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Glucocorticoid induces mesenchymal-to-epithelial transition and inhibits TGF-β1-induced epithelial-to-mesenchymal transition and cell migration

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ABSTRACT

Epithelial-to-mesenchymal transition (EMT) has been implicated in various physiological and pathological events. In this study, we found that the synthetic glucocorticoid dexamethasone (Dex) can inhibit transforming growth factor-beta1-induced EMT and cell migration. We also demonstrated that Dex inhibits EMT through a mechanism involving the suppression of ROS generation. Surprisingly, Dex alone induced mesenchymal-to-epithelial transition (MET). Dexamethasone treatment abolished Snail1 binding to the E-cadherin promoter, suggesting that suppression of Snail1 contributes to the above roles of Dex. Our findings demonstrate that Dex functions as both a suppressor of EMT and as an inducer of MET and therefore may be implicated in certain pathophysiological events.

1. Introduction

Epithelial-to-mesenchymal transition (EMT) is a trans-differentiation process by which epithelial cells lose their epithelial cell characteristics and acquire a mesenchymal phenotype. EMT is characterized by changes in cell morphology, the disruption of tight junctions and adherent junctions, and decreased expression of E-cadherin, zonula occludens-1 (ZO-1) and other molecules. In addition, EMT is associated with increased expression of mesenchymal markers, such as fibronectin and vimentin. EMT plays an important role in embryogenesis, wound healing, tissue remodeling, fibrosis, and tumor metastasis [1,2]. The inverse process, mesenchymal-to-epithelial transition (MET), has also been implicated in development and other biological events. However, very little is known about the regulatory mechanisms underlying the MET.

The multifunctional cytokine transforming growth factor-β (TGF-β) regulates cell proliferation, differentiation, migration, extracellular matrix production, apoptosis and tumorigenesis [3]. TGF-β is also a potent inducer of EMT, and it has long been recognized that through EMT induction, TGF-β can promote tumor metastasis and invasion. Blockade of TGF-β signaling can decrease tumor cell motility and metastasis [4].

A growing number of molecules have been found to be involved in the EMT process. Among them, the zinc-finger transcription factor Snail, one member of the Snail superfamily of transcriptional repressors, plays a major role in triggering EMT [5]. Ectopic expression of Snail suppresses E-cadherin expression, leading to a full EMT phenotype, whereas silencing of Snail expression reverses this process [6,7]. Snail expression has been detected in a number of different human carcinoma and melanoma cell lines [8]. More importantly, Snail is expressed at the invasive front of epidermoid carcinomas and has been associated with breast carcinoma metastasis [9]. These data support a key role for Snail as an inducer of tumor metastasis.

Glucocorticoids are important signaling molecules involved in a variety of physiological and pathological responses [10]. Synthetic glucocorticoids are widely used drugs with broad anti-inflammatory effects. The biological effects of glucocorticoids are mediated by the glucocorticoid receptor (GR), a member of the nuclear receptor superfamily [11,12]. The glucocorticoid receptor regulates target gene expression through a glucocorticoid response element (GRE)-dependent mechanism. Depending on the nature of the GRE, glucocorticoid receptor binding can result in activation or repression of genes containing GR-binding sites. Alternatively, the glucocorticoid receptor can induce or suppress gene expression

Abbreviations: Dex, dexamethasone; TGF-β, transforming growth factor-beta; EMT, epithelial-to-mesenchymal transition; MET, mesenchymal-to-epithelial transition; ROS, reactive oxygen species

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through GRE-independent mechanisms, which are mediated through protein–protein interactions of the glucocorticoid receptor with other transcription factors. RU486 is a glucocorticoid antagonist that can compete with glucocorticoids and prevent glucocorticoid-receptor binding and is widely used in steroid hormone research and for the treatment of Cushing's syndrome [13].

The TGF-β and glucocorticoid signaling pathways interact both positively and negatively. Glucocorticoids inhibit the TGF-β-induced expression of extracellular matrix proteins, collagen, and tissue inhibitors of metalloproteinases [14–16]. TGF-β has also been shown to antagonize the effects of glucocorticoids during wound healing and fibrosis [17–19]. In addition, glucocorticoids and TGF-β have been shown to have opposite effects on the regulation of bone formation [20]. Conversely, TGF-β and glucocorticoid signaling pathways interact positively in some processes [21–24]. However, it remains unclear whether glucocorticoid signaling is involved in TGF-β-induced EMT.

2. Materials and methods

2.1. Cell culture and transfection

MvlLu cells (Mink lung epithelial cell line) were cultured in MEM medium containing 10% FBS, penicillin (100 U/mL), streptomycin (100 μg/mL) and non-essential amino acids. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 until 30–50% of confluence was reached. Transfection was carried out using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

2.2. Cell lysate preparation and immunoblotting

Cells were lysed in lysis buffer containing 50 mmol/L HEPES (pH 7.4), 5 mmol/L EDTA, 50 mmol/L NaCl, 1% Triton X-100, 50 mmol/L NaF, 10 mmol/L $Na_4P_2O_7{\cdot}10H_2O$, 5 μg/mL aprotinin, 5 μg/mL leupeptin, 1 mmol/L Na_3VO_4, and 1 mmol/L phenylmethylsulfonyl fluoride (PMSF). Proteins (30 μg) were separated by SDS–polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The membranes were subsequently blocked with 5% skim milk and incubated with the indicated antibodies. Protein bands were visualized using ECL reagents.

2.3. Measurements of cellular reactive oxygen species (ROS)

Cells were trypsinized, suspended in 1 mL of serum-free DMEM, incubated with 10 μmol/L 2′,7′-dichlorofluorescein-diacetate (DCFDA) at 37 °C for 30 min, and then washed 3 times with serum-free DMEM medium. DCFDA was excited at 488 nm, and fluorescence was measured at 525 nm with a flow cytometer (Becton Dickinson FACSCalibur). The mean fluorescence per cell was used for comparison.

