Fibroblast Growth Factor 2 Augments Transforming Growth Factor Beta 1 in Inducing Epithelial-Mesenchymal Transition in Human Lung Epithelial Cells

CURRENT STATUS: POSTED

Lamis M.F. El-Baz
Suez Canal University Faculty of Science

Nahla M. Shoukry
Suez Canal University Faculty of Science

Mohamed L. Salem
Tanta University

Hani S. Hafez
Suez Canal University Faculty of Science

Robert D. Guzy  rguzy1@bsd.uchicago.edu
University of Chicago
Corresponding Author
ORCiD: 0000-0001-8420-6177

DOI:
10.21203/rs.2.10955/v1

SUBJECT AREAS
General Cell Biology & Physiology  Molecular Biology

KEYWORDS
Fibroblast Growth Factor, Transforming growth factor, Epithelial-mesenchymal transition, Lung injury, Epithelial cells.
Abstract

Background: Epithelial-mesenchymal transition (EMT) is a critical event in wound healing and tissue repair following injury. Transforming growth factor beta-1 (TGFβ1) plays an important role in inducing EMT in lung epithelial cells in vitro and in vivo. As fibroblast growth factor-2 (FGF2) reverses TGFβ1-induced collagen I (COL1A1) and α-smooth muscle actin (Actin alpha 2; ACTA2) expression in primary mouse and human lung fibroblasts, we set out this study to determine the effect of FGF2 on TGFβ1-induced EMT in human lung epithelial cells. Methods: BEAS-2B and A549 cells were treated with recombinant FGF2 (2 nM) with or without TGFβ1 (2 ng/ml) for up to 4 days. The phenotypic alterations associated with EMT were assessed by quantitative real-time PCR and E-cadherin protein expression levels was assayed by western blot and immunofluorescence staining. Cell migration was confirmed using wound-healing assay. Results: TGFβ1 treatment led to significantly reduced expression of E-cadherin (CDH1) and markedly induced expression of mesenchymal proteins such as N-cadherin (CDH2), tenasin C (TNC), fibronectin (FN), ACTA2 and COL1A1. TGFβ1 also induced a morphological change and a significant increase in cell migration. FGF2 did not significantly alter EMT gene expression markers on its own, however enhanced TGFβ1-induced suppression of CDH1 and upregulation of ACTA2, but did not alter TNC, FN and CDH2 gene expression levels induced by TGFβ1. FGF2 maintained TGFβ1-induced morphologic changes as well as increased the migration of TGFβ1-treated cells. Furthermore, FGF2 treatment significantly inhibited TGFβ1-induced COL1A1 expression in both BEAS-2B and A549 cells. FGFR-specific tyrosine kinase inhibitor PD173074 blocked the synergism between these two growth factors. Conclusions: This study suggests a synergistic effect between TGFβ1 and FGF2 in inducing EMT, which may play an important role in wound healing and tissue repair after injury. Our findings provide insight into the effects of FGF2 following lung injury and in pulmonary fibrosis.
Background

Lung epithelial cells are a frequent target of injury, and abnormalities in epithelial repair occur in many lung diseases such as asthma, chronic obstructive pulmonary disease (COPD), and pulmonary fibrosis, leading to accumulation of pathologic fibroblasts and myofibroblasts, collagen overproduction and tissue fibrosis [1]. Additionally, abnormalities in epithelial cell survival, apoptosis, proliferation, and migration contribute to pulmonary fibrosis [2].

Epithelial-mesenchymal transition (EMT) is a process by which differentiated epithelial cells lose their epithelial characteristics and reversibly acquire a migratory mesenchymal phenotype. EMT is characterized by a loss of polarity and loss of epithelial markers including junctional and cell-cell adhesion proteins such as zonula occludens-1 (ZO-1) and E-cadherin (CDH1), cytoskeletal reorganization, a transition to a spindle-shaped morphology, and acquisition of mesenchymal markers. Mesenchymal markers used to define EMT are α-smooth muscle actin (α-SMA), vimentin (VIM), collagen I (COL1A1), fibronectin (FN), connective tissue growth factor (CTGF), N-cadherin (CDH2), fibroblast-specific protein 1 (FSP1), the transcription factors Snail (SNAI1) and Slug (SNAI2), and matrix metalloproteinases (MMPs) [3–5].

EMT is classified into three subtypes based on different biological and functional consequences. Type I EMT occurs during organ development and is essential in gastrulation and neural crest cell migration. Type II EMT is associated with wound healing and organ fibrosis, where epithelial cells acquire a “myofibroblast” phenotype and migrate to heal injured tissues after trauma and/or inflammatory injury. If the injury is mild and acute, this process ends once the tissue is repaired and inflammation is attenuated. However, persistent EMT has been argued to promote myofibroblast differentiation leading to fibrosis [6, 7]. Type III EMT occurs in cancer metastasis, where neoplastic epithelial
cells are transformed into invasive metastatic mesenchymal cells that underlie cancer progression [8, 9]. The mechanism underlying EMT is not well understood in the context of pulmonary fibrosis, and the role of EMT in the pathogenesis of different respiratory diseases such as asthma, COPD and pulmonary fibrosis is under debate [10, 11].

There are several growth factors that have been demonstrated to induce EMT in various cell types, including transforming growth factor beta-1 (TGFβ1), fibroblast growth factor-2 (FGF2), epidermal growth factor (EGF), insulin-like growth factor-2 (IGF-II), interleukin-1 (IL-1), hepatocyte growth factor (HGF), and Wnt ligands [12]. In addition, other factors that can enhance EMT include hypoxia [13, 14], high glucose, angiotensin II, albumin [15, 16], inflammatory cytokines [17] and matricellular proteins [18, 19].

TGFβ1 was first defined as a major inducer of EMT in normal mouse mammary epithelial cells in vitro [20], and has been implicated in mediating EMT in vitro in epithelial cells from kidney [21–23], eye [24, 25], and lung [26–37]. Among various FGFs, FGF2 and FGF4 are key regulators of EMT during development and cancer progression in the lung [38, 39]. It has been reported that FGF2 reduces E-cadherin in human ovarian cancer cells [40], and induces the expression of mesenchymal markers (VIM, FSP1, α-SMA and SNAI1) in corneal endothelial cells [41] and proximal tubular epithelial cells [39, 42]. A number of studies have shown the synergistic effect of combined treatment of TGFβ1 and FGF2 in inducing EMT in mouse normal mammary epithelial (NMuMG) cells [43], rat Hertwig’s epithelial root sheath (HERS) cells [44], mouse lung epithelial type II cell line MLE-12 [45], human non-small cell lung cancer cell lines NCI-H1975 and NCI-H165 [46], and human lung adenocarcinoma cell lines PC-9, HCC-827 and A549 [47, 48]. These studies only used the combination of TGFβ1 and FGF2 as profibrotic cytokines to induce type III EMT which is a key component of carcinogenesis. Therefore, inhibition of EMT induction may modify tumor progression and responsiveness to chemotherapy and/or immunotherapy for cancer.
No studies have shown the synergistic effect of FGF2 and TGFβ1 in inducing type II EMT in lung epithelial cells that is associated with wound healing and tissue regeneration after injury.

