Transcriptional Repressor Activating Transcription Factor 3 Protects Human Umbilical Vein Endothelial Cells from Tumor Necrosis Factor-\(\alpha\)-induced Apoptosis through Down-regulation of \(p53\) Transcription*

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Activating transcription factor 3 (ATF3) is a transcriptional repressor that is rapidly induced in cells exposed to a wide range of stress stimuli. To clarify the role of ATF3 in determining cell fate, we overexpressed it in human umbilical vein endothelial cells (HUVECs) by adenovirus-mediated gene transfer. ATF3 protected these cells from tumor necrosis factor (TNF-\(\alpha\))-induced apoptosis, as measured by flow cytometric analysis, trypan blue exclusion assay, and cleavage of procaspase 3 and poly(ADP-ribose) polymerase. Northern blot and nuclear run on assay showed that the transcription of tumor suppressor gene \(p53\) was down-regulated in the ATF3-overexpressing cells. In the transient expression assay, ATF3 suppressed the \(p53\) gene promoter activity through its specific binding to an atypical AP-1 element, PF-1 site, in the \(p53\) gene promoter. Furthermore, the cell-protecting effect of ATF3 was remarkably reduced in \(p53\)-deficient cells. These results demonstrate that overexpression of ATF3 suppresses TNF-\(\alpha\)-induced cell death of HUVECs, at least in part, through down-regulating the transcription of \(p53\) gene. ATF3 may function as a cell survival factor of endothelial cells during vascular inflammation and atherogenesis.

Non-adhesive and non-thrombotic feature of vascular endothelial surface is essential to maintaining physiological homeostasis of vascular function. When this function is perturbed, a cascade of thrombogenic and atherogenic reaction may occur. Injured endothelial cells become procoagulant and promote the formation of thrombi. They also attract phagocytes and produce cytokines and growth factors that act on adjacent vascular cells to promote their growth. Thus, endothelial cell death may play a pivotal role in the pathogenesis of vascular diseases such as vascular inflammation, thrombosis, and atherosclerosis (1).

Tumor necrosis factor (TNF)-\(\alpha\) is a cytokine produced by many cell types, including macrophages, monocytes, lymphoid cells, and fibroblasts, in response to inflammation, infection, and various environmental stimuli (2, 3). This cytokine is associated with a variety of cellular defense responses and activation of beneficial and cell-protecting genes. Simultaneously, TNF-\(\alpha\) also causes a myriad of lethal effects such as septic shock, tissue injury, inflammation, and cachexia, and these effects may be associated with apoptosis in susceptible cells. Although intact endothelial cells are rather resistant to TNF-\(\alpha\), they clearly undergo apoptotic cell death when exposed to TNF-\(\alpha\) (4–6). This becomes more significant in the presence of a low amount of RNA or protein synthesis inhibitors such as actinomycin D (5, 6). Thus, TNF-\(\alpha\) is considered to play roles in the pathogenesis and progression of vascular diseases. In TNF-\(\alpha\)-induced apoptosis, factors such as TRADD, FADD/MORT, RIP, FLICE/MACH, and TRAF5's associate with cell surface receptor TNFR1 in initiating the TNF-\(\alpha\)-induced signaling pathway (7, 8). In contrast, downstream events connecting the initial receptor binding and the final apoptotic process are relatively unknown, although the sphingomyelin pathway and ceramide are reported to be involved.

Tumor suppressor protein \(p53\) is activated in response to DNA damage or a wide range of stress stimuli. This leads to growth arrest and provides sufficient time for cells to repair damage. Alternatively, \(p53\) can trigger apoptosis and thus eliminate cells that have been damaged beyond repair. In TNF-\(\alpha\)-induced apoptosis, \(p53\) is also implicated in the death process. For instance, \(p53\) is involved in cytotoxic activity of TNF-\(\alpha\) in c-Myc-expressing cells (9), and its loss of function is associated with resistance of MCF7 human breast carcinoma cells to TNF-\(\alpha\) (10). Furthermore, \(p53\) is activated in TNF-induced apoptosis of human promonocytic cells (11) or ME-180 cells (12). Many studies show that translational and post-translational events are major regulatory steps in \(p53\) activation (13). Recently, however, it has been shown that \(c-jun\) null cells express elevated levels of \(p53\) mRNA, and reintroduction of a constitutive \(c-jun\) allele represses \(p53\) transcription (14, 15). Furthermore, AP-1 repressor protein JDP-2 inhibits UV-mediated apoptosis through down-regulation of \(p53\) (16). Therefore,
the transcriptional regulation of p53 also contributes to its accumulation in response to stress. However, at this moment, our knowledge regarding one or more transcription factors that control the expression of p53 gene in response to stress stimuli is rather limited.

