Endoplasmic Reticulum Stress Response is Transmissible *in vitro* from Epithelial Cells to Fibroblasts *via* the Mediation of Unfolded Protein Response

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The unfolded protein response (UPR) can restore cellular homeostasis and induce apoptosis during endoplasmic reticulum (ER) stress. Its activation has been observed during inflammation and fibrogenesis, which undermine tissue architecture and organ function. Nonetheless, the escalation from cell-intrinsic UPR to tissue-wide damage remains unclear. This study investigated whether UPR-induced signals in epithelial cells are transmissible to fibroblasts and if they could alter fibroblast gene expression towards fibrotic phenotype. An engineered epithelial cell line HEK^{DAX} in which UPR pathways can be activated is co-cultured with fibroblasts to mimic the tissue environment. Fibroblast gene expression was analyzed using fluorescent stress bioassay, Western blot, and immunocytochemistry. Changes in the expression of ATF6- and XBP1s-induced genes and altered fibroblast endosomal and secretory compartments were observed. Increased fibroblast proliferation was measured. These findings suggested that this epithelial UPR-initiated stress communication has direct influences on fibroblast, providing new insights into the pathophysiological roles of the UPR in fibrogenesis.

Key Words: Transmissible ER stress, unfolded protein response, fibrotic disease, intercellular stress communication

1 Introduction

As the "protein factory" for cells, the endoplasmic reticulum (ER) facilitates protein translation and folding and initiates the trafficking of properly folded proteins to the cell membrane or out of the cell (Schwarz & Blower, 2016). However, mutated or misfolded proteins accumulate in the ER and disrupt ER homeostasis. This disruption of ER balance is referred to as ER stress. A natural intracellular adaptive network called the unfolded protein response (UPR) is then activated. Like a double-edged sword, the UPR can restore homeostasis and also lead to apoptosis. Extensive research in the past two decades has identified the pathways and target genes of the UPR (Karagöz, Aragón, & Acosta-Alvear, 2019; Mori, 2009; Ron & Walter, 2007; Schroder & Kaufman, 2005; Walter P., 2011).

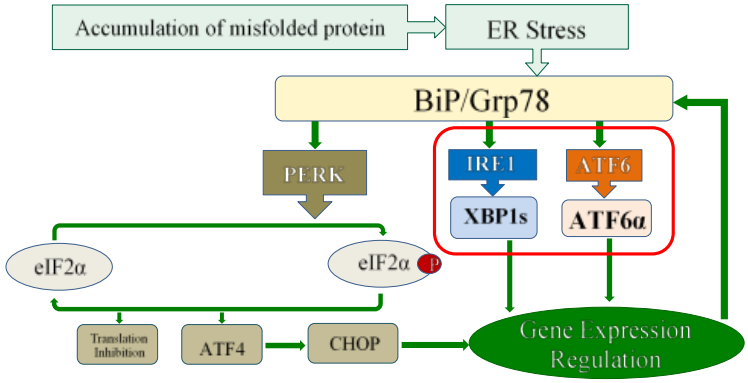

Figure 1. The unfolded protein response is an adaptive network that can restore homeostasis and activate apoptosis. Upon ER stress, BiP disassociates from the three UPR sensors PERK, IRE1, and ATF6, thereby activating them. PERK phosphorylates eIF2α, which inhibits mRNA translation except that of ATF4, a transcription factor upstream of the apoptosis pathway. IRE1 splices XBP1. ATF6 is trafficked to the Golgi, and the cytosolic side is cleaved to be ATF6α (still referred to be ATF6). Transcription factors XBP1s and ATF6 go back to the nucleus and promote genes enhancing ER capacity such as upregulated BiP expression