We have previously shown that FGF2 is crucial for epithelial repair and recovery after bleomycin-induced lung injury in mice [49]. We have also found that FGF2 overexpression is protective against bleomycin-induced lung injury in vivo and inhibits TGFβ1-induced collagen I and α-SMA expression in primary mouse and human lung fibroblasts in vitro, suggesting that FGF2 is antifibrotic and protective against lung injury [50]. In this study we investigated the effect of FGF2 on TGFβ1-induced gene expression in both bronchial and alveolar lung epithelial cells in vitro. We hypothesized that FGF2 would induce EMT and may play an important role in wound healing and repair of lung epithelial cells after injury. In vitro, we found that FGF2 did not inhibit the majority of TGFβ1-induced expression of EMT markers in human lung epithelial cells. This study provides insight into the potential use of FGF2 following lung injury and in pulmonary fibrosis.

Methods

Cell culture

Human virus-transformed bronchial epithelial cell line (BEAS-2B) and Human alveolar type II epithelial carcinoma cell line (A549) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA; #CRL9609, #CCL185, respectively). BEAS-2B cells were plated on pre-coated plates with a mixture of 0.01 mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01 mg/ml bovine serum albumin (BSA) dissolved in BEBM medium and were grown in bronchial epithelial growth medium bullet kit (BEGM; Lonza, Walkersville, MD, USA). A549 cells were plated in Ham’s F-12 medium (Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (10,000 U/ml). Cells were incubated in a humidified incubator at 37°C with
5% CO₂.

**Epithelial-mesenchymal transition induction**

To induce epithelial-mesenchymal transition, cells were plated at ~30-40% confluence in 6-well plates. After overnight culture, cells were treated with 2 ng/ml of TGFβ1 (Fisher Scientific, Fair Lawn, NJ, USA, #PHG9204), 2 nM of recombinant human low-molecular-weight FGF2 (PeproTech, Rocky Hill, NJ, USA, #100-18B) + 1 nM heparin sulfate (Fisher Scientific, #BP2524), or TGFβ1 + FGF2 + heparin for 4 days in complete medium. Medium with or without treatments was changed after 48 hours. The experiments were designed so that the cells reached confluence one day prior to harvesting and were conducted independently 3-6 times each in duplicate.

**EMT assay in the presence of FGFR-specific tyrosine kinase inhibitor**

BEAS-2B cells were incubated with TGFβ1 (2 ng/ml) alone, FGF2 (2 nM) alone, PD173074 (0.1 µM, Cayman Chemical, Ann Arbor, MI, USA, #13032) alone or FGF2 (2 nM) + TGFβ1 +/- PD173074 for 4 days prior to collection of RNA.

**RNA isolation and quantitative real-time PCR**

Cells were lysed in RLT buffer and total RNA was prepared from the cells using the RNeasy plus mini kit (Qiagen, Valencia, CA, USA, #74136) according to the manufacturer’s instructions. RNA concentration was determined utilizing a Nanodrop spectrophotometer. cDNA was made using the BioRad iScript Reverse Transcription Supermix for RT-qPCR kit (BioRad, Hercules, CA, USA, #170-8841). Quantitative RT-PCR was performed on an Applied Biosystems StepOne thermocycler using ABI Taqman® Fast Advanced Master Mix (Applied Biosystems, Foster City, CA, USA, #4444557) and Taqman® gene expression assays. All samples were normalized to GAPDH and then scaled relative to controls using the standard delta-Ct (ΔCt) method. Data are reported as fold change over control.

**Protein isolation and immunoblotting**
Protein was extracted from cultured epithelial cells in radioimmunoprecipitation assay (RIPA) lysis buffer with freshly added 2% Protease Inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO, USA, #P8340) and Phosphatase Inhibitor Cocktail I and II (Sigma-Aldrich, #P2850 and #P5726, respectively). Protein concentration was determined utilizing a Pierce Bicinchoninic acid (BCA) assay kit (Thermo Scientific, Rockford, IL, USA, #23225). Total protein (20-40 µg) was separated on 4-20% polyacrylamide gels (BioRad) and transferred to PVDF membranes using a Trans-Blot Turbo transfer system (BioRad). Membranes were blocked for one hour at room temperature with gentle shaking in Tris-buffered saline with Tween-20 (TBST; 50 mM Tris, pH 7.4, 150 mM NaCl, 0.1% Tween-20) containing 5% BSA, and then probed with primary antibodies against E-Cadherin (BD Transduction Laboratories, Lexington, KY, USA, #610181) overnight at 4°C. Immunoblotting for β-tubulin (Abcam, Cambridge, MA, USA,#ab6046) was used as loading control. After three rinses in TBST, membranes were incubated for one hour at room temperature in horseradish peroxidase-linked secondary antibodies in TBST with 5% nonfat milk, rinsed again in TBST, and developed using SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Scientific, #34096) or Pico (Thermo Scientific, #34580).

Migration assay

BEAS-2B cells were cultured in 6-well plates and grown in complete media. Cells were ~90% confluent at the time of scratch wounding. Five 1 mm diameter circular wounds were created using a custom-made rubber tool [51]. The non-adherent cells washed off with medium and fresh medium with the same treatments as described previously was added to the wells. The wound closure was measured immediately after scratch wounding (0 h), at 24 h, 48 h and 72 h or measured at 0 h, 24 h, 40 h and 48 h after the cells were treated for 3 days prior to wounding. Phase contrast light microscope pictures were taken using a digital camera attached to an inverted-stage microscope (Nikon, Morton Grove,
IL). Wound areas were measured using Image J (NIH), and values were normalized to time = 0 values. Experiments were conducted independently 3 times each in triplicate.

**Immunofluorescence staining for E-cadherin**

BEAS-2B cells were grown on coated glass coverslips and stimulated with the same treatments for 4 days as described above. Cells were fixed with 4 % paraformaldehyde for 10 minutes at room temperature. After washing with ice-cold PBS, cells were blocked in 0.1% BSA and 5% serum prepared in PBS + 0.2% Tween-20 for 1h at room temperature. Coverslips were stained with monoclonal mouse anti-E-cadherin antibody (BD Transduction Laboratories, #610181) in a dilution of 1:50 made in blocking buffer and incubated overnight at 4°C, followed by the secondary antibody (goat anti-mouse conjugated with Alexa488 Abcam, #ab150113) in a dilution of 1:500. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (Thermo Scientific, #D1306) and coverslips mounted with SlowFade™ Gold Antifade Mountant (Thermo Scientific, #S36936). Images were captured with 3i Marianas Yokogawa-type spinning disk confocal microscope and the two channels merged using Image J software.

**Statistical Analysis**

The data showed the mean ± standard deviation and the significant differences in mean values were determined using one-way ANOVA. A $p$-value of less than 0.05 was considered to be significant. Statistical analysis was performed using GraphPad Prism software.