Activating transcription factor 3 (ATF3), a member of the ATF/CREB subfamily, is a bZIP transcription factor (17, 18). It forms a homodimer that represses transcription from promoters with ATF sites (17), TNF-α-induced E-selectin gene expression (19), and arsenite-responsive activation of GADD153 gene (20). Heterodimers of ATF3 with c-Jun and JunB, on the other hand, activate transcription in transient transfection assays (21). ATF3 therefore can repress or activate target genes by forming homo- or heteromeric complexes. ATF3 is rapidly induced by ischemia-coupled reperfusion in heart and kidney (22, 23) and several stimuli such as anisomycin (17), anti-cancer drugs (24), genotoxic agents, or ionizing radiation (25), which can all induce cell cycle arrest and apoptotic cell death. We previously reported that ATF3 is rapidly induced in human vascular endothelial cells in response to TNF-α, oxidized low density lipoprotein, and homocysteine (26, 27). However, it remains unclear whether ATF3 functions as proapoptotic or antiapoptotic factor or which one or more target genes are regulated by ATF3 in this process.

In this study, we overexpressed ATF3 in human umbilical vein endothelial cells (HUVECs) by adenovirus-mediated gene transfer and found that ATF3 protected them from TNF-α-induced apoptosis. Furthermore, ATF3 was shown to repress transfer and found that ATF3 protected them from TNF-α regulated by ATF3 in this process.

Whole Cell Extract Preparation and Western Blot Analysis—HUVECs (3 × 10⁵ cells) treated as indicated were washed in PBS, resuspended in 50 μl of lysis buffer (50 mM Heps-KOH, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1.5 mM MgCl₂, 1 mM EDTA, 0.1% SDS, 10 μg/ml leupeptin and aprotinin, 200 μg/ml sodium vanadate, 100 μg/ml NaF, and 10% glycerol), and incubated on ice for 10 min. The cells were collected by centrifugation at 10,000 × g for 10 min, and the supernatant was then taken as whole cell extract. The amounts of protein were measured by Lowry method using bovine serum albumin as standard (29). Whole cell extracts (20 μg of protein) were separated on an SDS-PAGE, transferred onto a nitrocellulose membrane, and subjected to Western blot using the protocol of ECL kit (Amersham Biosciences) as previously described (22).

RNA Isolation and Northern Blot Analysis—Total RNA was isolated by the guanidinium method using IsoGen (Nippon Gene, Japan), fractionated on a formaldehyde-agarose gel, transferred to a Hybond-N membrane, and hybridized to random-primed cDNA probe for the human p53 gene as described (27). The membrane was exposed and analyzed by Bas 2500 Bio-image analyzer (Fujiﬁlm Co., Tokyo, Japan). DNA fragment for the human p53 cDNA, 2.0 kb, was radiolabeled with [α-32P]dATP (6000 Ci/mmol, Amersham Biosciences) using a random primer-labeling kit from Takara and used as probe.

Nuclear Run-on Assay—HUVECs (1 × 10⁶ cells) were infected with AdATF3 or AdLacZ viruses for 48 h, and their nuclei were prepared and frozen in liquid nitrogen as described previously (30). Elongation of nascent RNA chains was initiated by mixing the nuclei with 100 μl of reaction buffer (10 mM Tris-HCl, pH 8.0, 5 mM MgCl₂, 300 mM KCl, 0.5 mM each of ATP, CTP, and GTP; and 100 μl of [α-32P]UTP (3000 Ci/mmol, Amersham Biosciences) and incubating at 30 °C for 30 min. RNA synthesis was terminated by incubating with 5 μg/ml RNase-free DNase I (Roche Molecular Biologicals) at 30 °C for 10 min. The mixture was then digested with proteinase K (200 μg/ml) in 10 mM Tris-HCl, pH 7.4, 5 mM EDTA, and 1% SDS at 50 °C for 1 h, and radiolabeled nuclear transcripts were separated from unincorporated nucleotides on Sephadex G-50 column equilibrated with 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.5 mM NaF, and 10% glycerol. The labeled RNA was boiled for 3 min, chilled on ice, and hybridized to human glyceraldehyde-3-phosphate dehydrogenase cDNA, human p53 cDNA, and plasmid pBR322 (10 μg each) immobilized on a Hybond-N membrane at 42 °C for 48 h. The membrane filters were analyzed by Bas 2500 Bio-image analyzer (Fujiﬁlm).

