3 expression is upregulated by a variety of signals: its steady-state mRNA level is low or nondetectable in most cell lines and tissues, but greatly increases upon stimulation at early stage (usually within hours) of induction (21,25). One striking feature is that the induction of ATF3 is neither stimulus nor cell type specific. The common feature for all the ATF3-inducing stimuli reported in the 1990s was their ability to damage cells; thus, we referred to ATF3 as a stress-inducible gene (25). However, with the wide use of cRNA array, the list of stimuli that can induce ATF3 expression continued to grow and the term stress-inducible gene no longer encompassed all the stimuli that can induce ATF3. Some of the ATF3-inducing stimuli, such as adipokines and G_1 to S transition, do not fit the traditional definition of stress. Thus, we suggested that an adaptive-response gene is a better term to describe ATF3 (21). Expanding on this idea, we now

[1]For nomenclature, history, and interplay with other bZip proteins, see (21,22,24,25); for interacting proteins and target genes, see (48); for ATF3 in cancer and immunity, see (64).

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put forth the idea that ATF3 is a hub of the cellular adaptive-response network to respond to signals perturbing homeostasis. The following lines of evidence and clues prompted this idea.

The Signaling Pathways. In addition to the broad spectrum of stimuli that can induce ATF3 (reviewed previously), various signaling pathways have been linked to ATF3. Figure 1A shows a schematic representation. In making this figure, we excluded the reports that are only correlative in nature and focused on the reports that demonstrated the link by either gain-of-function or loss-of-function approach, or both.

The ATF3 Promoters. In 1996, we reported that an approximately 2-kilobase (kb) fragment of the human ATF3 promoter responded to stress signals in a reporter assay (40). Since then, this fragment has been used as the so-called "full-length" ATF3 promoter. However, as with all reporter assays, caveats must be taken in the interpretation of data derived from this reagent. This 2-kb fragment by no means contains all the regulatory elements controlling ATF3 promoter activity, and the lack of chromosomal structure in the reporter assay is another limitation. Nevertheless, inspection of the sequence revealed that this fragment is packed with transcription factor binding sites, many of which are recognized by factors downstream of the signaling pathways shown in Figure 1A, consistent with the idea that ATF3 is a downstream target of these pathways. We highlighted some of the binding sites in Figure 1B.

With the completion of genomic sequencing and the availability of expressed sequence tags (ESTs), two potential promoters in addition to the one we reported in 1996 have been denoted on the ATF3 Genome Browser sites: one ~2 kb upstream from the original promoter and the other one ~43 kb upstream in the human ATF3 gene (~35 kb for the mouse ATF3 gene) (37). Recently, Miyazaki et al. provided experimental data supporting the biological relevance of the −43 kb promoter (49). They showed that serum stimulation primarily upregulates this promoter, with slight induction of the original promoter. Interestingly, this promoter is constitutively active in several cancer cell lines and contributes to the high levels of ATF3 protein in these cells (49). Figure 2 shows a schematic of the mouse ATF3 gene structure with multiple promoters. According to the nomenclature by Miyazaki et al. (49), the −35 kb promoter is denoted as P1 and the original promoter P2. The −2 kb promoter is indicated as putative without a designation, because it is not clear whether it is a true promoter.

Analyses of all three promoters using the ClusterBuster program revealed clusters of transcription factor binding sites on all three of them. However, the cluster for P2 is more proximal to the transcriptional start site (TSS) than the clusters for P1 and the putative promoter (at −2 kb). We also analyzed the ATF3 gene region (spanning between the genes immediately upstream or downstream from ATF3) using the CpG Island Searcher program (61) and found potential CpG islands covering the exon immediately downstream from the P2 promoter, designated as A2 in this review (Fig. 2). The primary sequence of exon A2 between human and mouse is ~72% homologous and the CpG islands are conserved. It is intriguing that no matter which promoter is utilized, the resulting open reading frame is the same. This is because the first methionine codon is located in the second exon (exon B); thus, transcription from different promoters gives rise to different first exons. These untranslated regions (5′ UTR) may provide different regulation of translational initiation. Using a translational reporter assay, Miyazaki et al. showed that exon A1 (derived from the P1 promoter) is regulated by stress signals (49). Taken together, ATF3 is regulated by many transcription factors via multiple promoters, and can potentially be regulated by both CpG islands and at the translational level. All these suggest that ATF3 acts as an integration point for a variety of controls, supporting the hub idea.