**Results**

**Recombinant FGF2 does not alter morphological changes in bronchial epithelial cells induced by TGFβ1.** Addition of FGF2 to BEAS-2B cells did not alter their cobblestone-like morphology (Figure 1A, B). Upon treatment with TGFβ1, cells developed a fibroblast-like shape (Figure 1C). Compared to cells treated with TGFβ1 alone, the addition of FGF2 maintained the fibroblast-like shape of BEAS-2B cells (Figure 1D).
Recombinant FGF2 enhances TGFβ1 induced EMT-related gene expression. To determine the minimal concentration of TGFβ1 and the time point required to induce EMT in BEAS-2B cells, BEAS-2B cells were treated with 2 ng/ml or 5 ng/ml of TGFβ1 for 3, 4 and 5 days and total RNA was collected. Expression of CDH1, ACTA2, and COL1A1 mRNA were then assessed by qRT-PCR. We found that 2 ng/ml was sufficient to repress CDH1 and induce ACTA2 and COL1A1, but ACTA2 started to be only detectable after 4 days of treatment (data not shown). We then determined whether the addition of FGF2 to BEAS-2B cells leads to altered gene expression. BEAS-2B cells were treated for 4 days with FGF2 (2 nM), TGFβ1 (2 ng/ml), or TGFβ1 + FGF2. FGF2 treatment alone led to a non-significant decrease in CDH1 ($P$ value = 0.2225) and a non-significant increase in both ACTA2 ($P$ value = 0.2239) and CDH2 ($P$ value = 0.7515) when compared to control. We observed a significant decrease in CDH1 ($P$ value = <0.0001) (Figure 2A) and a significant increase in ACTA2 ($P$ value = 0.0142) (Figure 2B) and CDH2 ($P$ value = 0.0065) (Figure 2C) mRNA expression levels after treating BEAS-2B cells with TGFβ1. Addition of FGF2 to TGFβ1 resulted in a further significant decrease in CDH1 expression ($P$ value = 0.0470) (Figure 2A) and significant increase in ACTA2 ($P$ value = 0.0160) compared to TGFβ1 treatment alone (Figure 2B). Addition of FGF2 to TGFβ1 did not alter expression of CDH2 ($P$ value = 0.9950) induced by TGFβ1 alone (Figure 2C).

To confirm these mRNA changes, we assessed the effects of TGFβ1 and FGF2 on E-cadherin protein levels in BEAS-2B cells. Immunoblotting of total cell lysates obtained after 4 days of incubation with TGFβ1, FGF2, or TGFβ1 + FGF2 demonstrated that E-cadherin protein levels were not significantly altered by FGF2 treatment alone and were significantly decreased ($P$ value = 0.0012) in response to TGFβ1 (Figure 3A, B). Addition of FGF2 to TGFβ1 led to further suppression of E-cadherin (Figure 3A, B). Immunofluorescence for E-cadherin revealed a loss of grid-like localization of E-cadherin
at the cell-cell contact surface following FGF2 treatment and further loss of cell-cell contact induced by TGFβ1 that was not altered by the addition of FGF2 (Figure 3C).

**FGF2 inhibits TGFβ1-induced collagen, but not fibronectin or tenascin C.** We then tested whether FGF2 has an effect on expression of extracellular matrix (ECM) proteins such as fibronectin (*FN*), tenasin C (*TNC*) and collagen I (*COL1A1*). BEAS-2B cells were treated with FGF2, TGFβ1, or TGFβ1 + FGF2 for 4 days prior to total mRNA collection. Treatment with FGF2 alone did not significantly alter expression of *FN*, *TNC*, or *COL1A1*, but TGFβ1 treatment led to a highly significant induction of each of these genes (*P* values = <0.0001) (Figure 4A-C). FGF2 treatment had no effect on TGFβ1-induced expression of *FN* (Figure 4A) and *TNC* (Figure 4B), but interestingly there was a significant decrease in the expression of *COL1A1* (*P* value = 0.0008) (Figure 4C) compared to TGFβ1 treatment alone unlike other EMT genes studied in this report.

**Recombinant FGF2 has similar effects on EMT gene expression induced by TGFβ1 in A549 cells.** We then tested whether the effect of FGF2 on TGFβ1-induced gene expression in BEAS-2B cells was unique to bronchial epithelial cells or conserved in multiple epithelial cell types in the lung. To do this we treated the alveolar epithelial A549 cells with FGF2, TGFβ1, or TGFβ1 + FGF2 for 4 days prior to collection of total mRNAs. We observed that FGF2 enhanced TGFβ1-induced downregulation of *CDH1* (*P* value = <0.0001) (Figure 5A) and upregulation of *ACTA2* (*P* value = 0.0013) (Figure 5B) compared to control. In addition, FGF2 suppresses TGFβ1-induced *COL1A1* mRNA expression (*P* value = 0.0011) in A549 cells compared to TGFβ1 treatment alone similar to what was observed in BEAS-2B cells (Figure 5C).

**Effect of FGF2 on TGFβ1-treated cells is inhibited by PD173074 in both types of epithelial cells.** To confirm that the effect of FGF2 is mediated by FGF receptor (FGFR) signaling, BEAS-2B and A549 cells were treated with the FGFR-specific tyrosine kinase
inhibitor PD173074 (0.1 µM) in combination with FGF2, TGFβ1, or TGFβ1 + FGF2. We found that PD173074 significantly blocked the effect of FGF2 on TGFβ1-induced repression of CDH1 (P value = 0.0002) (Figure 6A) and induction of ACTA2 (Figure 6B), and reversed FGF2-mediated inhibition of TGFβ1 induction of COL1A1 expression non-significantly (P value = 0.8644) in BEAS-2B cells (Figure 6C). Similarly, PD173074 inhibited FGF2 effect on TGFβ1-induced EMT in A549 cells significantly for CDH1 (P value = <0.0001) (Figure 6D) and non-significantly for ACTA2 (P value = 0.5619) (Figure 6E) and COL1A1 (P value = 0.0641) (Figure 6F). PD173074 alone did not alter the expression of the above genes in both types of epithelial cells.

**FGF2 promotes epithelial cell migration alone or in combination with TGFβ1.** As FGF2 appears to enhance EMT gene expression induced by TGFβ1, we then determined whether FGF2 enhances migration of BEAS-2B cells after injury. BEAS-2B cells were treated with FGF2, TGFβ1, or TGFβ1 + FGF2 immediately after wounding with a 1 mm circular rubber stylet. Treatment with TGFβ1 significantly reduced migration rates of BEAS-2B cells when given immediately after wounding at 48 h (P value = 0.0345) and 72 h (P value = 0.0422), however, both FGF2 alone and the addition of FGF2 to TGFβ1-treated cells caused a non-significant increase in migration rate (Figure 7A, B). Therefore, we treated BEAS-2B cells with FGF2, TGFβ1, or TGFβ1 + FGF2 for 3 days prior to wounding. Both FGF2 and TGFβ1 alone significantly increased migration at 24 h (P values = <0.0001), 40 h (P values = <0.0001 and 0.0222, respectively) and 48 h (P values = <0.0001 and 0.0042, respectively) after wounding compared to control, and this effect was substantially potentiated by addition of FGF2 to TGFβ1 (P values = <0.0001) (Figure 8A, B).