Binding immunoglobulin protein (BiP), also referred to as Grp78 or HSPA5, is an ER chaperone protein and the "master regulator" of the UPR. Three ER membrane proteins act as ER stress sensors: double-stranded RNA-activated protein kinase-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6). They each trigger a unique cascade of signaling pathways and are collectively termed the unfolded protein response (Figure 1). The restorative responses of the UPR improve cellular proteostasis through the degradation of misfolded proteins, reduced translation, and increased ER folding capacity. On the other hand, maladaptive UPR results in cell death and leads to tissue damage (Mori, 2009; Schroder & Kaufman, 2005; Wong et al., 2018). Although the cell-intrinsic pathways of the UPR are well understood, only limited data are available concerning the signals that cells with UPR activation may transmit to other cells in tissues and organs (Mardones, Dillin, & Hetz, 2014; Taylor, Berendzen, & Dillin, 2014). Since the UPR plays an indispensable role in regulating the proteostasis of the secretory pathway, the UPR is a prominent candidate capable of initiating the enigmatic tissue-wide signaling during fibrogenesis (Moore & Hollien, 2012; Plate & Wiseman, 2017; Wong et al., 2018).

Recent studies shed light on the intercellular signaling capability of the UPR. *In C. elegans*, elevated XBP1s levels in Pomc neurons could increase longevity and activate the UPR in other cell types through a cell-nonautonomous mechanism (Taylor & Dillin, 2013; Williams et al., 2014). While studying the drug resistance of cancer cells, a group observed intercellular transmission of the ER stress in a prostate cancer cell line (Rodvold et al., 2017; K. S. Rodvold JJ, Zanetti M, 2017). They found that by inducing the UPR in some cells, these cells were able to elicit *de novo* UPR activation in the surrounding cells, providing an explanation for drug resistance as well as an example of the UPR-initiated intercellular communication (M. N. Rodvold JJ, Zanetti M., 2016 ; Rodvold & Zanetti, 2018). Confirming the results of this study, another group observed transmissible ER stress from Acute Myeloid Leukemia (AML) cancer cells to mesenchymal stromal cells and osteoprogenitor cells (Butler J, 2019; Doron et al., 2019). More recently, it was found that this transmission of ER stress was not limited to cancer but also appeared among cells in the central nervous system (Sprenkle, Lahiri, Simpkins, & Meares, 2019). In the present project, we seek to

answer whether UPR activation in epithelial cells can be transmitted to fibroblasts and elicit fibrotic responses.

Collectively speaking, fibrosis is a scarring and remodeling event that occurs after injuries. Fibroblasts produce and depose collagens into the extracellular matrix (ECM). The accumulation of excessive fibrotic tissue deprives organs of their function and ultimately leads to organ failure (Rockey, Bell, & Hill, 2015). In chronic fibrotic diseases such as cystic fibrosis and polycystic kidney disease, fibrosis is likely triggered by respiratory epithelium damage (Bebok & Fu, 2018) or kidney tubular defects (Hill et al., 2016). In both cases, epithelial cells are defective due to mutations, resulting in a tissue wide injury. The damages or mutant proteins cause ER stress, activate the UPR, and elicit signals that may lead to fibrosis (Bebok & Fu, 2018). An analogous study on cardiac fibrosis showed a connection between the UPR and fibrogenesis (Groenendyk et al., 2016), where the authors demonstrated that by inhibiting the UPR, cardiac fibrosis was significantly ameliorated, implicating that the UPR played a role in tissue-wide communication leading up to fibrosis. Although other signaling pathways may also activate fibroblasts, there are gaps in our current knowledge that obscure the degree of contribution by different signaling pathways. We hereby study the role of epithelial UPR in the process of developing fibrosis. We report that the epithelial UPR-initiated signals are transmissible to fibroblasts, and these signals are capable of promoting fibroblast proliferation and altering endosomal and secretory activity.