Potential Posttranslational Modifications. Amino acid sequence analyses of ATF3 revealed many potential sites for modification. Table 1 lists some of them based on the known consensus sequence. In making the table, we only considered four residues: (i) serine, threonine, and tyrosine residues—potential phosphorylation sites, and (ii) lysine residue—potential sites for acetylation, methylation, ubiquitination, and sumoylation. We emphasize that they are listed here as a reference, not to imply any experimental evidence or functional significance, because rigorous evidence to address these issues is lacking. Nevertheless, it is interesting to note that ATF3 has 21 serine/threonine, 1 tyrosine, and 17 lysine residues, accounting for ~20% of the molecule (39 out of 181 residues). This abundance of potential posttranslational modification sites is consistent with the hub idea that ATF3 is a target for regulation by many signaling pathways.

The Context Dependency of the Hub

Taken together, ATF3 functions as an early responder to link various signaling pathways to downstream events, resulting in the alteration of cellular bioactivity. By its nature as an immediate-early gene,

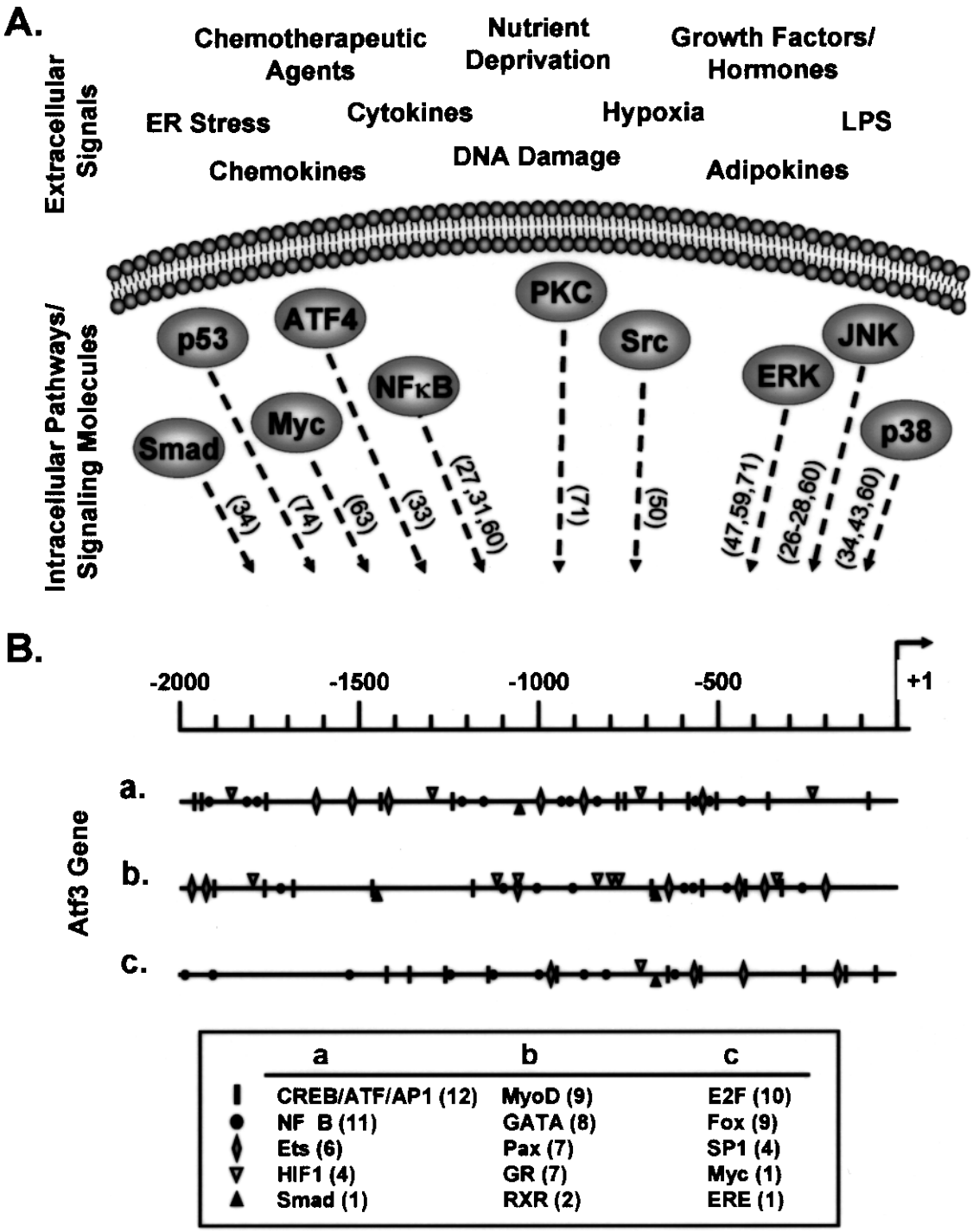

Figure 1. ATF3 as a hub of the cellular adaptive-response network. (A) Various signaling pathways were linked to ATF3 induction by gain-of-function or loss-of-function approach, or both. Numbers in parentheses indicate the references. Due to space limitations, the citation is not comprehensive and we apologize to those whose work is not cited here. LPS, lipopolysaccharide. (B) Analyses of the two kb region of the most proximal promoter (designated as P2 in Fig. 2) by several programs (Jaspar, MotifMogul, PROMO) (9,12,16) revealed numerous transcription factor binding sites.

