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Absence of typical unfolded protein response in primary cultured cystic fibrosis airway epithelial cells ☆

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Abstract

We examined whether the unfolded protein response is activated in cells expressing incorrectly folded cystic fibrosis transmembrane conductance regulator. Airway epithelial cells from three control and three CF patients homozygous for the ΔF508 mutation were tested. There were no differences in protein expression of the pro-apoptotic factor C/EBP homologous protein (CHOP) or the endoplasmic reticulum (ER) chaperone binding Ig protein. Nor were there differences in phosphorylation of protein kinase R-like ER kinase or eukaryotic initiation factor-2α, or the splicing of X-box binding protein (XBP)-1. However, CF cells showed increased mRNA expression of CHOP and XBP-1. A proteasome inhibitor increased CHOP expression in CF cells, suggesting that enhanced proteasome activation is responsible for the observed post-transcriptional regulation. Finally, CF cells were resistant to apoptosis, suggesting that post-transcriptional regulation of CHOP prevents apoptosis. While CHOP and XBP-1 mRNA expression is increased in CF cells, the classic UPR is not present.

Keywords: ATF6; BiP; CHOP; Endoplasmic reticulum; GADD153; PERK; XBP1

Deletion of phenylalanine at position 508 (ΔF508) produces an incorrectly folded cystic fibrosis transmembrane conductance regulator (CFTR) that accumulates in part in the lumen of the endoplasmic reticulum (ER). Expression of ΔF508 in COS-7 cells yields an incompletely glycosylated protein which accumulates in the ER and is subsequently degraded [1]. CFTR is distributed diffusely throughout the ER in the surface epithelial cells of human bronchial xenografts transduced with adenoviral vector expressing ΔF508 [2]. In pancreatic adenocarcinoma cells naturally expressing ΔF508, CFTR has been shown to accumulate in both the ER-Golgi intermediate compartment as well as the ER [3].

Accumulation of unfolded proteins in the ER lumen induces the transcription of a large set of genes whose products increase the volume of the ER or its capacity for protein folding, promote the degradation of misfolded proteins through the process of ER-associated protein degradation (ERAD), and promote apoptotic cell death. This homeostatic process, the unfolded protein response (UPR), is initiated by binding of the most abundant ER chaperone protein, binding Ig protein/glucose-regulated protein of molecular weight 78 kDa (BiP/GRP78), to hydrophobic exposed patches on the surfaces of unfolded proteins and interactive sites on unassembled protein

☆ *Abbreviations:* CFTR, folded cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; UPR, unfolded protein response; CF, cystic fibrosis; BiP/GRP78, binding Ig protein/glucose-regulated protein of molecular weight 78 kDa; CHOP/GADD153, C/EBP homologous protein/DNA damage-inducible gene 153; PERK, protein kinase R-like ER kinase; eIF, eukaryotic initiation factor; XBP, X-box binding protein; IRE, inositol requiring; ATF, activating transcription factor; AARE, amino-acid-regulatory element; NF, nuclear factor; ERSE, ER stress response element.

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subunits. Binding to unfolded proteins displaces BiP's usual ER membrane binding partners, protein kinase R (PKR)-like ER kinase (PERK), the basic leucine-zipper type activating transcription factor (ATF)-6, and the kinase and endoribonuclease inositol requiring (IRE)-1α and β, each of which contains a luminal domain that interacts with BiP.

Activated PERK phosphorylates Ser^{51} on eukaryotic initiation factor (eIF)-2α, which in turn blocks the binding of the initiator Met-tRNA to the ribosome by inhibiting turnover of eIF2B, thereby reducing the influx of nascent proteins into the ER. However, under these conditions, the mRNA of activating transcription factor (ATF)-4, a transcription factor which regulates the expression of genes involved in amino acid metabolism, is efficiently translated by virtue of its cluster of upstream open-reading frames [4]. ATF4 binds both the consensus ATF/cAMP response element as well as the amino-acid-regulatory element (AARE) core sequence [5]. ATF6 is transported to the Golgi apparatus and cleaved by Site-1 and Site-2 proteases [6]. The released N-terminal cytosolic domain is transported to the nucleus where, in interaction with nuclear factor (NF)-Y, it binds and activates the ER stress response element (ERSE) [7]. Activated IRE1 cleaves the ATF6-inducible precursor X-box binding protein (XBP)-1 mRNA to form mature XBP1 mRNA, which encodes a transcription factor that can also bind the ERSE [8]. ATF4, ATF6, and XBP1 each regulate the expression of the pro-apoptotic transcriptional factor C/EBP homologous protein/DNA damage-inducible gene 153 (CHOP/GADD153), the promoter of which contains both AARE and ERSE transcriptional elements [5,7]. ERSEs are also present in the promoters of other UPR target genes, including BiP itself [9].