2 Materials and Methods

Materials

Dulbecco's Modified Eagle Medium (DMEM; Gibco™ 11965092), fetal bovine serum (FBS; Gibco™ A3840002), radioimmunoprecipitation assay buffer (RIPA; #89900) were purchased from Thermo Fisher Scientific. Blocking solution (P/N 927-60001) and antibody diluent solution (P/N: 927-65001) were purchased from LI-COR. Polysorbate 20 (Tween 20) was purchased from Sigma-Aldrich (P1379-1L). Phosphate-buffered saline (PBS; Gibco™ 10-010-023), paraformaldehyde (PFA; #50-980-487) were purchased from Fisher Scientific. Antibodies for BiP/Grp78 (CST 3177), XBP1s (83418), PERK (CST 3192), P-PERK (SCT 3179S), ATF4 (CST 11815), CHOP (CST 2895) were from Cell Signaling Technology. Antibodies for ATF6 (ab122897) and Ki67 (ab16667) were from Abcam. Antibodies for FAP (PA 5-51059), collagen I (PA 5-29569), and CD63 (MA 1-19281) were from Invitrogen. The antibody for β-actin (sc-47778) was from Santa Cruz Biotechnology. Thapsigargin (Thg; 586005–1MG) and tunicamycin (Tum; 65–438-010MG) were from Calbiochem.

Cell Culture

IMR90 is a human lung fibroblast cell line (ATCC CCL-186). HEK^{DAX} is an engineered human embryonic kidney cell line from HEK293 developed in the Wiseman Lab at the Scripps Research Institute (Shoulders et al., 2013), in which spliced XBP1 (XBP1s) from the IRE1 pathway and cleaved ATF6 expression can be induced without two drugs doxycycline and trimethoprim (TMP) respectively, without causing ER stress. All cell lines were cultured in DMEM media with 10% FBS in a 37 ^{0}C incubator with 5% CO_2.

Western Blot Analysis

Western blot analysis was performed to identify changes in protein levels; specifically, the expression of BiP/Grp78, XBP1s, ATF6, PERK, P-PERK, FAP, ATF4, CHOP, collagen I, and CD63. Protein isolates were separated by SDS-PAGE and followed by Western transfer to nitrocellulose membranes (LI-COR P/N 926-31092). Total proteins were stained by REVERT protein stain kits (LI-COR P/N 926-11016). The membrane was blocked for one hour at room temperature (LI-COR P/N 927-60001). Following blocking, the membrane was incubated with primary antibodies overnight or three hours at room temperature and then secondary antibodies for one hour at room temperature (antibody and dilution were listed in the Materials section). Western blot assay data was collected by the LI-COR Odyssey CLx imaging system (700nm and 800nm). The presence of proteins of interest was indicated and quantified by the intensity of the bands in Image Studio Light software. Densitometry was performed in Microsoft Excel software. Band densities were determined relative to the internal control β-actin to determine protein expression changes.

Immunocytochemistry Analysis

After co-culturing with HEK^{DAX} cells, IMR90 cells were washed three times with filtered PBS before fixation with 4% paraformaldehyde. 0.1% Triton was used to permeabilize the cell membranes. After washing three times with filtered PBS, 2.5% goat serum was used to block the samples. BiP/Grp78, XBP1s, ATF6, collagen I, FAP, paxillin, and CD63 primary antibodies were used to stain the samples. After washing five times with PBS plus 0.1% Tween20, the samples were incubated with secondary antibodies. DAPI staining was used to stain the nuclei. Assay data were collected by epifluorescent microscopy. The localization and intensity of fluorescence of targeted proteins in IMR90 were documented and compared among experimental groups.

Experimental Design and Data Analysis

To mimic the activation of UPR in epithelial cells, the transcription factors ATF6 and XBP1s were induced in HEK^{DAX} cells by doxycycline (Dox) and trimethoprim (TMP) (as shown in Figure 2A). They were direct downstream products of the IRE1 and ATF6 pathways of the UPR. IMR90 cells were grown in 6-well plates. HEK^{DAX} cells were grown on permeable