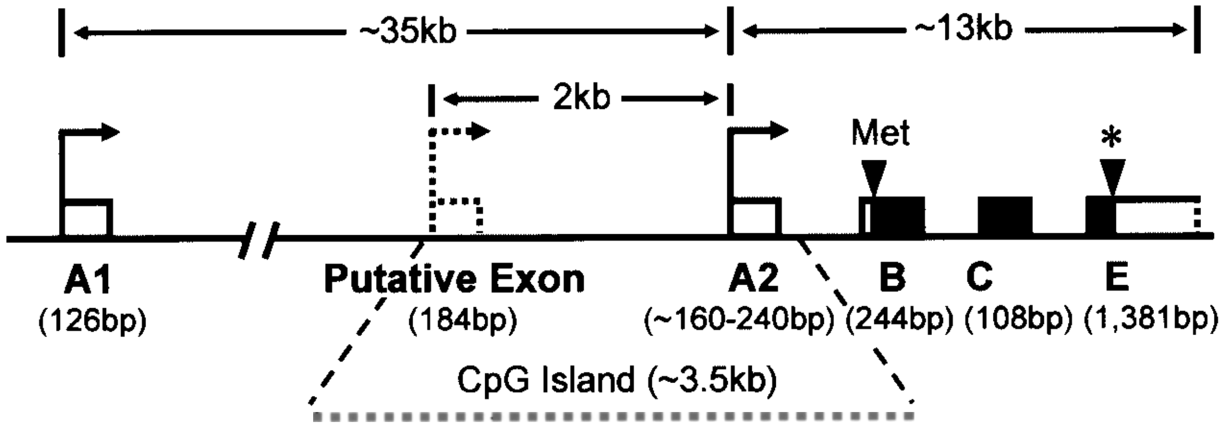

Figure 2. A schematic of the mouse ATF3 gene structure. Arrows indicate the transcriptional start site (TSS); open boxes indicate exons and lines between the boxes represent introns. The P1, P2, and a putative promoter are indicated (see text for description). The P2 promoter has multiple start sites, resulting in variable size of exon A2. The end of exon E is indicated by a dotted line, because the transcriptional termination site is not clear. For the purpose of illustration here, it is arbitrarily defined as the point where polyA is added. CpG islands identified by the CpG Island Searcher program (61) is indicated by the gray dotted line. Note that the figure is not proportional to scale and the intron between exons A2 and B is about 5 kb. Met: methionine. *: Termination condon. The human ATF3 gene has similar genomic organization and CpG islands spanning exon A2.

ATF3 has far-reaching effects. Immediate-early genes encode transcription factors and are known to turn on/off genes encoding transcription factors, which in turn regulate downstream genes, leading to a cascade of changes in transcriptional programs. We note that, despite the involvement of ATF3 in various pathways, ATF3 knockout (KO) mice develop normally and do not display any discernable phenotypes under normal conditions (27). Thus far, all phenotypes described for ATF3 deficiency were revealed when the KO mice or cells were examined under stimulation. Thus, ATF3 is apparently not essential for embryonic development, despite the importance of cellular response to intra- and extracellular cues during this process. Therefore, if the hub concept is correct, ATF3 is a context-dependent hub.