2.4. Scratch assays

Confluent monolayers of cells were scratched by a pipette tip and further incubated with 10% FBS medium in the presence or absence of TGF-β1 for 48 h. Cell migration images were taken under a microscope.

2.5. E-cadherin promoter analysis

The E-cadherin promoter luciferase reporter plasmid was a generous gift from Dr. Amparo Cano. Mv1Lu cells were seeded in 24-well tissue culture plates and transiently transfected with human E-cadherin promoter reporter and pRL-CMV Renilla reporter using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Where indicated, cotransfections were carried out in the presence of empty vector or human Snail1 expression plasmids. Twenty-four hours after transfection, cells were treated with Dex or TGF-β1. Luciferase activities (firefly luciferase and Renilla luciferase) were determined by a dual luciferase reporter assay system (Promega). Luciferase activity was normalized to E-cadherin promoter activity in control cells. All experimental values were determined from triplicate wells.

2.6. Immunofluorescence analysis of actin remodeling

Cells were grown on glass slides and treated with TGF-β1 and Dex. To terminate the reactions, slides were quickly washed with PBS followed by fixing in 4% polyoxymethylene for 10 min. The samples were stained with Texas red-phalloidin to visualize F-actin and nuclei were stained with DAPI (blue fluorescence). Images were taken at 1000× magnification under a confocal microscope.

2.7. Antibodies and plasmids

Antibodies against the following were obtained: E-Cadherin (BD Biosciences), Snail1 (Cell Signaling Technology), α-SMA (Millipore), fibronectin and Flag (Sigma), actin, glucocorticoid receptor, vimentin and Cytokeratin 18 (Santa Cruz). Human Snail1 sequence was cloned into the pCMV-Tag2B plasmid.

2.8. Chromatin immunoprecipitation (ChIP) assays

Chromatin preparation and ChIP experiments were performed according to the protocol from Upstate Biotechnology. E-Cadherin promoter was amplified with the primers described in Supplementary data. PCR was carried out according to the following program: 40 cycles at 94 °C for 40 s, 62–65 °C for 40s, and 72 °C for 40s. Amplified DNA was separated on a 2% agarose gel and visualized with ethidium bromide.

2.9. Determination of Snail1 mRNA levels by reverse transcription-PCR

Total cellular RNA was isolated using Trizol reagent according to the manufacturer's instructions. RNA was reverse transcribed and amplified by PCR with the following primers described in Supplementary data. The following PCR conditions were used for Snail1: 30 s at 94 °C, 40 s at 60 °C, and 30 s at 72 °C. The amplification products obtained in 35 cycles were analyzed on 2% agarose gels.

2.10. Materials

Dexamethasone (Dex), LY29402, SB203580 and RU486 were purchased from Sigma. Compound C was from Calbiochem. NAC and 2′,7′-dichlorofluorescein-diacetate (DCFDA) were from Beyotime.

2.11. Statistical analysis

Data are expressed as means ± S.E.M. from at least three independent experiments. Statistical analysis was performed using Student's *t*-test or one way ANOVA, followed by the LSD-*t* test for multiple comparisons. P values < 0.05 were considered statistically significant.

3. Results

3.1. Dex inhibits TGF-β1-induced EMT and cell migration

Upon treatment with TGF-β1 (2 ng/mL), Mv1Lu cells acquired a spindle-like cell morphology (Fig. 1A, upper panel), which is