Given the key role of BiP in the UPR, misfolded proteins that do not bind BiP are unlikely to activate the UPR. Calnexin, another ER lumenal chaperone, and the cytosolic chaperones heat shock protein (Hsp)-70 and Hsp90 each associate with CFTR in mammalian cells [10–13], but association of CFTR with BiP has not been demonstrated [10–12]. Thus, ΔF508 is not thought to activate the UPR. Similarly, the insoluble Z allele of the α-1-antitrypsin (α-1-AT) gene, which produces a protein that polymerizes and is retained in the ER for degradation by the proteasome, neither binds BiP nor activates the UPR [14]. However, after treatment with additional stresses such as thapsigargin, which causes calcium depletion, and increased temperature, expression of Z α-1-AT increases BiP promoter activity, whereas expression of the M allele does not [15]. Thus, it is conceivable that under stressful conditions which further slow degradation of misfolded protein in the ER, misfolded proteins such as Z α-1-AT and ΔF508 CFTR could induce the UPR. We therefore sought to characterize the UPR in primary CF cells, elements of which have only recently been described [16].

Materials and methods

Cell culture. Airway segments from three non-CF donors and three CF patients homozygous for the ΔF508 mutation were collected at the time of double lung transplantation at the University Health Network (Toronto). Thawed passage 1 cells were seeded into 12-mm collagen-coated Transwell-clear inserts (Costar, Cambridge, MA). Cells were grown under submerged conditions in bronchial epithelial cell growth medium (BEGM, Clonetics) containing epidermal growth factor (EGF, 25 ng/ml), bovine pituitary extract (65 ng/ml), all-trans retinoic acid (5×10^8 M), bovine serum albumin (1.5 μg/ml), nystatin (20 IU/ml), hydrocortisone (0.5 μg/ml), insulin (5 μg/ml), transferrin (10 μg/ml), epinephrine (0.5 μg/ml), triiodothyronine (6.5 ng/ml), gentamicin (50 μg/ml), and amphotericin (50 μg/ml). At confluence, usually 5 days after plating, cells were shifted to a 1:1mixture of BEGM and Dulbecco's modified Eagle's medium containing all the above growth factors and hormones, and grown at air–liquid interface to promote mucociliary differentiation. The epithelium is pseudostratified with ciliated cells interspersed among mucus secreting cells [17]. Periodic acid–Schiff staining shows the presence of goblet cells and luminal glycoproteins, and immunostaining with antibody to tracheobronchial mucin has confirmed that the PAS-positive material is mucin. These cultures also show an acceptable transepithelial electrical resistance (500–540 Ω/cm^2). Cells were harvested for analysis after three weeks at air–liquid interface. In selected experiments, cells were incubated with thapsigargin or MG-132 (each from Sigma Chemical, St. Louis, MO).

Immunohistochemistry. Cultures were embedded in 2% agar, fixed in 10% buffered formalin overnight at 4 °C, dehydrated, and embedded in paraffin. Five micromolar thick sections were incubated with monoclonal antibody to the C-terminal domain of CFTR (1:100 dilution, R&D Systems, Minneapolis, MN). Bound antibody was detected by using a second antibody conjugated with biotin followed by streptavidin-Texas red. Sections were then examined under a fluorescence microscope. As we have shown previously [17], analysis of these cultures indicated the presence of CFTR at the apical surface of normal but not CF cells (Fig. 1), a characteristic of CF airway epithelia in vivo [18].

Immunoblotting. Primary antibodies used for immunoblotting were CHOP/GADD153, GRP78/BiP, calnexin, XBP-1 (Santa Cruz Biotechnology, Santa Cruz, CA), phospho-PERK, phospho-eIF2α (Cell Signaling Technology, Beverly, MA), hemagglutinin (HA, Covance, Berkeley, CA), ATF6 (generously provided by Dr. Randall Kaufman, University of Michigan), and CFTR (R&D Systems). Cell lysates were resolved by 10% SDS–PAGE and transferred to nitrocellulose by semidry transfer. After incubation with antibody, signals were amplified and visualized by enhanced chemiluminescence.

Northern blotting. Total RNA, prepared from primary cells using Trizol reagent, was electrophoresed on 1.25% SeaKem Gold Agarose gels (Reliant RNA Gel System, Cambrex, Rockland, MN) and then transferred onto Immobilin-NY Plus membranes (Millipore, Bedford, MA). Probe template was prepared from RNA by RT-PCR with the CHOP primers (sense) 5′-GCACCTCCCAGAGCCCTCTCC-3′ and (antisense) 5′-GTCTACTCCAAGCCTTCCCCCTGCG-3′; BiP primers (sense) 5′-ATGGTATTCTCCGAGTGACA-3′ and (antisense) 5′-TTGGCTTTAAAGTCTTCAAT-3′; and XBP1 primers (sense) 5′-CTGGAACAGCAAGTGGTAGAG-3′ and (antisense) 5′-CTGGGTCCTTCTGGGTAGAC-3′ (NM_005080). ^{32}P-radiolabeled probes were prepared by using the Ready-To-Go DNA labeling beads (-dCTP) (Amersham Pharmacia, Piscataway, NJ). Probe hybridization was performed with Ultrahyb buffer as recommended by the manufacturer (Amersham-Pharmacia).