Luciferase Assay—Reporter plasmid containing a 2.4-kb fragment of the human p53 gene promoter, pLuc-2400, was as described (30). The 5′-deletion mutants of reporter genes were prepared by PCR-amplifying and subcloning of DNA fragments from −460, −290, and −110 to +6 from the XhoI and BgII sites of pICGurance pGVB2 (Toyobo, Osaka, Japan), to produce pLuc-460, −290, and −110, respectively. pLuc-460mFP-1 and pLuc-460mCRE-BPI containing the mutant FP-1 site from −184 to −177 and the mutant CRE-BPI site from −402 to −395, respectively, were prepared from pLuc-460 using a G-C/Challenger mutagenesis kit from Stratagene (La Jolla, CA). The mutant FP-1 and CRE-BPI sites contained the sequence of 5′-TGAAATTC-3′ and 5′-TTTGGGAA-3′ (mutated nucleotides underlined) compared with wild type FP-1 sequence, 5′-TGACTCTC-3′, and CRE-BPI sequence, 5′-TTCAGGAA-3′, respectively. HUVECs (3 × 10⁵ cells in a 35-mm dish) were transfected with 1 μg of human p53 reporter plasmid along with pCI-Neo and Actinomycin D (10 μg/ml) using Lipofectamine. Cells were harvested and washed once in phosphate-buffered saline, and cell extracts were assayed for basal p53 promoter activity. For the activated p53 promoter activity, cells were further stimulated by 10 ng/ml TNF-α in the presence of 0.1 μg/ml actinomycin D for another 10 h. Both ﬁreﬂy and sea pansy luciferase activities were measured using a dual luciferase reporter assay system according to the manufacturer’s protocol (Promega, Madison, WI), pRL-TR (Toy Ink, Tokyo, Japan) containing the sea pansy luciferase.
ATF3 Protects HUVECs from TNF-α-induced Cell Death—To study the functional role of ATF3 in endothelial cell apoptosis, we expressed ATF3 in HUVECs by infecting cells with adenoviral expression vector AdATF3. Fig. 1 showed that the increasing titer of AdATF3 expressed ATF3 protein in a dose-dependent manner, while AdLacZ, which encoded β-galactosidase, did not express ATF3 and served as control. The expression of ATF3 did not change the cell viability for at least 4 days after infection, suggesting that overexpression of ATF3 by itself was not capable of causing apoptosis. We then treated HUVECs by TNF-α in the presence of actinomycin D, under which HUVECs undergo significant apoptotic cell death (5, 6). This treatment caused a rapid induction of ATF3 expression as significantly as AdATF3 (Fig. 1 and Ref. 26). Next, the effect of ATF3 expression on TNF-α-induced apoptosis was examined. As shown in the upper panel of Fig. 2A, FACS analysis revealed that the number of cells at the subG1 region, i.e. hypodiploid, increased after treatment with TNF-α for 6 and 18 h in the AdLacZ-infected cells. Under this condition, overexpression of ATF3 remarkably suppressed the number of subG1 cells (Fig. 2A, lower panel). This inhibition of apoptosis was observed by 3 times higher level of ATF3 than in the TNF-treated HUVECs (compare the ATF3 expression at m.o.i. of 50 with that at 6 h of TNF-treated cells in Fig. 1). These data indicate that ATF3 may protect HUVECs from TNF-α-induced cell death.