**Discussion**

This study has demonstrated that TGFβ1 induces EMT in human bronchial epithelial cells
(BEAS-2B) and alveolar type II epithelial cells (A549), as shown by morphological and EMT-related gene expression. We found that FGF2 enhances TGFβ1 induced EMT-related gene expression except for collagen I, which is inhibited by FGF2. Additionally, epithelial migration was enhanced by pre-treatment with FGF2 and/or TGFβ1.

Several studies have demonstrated that human alveolar epithelial cells [26, 27, 30–36] and human bronchial epithelial cells [28, 29, 32, 37] undergo EMT in response to TGFβ1 in vitro. A number of studies have reported that FGF2 induces EMT in malignant pleural mesothelioma cells [52], tubular epithelial cells [39, 42] and lens epithelial cells [53]. Although the synergistic effect between TGFβ1 and FGF2 in inducing EMT [43, 44, 47] and proliferation [54–56] has been described in other cell types, to our knowledge, the synergistic effect between FGF2 and TGFβ1 in inducing type II EMT in lung epithelial cells in vitro had not been described previously.

In this study, we stimulated both BEAS-2B and A549 cells with TGFβ1, FGF2 or both for up to 4 days. In response to TGFβ1, BEAS-2B cells lost their cobblestone morphology and adopted an elongated spindle-like shape, and this shape was unaltered by the addition of FGF2. TGFβ1 treatment also led to downregulation of the epithelial cell-specific adherence junction protein CDH1 and upregulation of the mesenchymal markers ACTA2 and CDH2. FGF2 alone did not significantly alter CDH1, ACTA2, or CDH2 expression, but had a significant additive effect on the changes in expression of these genes when added to TGFβ1-treated cells. Additionally, FGF2 augments the increase in the ECM mRNA expression of FN and TNC induced by TGFβ1. Similarly, we observed an identical pattern of EMT gene expression following stimulation with TGFβ1 +/- FGF2 in A549 cells. These results establish that FGF2 augments TGFβ1 in the induction of EMT in multiple lung epithelial cell lines in vitro.

In accordance with the present results, Shirakihara et al. [43] showed that there was a
synergistic effect between FGF2 and TGFβ1 on the induction of EMT in the mouse normal mammary epithelial (NMuMG) cells, without evidence of induction of EMT by FGF2 alone. They found that the morphology of NMuMG cells clearly changed from a cobblestone-like shape to a fibroblastic spindle shape with TGFβ1 treatment. Although FGF2 alone did not alter this shape, the addition of FGF2 to TGFβ1-treated cells maintained the spindled-shape morphology. Li et al. [46] and Kurimoto et al. [48] indicated that treatment with FGF2 + TGFβ1 downregulated E-cadherin, upregulated vimentin and N-cadherin and increased migration ability in A549 cells. These findings suggest that the combination of FGF2 and TGFβ1 treatment and not FGF2 alone is an effective way of promoting the induction of an EMT phenotype.

The present study showed that FGF2 alone or in combination with with TGFβ1, increased the migratory capacity of BEAS-2B cells after 24 h, 40 h and 48 h that were pre-treated for 3 days prior to wounding, however, the cell motility of immediately treated cells after wounding was not accelerated significantly when measured after 24 h, 48 h and 72 h. These results show that treatment with TGFβ1, FGF2, or both requires at least 3 days to promote increased motility and almost complete wound closure after injury. These findings are consistent with a previous study which showed that TGFβ1 treatment alone increased cell motility, and the addition of FGF2 to TGFβ1-treated cells treated for 4 days strongly enhanced the motility of NMuMG cells [43]. These results match those observed in Chen et al. [44] study who also reported that treatment with TGFβ1, FGF2, or both generate EMT phenotype in rat Hertwig’s epithelial root sheath (HERS) cells. They found that there was no significant difference in the EMT markers mRNA expression levels after 3 days and highly appeared after 7 days of induction. Also, they found the migratory capacity highly increased after 48h and 72 h of the pretreated cells with TGFβ1, FGF2, or both for 3 days. These findings suggest that generating a well-established EMT phenotype in epithelial
cells using the combined treatments of TGFβ1 and FGF2 require prolonged induction. Several studies have shown increased COL1A1 expression in BEAS-2B [28, 29, 57] as well as in A549 [26, 32, 35] epithelial cells in response to TGFβ1. This also accords with our observations, which showed that TGFβ1 treatment significantly increases COL1A1 expression in both BEAS-2B and A549 cells. However, interestingly, COL1A1 expression was dramatically suppressed with the addition of FGF2 to TGFβ1-treated BEAS-2B and A549 cells. These findings mirror those of our previous study demonstrating inhibition of TGFβ1-induced collagen expression by FGF2 in primary mouse and human lung fibroblasts in vitro [50]. These results may provide an important insight into the antifibrotic effect of FGF2 through suppression of TGFβ1-induced collagen expression in both lung fibroblast and epithelial cells.

The FGFR-specific tyrosine kinase inhibitor PD173074 was used to block the inductive effect of FGF2 on TGFβ1-treated cells. PD173074 inhibitor was reported to show both high affinity and selectivity for the FGF receptor (FGFR) family [58]. We found that the addition of PD173074 attenuates the inductive effect of FGF2 as indicated by reversing reduction of CDH1 expression and COL1A1, and the induction of ACTA2. This finding suggests that the effect of FGF2 is dependent upon canonical signaling through FGFRs.

Numerous studies have demonstrated that EMT is implicated in pulmonary fibrosis and cancer metastasis in mouse models [59–61] and in humans [62, 63]. The importance of EMT in pulmonary fibrosis has been challenged by other studies in animal models [64–66] and humans [67]. Whilst this study did not investigate the cellular mechanism underlying the effect of FGF2 in lung epithelial cells, it does demonstrate that FGF2 has an antifibrotic effect in part through decreasing collagen expression in epithelial cells and promotion of TGFβ1-induced gene expression required for migration of epithelial cells in wound repair after injury.
Conclusions

In conclusion, the data presented in this report suggest that FGF2 is synergistic with TGFβ1 to drive type II EMT associated with wound healing and tissue repair after injury. The mechanism driving this synergy needs more investigation to identify possible therapeutic targets and the future uses of FGF2 following lung injury and in pulmonary fibrosis.

Abbreviations

ACTA2: Actin alpha 2; BCA: Bicinchoninic acid; BEGM: Bronchial epithelial growth medium; BSA: Bovine serum albumin; CDH1: E-cadherin; CDH2: N-cadherin; COL1A1: Collagen I; CTGF: Connective tissue growth factor; DAPI: 4′,6-diamidino-2-phenylindole; EGF: Epidermal growth factor; EMT: Epithelial-mesenchymal transition; FGF2: Fibroblast growth factor-2; FN: Fibronectin; FSP1: Fibroblast-specific protein 1; HGF: Hepatocyte growth factor; IGF-II: Insulin-like growth factor-2; IL-1: Interleukin-1; MMPs: Matrix metalloproteinases; RIPA: Radioimmunoprecipitation assay; SNAI1: Snail; SNAI2: Slug; TBST: Tris-buffered saline with Tween-20; TGFβ1: Transforming growth factor beta-1; VIM: Vimentin; ZO-1: Zonula occludens-1; α-SMA: α-smooth muscle actin.