RT-PCR analysis. Total RNA extracted from confluent primary cells was ethanol precipitated and treated with DNase I before RT-PCR. Each RT reaction was carried out by combining 5–10 μl of total RNA mix with 2 μl of 10× RT buffer, 3 μl dNTP mix, 2 μl of random primer, 1 μl of Moloney murine leukemia virus RT, and 1 μl of RNase inhibitor (42 °C for 1 h). The PCR was performed by combining 10 μl cDNA, 2 μl of

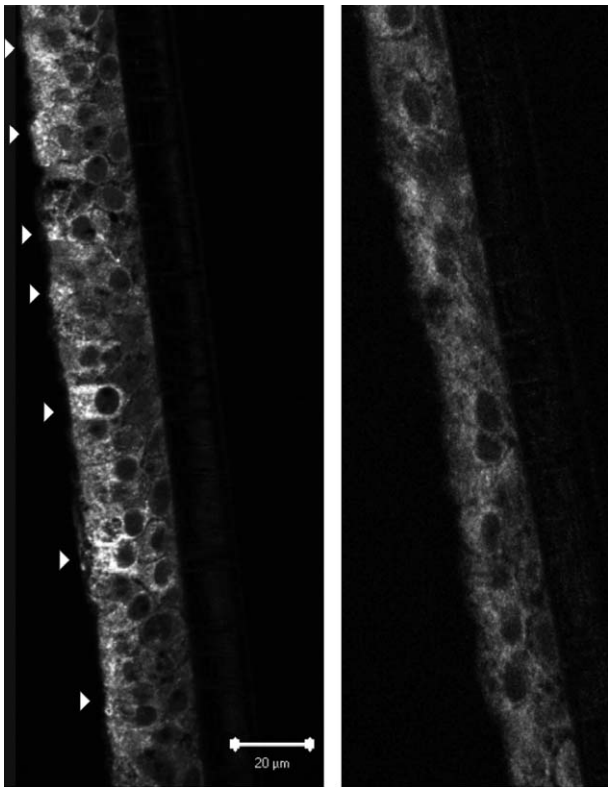

Fig. 1. Localization of CFTR detected with an antibody to the C-terminal domain indicated the presence of CFTR at the apical surface of normal (arrowheads, left panel) but not CF (right panel) epithelium.

primer mix (1 μl of each 5′ and 3′ primer), 5 μl of 10× buffer, 1 μl of 50× *Taq* DNA polymerase, and 5 μl of 10× dNTPs. PCR primers for human XBP1 were 5′-CTGGGTCCTTCTGGGTAGAC-3′ and 5′-CTGGGTCC TTCTGGGTAGAC-3′. Conditions for PCR consisted of incubation at 94 °C for 3 min; 30 cycles each of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and incubation at 72 °C for 7 min. Samples were analyzed by electrophoresis on a 2% agarose gel. GAPDH was used as a normalization marker.

Transfection of C38 airway epithelial cells. To facilitate identification of CHOP protein by immunoblotting, cDNA encoding human CHOP (provided by A. Fornace, National Cancer Institute, Bethesda, MD), was subcloned into pcDNA3.1/Zeo(+) and transiently transfected into C38 cells, an adeno12-SV40 immortalized human bronchial epithelial cell line derived from a CF patient (ΔF508/W1282X) which expresses an episomal copy of a truncated but functional CFTR (obtained from P. Zeitlin, Johns Hopkins University, Baltimore, MD) [19,20]. Cells were grown in LHC-8 (Biofluids, Rockville, MD) supplemented with 5% FBS. Cells were transiently transfected using Lipofectamine and Opti-MEM transfection medium (each from Invitrogen, Gaithersburg, MD), as described previously [21].

Similarly, to determine the electrophoretic mobility of ATF6, C38 cells were transiently transfected with cDNA encoding HA-tagged forms of cleaved (pCGN HA-ATF6 E.1) and uncleaved (pCGN HA-ATF6 1.373) ATF6α (provided by Ron Prywes, Columbia University) [22].

Detection of apoptosis. Primary control and CF cells were treated with thapsigargin (2 μM for 24 h), and apoptotic, non-necrotic cells detected by flow activated cell sorting with FITC-conjugated annexin V and propidium iodide (Apoptosis Detection Kit, Sigma Chemical).

Statistical analysis. Group data were expressed as means ± SEM. Statistical significance was assessed by analysis of variance (ANOVA).