ATF3 Prevents Decrease of Cell Viability in TNF-α-stimulated HUVECs—Next, we further examined the effect of ATF3 on cell viability in the TNF-α-treated HUVECs. For this purpose, HUVECs infected with AdATF3 or AdLacZ were treated with TNF-α, and their viability was determined. As shown in Fig. 2B, treatment with increasing amounts of TNF-α decreased cell viability of the control and AdLacZ-infected HUVECs, as measured by trypan blue exclusion assay. On the other hand, overexpression of ATF3 significantly prevented the decrease of cell viability. These cells were also examined on microscopy. Most of the uninfected control and AdLacZ-infected HUVECs exhibited a round shape change and became detached from the dish (Fig. 2C, panels d and f), indicative of cell death. In contrast, the AdATF3 cells retained the normal shape and remained attached to the dish (Fig. 2C, panel e). Thus, it is indicated that ATF3 prevents the decrease of cell viability of HVECs induced by TNF-α.

Activation of Caspase 3 and Cleavage of PARP Is Suppressed in AdATF3-infected HUVECs—The caspase family of cysteine proteases is implicated in the apoptotic process of numerous cells, and its target substrate such as PARP is proteolytically cleaved. To obtain biochemical evidence for the protective effect of ATF3 in the TNF-α-induced apoptosis, we examined the activation and cleavage of procaspase 3 and PARP in HUVECs that overexpressed ATF3. Fig. 3 (upper panel) showed that procaspase 3 was activated to yield a 16-kDa fragment in the uninfected control and AdLacZ-infected cells after exposure to TNF-α, whereas its activation was suppressed in the AdATF3-infected cells (upper panel). PARP was cleaved after TNF-α treatment in the control and AdLacZ cells and this cleavage was also suppressed in the ATF3-expressing cells (Fig. 3, lower panel). These data indicate that ATF3 suppresses the activation and cleavage of procaspase 3 and PARP in the TNF-α-stimulated HUVECs.

Transcription of p53 Gene Is Down-regulated in AdATF3-infected HUVECs—The data above suggested that the expression of proapoptotic or antiapoptotic genes may be affected by ATF3, which leads to suppression of endothelial cell death in response to TNF-α. As a first step to elucidate the mechanism of action of ATF3, we examined for such genes whose regulation is correlated with inhibition of apoptosis. Fig. 4A showed that p53 mRNA was significantly down-regulated in the AdATF3-infected HUVECs, although it was not affected in the AdLacZ cells. In Fig. 4B, we performed nuclear run-on assay to determine whether the reduced level of p53 mRNA was due to the decreased transcription. Results showed that the p53 transcription was significantly suppressed in the AdATF3-infected cells. This effect appeared to be specific, because AdLacZ cells showed no such suppression and
the activity of glyceraldehyde-3-phosphate dehydrogenase gene transcription was comparable between these cells. These data clearly shows that overexpression of ATF3 down-regulates p53 mRNA at the level of transcription.

**Activation of p53 in Response to TNF-α Is Suppressed in AdATF3-infected HUVECs**—We next studied the expression of p53 mRNA of the AdATF3-infected HUVECs in response to TNF-α. Fig. 5A showed that p53 mRNA was induced by ~2.3
fold in the AdLacZ cells at 2 h post-stimulation by TNF-α. In the AdATF3-cells, basal level of p53 mRNA was remarkably reduced, and its activation was almost abolished at 2 h post-stimulation, although it appeared to slightly increase at 24 h post-stimulation. The activation of p53 protein of these cells was also examined. As shown in Fig. 5B, p53 protein was rapidly accumulated in the AdLacZ cells in response to TNF-α. In the AdATF3 cells, both basal and stimulated level of p53 protein was significantly reduced compared with that of AdLacZ cells. However, the extent of activation by TNF-α appeared comparable between AdLacZ and AdATF3 cells, 2.98- and 2.85-fold, respectively, suggesting that stability of p53 protein was not affected by ATF3. These data together indicate that the expression of ATF3 down-regulates both basal and stimulated level of p53 mRNA and this leads to decreased activation of p53 protein.