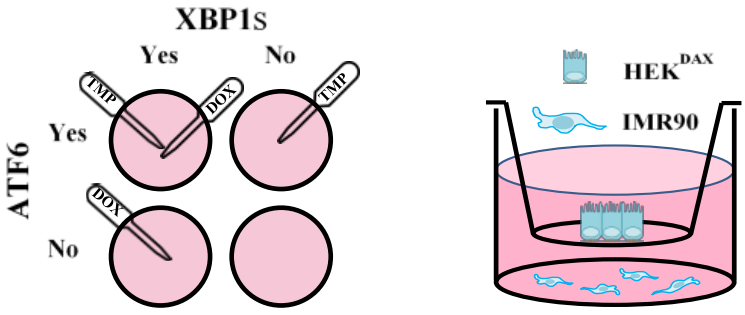

Figure 2. Schematic representation of the experimental design. A: Induction of XBP1s, ATF6, or both in HEK^{DAX} cells to create three experimental groups (ATF6+/XBP1s+, ATF6+/XBP1s-, ATF6-/XBP1s+) and one negative control (ATF6-/XBP1s-). B: HEK^{DAX} cells were grown on permeable supports and IMR90 cells were grown on cell culture plates. Co-culture was done by inserting the permeable support into the wells of cell culture plates.

supports that could be inserted into the wells of 6-well plates. When both cell lines reached 70%-90% confluency, permeable supports with HEK^{DAX} cells were transferred to IMR90 plates for 12-hour co-culturing (as illustrated in Figure 2B). UPR products (ATF6 and XBP1s) and UPR-targeted gene products were tested *via* Western blot analysis, immunocytochemistry analysis, and epifluorescent microscopy to measure fibroblast protein expression changes. All Western blot analysis experiments were replicated at least three times and analyzed in Image Studio Lite software, and immunocytochemistry at least twice and analyzed by ImageJ and Microsoft Excel software. Comparisons between two groups were performed with a two-tailed t-test. For all experiments, statistical significance was labeled with *= p <0.05.

3 Results

UPR signals are transmitted from HEKDAX to IMR90 cells

In the past studies of transmissible ER stress (Doron et al., 2019; Rodvold et al., 2017; Sprenkle et al., 2019), tunicamycin (Tum) or thapsigargin (Thg) were commonly used to induce ER stress. After washing the drugs away, the cells are left in fresh media, allowing the cells to deposit any substances to the media. The media is then collected as "conditioned" media and applied to other cells to test if there was UPR-transmission by factors released into the conditioned media (Rodvold et al., 2017).