Results

Protein expression of CHOP, BiP, and calnexin is not increased in CF airway epithelial cells

Primary airway cells from non-CF donor and CF patients homozygous for the ΔF508 mutation were obtained from lung transplant donor tracheas. Thawed passage 1 cells were grown at air–liquid interface to promote mucociliary differentiation. Cell lysates were resolved by 10% SDS–PAGE, transferred to nitrocellulose, and probed with antibodies against BiP, CHOP, and calnexin. There were no differences in BiP, CHOP or calnexin protein expression between normal and CF cells (Figs. 2A–C). As expected, treatment with thapsigargin, an inhibitor of sarcoplasmic/endoplasmic reticulum calcium (SERCA) pump which causes calcium release from the ER, increased BiP and CHOP expression in both normal and CF cells; however, the relative increase in CHOP protein expression was reduced in CF cells.

mRNA expression of CHOP, but not BiP, is increased in CF airway epithelial cells

BiP expression is tightly controlled at the post-transcriptional level [23]. To determine BiP and CHOP steady-state

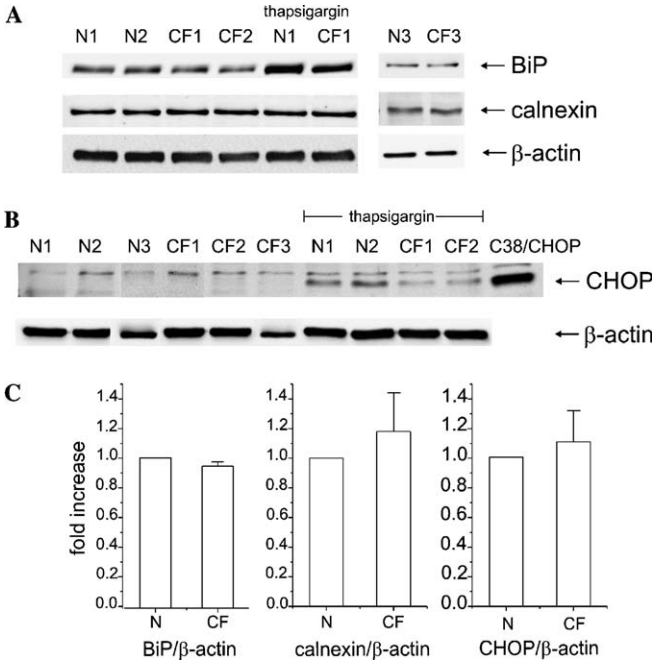

Fig. 2. Protein expression of BiP, calnexin, and CHOP in control and CF airway epithelial cells. Western blot analysis of total cellular extracts from normal (N1, N2, and N3) and CF (CF1, CF2, and CF3) primary airway epithelial cells grown in liquid–air interface for BiP and calnexin (A) and CHOP (B). Thapsigargin treatment (2 μM for 24 h) was used as a positive control to stimulate the unfolded protein response, and C38 cells overexpressing recombinant CHOP were used as a positive control for CHOP expression. (C) Group mean data for BiP, calnexin, and CHOP protein expression. Data were normalized to expression of β-actin ($n = 3$, means ± SD).

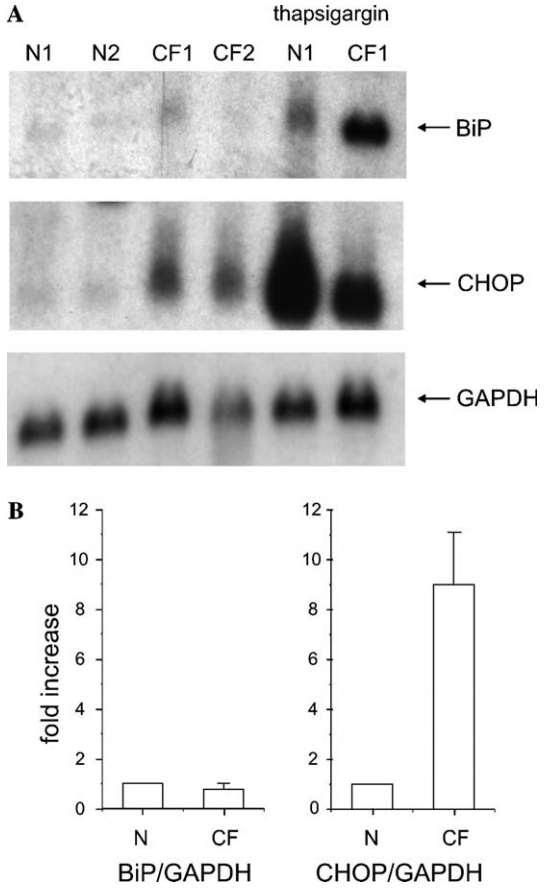

Fig. 3. mRNA expression of BiP and CHOP in control and CF airway epithelial cells. (A) Northern blot analysis of normal (N1 and N2) and CF (CF1 and CF2) primary airway epithelial cells grown in liquid–air interface for BiP and CHOP mRNA. Normal (N1) and CF (CF1) primary airway epithelial cells exposed to thapsigargin (2 μM for 24 h) were used as a positive control. (B) Group mean data for BiP and CHOP mRNA expression. Data were normalized to GAPDH mRNA expression ($n = 2$, means ± SD).

mRNA levels, total RNA from primary cells was resolved on an agarose gel, transferred to a nylon membrane, and probed using the appropriate ^{32}P-radiolabeled sequences (Fig. 3A). As with protein abundance, there was no difference in BiP steady-state mRNA level between CF and non-CF cells (Figs. 3A and B). However, CHOP mRNA was increased nearly 8-fold in primary CF cells relative to controls. Together with the above immunoblots (Fig. 2B), these data suggest that CHOP protein expression is blocked at the post-transcriptional level in CF cells.