TABLE 1
SOME POTENTIAL POSTTRANSLATIONAL MODIFICATION SITES ON ATF3

Enzyme/ Modification	Consensus Motif	Putative Site(s) on ATF3	Ref.
Akt	RXRXX(**S/T**)	CHRMS**S**$_{60}$	51
AMPK	IXHRX**S**XXEI MKKSX**S**XXDV LRRVX**S**XXNL	LCHRM**S**$_{59}$	20
CK1	(pS/pT)XX(**S/T**)	TLQ**S**$_{181}$	15
CK2	(**S/T**)XX(E/D)	**S**$_{10}$ASE **S**$_{60}$ALE **T**$_{162}$PED	69
GSK	(**S/T**)XXX(pS/pT)	**S**$_{24}$PPGS **S**$_{60}$ALES **S**$_{64}$VTVS	14,54
MAPK	(**S/T**)P	**S**$_{24}$P **T**$_{38}$P **T**$_{162}$P	58
Sumoylation	Ψ**K**X(D/E)	V**K**$_{42}$EE L**K**$_{136}$NE I**K**$_{175}$EG	65

The references listed here are for the consensus motifs, not for potential modification of ATF3. Ψ: any hydrophobic residue.

REGULATION OF INFLAMMATORY RESPONSE BY ATF3

Regulation of Inflammatory Genes by ATF3 in Immune Cells

Recently, ATF3 was shown to play an important role in innate immunity [see a review (64)]. Using a combination of systems biology and molecular biology approaches, Aderem and colleagues identified ATF3 to be a negative regulator of the Toll-like receptor 4 (TLR4) signaling in macrophages (19). Activation of TLR4 by lipopolysaccharide (LPS) induces the expression of ATF3, which in turn dampens the expression of various inflammatory genes induced by TLR4 signaling, including IL-6, IL-12b, and TNF-α. This dampening effect is protective to the whole organism in the LPS-induced septic shock model: ATF3 KO mice succumbed to death upon high-dose LPS injection (20 mg/kg body weight) with a faster and higher frequency than wild-type (WT) mice. This is echoed in a low-dose LPS injection (50 μg/kg body weight) model that induces febrile response: ATF3 KO mice had higher body temperature than the WT mice (62). Thus, by dampening the expression of pro-

inflammatory genes, ATF3 prevents the immune system from overreacting. Strikingly, this effect is not limited to TLR4 signaling; ATF3 acts as a negative regulator in TLR2/6, TLR3, TLR4, TLR5, TLR7, and TLR9 signaling in macrophages, as shown by Williams and colleagues (68). ATF3 also plays a similar role in dendritic cells (68)—the major antigen presenting cells (APCs) of the mammalian immune system. In addition to suppressing cytokine gene expression, ATF3 suppresses the expression of the chemokine gene CCL4 (MIPβ) in macrophages (35). All the above studies used the loss-of-function approach. A recent study took a gain-of-function approach and showed that peritoneal macrophages expressing the ATF3 transgene driven by the scavenger receptor-A (SR-A) promoter had lower TNF-α mRNA level than the nontransgenic counterpart (60), a result that is consistent with the above data using the loss-of-function approach. Table 2 summarizes these gene expression results. We note that the number of innate immunity genes regulated by ATF3 in macrophages is much higher than those listed in the table. Using systems biology approach combined with ChIP-on-chip and microarray data, Aderem and colleagues revealed that ATF3 may modulate a wide array of genes in macrophages (38,41). Due to the long list of genes, they are not listed here.

In addition to macrophages, ATF3 also represses the expression of various cytokine and chemokine genes in other immune cells under various stress models (Table 2). These include natural killer (NK) cells in a mouse cytomegalovirus (MCMV)-infection model (57), $CD4^+$ T cells in an ovalbumin-induced asthma model (17), and mast cells in a differentiation model (18). Using a ventilation-induced lung injury model, dos Santos and colleagues showed that ATF3 KO mice had increased lung injury, increased cell infiltration and proinflammatory cytokines in the lung (1). Thus, ATF3 again functions as a negative regulator; however, the immune cell types in which ATF3 plays a role in this model are not specifically addressed in the report.