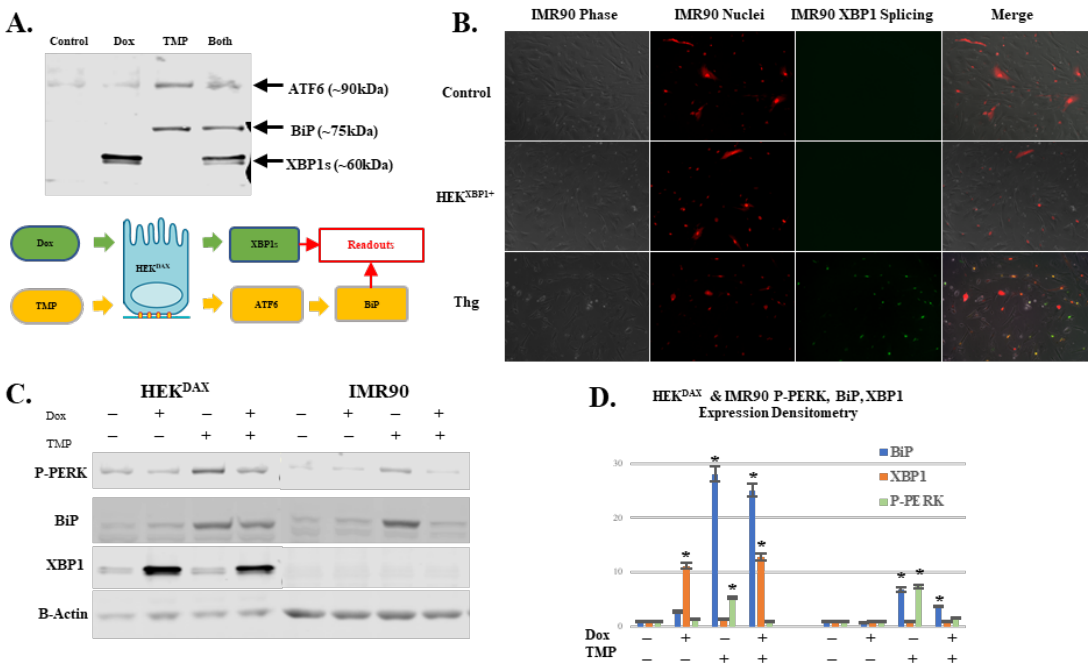

*Figure 3. ER stress was transmissible from HEK^{DAX} cells to IMR90 cells and HeLa cells in vitro. A: Western blot analysis showing that XBP1s was upregulated after treating the HEK^{DAX} cells with Dox while ATF6 was upregulated after treating with TMP. B: Cell stress assay. Red fluorescence indicated IMR90 Nuclei. Green fluorescence indicated XBP1 splicing. Clear green fluorescence was detected when IMR90 was stressed with Thapsigargin (Thg). No fluorescence was observed in the negative control and when IMR90 cells were co-cultured with XBP1s-expressing HEK^{DAX} cells. C: Western blot assay of the UPR downstream products. D: Densitometry of the Western blot analysis. BiP and P-PERK had increased expression in IMR90 cells when they were co-cultured with TMP-treated HEK^{DAX} cells (n=3; *=p<0.05). E: Immunocytochemistry of XBP1s in HEK^{DAX} cells and HeLa cells. Expression of XBP1s was captured in HeLa cells after co-culturing with XBP1s-expressing HEK^{DAX} cells.*

However, if Tum and Thg were not washed out completely, they would induce the UPR in target cells and skew the experimental results. To pinpoint whether the UPR acted as the initiator of the intercellular stress transfer, we took advantage of the HEK^{DAX} cells in which the immediate products of the UPR (XBP1s and ATF6) can be directed induced without the use of a stress-causing agent such as Tum and Thg (Shoulders et al., 2013). XBP1s was induced by doxycycline (Dox), and ATF6 was induced by trimethoprim (TMP) (Figure 3A). Dox and TMP do not have any direct impact on target cells (Shoulders et al., 2013).

To answer the question of whether the UPR in inducer HEK^{DAX} cells can elicit the UPR in target IMR90 cells, a live cell stress assay was used to visualize XBP1 splicing in the target IMR90 cells after co-culturing with Dox-induced HEK^{DAX} cells (Figure 3B). XBP1 splicing was observed in the positive control, where Thg was added to cause ER stress. XBP1 splicing was not observed in the target IMR90 cells when they were co-cultured with the negative control HEK^{DAX} cells or with Dox-treated HEK^{DAX} cells (HEK^{XBP1+}). The lack of endogenous XBP1 splicing in the IMR90 cells might suggest a transfer of XBP1s from the HEK^{DAX} cells or a transfer of the products of XBP1s-targeted genes. In support of this idea, it was reported in the literature that XBP1 mRNA and the mRNA of its targeted gene products were sent out of the stressed cells (Hosoi, Nakashima, & Ozawa, 2018; Kanemoto et al., 2016). The results of cell stress assay were reaffirmed by the Western blot analysis (Figure 3C) in which target IMR90 cells did not show a high level of XBP1s expression.

Next, we tested changes in the protein expression of XBP1s and ATF6-targeted downstream genes (Figure 3C). BiP/Grp78 was chosen as the readout for the activation of ATF6 pathway (Figure 3A) (Maiuolo, Bulotta, Verderio, Benfante, & Borgese, 2011; Shoulders et al., 2013). Phosphorylated-PERK (p-PERK) and BiP/Grp78 expression had noticeable increases when inducer HEK^{DAX} cells were treated with TMP (Figure 3C), suggesting the activation of PERK and ATF6 pathways in the IMR90 cells. This result supported our hypothesis of transmissible ER stress from epithelial cells to fibroblasts. Furthermore, it was the ATF6 pathway in the HEK^{DAX} cell that mediated this transfer of signals to IMR90 cells. This might imply that the signal(s) were products of ATF6-targeted genes, significantly reducing the pool of candidates to screen if we were to unveil the signaling molecules.