Thapsigargin treatment substantially increased BiP and CHOP mRNA in both normal and CF cells. However, the CHOP mRNA response was greater in normal cells (Figs. 3A and B), consistent with the observed protein levels.

Absence of phosphorylation of PERK and eIF2α in CF airway cells

The UPR is classically associated with activation of PERK and Ser^{51} phosphorylation of eIF2α, which in turn

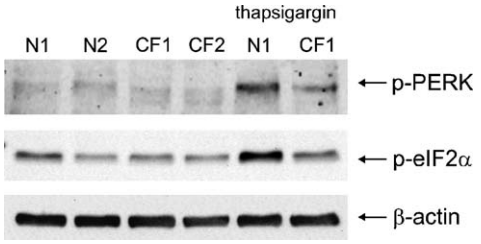

Fig. 4. Absence of phosphorylation of PERK and eIF-2α in CF airway cells. Western blot analysis of normal (N1 and N2) and CF (CF1 and CF2) airway epithelial cells for phospho-PERK (upper panel) and phospho-eIF2α (middle panel). Normal and CF airway cells exposed to thapsigargin (2 μM for 24 h) show phosphorylation of PERK and eIF-2α.

blocks mRNA translation and the influx of nascent proteins into the ER. Phosphorylation of PERK and eIF2α was monitored using the appropriate anti-phospho antibodies (Fig. 4). Phosphorylation of PERK and eIF2α was minimal in both CF and non-CF cells. As expected, treatment with thapsigargin increased phosphorylation of PERK and eIF2α.

XBP1 mRNA expression is increased in CF cells

In the classic UPR, activated IRE1 cleaves the ATF6-inducible precursor X-box binding protein (XBP)-1 mRNA to form mature XBP1 mRNA, which encodes a transcription factor that can bind the ERSE [8]. To determine whether XBP1 expression was increased in CF cells, we performed a Northern blot analysis of total RNA from primary cells (Figs. 5A and B). Steady-state XBP1 mRNA levels were increased in CF cells ($p = 0.033$, ANOVA). We were unable to identify XBP-1 protein using a commercially available antibody.

We sought to determine whether cleavage of XBP1 mRNA was altered in CF cells using RT-PCR. Thapsigargin treatment of both CF and non-CF cells appeared to induce the formation of a smaller XBP1 mRNA product which likely represents the cleaved form (Fig. 5C). However, we could not reliably identify the cleaved form in unstimulated CF or control cells.

Protein abundance of ATF6

In the canonical UPR, binding of BiP to unfolded proteins displaces ATF6, which is then transported to the Golgi apparatus and cleaved by Site-1 and Site-2 proteases [6]. The released N-terminal cytosolic domain is transported to the nucleus where, in interaction with NF-Y, it may bind and activate the ER stress response element (ERSE) [7]. To determine the precise electrophoretic mobility of cleaved and uncleaved ATF6, C38 cells were transfected with cDNAs encoding HA-tagged forms of these proteins, and cell lysates probed with an anti-HA antibody (Fig. 6). Immunoblots of primary cells from non-CF individuals appeared to show increased protein abundance of full-length ATF6 compared to CF cells. As expected,