Taken together, it is clear that ATF3 plays a role in a broad range of immune cells. Here, we note two interesting lessons from these and other studies. (a) The action of ATF3 is context dependent. As an example, ATF3 represses the expression of the IFN-γ gene in NK cells (57) but not in $CD4^+$ T cells (17); in addition, it represses the expression of TNF-α in macrophages (19,68) but not in NK cells (57). The difference could be due to the different cell types or the different stress signals used to induce ATF3 in these models. (b) The functional consequences of ATF3 for the whole organism vary, depending on the model. In the LPS-induced septic shock (19) and ovalbumin-induced asthma models (17), the dampening of the immune response by ATF3 reduces inflammation and is beneficial to the host. Similarly, Williams and colleagues showed that WT mice regained their body weight faster than the ATF3 KO mice upon influenza virus infection (68), suggesting a beneficial role of ATF3 to the host. However, ATF3 appears to be detrimental to the host in the context of the MCMV-infection model, where the WT mice developed higher hepatic viral load and more severe liver histopathology (57). In this model, NK cells play an important role in controlling the viral load by

TABLE 2
REGULATION OF GENES ENCODING CYTOKINES, CHEMOKINES, AND OTHER IMMUNE-MODULATING MOLECULES BY ATF3

Gene	Cell Type(s)	Ref.
Cytokines		
IL-1β	Islets	76
IL-4	$CD4^+$ T cells	17
	Mast cells	18
IL-5	$CD4^+$ T cells	17
IL-6	Islets	76
	Mouse embryonic fibroblast	62
	Macrophages	19,62
	Mast cells	18
	Dendritic cells	68
	Whole lung	1
IL-10	Dendritic cells	68
IL-12b	Macrophages	19
	Dendritic cells	68
	Whole lung	1
IL-13	$CD4^+$ T cells	17
IFN-γ	Natural killer	57
	$CD4^+$ T cells	13
	Whole lung	1
TNF-α	Islets	76
	Macrophages	19,60
	Dendritic cells	68
	Splenocytes	68
TGF-β	Epithelial cells	73
Chemokines		
CCL2	Islets	76
CCL2, 7, 8, 11	Whole lung	17
CCL4	Macrophages	35
	Whole lung	1
CCL5	Mouse embryonic fibroblast	62
	Macrophages	7
CXCL1, 2, 5	Whole lung	17
CX3CL1	Mouse embryonic fibroblast	62
CXCL16	Mouse embryonic fibroblast	62
Other		
iNOS	Macrophages	19
	Mouse embryonic fibroblast	62
Adiponectin	3T3-L1 adipocytes	36

secreting IFN-γ, and the ability of ATF3 to repress IFN-γ gene expression hampers the ability of the host to fight against MCMV infection. Thus, as a built-in brake, ATF3 can dampen the deleterious effects of prolonged innate immune response. However, the price for this feedback brake is that it weakens the host defense system, making it more vulnerable under certain conditions. This underscores the double-edge sword nature of ATF3.

Regulation of Inflammatory Genes by ATF3 in Nonimmune Cells

Although immune cells are well recognized to be the major source of cytokines and chemokines, nonimmune cells can also produce them. In fact, it appears that all cell types have the potential to produce them under proper conditions. Thus, it is interesting to note that ATF3 has also been demonstrated to modulate the expression of various cytokines and chemokines in nonimmune cells. Nakai and colleagues demonstrated that ATF3 mediates heat shock-induced repression of IL-6, CCL5 (RANTES), CXCL16, and other genes in mouse embryonic fibroblasts (62). Recently, we found that ATF3 regulates the expression of IL-1β, IL-6, TNF-α, and CCL2 (MCP-1) in the pancreatic islets upon stress stimulation (76). However, ATF3 upregulates, rather than downregulates, their expression. One implication is that ATF3 would be proinflammatory in this context, opposite from the anti-inflammatory function shown in other systems. Consistent with this idea, WT islets are more effective in recruiting macrophages than the ATF3 KO islets upon transplantation or in an in vitro recruitment assay (76). Because IL-1β, IL-6, and TNF-α can induce the expression of ATF3, this means that ATF3 forms a positive feedback loop with these cytokines to amplify them. The proinflammatory function of ATF3 is also implied by a finding using adipocytes, where ATF3 was shown to repress the expression of adiponectin (36). Adiponectin encodes a cytokine-like hormone with anti-inflammatory effects (53); thus, its downregulation by ATF3 points to a proinflammatory role of ATF3 (see more below). Table 2 summarizes these results.

IS MODULATION OF INFLAMMATION A KEY COMPONENT FOR THE ROLES OF ATF3 IN VARIOUS DISEASES?