Altered endosomal and secretory compartments and increased fibroblast proliferation in IMR90 cells

Collagen I is the main contributing factor in fibrotic tissues, and fibroblast activation protein (FAP) expression is unique and strong during tissue remodeling and fibrosis (Acharya PS, 2006; Levy MT, 1999; Wang, Yu, McCaughan, & Gorrell, 2005). They are both fibroblast-specific proteins, and therefore we proceeded to examine their protein expression after screening UPR-related proteins in IMR90 cells. No variable of collagen I expression was observed among the experimental groups, and a significant decrease in its expression was observed in the positive control (Tum treatment) (Figure 4A & 4B). Based on the interaction between FAP and integrin proteins on the cell surface, it was postulated that FAP mediates cell migration and invasion (Kalluri & Zeisberg, 2006). We hence tested both FAP protein expression levels and intercellular localization. No significant change in FAP protein expression level was observed by Western blot analysis (Figure 4A & 4B). To test if the intracellular localization altered following co-culture with HEK^{DAX} cells, immunocytochemistry was used to co-stain FAP and Paxillin (an indicator of cell

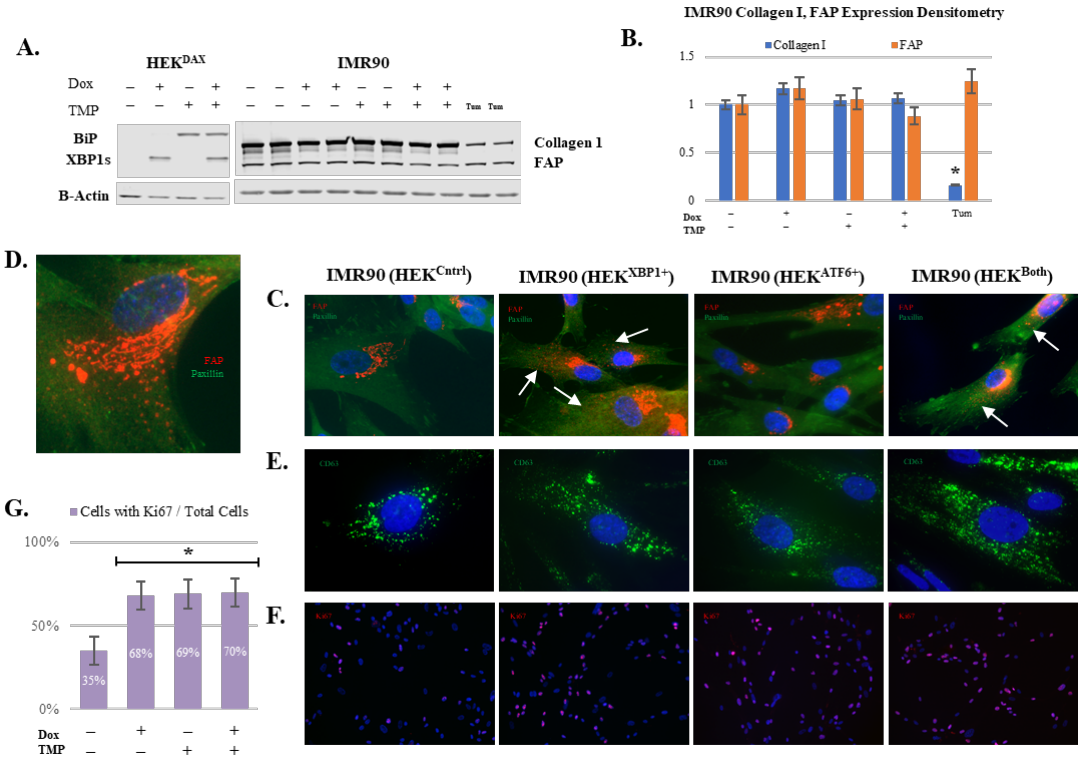

Figure 4. Epithelial ER stress transfer altered fibroblast endosomal and secretory compartments and increased fibroblast proliferation. A: Western blot analysis on fibroblast-specific proteins collagen I and fibroblast activation protein (FAP) in IMR90 cells. The HEK^{DAX} blot demonstrated the expression of XBP1s and ATF6 in the inducer HEK^{DAX} cells. B: Densitometry of the Western blot analysis. Collagen I expression was significantly reduced upon direct stress from tunicamycin (Tum) (n=7; p<0.05). No significant variation in the expression of FAP was observed (n=6). C&D: Co-staining of FAP (red) and Paxillin (green). FAP accumulated in a Golgi apparatus-like complex. When IMR90 cells were co-cultured with XBP1+ HEK^{DAX} cells, FAP appeared to leave the Golgi apparatus-like complex and was trafficked in the secretory pathway (white arrows). E: Immunocytochemistry staining of multivesicular body (MVB) marker CD63. The localization of MVBs appeared to be more dispersed and scattered throughout the cell, and the number of MVBs seemed to be greater based on the intensity of overlapping fluorescent signals when IMR90 cells were co-cultured with HEK^{DAX} cells expressing UPR products (XBP1s, ATF6, or both). F&G: Immunocytochemistry stain of proliferation marker Ki67 showed a twofold increase in IMR90 cells that were co-cultured with HEK^{DAX} cells expressing XBP1s, ATF6, or both (n>400 cells; p<0.05).