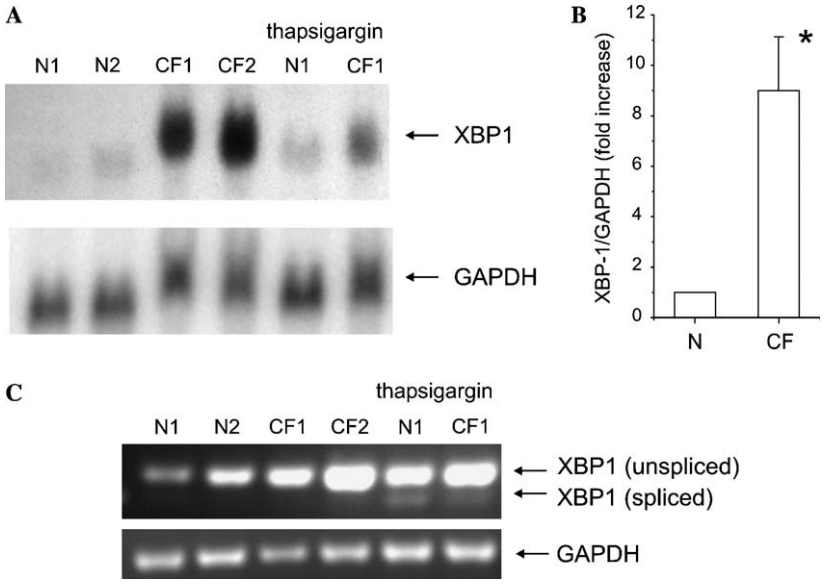

Fig. 5. XBP1 expression is increased in CF cells. (A) Northern blot analysis of normal (N1 and N2) and CF (CF1 and CF2) primary airway cells for unspliced XBP1 mRNA expression (upper panel). (B) Group mean data. GAPDH was used as loading control ($n = 2$, means ± SD, $^*p = 0.033$, ANOVA). (C) RT-PCR analysis of normal (N1 and N2) and CF (CF1 and CF2) primary cells for the spliced form of XBP1. Primary airway cells treated with thapsigargin appear to show a smaller XBP1 mRNA product which likely represents the cleaved form of XBP1 mRNA.

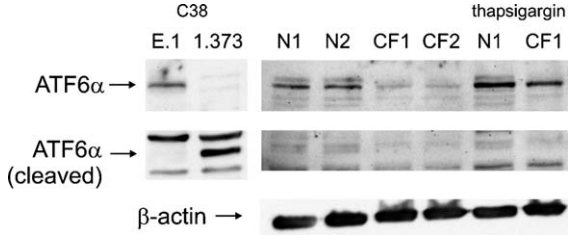

Fig. 6. ATF6α in CF airway cells. To identify intact and cleaved ATF6, C38 cells were transiently transfected with either HA-tagged p90 ATF6α (pCGN HA-ATF6 E.1) or HA-tagged p50 ATF6α (pCGN HA-ATF6 1.373), respectively. Cell lysates from these cells were resolved by SDS–PAGE, transferred to nitrocellulose, and probed with an anti-HA antibody. Lysates from normal (N1 and N2) and CF (CF1 and CF2) primary airway cells were probed with an anti-ATF6 antibody. Thapsigargin-treated primary airway cells showed increased amounts of full-length ATF6α. On the other hand, we did not detect any band corresponding to the electrophoretic mobility of cleaved ATF6α. Expression of β-actin was used as loading control.

thapsigargin pre-treatment increased the protein abundance of full-length ATF6 in primary cells from control and CF patients. We could not identify a band with the same electrophoretic mobility of cleaved ATF6 in primary cells or cell lines from either control or CF patients.

Mechanism of CHOP post-transcriptional control

CHOP mRNA, but not protein, levels were increased in unstimulated CF cells, suggesting that CHOP protein expression in CF cells is regulated at a post-transcriptional level. We hypothesized that increased proteasome activation was responsible for the absence of CHOP protein expression. To test this, we treated control and CF cells with the chemical proteasome inhibitor MG-132. Proteasome inhibition significantly increased CHOP expression in CF but not control cells (Figs. 7A and B, $p < 0.001$, ANOVA), suggesting that proteasome activity is at least in part responsible for repressed CHOP protein levels in CF cells. Proteasome inhibition had no effect on CFTR protein abundance (Fig. 7C), demonstrating that the observed increase in CHOP expression was not due to reduced CFTR degradation.

Apoptosis in control and CF epithelial cells

We reasoned that the absence of CHOP protein expression in CF cells would prevent apoptosis. To test this, we exposed control and CF cells to thapsigargin and measured apoptosis by annexin V staining. Consistent with previous reports [24,25], we found that CF cells were relatively resistant to apoptosis (Figs. 8A and B; $p < 0.001$, ANOVA).

Discussion

Several autosomal recessive diseases are due to mutations that disturb protein folding, leading to the abnormal retention of protein in the ER. However, in only a subset of these is UPR activation thought to contribute to disease progression. In the most common genetic form of cystic fibrosis (ΔF508/ΔF508), incorrectly folded CFTR is retained in the ER and susceptible to ERAD. However, an association of CFTR with BiP, the most abundant ER chaperone protein, has not been demonstrated [10–12], and therefore it has been assumed that the UPR is not activated in cystic fibrosis. Similarly, the insoluble Z allele of the α-1-antitrypsin (α-1-AT) gene, which produces a protein that polymerizes and is