Taken together, ATF3 modulates the expression of various genes regulating inflammatory response in both immune cells and nonimmune cells. Although much more investigation is required, based on the current literature we propose the following speculation on ATF3.

(a) One lesson from various studies involving immune cells is that they are a heterogeneous population of cells. As an example, macrophages were grouped into type 1 and type 2 macrophages (M1 vs. M2), depending on their gene expression (45). However, an emerging picture is that this M1/M2 polarization is an overly simplified framework. A more nuanced view is that macrophages are a heterogeneous population of cells with a continuum of functional states in which M1 and M2 represent the extremes of the spectrum (45). With this as a backdrop, we propose the following role of ATF3 in immune modulation. As a hub of cellular adaptive-response network and as a master transcription factor regulating a wide array of immune response genes, ATF3 is a key modulator of local and systemic inflammation in order to help cells respond/adapt to disturbance of homeostasis. Because it can positively or negatively regulate the functional state or bioactivity of various immune and nonimmune cells, the net effect of ATF3 will be complex. Thus, although characterizing ATF3 as pro- or anti-inflammatory has its utility, it is likely to be too simplistic. A more nuanced view is that ATF3 is an immune modulator that functions in a context-dependent manner. It may be pulling the immune response in all different directions and could paradoxically enhance and inhibit inflammation simultaneously by regulating various target genes. The final readout or functional consequence to the host will differ depending on the nature of the stress conditions (such as the type and duration of the stress) and the target tissue/cell types that the stress signals affect.

(b) Low-grade, chronic systemic inflammation has been associated with many diseases, especially chronic diseases such as obesity/metabolic syndrome, atherosclerosis, heart disease, arthritis, cancer, and Alzheimer's disease (6,10). Interestingly, a survey of the literature indicates that ATF3 is implicated in a diversity of diseases based on the following criteria: (i) deficiency (knockout mice) or ectopic expression (transgenic mice or injection models) of ATF3 causes disorders in animal models, and (ii) expression of ATF3 in human samples is associated with certain diseases. Tables 3 and 4 summarize some of these papers. Considering the importance of inflammation in disease development, we speculate that the ability of ATF3 to modulate inflammatory response genes—either in the immune cells or nonimmune cells—is a key component for the potential implication of ATF3 in various diseases.

FUTURE PERSPECTIVES

The cDNA for ATF3 was first isolated more than two decades ago (23). Despite its well-documented

TABLE 3
PLEIOTROPIC EFFECTS OF ATF3 REVEALED BY MOUSE MODELS THAT MODULATE THE EXPRESSION OF ATF3

	Ref.
Gain-of-function	
Deleterious effects of ATF3 expression in various tissues	
Transgenic mice expressing ATF3 in the liver and pancreas (by the transthyrectin promoter) displayed liver dysfunction and defects in glucose homeostasis	2,3
Transgenic mice expressing ATF3 in the islets (by the PDX promoter) had small, abnormal islets and defects consistent with β-cell deficiency	27
Transgenic mice expressing ATF3 in the pancreatic β-cells (by the rat insulin promoter) had reduced β-cell mass and defects in glucose homeostasis	39
Transgenic mice expressing ATF3 in the heart (by the α-myosin heavy chain promoter) displayed conduction abnormalities and contractile dysfunction	52
Oncogenic effects of ATF3*	
Transgenic mice expressing ATF3 in the basal epithelial cells (by the bovine cytokeratin 5 promoter) developed epidermal hyperplasia, oral carcinoma, and mammary carcinomas (in biparous mice)	66,67
Ectopic expression of ATF3 in prostate cancer cells (AT2.1) increased their metastatic potential in a subcutaneous injection model using SCID mice	4
Ectopic expression of ATF3 in breast cancer cells (MCF10CA1a) increased their tumor initiation frequency as assayed by serial dilution combined with subcutaneous injection into nude mice	73
Ectopic expression of ATF3 in keratinocytes (HKC and SCC cells) reduced the expression of p53 and senescence-associated genes, and increased their tumorigenicity upon injection into the SCID mice	70
Ectopic expression of ATF3 in melanoma cells (B16F1) increased their lung colonization ability in an intravenous injection model using the syngeneic C57BL/6 mice	30
Antioncogenic effects of ATF3*	
Ectopic expression of ATF3 in colon cancer cells (HCT-116) reduced primary tumor growth in a subcutaneous injection model using nude mice	8
Loss-of-function	
Inhibition of overreactive inflammatory response in the organisms by ATF3 due to its immune suppression effects	
ATF3 KO mice demonstrated increased airway hyperresponsiveness and pulmonary inflammation in response to allergen	17
ATF3 KO mice demonstrated delayed recovery from an intranasal model of influenza A infection	68
ATF3 KO mice showed increased sensitivity to ventilator-induced lung injury	1
ATF3 KO mice displayed a hyperinflammatory response and rapidly succumb to death upon LPS-induced septic shock	19
Increased vulnerability of the organisms to infection by ATF3 due to its immune suppression effects	
ATF3 KO mice displayed decreased viral load in a MCMV infection model	57
Oncogenic effect of ATF3*	
Knockdown of ATF3 in Ras-transformed keratinocytes reduced the tumor-promoting effect of cyclosporin A, a calcineurin inhibitor	70
ATF3 antisense treatment of mice subcutaneously injected with colon cancer cells (HT29) resulted in reduced tumor growth and longer survival time	29
Antioncogenic effect of ATF3*	
Upon Ras transformation, immortalized ATF3 KO MEFs had enhanced tumorigenicity than the WT counterpart	44
Beneficial effects of ATF3 in the adaptation of β-cells to metabolic demands	
ATF3 KO mice displayed increased glucose intolerance upon high-fat diet feeding, presumably due to the decreased ability of their pancreatic β-cells to produce insulin and thus decreased ability to cope with higher metabolic demands	75