**ATF3 Down-regulates the Promoter Activity of p53 Gene in Reporter Assay**—Transcriptional regulation of p53 gene contributes to its activation in response to stress stimuli (14–16). Because the data above suggested that p53 gene transcription might be regulated by ATF3, we transfected the human p53 reporter gene, pLuc-2400, into HUVECs and examined whether ATF3 affected the p53 promoter activity. Fig. 6A showed that the promoter activity of pLuc-2400 was suppressed by co-expression of ATF3. Reporter plasmids pLuc-460 and pLuc-290 that deleted 5′-upstream sequence to −460 and −290, respectively, also exhibited the similar degree of repression by ATF3. In contrast, pLuc-110 almost abrogated the ATF3-dependent repression. Computer analysis of sequence from −460 to −110 revealed two candidate elements for ATF3 binding, CRE-BP1 and PF-1 sites, respectively. We therefore prepared mutant reporter gene for each site and assayed the promoter activity. Fig. 6A showed that the CRE-BP1 mutant was repressed by ATF3 as much as wild type reporter gene pLuc-2400, whereas the PF-1 mutant significantly diminished the ATF3-dependent repression. These data altogether indicated that the p53 promoter activity was repressed by ATF3, at least in part, through the PF-1 site of the 5′-upstream region. We next performed reporter assay in the presence of TNF-α. As shown in Fig. 6B, the promoter activity in the presence of TNF-α was inhibited by ATF3 in a dose-dependent manner,
whereas the inhibitory effect of ATF3 was significantly reduced with reporter gene containing the mutant PF-1 site. It was not apparent whether ATF3 affected the TNF-activated promoter activity, because the TNF-α-dependent component of promoter activity was extremely modest in this assay. These results indicate that the promoter activity of p53 gene, both in the absence and presence of TNF-α, is suppressed by ATF3 in a PF-1 site-dependent manner.

**ATF3 Is a Component of Complex(es) Recognizing PF-1 Site in AdATF3-infected HUVECs and Directly Binds to PF-1 Sequence**—The data above suggested that ATF3 could recognize the AP-1-like PF-1 sequence of p53 gene promoter, since ATF3 specifically binds to AP-1 site (33). Thus, we performed an electrophoretic mobility shift assay of the AdATF3-infected HUVECs. Fig. 7A showed that nuclear extracts of the AdATF3-cells produced at least three bands, a major band C and more slowly migrating bands A and B (lane 2). These complexes was also detected in AdLacZ-cells (lane 5) and appeared specific for PF-1 sequence, because wild type, but not mutant type PF-1 sequence, significantly abolished the formation of these complexes (lanes 9–12). Under this condition, anti-ATF3 antibody suppressed the formation of the bands A and B, and produced the supershifted band (lane 3), although the band C was less affected. This was not observed when control IgG was used (lane 4) or nuclear extract from the AdLacZ-cells was assayed (lanes 6 and 7). These data indicate that the PF-1 sequence binds a heterogeneous group of factors in HUVECs and that ATF3 appears to be recruited into bands A and B in AdATF3-cells. Next, we examined whether ATF3 could directly bind to PF-1 motif using the bacterially expressed recombinant GST-ATF3 protein. As shown in the left panel of Fig. 7B, GST-ATF3 protein produced band A (lane 2), whereas GST protein did not (lane 3). The band was specifically competed out with wild type but not with mutant PF-1 oligonucleotide (lanes 6 and 7).

Under this condition, anti-ATF3 antibody, but not control IgG, specifically inhibited the band formation and produced a supershifted band (lanes 4 and 5). These data indicate that the ATF3 protein is capable of directly binding the PF-1 motif of the human p53 gene promoter, and it is recruited into the DNA-protein complexes formed at PF-1 site in the ATF3-overexpressing HUVECs.

**ATF3 Suppresses TNF-α-induced Apoptosis of Cells with Wild Type p53 Allele, but Not Cells with p53 Null Allele**—Data above strongly suggested that the protection from TNF-α-induced apoptosis by ATF3 was partly mediated through down-regulation of p53 transcription. To examine whether this repression was important for the protection from apoptosis in vivo, we overexpressed ATF3 in p53-deficient Saos-2 cells and examined its effect on the TNF-α-induced apoptosis. Fig. 8A showed that TNF-α caused a significant degree of apoptosis of control and AdLacZ-infected cells. Under this condition, the AdATF3-infected cells also showed the similar extent of apoptosis, indicating that the inhibitory effect of ATF3 was almost abrogated in p53-deficient Saos-2 cells. As control, we also examined the effect of ATF3 in MCF-7 cells with wild type p53 allele (lower panel). Result showed that ATF3 significantly suppressed the TNF-α-induced apoptosis, when compared with the control and AdLacZ-infected cells. These data strongly indicates that the protection from TNF-α-induced apoptosis by ATF3 is mediated by p53 and this mechanism plays a functional role in vivo.