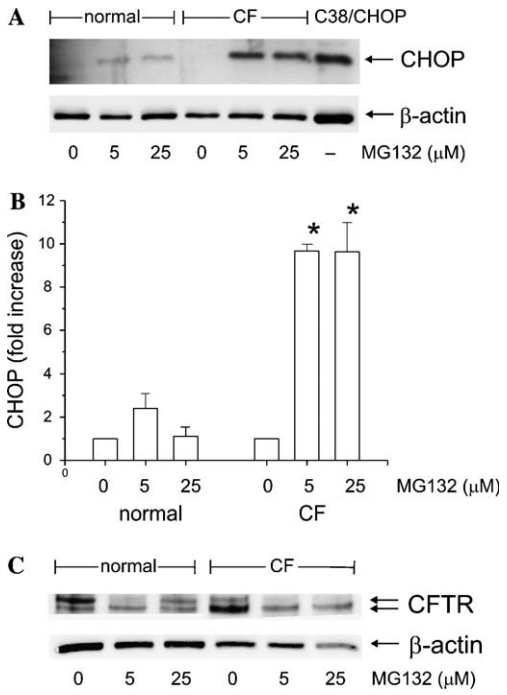

Fig. 7. Mechanism of CHOP post-transcriptional control. (A) Control and CF cells were treated with the chemical proteasome inhibitor MG-132. CHOP expression was increased in CF but not control cells. The blot shown is representative of two experiments. (B) Group mean data (±SD, $*p < 0.001$, ANOVA). (C) Proteasome inhibition did not increase CFTR protein abundance, suggesting that the effect on CHOP expression was not due to an inhibition of CFTR degradation. Arrows indicate the position of the fully glycosylated mature (upper band) and immature (lower band) forms of CFTR.

retained in the ER for degradation by the proteasome, neither binds BiP nor activates the UPR [14]. On the other hand, in osteogenesis imperfecta, misfolding mutations in procollagen produce molecules that bind BiP and may therefore activate the UPR [26]. The UPR may also play a role in the pathogenesis of neurodegenerative diseases. The accumulation of misfolded proteins in the leukodystrophy Pelizaeus-Merzbacher disease activates this stress response, resulting in apoptosis of oligodendrocytes [27]. Accumulation of abnormal protein aggregates also occurs in Alzheimer's disease and Parkinson's disease, and therefore the UPR may also be involved in these disorders. In Alzheimer's disease, BiP binds the amyloid precursor protein in healthy cells, thereby limiting production of fibrillar β-amyloid [28]. β-Amyloid-mediated cytoxicity might depend on caspase-12, which is implicated in UPR-induced apoptosis [29]. Mutations in presenilin 1 associated with familial Alzheimer's disease perturb IRE1 function and reduce BiP levels in neuroblastoma cell lines, and neurons from transgenic mice engineered to express mutant presenilin 1 are more prone to undergo apoptosis when exposed to ER stress [30,31]. However, this finding has not been confirmed by other laboratories [32].

Recently, aspects of the UPR were examined in short- (6–11 days) and long-term (30–40 days) primary cultures of ΔF508 bronchial epithelia [16]. In this study, the "hyper-inflammatory" response of freshly isolated primary CF airway epithelial cells in short-term culture resolved with long-term culture [16], suggesting that observed differences between control and CF cells in short-term culture may be a result of chronic infection in vivo. Consistent with this idea, unstimulated long-term cultures of CF cells showed negligible splicing of XBP-1, which when cleaved encodes an ERSE-binding transcription factor involved in the transcription of BiP and other UPR-related genes. Exposure to supernatant from mucopurulent material from CF airways increased XBP-1 splicing as well as BiP mRNA and protein expression. Finally, immunohistochemistry of freshly isolated tissue showed increased BiP immunoreactivity in CF epithelia compared to controls, suggesting that exposure to chronic inflammation and infection might initiate the UPR in vivo.

In the present study, we confirm that primary CF airway epithelial cells fail to show manifestations of the classic UPR under basal conditions. Using primary passage 1 cells that were frozen, thawed, and cultured for an additional 26 days, we found no increase in BiP expression, PERK or eIF2α phosphorylation, or XBP-1 splicing in cells isolated from CF patients compared to those from control individuals. Analogous to stimulation of long-term CF cultures with mucopurulent material from CF airways [16], we found that BiP expression and XBP1 splicing were increased after stimulation with thapsigargin. There were, however, more subtle differences between CF and control cells. Primary airway epithelial cells from patients with CF showed increased mRNA expression of CHOP, a pro-apoptotic transcription factor implicated in the UPR. In addition, increased CHOP mRNA expression in CF cells was accompanied by increased mRNA expression of XBP1. Together, these data suggest that a subset of elements of the UPR is activated in ΔF508/ΔF508 CF cells. We also observed increased CHOP and XBP1 mRNA expression in highly passaged IB-3 cells, an adeno12-SV40 immortalized human bronchial epithelial cell line derived from a ΔF508/W1282 CF patient (not shown), suggesting that the observed changes in CHOP and XBP1 mRNA expression are not a result of chronic infection.

While we have demonstrated increased mRNA expression of XBP1 in CF cells, the precise mechanism by which mRNA expression of CHOP, but not BiP, is increased in CF cells remains unknown. We did not detect an increase in the spliced form of XBP-1, nor did we detect an increase in the protein level of ATF6, each of which regulates transcription from the CHOP promoter. Arsenite exposure of primary neuronal cells affects the expression and translation of genes encoding BiP and CHOP to different extents [33], suggesting that their expression may be dissociated under certain conditions. As in our study, arsenite-induced CHOP expression occurred in the absence of XBP1 processing. The limited response may also relate to the quantity of unfolded CFTR in the ER, which may be efficiently reduced by ERAD.