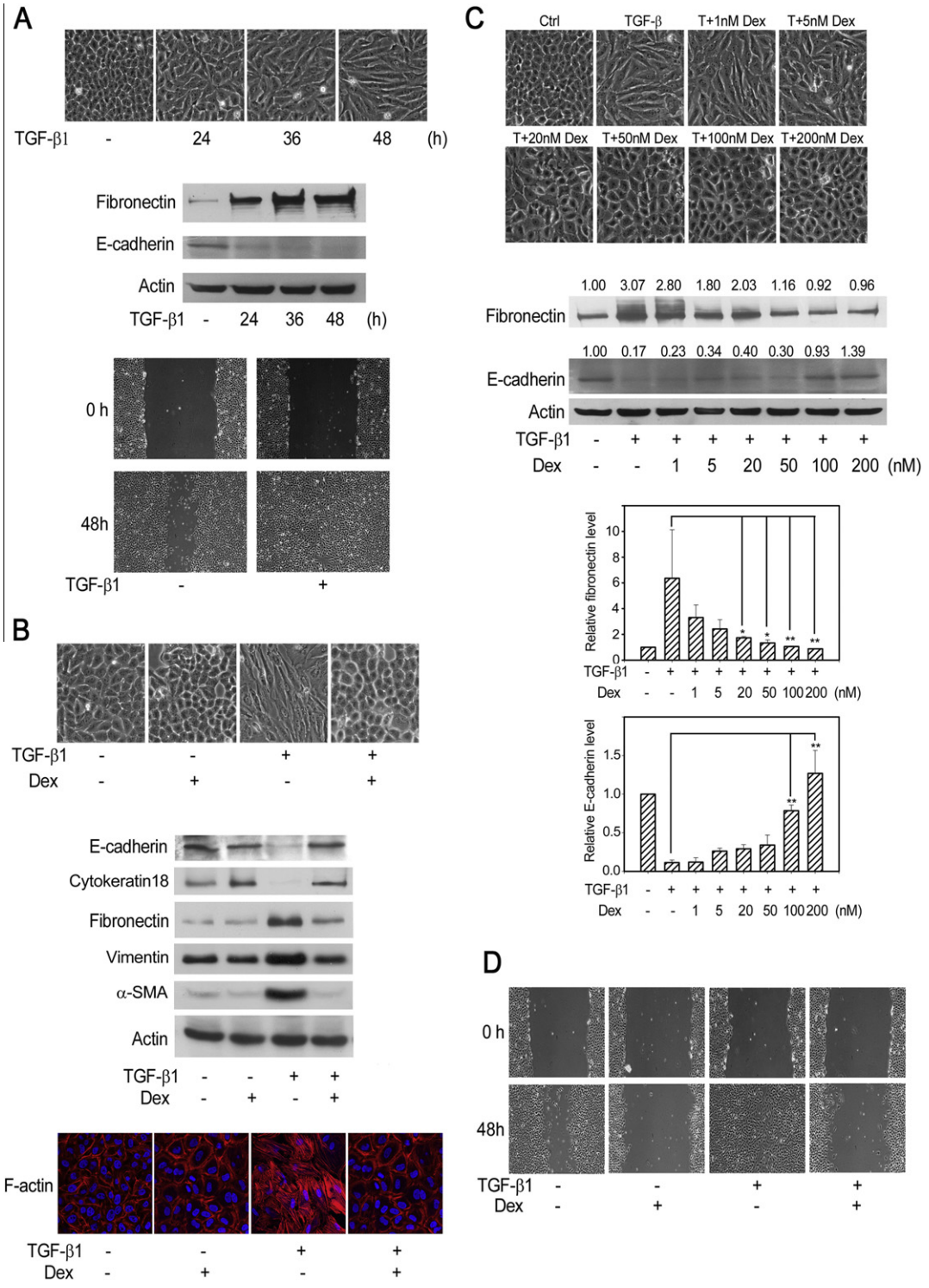

Fig. 1. Dexamethasone (Dex) inhibits TGF-β1-induced EMT and cell migration. Mv1Lu cells were treated with TGF-β1 (2 ng/mL) for the indicated times. (A) EMT was assessed by observing cell morphological changes under a phase-contrast microscope (top). The expression levels of EMT markers E-cadherin and fibronectin were examined by western blot (middle). To examine cell motility, confluent Mv1Lu cells were scratched by a pipette tip and treated with TGF-β1 for 48 h in 10% FBS-containing medium. Cell migration was observed under a phase-contrast microscope (bottom). (B) Cells were treated with TGF-β1 (2 ng/mL) and/or Dex (100 nM) for 48 h in 10% FBS-containing medium, EMT was examined by examining the cell morphological changes (upper) and the expression levels of E-cadherin, α-SMA, vimentin, Cytokeratin 18 and fibronectin (lower) were determined. The effect of Dex (100 nM) on actin remodeling induced by TGF-β1 (48 h) was examined by immunofluorescence (bottom). (C) Dose response of Dex-induced inhibition of TGF-β1-induced EMT. Cells were treated with TGF-β1 (2 ng/mL) and the indicated doses of Dex. EMT was examined by cell morphological phenotype (upper) and the levels of fibronectin and E-cadherin (middle). Data are presented as means ± S.D. from at least three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$. (D) Cells were treated with or without TGF-β1 in the presence or absence of Dex (100 nM) as described above. Cell migration was determined by scratch assay.

consistent with the observed decrease in the epithelial marker E-cadherin and upregulation of the mesenchymal marker fibronectin (Fig. 1A, middle panel). TGF-β1 also increased the motility of Mv1Lu cells as shown by a scratch assay (Fig. 1A, lower panel). Interestingly, treatment of cells with the synthetic glucocorticoid dexamethasone significantly inhibited TGF-β1-induced EMT, as