Declarations

Availability of data and materials

The datasets used and analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

No conflicts of interest, financial or otherwise, are declared by the authors.

Funding

This work was funded by NIH grant K08HL125910, and AHA grants 14FTF19840029. LMFB
was supported by The Culture Affairs and Mission Sector, Ministry of Higher Education and Scientific Research, Egypt.

**Author Contributions**

LMFEB performed all experiments, analyzed data, and wrote the manuscript. NMS, MLS, and HSH contributed to manuscript revision. RDG designed, supervised all experiments, and contributed to manuscript revision.

**Acknowledgments**

The authors are grateful for Dr. Steven White (University of Chicago, Chicago, IL) and his lab members for helping in epithelial cell cultures.

**References**

1. Horowitz JC, Thannickal VJ. Epithelial–Mesenchymal Interactions in Pulmonary Fibrosis. Semin Respir Crit Care Med. 2006;27:600. doi:10.1055/S-2006-957332.

2. Selman M, King TE, Pardo A, American Thoracic Society, European Respiratory Society, American College of Chest Physicians. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. Ann Intern Med. 2001;134:136–51. http://www.ncbi.nlm.nih.gov/pubmed/11177318. Accessed 7 Apr 2018.

3. Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest. 2003;112:1776–84. doi:10.1172/JCI20530.

4. Lee JM, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. J Cell Biol. 2006;172:973–81. doi:10.1083/jcb.200601018.

5. Li M, Luan F, Zhao Y, Hao H, Zhou Y, Han W, et al. Epithelial-mesenchymal transition: An emerging target in tissue fibrosis. Exp Biol Med (Maywood). 2016;241:1–13. doi:10.1177/1535370215597194.
6. Kalluri R. EMT: When epithelial cells decide to become mesenchymal-like cells. J Clin Invest. 2009;119:1417–9. doi:10.1172/JCI39675.

7. Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. J Clin Invest. 2009;119:1429–37. doi:10.1172/JCI36183.

8. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119:1420–8. doi:10.1172/JCI39104.

9. Tennakoon A, Izawa T, Kuwamura M, Yamate J. Pathogenesis of Type 2 Epithelial to Mesenchymal Transition (EMT) in Renal and Hepatic Fibrosis. J Clin Med. 2015;5:4. doi:10.3390/jcm5010004.

10. Willis BC, Borok Z. TGF-β-induced EMT: mechanisms and implications for fibrotic lung disease. Am J Physiol Cell Mol Physiol. 2007;293:L525–34. doi:10.1152/ajplung.00163.2007.

11. Bartis D, Mise N, Mahida RY, Eickelberg O, Thickett DR. Epithelial-mesenchymal transition in lung development and disease: does it exist and is it important? Thorax. 2014;69:760–5. doi:10.1136/thoraxjnl-2013-204608.

12. Zavadil J, Böttinger EP. TGF-β and epithelial-to-mesenchymal transitions. Oncogene. 2005;24:5764–74. doi:10.1038/sj.onc.1208927.

13. Manotham K, Tanaka T, Matsumoto M, Ohse T, Inagi R, Miyata T, et al. Transdifferentiation of cultured tubular cells induced by hypoxia. Kidney Int. 2004;65:871–80. doi:10.1111/j.1523-1755.2004.00461.x.

14. Du R, Sun W, Xia L, Zhao A, Yu Y, Zhao L, et al. Hypoxia-Induced Down-Regulation of microRNA-34a Promotes EMT by Targeting the Notch Signaling Pathway in Tubular Epithelial Cells. PLoS One. 2012;7:e30771. doi:10.1371/journal.pone.0030771.

15. Ibrini J, Fadel S, Chana RS, Brunskill N, Wagner B, Johnson TS, et al. Albumin-Induced Epithelial Mesenchymal Transformation. Nephron Exp Nephrol. 2012;120:e91–102.
16. Lee JH, Kim JH, Kim JS, Chang JW, Kim SB, Park JS, et al. AMP-activated protein kinase inhibits TGF-β-, angiotensin II-, aldosterone-, high glucose-, and albumin-induced epithelial-mesenchymal transition. Am J Physiol Physiol. 2013;304:F686–97. doi:10.1152/ajprenal.00148.2012.

17. Liang M, Wang J, Chu H, Zhu X, He H, Liu Q, et al. Interleukin-22 inhibits bleomycin-induced pulmonary fibrosis. Mediators Inflamm. 2013;2013:209179. doi:10.1155/2013/209179.

18. DeMaio L, Buckley ST, Krishnaveni MS, Flodby P, Dubourd M, Banfalvi A, et al. Ligand-independent transforming growth factor-β type I receptor signalling mediates type I collagen-induced epithelial-mesenchymal transition. J Pathol. 2012;226:633–44. doi:10.1002/path.3016.

19. Schneider DJ, Wu M, Le TT, Cho S-H, Brenner MB, Blackburn MR, et al. Cadherin-11 contributes to pulmonary fibrosis: potential role in TGF-β production and epithelial to mesenchymal transition. FASEB J. 2012;26:503–12. doi:10.1096/fj.11-186098.

20. Miettinen PJ, Ebner R, Lopez AR, Derynck R. TGF-beta induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. J Cell Biol. 1994;127 6 Pt 2:2021–36. http://www.ncbi.nlm.nih.gov/pubmed/7806579. Accessed 15 Oct 2018.

21. Fan J-M, Ng Y-Y, Hill PA, Nikolic-Paterson DJ, Mu W, Atkins RC, et al. Transforming growth factor-β regulates tubular epithelial-myofibroblast transdifferentiation in vitro. Kidney Int. 1999;56:1455–67. doi:10.1046/j.1523-1755.1999.00656.x.

22. Kaimori A, Potter J, Kaimori J-Y, Wang C, Mezey E, Koteish A. Transforming growth factor-beta1 induces an epithelial-to-mesenchymal transition state in mouse hepatocytes in vitro. J Biol Chem. 2007;282:22089–101.
Zheng G, Lyons JG, Tan TK, Wang Y, Hsu T-T, Min D, et al. Disruption of E-Cadherin by Matrix Metalloproteinase Directly Mediates Epithelial-Mesenchymal Transition Downstream of Transforming Growth Factor-β1 in Renal Tubular Epithelial Cells. Am J Pathol. 2009;175:580-91. doi:10.2353/AJPATH.2009.080983.

Hales AM, Schulz MW, Chamberlain CG, McAvoy JW. TGF-beta 1 induces lens cells to accumulate alpha-smooth muscle actin, a marker for subcapsular cataracts. Curr Eye Res. 1994;13:885-90. http://www.ncbi.nlm.nih.gov/pubmed/7720396. Accessed 11 Oct 2018.