morphology and attachment *via* focal adhesion) (Figure 4C). The FAP staining in IMR90 cells appeared to aggregate in a Golgi apparatus-shaped organelle, and the staining pattern suggested Golgi's trafficking function (Figure 4D). Golgi marker will be used to verify this postulation. When the inducer HEK^{DAX} cells were treated with Dox, FAP in IMR90 cells seemed to leave the Golgi-like organelle and was trafficked towards cell membrane or Paxillin-rich focal adhesion complexes (indicated by bright green dots on the edge of the cell membrane in Figure 4C).

To obtain more insights into the endosomal and secretory compartments in the IMR90 cells, we did immunocytochemistry on a multivesicular body (MVB) marker CD63 (Figure 4E). MVBs seemed to concentrate around the IMR90 cell nucleus in the control group while more dispersed and scattered when XBP1s and ATF6 were expressed in the inducer HEK^{DAX}

cells. This observation correlated with previous research reporting an increased MVB number in epithelial cells following UPR activation (Kanemoto et al., 2016).

Epithelial and fibroblast proliferation along with macrophage recruitment were commonly observed in the pathophysiology of fibrotic conformational diseases (Wynn, 2008). However, the causal or sequential relationship among these phenomena remains unexplored. To determine whether the epithelial UPR signals were able to influence fibroblast proliferation, we stained IMR90 cells with a proliferation marker Ki67 (Figure 4F). The number of cells expressing Ki67 was counted as well as the total number of cells (Figure 4G). We observed significant increases in the number of IMR90 cells expressing Ki67 following co-culture with treated HEK^{DAX} cells. This data suggested that UPR-targeted gene products in HEK^{DAX} were capable of upregulating IMR90 cell proliferation, implicating transmissible UPR from epithelial cells.

4 Discussion

The findings reported herein support XBP1- and ATF6-mediated communication between HEK^{DAX} and IMR90 cells. When the ATP6 pathway is turned on in the inducer HEK^{DAX} cells, we observed increased BiP/Grp78 expression, an ATF6 target gene (Maiuolo et al., 2011), and PERK phosphorylation in the fibroblasts (Figure 3C & 3D). These changes implicated *de novo* activation of the PERK and ATF6 pathways. Previous studies reported that in the absence of XBP1, the PERK pathway is upregulated through ATF6 (Chalmers, van Lith, Sweeney, Cain, & Bulleid, 2017) to fine-tune and assist the ATF6 branch during ER stress as well (Gonen, Sabath, Burge, & Shalgi, 2019; Gupta et al., 2015). The pattern of PERK and ATF6 duo-activation in absence of IRE1 was also reported in the past (Song et al., 2019). Therefore, it was likely that the ATF6 pathway in the HEK^{DAX} cells triggered a cascade of reaction and initiated the transmission of ER stress to the IMR90 cells. Subsequently, IMR90 cells responded by turning on the ATF6 pathway, leading to PERK activation.