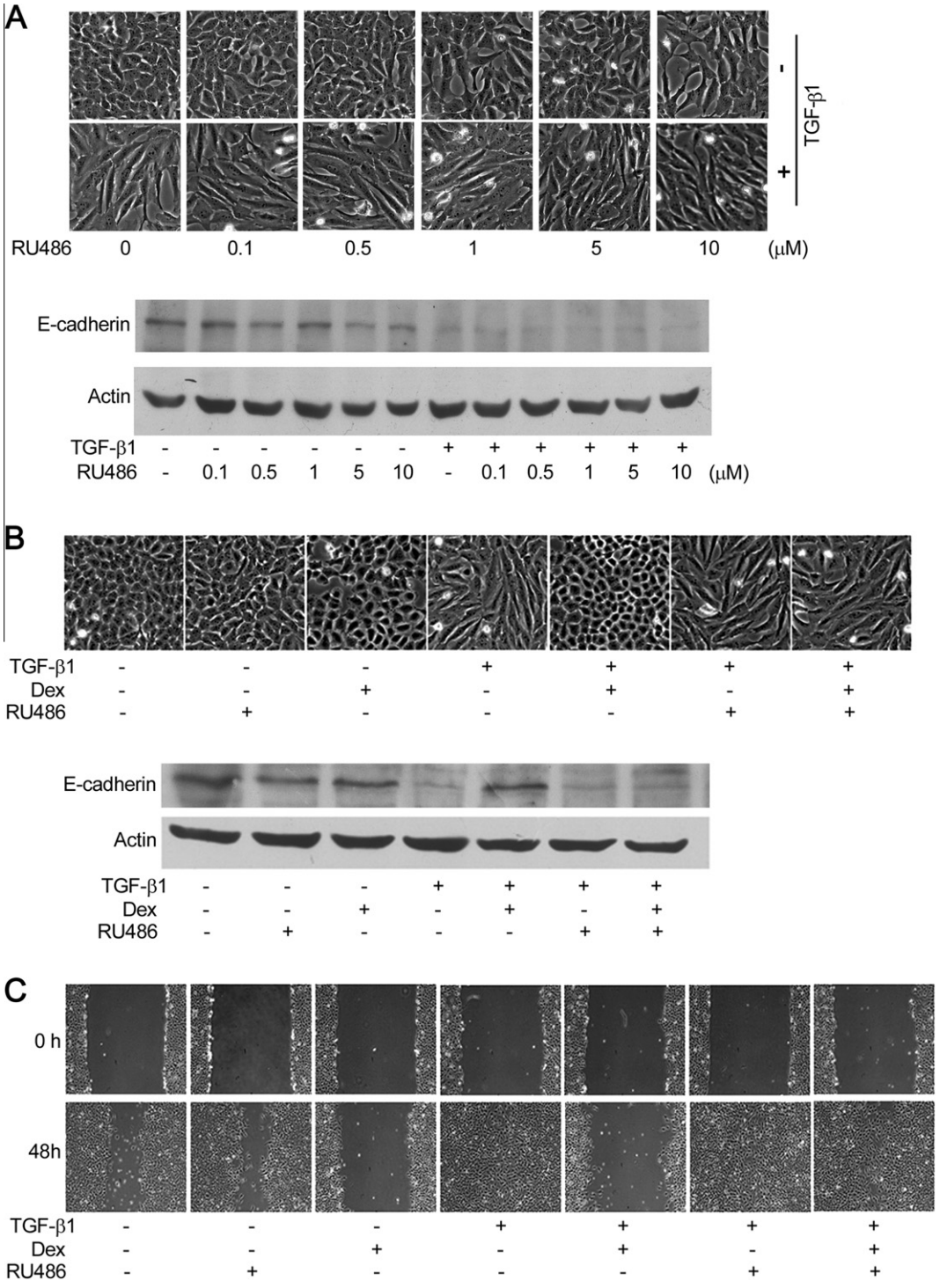

Fig. 2. RU 486 reverses the inhibitory effect of dexamethasone (Dex) on TGF-β1-induced EMT and cell migration. (A) Mv1Lu cells were treated with TGF-β1 (2 ng/mL) for 48 h in the presence of different concentrations of RU 486 and EMT was assessed by cell morphological changes and protein levels of E-cadherin. (B) Cells were treated with TGF-β1 for 48 h in the presence or absence of 100 nM Dex and RU 486 (0.5 μM). EMT was assessed by observing cell morphological changes and E-cadherin protein levels. Cell migration was measured by scratch assay (C).

indicated by changes in cell morphology, cytoskeleton rearrangement, and levels of E-cadherin, Cytokeratin 18, α-SMA, vimentin and fibronectin (Fig. 1B). Inhibition of EMT by Dex occurred in a dose-dependent manner (Fig. 1C). Consistent with its effects on EMT, Dex also inhibited TGF-β1-induced cell migration (Fig. 1D) in scratch assays. In addition, Dex inhibited cell migration in the absence of exogenous TGF-β1 (Fig. 1D). Interestingly, Dex had no effect on the proliferation of Mv1Lu cells, but could block TGF-β1-induced growth arrest (Fig. S1).

3.2. RU486 reverses the effect of Dex

To confirm the specific effects of glucocorticoids on these phenotypes, a glucocorticoid receptor antagonist, RU 486, was used