Saika S, Kono-Saika S, Ohnishi Y, Sato M, Muragaki Y, Ooshima A, et al. Smad3 signaling is required for epithelial-mesenchymal transition of lens epithelium after injury. Am J Pathol. 2004;164:651-63. doi:10.1016/S0002-9440(10)63153-7.

Kasai H, Allen JT, Mason RM, Kamimura T, Zhang Z. TGF-β1 induces human alveolar epithelial to mesenchymal cell transition (EMT). Respir Res. 2005;6:56. doi:10.1186/1465-9921-6-56.

Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, du Bois RM, et al. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: potential role in idiopathic pulmonary fibrosis. Am J Pathol. 2005;166:1321-32. http://www.ncbi.nlm.nih.gov/pubmed/15855634. Accessed 15 Oct 2018.

Doerner AM, Zuraw BL. TGF-beta1 induced epithelial to mesenchymal transition (EMT) in human bronchial epithelial cells is enhanced by IL-1beta but not abrogated by corticosteroids. Respir Res. 2009;10:100. doi:10.1186/1465-9921-10-100.

Kamitani S, Yamauchi Y, Kawasaki S, Takami K, Takizawa H, Nagase T, et al. Simultaneous Stimulation with TGF-β1 and TNF-α Induces Epithelial Mesenchymal
Transition in Bronchial Epithelial Cells. Int Arch Allergy Immunol. 2011;155:119–28. doi:10.1159/000318854.

30. Kim JH, Jang YS, Eom K-S, Hwang Y II, Kang HR, Jang SH, et al. Transforming Growth Factor β1 Induces Epithelial-to-Mesenchymal Transition of A549 Cells. J Korean Med Sci. 2007;22:898. doi:10.3346/jkms.2007.22.5.898.

31. Shintani Y, Maeda M, Chaika N, Johnson KR, Wheelock MJ. Collagen I Promotes Epithelial-to-Mesenchymal Transition in Lung Cancer Cells via Transforming Growth Factor-β Signaling. Am J Respir Cell Mol Biol. 2008;38:95–104. doi:10.1165/rcmb.2007-0071OC.

32. Câmara J, Jarai G. Epithelial-mesenchymal transition in primary human bronchial epithelial cells is Smad-dependent and enhanced by fibronectin and TNF-α. Fibrogenesis Tissue Repair. 2010;3:2. doi:10.1186/1755-1536-3-2.

33. Chen X-F, Zhang H-J, Wang H-B, Zhu J, Zhou W-Y, Zhang H, et al. Transforming growth factor-β1 induces epithelial-to-mesenchymal transition in human lung cancer cells via PI3K/Akt and MEK/Erk1/2 signaling pathways. Mol Biol Rep. 2012;39:3549–56. doi:10.1007/s11033-011-1128-0.

34. Kawata M, Koinuma D, Ogami T, Umezawa K, Iwata C, Watabe T, et al. TGF-β-induced epithelial-mesenchymal transition of A549 lung adenocarcinoma cells is enhanced by pro-inflammatory cytokines derived from RAW 264.7 macrophage cells. J Biochem. 2012;151:205–16. doi:10.1093/jb/mvr136.

35. O’Beirne SL, Walsh SM, Fabre A, Reviriego C, Worrell JC, Counihan IP, et al. CXCL9 Regulates TGF-β1-Induced Epithelial to Mesenchymal Transition in Human Alveolar Epithelial Cells. J Immunol. 2015;195:2788–96. doi:10.4049/jimmunol.1402008.

36. Kobayashi K, Koyama K, Suzukawa M, Igarashi S, Hebisawa A, Nagase T, et al. Epithelial-mesenchymal transition promotes reactivity of human lung
adenocarcinoma A549 cells to CpG ODN. Allergol Int. 2016;65:S45–52. doi:10.1016/j.alit.2016.06.010.

37. Hackett T-L, Warner SM, Stefanowicz D, Shaheen F, Pechkovsky D V., Murray LA, et al. Induction of Epithelial–Mesenchymal Transition in Primary Airway Epithelial Cells from Patients with Asthma by Transforming Growth Factor-β1. Am J Respir Crit Care Med. 2009;180:122–33. doi:10.1164/rccm.200811-1730OC.

38. Qi L, Song W, Li L, Cao L, Yu Y, Song C, et al. FGF4 induces epithelial-mesenchymal transition by inducing store-operated calcium entry in lung adenocarcinoma. Oncotarget. 2016;7:74015–30. doi:10.18632/oncotarget.12187.

39. Strutz F, Zeisberg M, Ziyadeh FN, Yang C-Q, Kalluri R, Müller GA, et al. Role of basic fibroblast growth factor-2 in epithelial-mesenchymal transformation. Kidney Int. 2002;61:1714–28. doi:10.1046/j.1523-1755.2002.00333.x.

40. Lau M-T, So W-K, Leung PCK. Fibroblast Growth Factor 2 Induces E-Cadherin Down-Regulation via PI3K/Akt/mTOR and MAPK/ERK Signaling in Ovarian Cancer Cells. PLoS One. 2013;8:e59083. doi:10.1371/journal.pone.0059083.

41. Lee JG, Jung E, Heur M. Fibroblast growth factor 2 induces proliferation and fibrosis via SNAI1-mediated activation of CDK2 and ZEB1 in corneal endothelium. J Biol Chem. 2018;293:3758–69. doi:10.1074/jbc.RA117.000295.

42. Masola V, Gambaro G, Tibaldi E, Brunati AM, Gastaldello A, D’Angelo A, et al. Heparanase and syndecan-1 interplay orchestrates fibroblast growth factor-2-induced epithelial-mesenchymal transition in renal tubular cells. J Biol Chem. 2012;287:1478–88. doi:10.1074/jbc.M111.279836.

43. Shirakihara T, Horiguchi K, Miyazawa K, Ehata S, Shibata T, Morita I, et al. TGF-β regulates isoform switching of FGF receptors and epithelial-mesenchymal transition. EMBO J. 2011;30:783–95. doi:10.1038/emboj.2010.351.
44. Chen J, Chen G, Yan Z, Guo Y, Yu M, Feng L, et al. TGF-β1 and FGF2 Stimulate the Epithelial-Mesenchymal Transition of HERS Cells Through a MEK-Dependent Mechanism. J Cell Physiol. 2014;229:1647–59. doi:10.1002/jcp.24610.

45. Chen P-Y, Qin L, Li G, Tellides G, Simons M. Fibroblast growth factor (FGF) signaling regulates transforming growth factor beta (TGFβ)-dependent smooth muscle cell phenotype modulation. Sci Rep. 2016;6:33407. doi:10.1038/srep33407.

46. Li F, Zhu T, Yue Y, Zhu X, Wang J, Liang L. Preliminary mechanisms of regulating PD-L1 expression in non-small cell lung cancer during the EMT process. Oncol Rep. 2018;40:775–82. doi:10.3892/or.2018.6474.