![Fig. 5. p53 activation in response to TNF-α is suppressed in AdATF3-infected HUVECs.](http://www.jbc.org/Downloadedfrom)
DISCUSSION

In this report, we demonstrated that transcriptional repressor ATF3 prevented HUVECs from programmed cell death induced by TNF-α. This effect was, at least in part, due to down-regulation of p53 transcription.

ATF3, a member of ATF/CREB subfamily of bZip transcription factors, is unique in that it represses the transcription of several genes in an ATF/CRE- or AP-1-dependent manner (17, 19, 21, 33). In the present study, ATF3 specifically bound the PF-1 motif of the p53 gene promoter and repressed its transcription (Figs. 6 and 7). The PF-1 sequence, 5'-TGACTCT-3', is conserved between mouse and human p53 gene promoter and differs in only one base from the consensus AP-1 motif 5'-TGACTCA-3' (31, 32, 34). Although this site is originally reported to be recognized by one or more factors other than AP-1 (31), we and others observed that c-Jun and c-Fos, therefore AP-1, could bind to PF-1 site with much less affinity than to AP-1 motif (data not shown) (16). ATF2, which is capable of forming heteromeric complex with ATF3, may also bind to PF-1 motif, because it binds to atypical AP-1 sequence of c-Jun gene promoter (35). It is further reported that AP-1 repressor JDP-2, highly homologous protein to ATF3, binds to the PF-1 site (16, 36). Thus, a group of factors can bind to PF-1 site. This might be related to the fact that several bands were detected in gel shift assay of the ATF3-overexpressing cells, and some of them were supershifted by anti-ATF3 antibody (Fig. 7A). However,

![Diagram](http://www.jbc.org/)
FIG. 7. ATF3 binds to PF-1 sequence of p53 gene promoter. A, nuclear extract was prepared from HUVECs infected with AdATF3 or AdLacZ for 48 h, and equal amount of proteins (2 μg) were employed for gel mobility shift assay using PF-1 sequence of the human p53 gene promoter as under "Experimental Procedures." Nuclear extract from the AdATF3 (lanes 2-4) or AdLacZ (lanes 5-7)-infected cells were assayed in the absence (lanes 2 and 5) or presence of anti-ATF3 antibody (lanes 3 and 6) or control IgG (lanes 4 and 7). Gel shift assay was also performed using nuclear extract from AdATF3-infected cells in the absence (lane 8) or presence of 50 and 100 times molar ratio of wild (lanes 9 and 10) or mutant (lanes 11 and 12) PF-1 oligonucleotide. Lane 1 shows probe only. Bands A and B represent specific complexes, and n.s. denotes nonspecific bands. The arrow indicates the supershifted band by anti-ATF3 antibody. B, GST-ATF3 protein was expressed and purified as under “Experimental Procedures.” In the right panel, GST and GST-ATF3 protein were analyzed by Western blot using anti-ATF3 (lanes 1 and 2) or anti-GST (lanes 3 and 4) antibody, respectively. In the left panel, GST-ATF3 (1 μg of protein) was incubated with radiolabeled PF-1 probe in the absence (lane 2) or presence of anti-ATF3 antibody (lane 4), control IgG (lane 5), 50 times molar amount of wild (lane 6), and mutant PF-1 oligonucleotide (lane 7). GST protein (1 μg of protein) was also assayed as control (lane 3). Lane 1 shows the probe only. Band A represents specific complex, and n.s. denotes nonspecific band. The arrow indicates the supershifted band formed in the presence of anti-ATF3 antibody.
at this moment, the nature of the PF-1 complex(es) responsible for ATF3-dependent repression of \( \text{p53} \) gene transcription remains unclear.