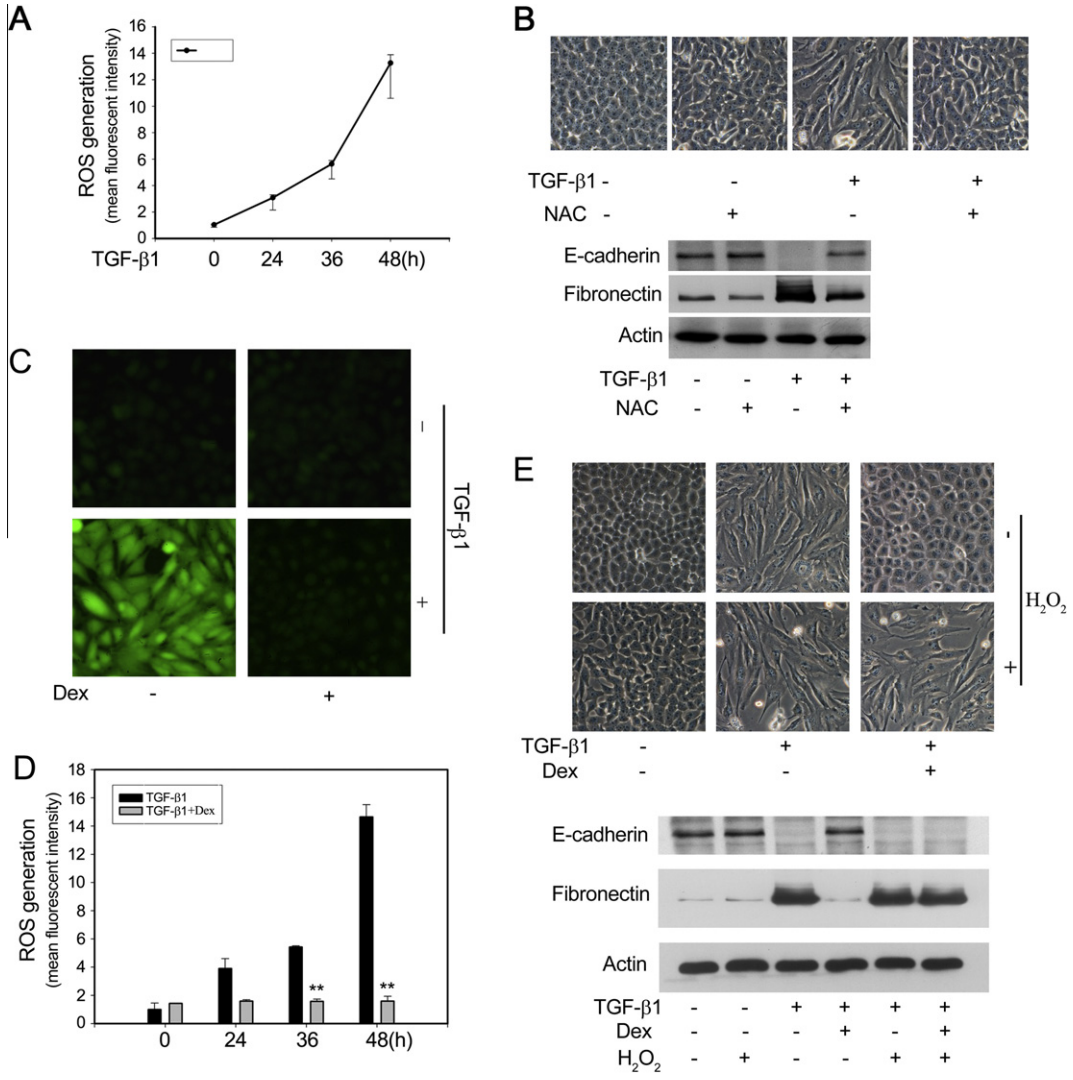

Fig. 3. Dexamethasone (Dex) inhibits EMT by suppressing ROS generation. (A) Mv1Lu cells were treated with TGF-β1 (2 ng/mL) for the indicated times. The level of cellular ROS was measured as described in Section 2. (B) NAC inhibits TGF-β1-induced EMT. Mv1Lu cells were treated with TGF-β1 for 48 h in the presence of 10 mmol/L NAC. EMT was determined by cell morphological changes (upper) and expression of E-cadherin and fibronectin (lower). (C) Dex inhibits TGF-β1-induced ROS generation. After treatment with TGF-β1 (2 ng/mL) and/or Dex (100 nM) for 48 h, cells were washed 3 times with serum-free medium, incubated for 30 min with 10 μM DCFDA, washed again 3 times with serum-free medium and imaged by fluorescence microscopy. (D) Cells were treated with TGF-β1 and/or Dex (100 nM) for the indicated times, and ROS generation was measured as described in Section 2. Data are presented as means ± S.D. from at least three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$. (E) Cells were treated with TGF-β1 and/or 100 nM Dex for 48 h in the absence or presence of 0.3 mmol/L H_2O_2, and EMT was examined by observing cell morphological changes (upper) and protein levels of E-cadherin and fibronectin (lower).

to block the effects of Dex. RU 486 does not noticeably affect TGF-β1-induced EMT (Fig. 2A), although high doses of RU 486 (5–10 μM) can induce cell phenotype changes and a decrease in E-cadherin. These changes may be due to non-specific effects, since high concentrations of RU 486 can also lead to decreased cell growth. In the presence of RU 486, Dex-mediated suppression of TGF-β1-induced EMT was abolished, as shown by cell morphology and expression of E-cadherin (Fig. 2B). RU 486 treatment also abolished the Dex-mediated inhibition of cell migration (Fig. 2C). These results indicate that glucocorticoid has strong inhibitory effects on TGF-β1-induced EMT and cell migration.

3.3. Dex inhibits EMT through suppression of ROS generation

Treatment of cells with TGF-β1 markedly increases the cellular ROS level, as measured by DCFDA fluorescence (Fig. 3A). *N*-Acetylcysteine (NAC) is a powerful antioxidant that reacts with several ROS. NAC can also suppress TGF-β1-induced ROS generation (Fig. S2). In the presence of NAC, TGF-β1-induced morphological changes, the increase in fibronectin, and the decrease in E-cadherin were blocked (Fig. 3B). Interestingly, Dex can also significantly suppress TGF-β1-induced ROS generation (Fig. 3C and D). Treatment with H_2O_2, a well-known oxidant that can increase cellular ROS levels, abolished the inhibitory effect of Dex on TGF-β1-induced EMT (Fig. 3E), which confirmed that Dex inhibits TGF-β1-induced EMT through a mechanism involving the suppression of ROS. Treatment with H_2O_2 alone did not induce EMT, suggesting that ROS is necessary but not sufficient to induce EMT.

3.4. Dex promotes MET

MET is the reverse process of EMT, and has been implicated in embryonic development [25]. Surprisingly, we observed that Dex not only blocked TGF-β1-induced EMT (Fig. 1), but also induced MET. Cells that underwent EMT 48 h after TGF-β1 induction (Fig. 4A) could be restored to their original epithelial phenotype