47. Kurimoto R, Iwasawa S, Ebata T, Ishiwata T, Sekine I, Tada Y, et al. Drug resistance originating from a TGF-β/FGF-2-driven epithelial-to-mesenchymal transition and its reversion in human lung adenocarcinoma cell lines harboring an EGFR mutation. Int J Oncol. 2016;48:1825–36. doi:10.3892/ijo.2016.3419.

48. Kurimoto R, Ebata T, Iwasawa S, Ishiwata T, Tada Y, Tatsumi K, et al. Pirfenidone may revert the epithelial-to-mesenchymal transition in human lung adenocarcinoma. Oncol Lett. 2017;14:944–50. doi:10.3892/ol.2017.6188.

49. Guzy RD, Stoilov I, Elton TJ, Mecham RP, Ornitz DM. Fibroblast Growth Factor 2 Is Required for Epithelial Recovery, but Not for Pulmonary Fibrosis, in Response to Bleomycin. Am J Respir Cell Mol Biol. 2015;52:116–28. doi:10.1165/rcmb.2014-0184OC.

50. Koo HY, El-Baz LMF, House SL, Cilvik SN, Dorry SJ, Shoukry NM, et al. Fibroblast growth factor 2 decreases bleomycin-induced pulmonary fibrosis and inhibits fibroblast collagen production and myofibroblast differentiation. J Pathol. 2018;246:54–66.

51. White SR, Fischer BM, Marroquin BA, Stern R. Interleukin-1beta mediates human
airway epithelial cell migration via NF-kappaB. Am J Physiol Lung Cell Mol Physiol. 2008;295:L1018-27. doi:10.1152/ajplung.00065.2008.

52. Schelch K, Wagner C, Hager S, Pirker C, Siess K, Lang E, et al. FGF2 and EGF induce epithelial–mesenchymal transition in malignant pleural mesothelioma cells via a MAPKinase/MMP1 signal. Carcinogenesis. 2018;39:534–45. doi:10.1093/carcin/bgy018.

53. Tanaka T, Saika S, Ohnishi Y, Ooshima A, McAvoy JW, Liu C-Y, et al. Fibroblast growth factor 2: roles of regulation of lens cell proliferation and epithelial-mesenchymal transition in response to injury. Mol Vis. 2004;10:462–7. http://www.ncbi.nlm.nih.gov/pubmed/15273655. Accessed 18 Oct 2018.

54. Strutz F, Zeisberg M, Renziehausen A, Raschke B, Becker V, Van Kooten C, et al. TGF-β1 induces proliferation in human renal fibroblasts via induction of basic fibroblast growth factor (FGF-2). Kidney Int. 2001;59:579–92. doi:10.1046/j.1523-1755.2001.059002579.x.

55. Bossé Y, Thompson C, Stankova J, Rola-Pleszczynski M. Fibroblast Growth Factor 2 and Transforming Growth Factor β1 Synergism in Human Bronchial Smooth Muscle Cell Proliferation. Am J Respir Cell Mol Biol. 2006;34:746–53. doi:10.1165/rcmb.2005-0309OC.

56. Xiao L, Du Y, Shen Y, He Y, Zhao H, Li Z. TGF-beta 1 induced fibroblast proliferation is mediated by the FGF-2/ERK pathway. Front Biosci (Landmark Ed. 2012;17:2667–74. http://www.ncbi.nlm.nih.gov/pubmed/22652804. Accessed 30 Oct 2018.

57. Hosper NA, van den Berg PP, de Rond S, Popa ER, Wilmer MJ, Masereeuw R, et al. Epithelial-to-mesenchymal transition in fibrosis: Collagen type I expression is highly upregulated after EMT, but does not contribute to collagen deposition. Exp Cell Res. 2013;319:3000–9. doi:10.1016/j.yexcr.2013.07.014.
58. Pardo OE, Latigo J, Jeffery RE, Nye E, Poulsom R, Spencer-Dene B, et al. The Fibroblast Growth Factor Receptor Inhibitor PD173074 Blocks Small Cell Lung Cancer Growth In vitro and In vivo. Cancer Res. 2009;69:8645–51. doi:10.1158/0008-5472.CAN-09-1576.

59. Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, et al. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. Proc Natl Acad Sci. 2006;103:13180–5. doi:10.1073/pnas.0605669103.

60. Tanjore H, Xu XC, Polosukhin V V., Degryse AL, Li B, Han W, et al. Contribution of Epithelial-derived Fibroblasts to Bleomycin-induced Lung Fibrosis. Am J Respir Crit Care Med. 2009;180:657–65. doi:10.1164/rccm.200903-0322OC.

61. Degryse AL, Tanjore H, Xu XC, Polosukhin V V., Jones BR, McMahon FB, et al. Repetitive intratracheal bleomycin models several features of idiopathic pulmonary fibrosis. Am J Physiol Cell Mol Physiol. 2010;299:L442–52. doi:10.1152/ajplung.00026.2010.

62. Ward C, Forrest IA, Murphy DM, Johnson GE, Robertson H, Cawston TE, et al. Phenotype of airway epithelial cells suggests epithelial to mesenchymal cell transition in clinically stable lung transplant recipients. Thorax. 2005;60:865–71. doi:10.1136/thx.2005.043026.

63. Harada T, Nabeshima K, Hamasaki M, Uesugi N, Watanabe K, Iwasaki H. Epithelial-mesenchymal transition in human lungs with usual interstitial pneumonia: Quantitative immunohistochemistry. Pathol Int. 2010;60:14–21. doi:10.1111/j.1440-1827.2009.02469.x.

64. Wu Z, Yang L, Cai L, Zhang M, Cheng X, Yang X, et al. Detection of epithelial to mesenchymal transition in airways of a bleomycin induced pulmonary fibrosis model
derived from an α-smooth muscle actin-Cre transgenic mouse. Respir Res. 2007;8:1. doi:10.1186/1465-9921-8-1.

65. Rock JR, Barkauskas CE, Cronce MJ, Xue Y, Harris JR, Liang J, et al. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. Proc Natl Acad Sci. 2011;108:E1475-83. doi:10.1073/pnas.1117988108.

66. Hoyles RK, Derrett-Smith EC, Khan K, Shiwen X, Howat SL, Wells AU, et al. An Essential Role for Resident Fibroblasts in Experimental Lung Fibrosis Is Defined by Lineage-Specific Deletion of High-Affinity Type II Transforming Growth Factor β Receptor. Am J Respir Crit Care Med. 2011;183:249-61. doi:10.1164/rccm.201002-0279OC.

67. Yamada M, Kuwano K, Maeyama T, Hamada N, Yoshimi M, Nakanishi Y, et al. Dual-immunohistochemistry provides little evidence for epithelial–mesenchymal transition in pulmonary fibrosis. Histochem Cell Biol. 2008;129:453-62. doi:10.1007/s00418-008-0388-9.