See text for the criteria for inclusion. The consequences of ATF3 expression are pleiotropic; in addition, they may vary depending on the context of the cell types and stress models. MEF, mouse embryonic fibroblasts.
*ATF3 can be either oncogenic or antioncogenic, depending on the models used. This dichotomy is reminiscent of the dichotomous roles of TGF-β in cancer development (11,46,56). Although the mechanisms behind the ATF3 dichotomy are not well understood, one potential mechanism is the degree of malignancy: in a pair of isogenic mammary epithelial cell lines, ATF3 enhances apoptosis in the untransformed cells, but protects the aggressive derivative cells from stress induced deleterious effects and enhances their cell motility and invasiveness in vitro (72).

induction by various stimuli and its function as a transcription factor, the mechanism of ATF3 actions—at the molecular level—is not well understood. Considering its emerging roles in immune response and its implication in many diseases, the mechanism of ATF3 actions is an important issue to address. Areas to investigate include the identification of important amino acid residues, the potential roles of posttranslational modifications, and ATF3-interacting proteins. Increasing our understanding in these areas can help us develop tools to modulate ATF3 and thus fine-tune its action. This, in turn, may help us design therapeutic agents to prevent or treat various diseases.

TABLE 4
IMPLICATION OF ATF3 IN HUMAN DISEASES

Disease	Association With ATF3	Ref.
Hypospadias	IHC of 48 human penile skin tissues revealed ATF3 upregulation in those with hypospadias	42
	Genotyping of 710 human patient samples revealed that the disorder is strongly associated with SNPs located within intron 1 of the *atf3* gene	5
Diabetes	IHC of pancreata from patients with type 1 or type 2 diabetes indicated elevated expression of ATF3 in the pancreatic β-cells	27
Cancer		
Prostate	IHC of 64 human prostate cancers revealed a strong positive correlation between nuclear ATF3 expression and bone metastasis	4
	IHC of 39 human prostate cancers revealed no ATF3 expression in benign samples and strong ATF3 expression in all malignant samples	55
Breast	Immunoblot analysis of 48 human breast cancers revealed that ATF3 expression is higher in the tumor than in the adjacent nontumor tissue in ~50% of the cases	72
	IHC of 127 tumor microarray cores showed strong nuclear stain of ATF3 in ~18% of the samples	67
SCC	IHC of tissue microarrays from patients with cutaneous SCCs showed elevated expression of ATF3 by the calcineurin inhibitor cyclosporin A, an agent that is commonly used to treat organ transplant patients but significantly increases cancer risk	70
Hodgkin lymphoma	IHC of 415 human lymphoma samples revealed that ATF3 is strongly expressed in almost all cases of classical Hodgkin lymphoma, but rarely in all other lymphomas assessed	32

IHC, immunohistochemistry; SCC, squamous cell carcinoma; SNP, single nucleotide polymorphish.

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