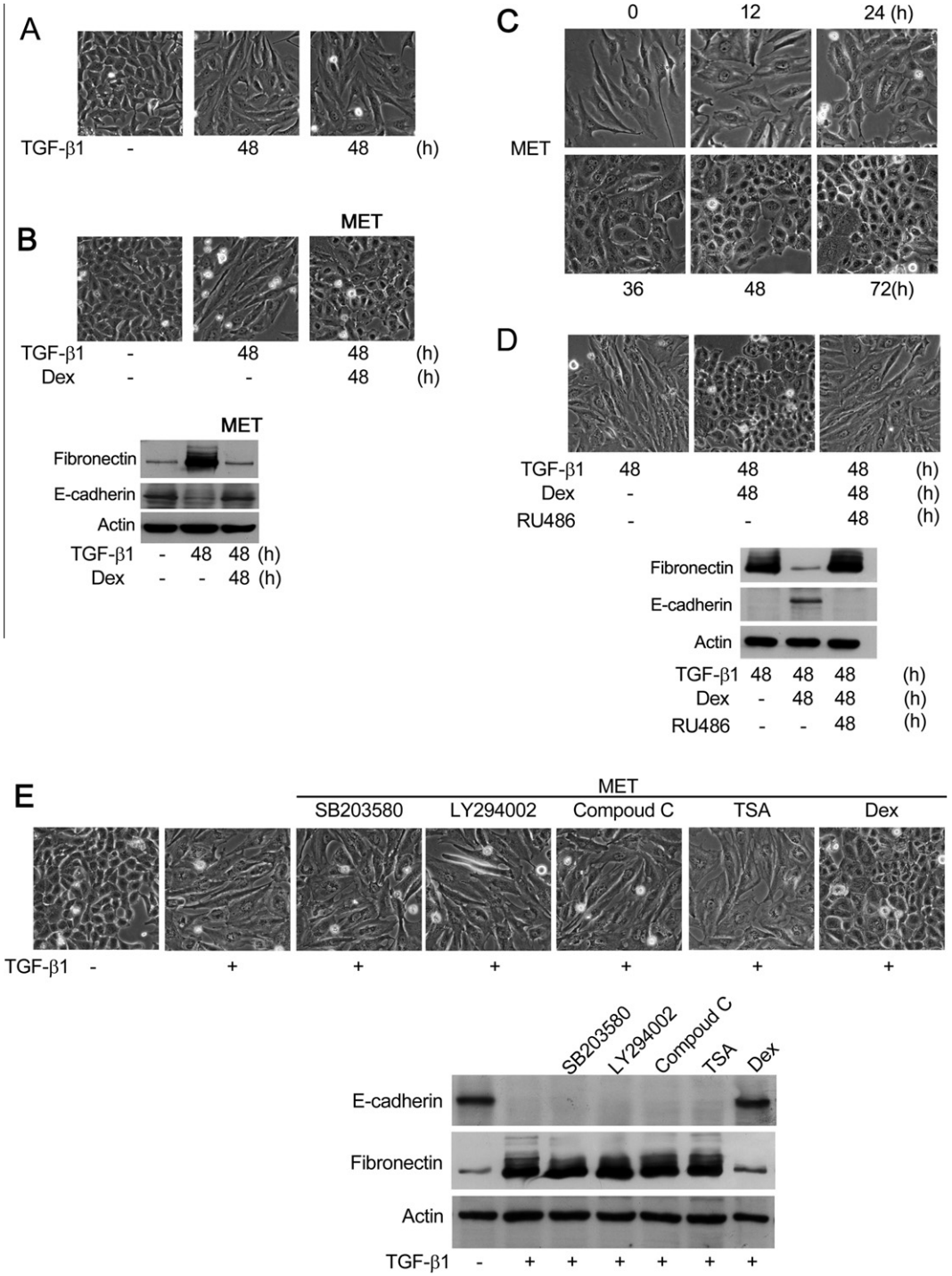

Fig. 4. Dexamethasone (Dex) promotes MET. After treatment with TGF-β1 (2 ng/mL) for 48 h, Mv1Lu cells acquired a mesenchymal phenotype (A). The mesenchymal-like cells (in A) were treated with Dex (100 nM) for another 48 h in the presence of TGF-β1. MET was assessed by cell morphology (B, upper) and the levels of mesenchymal markers fibronectin and epithelial maker E-cadherin were determined by Western blot (B, lower). (C) Cells were treated with TGF-β1 (2 ng/mL) for 48 h to induce EMT. Then, cells were treated with Dex (100 nM) for the indicated times in the presence of TGF-β1, and MET was determined by cell morphology. (D) RU 486 inhibits Dex-induced MET. After treatment with TGF-β1 (2 ng/mL) for 48 h to induce EMT, cells were treated with Dex (100 nM) for an additional 48 h in the presence or absence of RU 486 (0.5 μM). MET was assessed by observing cell morphological changes and the protein levels of E-cadherin and fibronectin. (E) SB203580, LY294002, Compound C, TSA did not induce MET. Mv1Lu cells were treated with TGF-β1 (2 ng/mL) for 48 h to induce EMT. Afterward, they were treated with SB203580 (10 μM), LY294002 (10 μM), Compound C (10 μM), TSA (20 ng/mL) or Dex (100 nM) for 48 h in the presence of TGF-β1, and MET was assessed by cell morphology and levels of fibronectin and E-cadherin.

after the addition of Dex for a further 48 h (Fig. 4B), as shown by cell morphology and expression levels of E-cadherin and fibronectin. Dexamethasone-induced MET exhibited a typical time-dependent progression (Fig. 4C). Furthermore, RU 486 treatment blocked Dex-induced MET in the presence of TGF-β1 (Fig. 4D), supporting a role for glucocorticoids in the induction of MET. To determine whether glucocorticoid promotes MET simply by virtue of inhibiting EMT, we examined the effects of other EMT inhibitors on MET induction: SB203580 (p38 inhibitor), LY294002 (PI3K inhibitor), TSA (HDAC inhibitor), and Compound C (AMP-activated kinase