Figures
Morphological changes induced by TGFβ1 and FGF2. BEAS-2B cells were grown in complete growth media (Control) or stimulated with TGFβ1 (2 ng/ml), FGF2 (2 nM) + heparin sulphate (1 nM), or TGFβ1 + FGF2 + heparin sulphate for 4 days. Representative phase contrast images (10× original magnification) show the morphological change of BEAS-2B cells from cobblestone-like shape as in the control (A) and FGF2 treatment alone (B) to the fibroblast-like shape in the presence of TGFβ1 (C) which is unaltered after adding FGF2 (D).
Recombinant FGF2 enhances TGFβ1-induced EMT gene expression in BEAS-2B cells. BEAS-2B cells were incubated for 4 days in the absence or presence of 2 ng/ml TGFβ1 alone, 2 nM FGF2 alone, or both TGFβ1 and FGF2. Quantitative real-time PCR analysis (n = 6) shows that FGF2 when in combination with TGFβ1, downregulates the epithelial marker CDH1 (A) and induces an increase in ACTA2 (B) and CDH2 (C) mRNA expression levels. Ct values were normalized to GAPDH and expressed as fold change from untreated controls in the same sample. Statistical significance was determined by one-way ANOVA followed by Tukey’s multiple comparisons test; ns = not significant, * indicates p < 0.05, ** indicates p < 0.01, *** indicates p < 0.001, and **** indicates p < 0.0001.
TGFβ1 induced decrease in E-Cadherin protein expression is augmented by FGF2.

(A) Total cell lysates from BEAS-2B cells stimulated for 4 days with or without TGFβ1 (2 ng/ml) alone, FGF2 (2 nM) alone or TGFβ1 and FGF2, were immunoblotted for E-cadherin. Blots were reprobed for β-tubulin as a loading control. (B) Densitometry for E-cadherin was normalized to β-tubulin and is expressed as fold change from untreated control. Statistical significance was determined by one-way ANOVA followed by Tukey’s multiple comparisons test; ns = not significant, * indicates p < 0.05, ** indicates p < 0.01, and *** indicates p < 0.001. (C) Representative images showing immunofluorescent staining for E-cadherin in BEAS-2B cells stimulated with or without TGFβ1 (2 ng/ml) alone, FGF2 (2 nM) alone or TGFβ1 and FGF2 for 4 days. The confocal images were obtained at 20× original magnification for E-cadherin (green) and DAPI (blue).
Recombinant FGF2 inhibits TGFβ1 induced collagen, but not fibronectin or tenascin-C. BEAS-2B cells were incubated for 4 days in the absence or presence of 2 ng/ml TGFβ1 alone, 2 nM FGF2 alone, or both TGFβ1 and FGF2. Quantitative real-time PCR analysis (n = 3-6) shows that FGF2 maintained the effect of TGFβ1-induced expression of FN (A) and TNC (B) and downregulate COL1A1 (C) mRNA expression levels. Ct values were normalized to GAPDH and expressed as fold change from untreated controls in the same sample. Statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparisons test; ns = not significant, *** indicates p < 0.001, and **** indicates p < 0.0001.
The effect of recombinant FGF2 on TGFβ1-induced EMT gene expression is conserved in A549 cells. A549 cells were grown in complete Ham’s F12 media (Control) or stimulated with TGFβ1 (2 ng/ml), FGF2 (2 nM) + heparin sulphate (1 nM), or TGFβ1 + FGF2 + heparin sulphate for 4 days. Quantitative real-time PCR analysis (n =3) shows that FGF2 has a similar effect of TGFβ1 in decreasing CDH1 (A) and increasing ACTA2 (B) as well as inhibiting COL1A1 (C) mRNA expression levels. Ct values were normalized to GAPDH and expressed as fold change from untreated controls in the same sample. Statistical significance was determined by one-way ANOVA followed by Tukey’s multiple comparisons test; ns = not significant, ** indicates p < 0.01, *** indicates p < 0.001, and **** indicates p < 0.0001.
The effect of recombinant FGF2 on TGFβ1 induced EMT gene expression in lung epithelial cells is blocked by the FGFR-specific tyrosine kinase inhibitor PD173074. BEAS-2B cells were incubated with TGFβ1 (2 ng/ml), FGF2 (2 nM) alone, PD173074 (0.1 µM) alone or FGF2 (2 nM) + TGFβ1 +/- PD173074 for 4 days. Quantitative real-time PCR analysis (n =3-6) shows that PD173074 inhibited the effect of FGF2 on TGFβ1-treated cells in repression of CDH1 (A), induction of ACTA2 (B) and blocking of COL1A1 (C) mRNA expression levels. A549 cells were incubated with TGFβ1 (2 ng/ml), FGF2 (2 nM) alone, PD173074 (0.1 µM) alone or FGF2 (2 nM) + TGFβ1 +/- PD173074 for 4 days. Quantitative real-time PCR analysis (n =3) shows that PD173074 inhibited the effect of FGF2 on TGFβ1-treated cells in repression of CDH1 (D), and partially inhibited the induction of ACTA2 (E) and blocking of COL1A1 (F) mRNA expression levels. ▼Ct values were normalized to GAPDH and expressed as fold change from untreated controls in the same sample. Statistical significance was determined by one-way ANOVA followed by
Tukey’s multiple comparisons test; ns = not significant, * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$, and **** indicates $p < 0.0001$. 
FGF2, but not TGFβ1, increases BEAS-2B migration of epithelial cells when added immediately after wounding. BEAS-2B were grown in complete media to confluence, and 1mm diameter circular wounds were generated and the cells were treated immediately with TGFβ1 (2 ng/ml), FGF2 (2 nM), or TGFβ1 + FGF2 (n = 3). (A) Wound area was imaged at 0 h, 24 h, 48 h and 72 h then the % of wound closure was measured using ImageJ software. Statistical significance was determined by one-way ANOVA followed by Tukey’s multiple comparisons test; Red asterisks mark significant differences for FGF2 vs. TGFβ1, blue asterisks mark significant differences for TGFβ1 + FGF2 vs. FGF2, and green asterisks mark significant differences for TGFβ1 vs. control. * indicates p < 0.05, *** indicates p < 0.001 and **** indicates p < 0.0001. (B) Representative phase contrast images (10× original magnification) of the same area were taken immediately after wounding (0 h) as well as 24 h, 48 h and 72 h later.
Pre-treatment with FGF2 and TGFβ1 significantly increases migration rates BEAS-2B cells after wounding. (A) BEAS-2B cells were treated with TGFβ1 (2 ng/ml), FGF2 (2 nM), or both TGFβ1 and FGF2 for 3 days (n = 3). The cells then wounded and the % of wound closure was measured at 0 h, 24 h, 40 h and 48 h later. Statistical significance was determined by one-way ANOVA followed by Tukey’s multiple comparisons test; Red asterisks mark significant differences for FGF2 vs. control, blue asterisks mark significant differences for TGFβ1 + FGF2 vs. FGF2, blue circles mark significant differences for TGFβ1 + FGF2 vs. TGFβ1, green asterisks mark significant differences for TGFβ1 vs. control and black asterisks mark significant differences for control vs TGFβ1 + FGF2. * indicates p < 0.05, ** indicates p < 0.01, *** indicates p < 0.001, and **** indicates p < 0.0001. (B) Representative phase contrast images (10× original magnification) of the same area were taken immediately after wounding (0 h) as well as 24 h, 40 h and 48 h later.