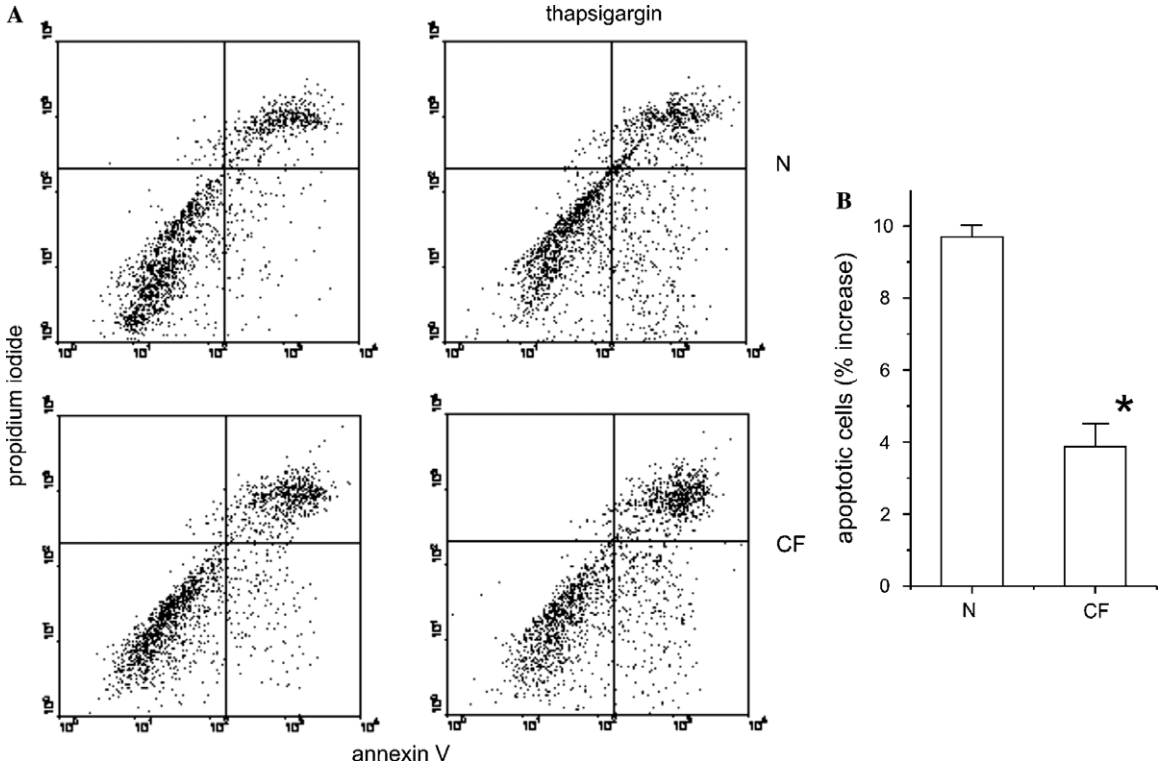

Fig. 8. Apoptosis in control and CF epithelial cells. Control and CF cells were treated with thapsigargin and apoptosis was measured by annexin V staining. Necrotic cells, as evidenced by propidium iodide staining, were excluded. Data shown are representative of three experiments. (B) Group mean data (±SD, $^{*}p < 0.001$, ANOVA).

BiP expression is tightly controlled at the post-transcriptional level [23]. Since CHOP mRNA but not protein expression was increased in CF cells, our data suggest that expression of CHOP is also regulated in this manner. In addition, the induction of CHOP mRNA and protein expression in response to thapsigargin stimulation was paradoxically reduced in CF cells compared to controls. Based on the information that abnormally folded CFTR is translocated back into the cytosol where it undergoes rapid degradation [34,35], we hypothesized that increased proteasome activation was responsible for the absence of CHOP protein expression. To test this, we treated control and CF cells with the chemical proteasome inhibitor MG-132. The increase in CHOP expression was significantly greater in CF than control cells, demonstrating that proteasome activity is at least in part responsible for repressed CHOP protein levels in CF cells.

CHOP has been shown to negatively regulate cell growth and induce apoptosis in a variety of cell types including fibroblasts, keratinocytes, HeLa cells, macrophages, and pancreatic β cells [36–40]. Thus, mechanisms limiting CHOP protein abundance, such as the post-transcriptional repression of CHOP protein expression, may serve to prevent apoptosis in response to chronic infection. Consistent with this, despite abnormal accumulation of incorrectly folded CFTR in the lumen of the ER, CF cells have been shown to be either no more susceptible to apoptosis than non-CF cells [41] or resistant to apoptosis [24,25]. In the present study, we found that CF cells were more resistant to thapsigargin-induced apoptosis than control cells.

We conclude that primary CF airway epithelial cells did not show manifestations of the classic UPR under basal conditions. However, these cells showed increased mRNA expression of CHOP and XBP1. Since the UPR could alter gene expression or cell survival, thereby affecting CF disease progression, further studies investigating the UPR in CF cells are warranted.

Acknowledgments

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