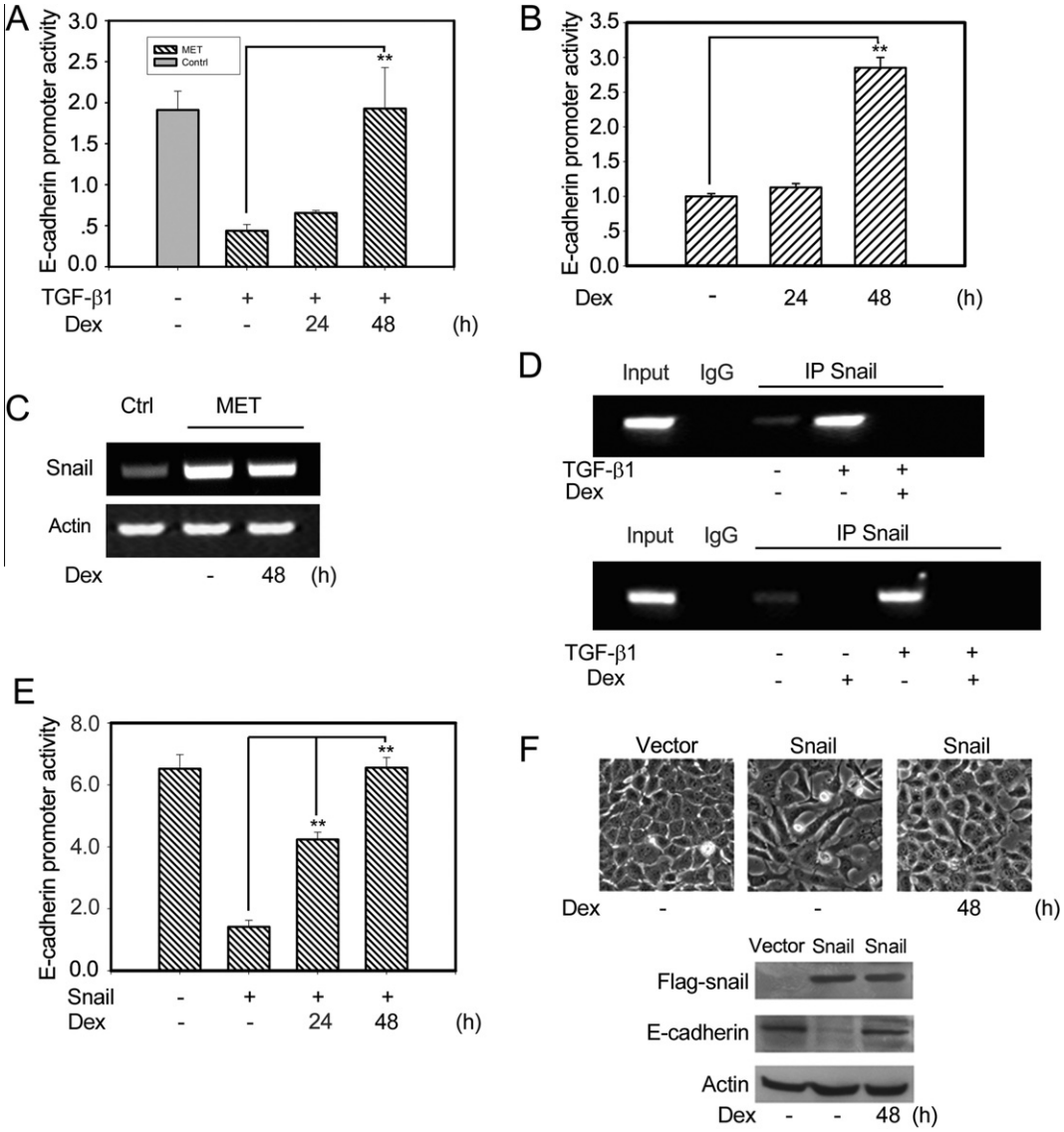

Fig. 5. Dexamethasone (Dex) promotes MET and inhibits EMT by blocking the inhibitory effect of Snail1 on E-cadherin promoter. (A) Dex increases E-cadherin promoter activity during MET. Twenty-four hours after transfection of the luciferase reporter plasmid, Mv1Lu cells were treated with TGF-β1 (2 ng/mL) for 48 h to induce EMT. The EMT cells were treated with 100 nM Dex for the indicated times in the presence of TGF-β1 and E-cadherin promoter activity was examined. Data are presented as means ± S.D. from at least three independent experiments. $^{**}P < 0.01$. (B) Dex stimulates E-cadherin promoter activity. Mv1Lu cells were treated with Dex for indicated times, and relative E-cadherin promoter activity was examined. Data are presented as means ± S.D. from at least three independent experiments. $^{**}P < 0.01$. (C) Dex induced MET (performed as above). Snail1 mRNA levels were examined by RT-PCR. (D) ChIP assays showed that Dex impaired Snail1 binding to the E-cadherin promoter during MET and EMT. Upper panel: cells were treated with TGF-β1 (2 ng/mL) for 48 h to induce EMT, and then treated with/without Dex (100 nM) for 48 h in the presence of TGF-β1. Lower panel: cells were treated with TGF-β1 (2 ng/mL) for 48 h in the presence or absence of Dex (100 nM). ChIP assays were carried out using antibodies against Snail1 and IgG. (E) Dex abolishes Snail1 overexpression-induced suppression of E-cadherin promoter activity. Twenty-four hours after transient transfection of Snail1, cells were treated with Dex for the indicated times, and relative E-cadherin promoter activity was examined. (F) Dex inhibits Snail1-induced EMT. After transient transfection of Snail1, cells were treated with/without 100 nM Dex for 48 h. EMT was assessed by cell morphological changes (upper panel) and the expression levels of E-cadherin (lower panel).

inhibitor) [26–29]. Although all these inhibitors could suppress TGF-β1-induced EMT (Fig. S3), they did not induce the MET (Fig. 4E, upper and lower panels). Similar results were obtained in cells co-treated with these inhibitors (Fig. S4).

3.5. Dex promotes MET and inhibits EMT by blocking the inhibitory effect of Snail1 on the E-cadherin promoter

To investigate the mechanism by which Dex promotes MET, we examined E-cadherin promoter activity. Dex could restore the E-cadherin promoter activity of cells with a mesenchymal phenotype to the normal level of epithelial cells (Fig. 5A). Dex treatment also abolished the inhibition of E-cadherin promoter activity by TGF-β1 during EMT (Fig. S5A). Moreover, Dex enhanced the E-cadherin promoter activity in a time-dependent manner in normal Mv1Lu cells (Fig. 5B). Snail1 is a key regulatory molecule for EMT that can bind to E-box elements of the E-cadherin promoter and suppress E-cadherin transcription. Snail1 expression increases during TGF-β1-induced EMT (Fig. S5B). The above results suggest that suppression of Snail1 may play a role in this MET process. Dex had no inhibitory effect on Snail1 mRNA levels during MET (Fig. 5C), and did not block the TGF-β1-induced increase in Snail1 expression levels (Fig. S5C). Dex treatment (48 h) of cells that have undergone EMT impaired the interaction of Snail1 with the E-cadherin promoter during MET, as shown by ChIP assay (Fig. 5D, upper panel). Dex also blocked Snail1 binding to the