The alteration in IMR90 endosomal and secretory compartments along with increased IMR90 proliferation warranted intercellular communication and suggested that the inducer HEK^{DAX} cells had a direct influence over IMR90 cells. Our qualitative observation of expanded localization and increased numbers of MVBs in IMr90 cells after co-culturing with Dox-treated HEK^{DAX} cells could be explained by previous reports that XBP1s mediates secretory pathway expansion (Lhomond et al., 2015; Wong et al., 2018). Another explanation for our observation could be due to the increased *de novo* trafficking in the IMR90 cells. Soluble factors—produced from the IRE1 pathway of the HEK^{DAX} cells—could be transferred to the IMR90 cells and mobilize its internal production of membrane proteins or secretory proteins (i.e. integrin, fibronectin, or collagen). Nonetheless, this alteration in IMR90 cells happened when XBP1s was induced in the HEK^{DAX} cells. It was reported in the literature that XBP1s-targeted genes could regulate and expand secretory compartments (Chalmers et al., 2017; Lhomond et al., 2015; Pramanik et al., 2018; Wong et al., 2018). We herein demonstrated this phenomenon intercellularly between epithelial cells and fibroblasts.

Different types of cells would have cell type-specific responses to transmissible ER stress (Murray et al., 2004). Hence, we tested some characteristic proteins during fibroblast activation and tissue remodeling such as collagen I and FAP. Because Tum inhibits

glycosylation; thus, it likely impeded the post-translational modification of collagen I, leaving it misfolded and degraded (DiChiara et al., 2016). Additionally, increased proteolysis during ER stress could also reduce the cytosolic collagen I, explaining our observation in the positive control (Figure 4A & 4B). The lack of variation in the experimental groups could be ascribed to the long production period of collagen I, whereby we did not culture the cells long enough to see a change in the protein level. As to FAP, although we did not see any deviation in the expression, we observed changes in the localization of FAP. In the control group, FAP appeared to localize in a Golgi-like organelle. When XBP1s was induced in the inducer HEK^{DAX} cells, FAP appeared to leave the Golgi-like organelle in vesicular pattern towards the cell membrane where focal adhesion complexes form. As previously reported in the literature, FAP could interact with integrins and was commonly regarded as a marker for fibroblast mobility and invasion (Kalluri & Zeisberg, 2006) as well as for active fibroblasts during tissue remodeling (Acharya PS, 2006; Levy MT, 1999; Wang et al., 2005). The observed trafficking of FAP towards focal adhesion could suggest the beginning of cell movement and activation. Since neither collagen I nor FAP had increased expression, IMR90 cells were not yet actively producing collagen I at this time point. A future experimental design that will prevent cell death over a longer incubation period under the same level of stress shall be performed to further explore the influence of transmissible ER stress on collagen I production.

In all experimental groups excluding the control, we measured increased IMR90 cell proliferation, a pathophysiological event in all fibrotic conformational diseases. This further implicated the role of transmissible ER stress in tissue-wide communication leading up to fibrosis. Therefore, it is pivotal to elucidate the UPR-mediated intercellular communication in order to modulate the yet enigmatic pathways and prevent lethal symptoms from developing.

5 Conclusion

In this study, we investigated whether ER stress response was transmissible from epithelial cells to fibroblasts. We used a modified HEK293 cell line, HEK^{DAX}, as our epithelial cells and IMR90 cells as our fibroblast. When we induced XBP1s and the IRE1 pathway in the HEK^{DAX} cells, we observed alteration in the endosomal or secretory compartments and increased proliferation in the IMR90 cells. When we induce the ATF6 pathway in the HEK^{DAX} cells, we observed upregulated p-PERK and BiP/Grp78 expression and increased proliferation in IMR90 cells. These results collectively demonstrated transmissible ER stress response from epithelial cells to fibroblasts through UPR mediated intercellular signaling. Future investigations into the mechanism of UPR-initiated intercellular communication will be critical to understanding the complex tissue-wide signaling network that leads to fibrotic diseases.

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