It is well established that the activation of \( \text{p53} \) is primarily regulated at post-translational level, by modulating the phosphorylation (37, 38), acetylation (39, 40), and protein degradation through the ubiquitin-proteasome system (41, 42). However, several studies support that the transcriptional control is also important in regulating the \( \text{p53} \) activity (14–16). Our demonstration that ATF3 down-regulated \( \text{p53} \) expression may also support the functional importance of transcriptional control. In the present study, 3 times higher amount of ATF3 than induced by TNF-\( \alpha \) was required to significantly suppress the \( \text{p53} \) transcription and TNF-induced apoptosis (Figs. 1, 2, and 4). Under this condition, \( \text{p53} \) protein was less down-regulated than \( \text{p53} \) mRNA (Fig. 5). These data may argue against the physiological role of ATF3 in vivo. However, the effect of ATF3 was significantly reduced in Saos-2 cells with \( \text{p53} \) null allele (Fig. 8), providing evidence that the cell-protecting effect of ATF3 was dependent on \( \text{p53} \) activity in vivo. Furthermore, the lower amount of ATF3 could suppress \( \text{p53} \) mRNA (Fig. 4A) and apoptosis (data not shown). Thus, it is possible that ATF3 may have a functional role in protecting or modifying the cell fate-determining process in vivo. The present study does not exclude the possibility that ATF3 down-regulates the trans-activating capacity of \( \text{p53} \). In this regard, it is intriguing to note that ATF3 directly binds \( \text{p53} \) and represses the \( \text{p53} \)-dependent trans-activation of the collagenase gene promoter (43). In their study, luciferase reporter gene activity driven by \( \text{p53} \)-response elements was also repressed by ATF3, providing the possibility that ATF3 could regulate a wide spectrum of \( \text{p53} \)-dependent gene transcription. These findings may further implicate the dual function of ATF3 in down-regulating the \( \text{p53} \) activity; one is due to the suppression of \( \text{p53} \) gene transcription, and the other is through antagonizing \( \text{p53} \)-dependent gene transactivation. This may function as a safety device in the tight regulation of the \( \text{p53} \) activity, ensuring to prevent the unscheduled or exaggerated activation of cell death signal.

It is argued that ATF3 has detrimental effects on determining cell fate in response to environmental stimuli (22, 23, 33). More recently, it is reported that transgenic mice expressing ATF3 in the pancreas and heart have malfunctions in the target tissues, developing symptoms characteristic of insulin-dependent diabetes (44) and conduction abnormality and contractile dysfunction (45), respectively. Therefore, ATF3 appears to be a part of the cellular response that leads to detrimental outcomes. However, ATF3 expression prevented HUVECs from TNF-\( \alpha \)-induced apoptosis in this study, in which ATF3 was overexpressed without any cellular context. Because ATF3 is immediately induced with concomitant response of a wide range of stress genes, the effect of ATF3 in this study may be different from that in vivo and awaits further investigation.

The activation of c-Jun NH\(_2\)-terminal kinase (JNK) contributes to cell survival response to stress signals, whereas this
and its expression is significantly induced in HUVECs. Its expression is correlated with apoptotic cell death of endothelial cells. What is the in vivo significance of protection of cell death in the ATF3-overexpressing HUVECs? We previously reported that ATF3 is expressed in atherosclerotic lesions, and its expression is significantly induced in HUVECs exposed to TNF-α, oxidized low density lipoprotein, and lysophosphatidylcholine (26). It is also reported that activation of JNK coincides with p53 in vascular cells of arteriosclerotic lesions (49), suggesting the close association of the activation of JNK and p53 in the pathogenesis of vascular diseases. Therefore, JNK and its downstream gene ATF3 are likely to be functionally implicated in determining cell fate of vascular cells. We speculate that ATF3 may protect endothelial cells from apoptosis induced by various stimuli and function as a cell survival factor during the pathogenesis and progression of vascular inflammation and atherosclerosis.

In summary, transcriptional repressor ATF3 prevented endothelial cells from TNF-α-induced apoptosis. This activity was, at least in part, due to down-regulation of the p53 gene transcription through the specific binding of ATF3 to PF-1 motif in the p53 gene. ATF3 may play a role in pathogenesis of vascular diseases and represent a novel therapeutic target in treating and preventing those diseases.

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