E-cadherin promoter during EMT (Fig. 5D, lower panel). We excluded the possibility that Dex inhibits the Snail–E-cadherin promoter interaction by inducing glucocorticoid receptor binding to the E-cadherin promoter. As shown in Fig. S6, the glucocorticoid receptor did not interact with the E-cadherin promoter. Furthermore, Dex treatment also significantly restored the E-cadherin promoter activity that was suppressed by Snail1 overexpression in Mv1Lu cells (Fig. 5E). These results demonstrate that blocking Snail1 binding to the E-cadherin promoter contributes to the Dex-mediated suppression of EMT and the induction of MET. To confirm this model, we generated a cell line transiently overexpressing Snail1. Overexpression of Snail1 induced the morphological phenotype characteristic of EMT. Treatment of Snail1-overexpressing cells with Dex completely restored the epithelial phenotype as shown by cell morphology (Fig. 5F, upper) and E-cadherin levels (Fig. 5F, lower).

4. Discussion

Glucocorticoids are among the most widely used anti-inflammatory drugs for the treatment of inflammatory disorders including rheumatoid arthritis, asthma, dermatitis, idiopathic pulmonary fibrosis and autoimmune diseases such as Crohn's disease [10]. Here, we found that the synthetic glucocorticoid Dex can block TGF-β1-induced EMT in Mv1Lu cells. Recently, Godoy et al. reported changes in the mRNA levels of 12 of 17 analyzed EMT markers in mouse primary hepatocytes in the presence of Dex [30]. This finding suggested that Dex may be a potent regulator of EMT, which is supported by our observations.

ROS are free radicals that contain an oxygen atom and include hydrogen peroxide, superoxide anion and hydroxyl radical. Cellular production of ROS has been implicated in various pathophysiological processes, such as carcinogenesis [31] and fibrotic diseases [32]. ROS have been reported to play an important role in TGF-β induced EMT [33,34]. In addition, ROS have also been shown to be involved in the mediation of matrix metalloproteinase 3-, hypoxia-, and aldosterone-induced EMT [35–37]. Suppression of cellular ROS signaling with antioxidants, such as NAC, can inhibit TGF-β-induced EMT [38]. In the current study, we have shown that Dex inhibited TGF-β1-induced EMT by suppressing TGF-β1-induced ROS generation. However, it remains unclear how glucocorticoids could block such ROS generation. Dex may suppress TGF-β-induced ROS generation by modulating mitochondrial function or the expression levels of certain redox-oxidation enzymes.

A growing body of evidence has been obtained concerning the mechanisms of EMT regulation. However, much less is known about MET. Auersperg et al. reported that MET could be induced by overexpression of the epithelial marker E-cadherin in cultured cells [39]. Das et al. showed that complete reversal of EMT required inhibition of both ZEB1 and Rho pathways [40]. Inhibition of p38 MAPK, PI3K, HDAC and AMP-activated kinase inhibited TGF-β-induced EMT, but could not reverse EMT or induce MET. These results indicate that inhibition of EMT alone is not sufficient to induce MET. Our novel finding that the glucocorticoid Dex can induce MET presents an important step toward understanding the regulation of MET.

The transcription factor Snail can suppress E-cadherin transcription by recognizing E-box elements in its target promoters, and it plays a critical role in EMT [6,7]. The finding that Dex stimulates E-cadherin promoter activity is consistent with previous reports that Dex can increase E-cadherin expression in human primary nasal epithelial cells and human osteoblastic Cells [41,42]. E-Cadherin re-expression is required for MET, which indicates that the suppression of Snail may be involved in Dex-mediated MET. Indeed, Dex blocked the binding of Snail1 to the E-cadherin promoter, which abolished the Snail1 overexpression-induced suppression of transcriptional activity of E-cadherin, contributing to the inhibition of EMT. These observations indicate that the same signaling components can be utilized both in the inhibition of EMT and in the induction of MET. The precise mechanism by which glucocorticoids inhibit Snail1 binding to the E-cadherin promoter has not yet been determined. Dex may induce the interaction of Snail1 with specific molecules that block E-cadherin promoter association. In addition, Dex may inhibit Snail1 binding to E-cadherin promoter by blocking the nuclear translocation of Snail1.

Inhibition of EMT is potentially of great importance in therapeutic practice, and the induction of MET may also be a promising strategy for medical treatment. The present study suggests that in the treatment of fibrosis, glucocorticoids can act not only through anti-inflammatory effects but also by inducing MET and inhibiting EMT. EMT defects have been reported to be involved in the induction of cleft palate [43], and glucocorticoids have been shown to induce cleft palate in mice [44–47]. Our data further suggest that glucocorticoid-induced cleft palate may be related to its inhibitory effect on EMT. It has been reported that Dex does not inhibit TGF-β1-induced changes in the mRNA levels of E-cadherin and fibronectin, but is able to suppress the increase of α-SMA in human bronchial epithelial cells [48]. The discrepancy between this report and our observations may be due to tissue or cell type specificity. In addition, mRNA levels may not correlate well with levels of protein molecules because the latter can be regulated at several different levels.

In summary, our data show that the synthetic glucocorticoid Dex inhibits TGF-β1-induced EMT and cell migration by suppressing TGF-β1-induced ROS generation. H_2O_2, a well-known oxidant that can increase cellular ROS, can block Dex-mediated suppression of EMT induced by TGF-β1. We have also demonstrated that Dex can induce MET. Treatment with Dex blocks Snail1 binding to the E-cadherin promoter, suggesting that suppression of Snail1 contributes to both the inhibition of EMT and the induction of MET. Further studies in other cellular models are needed to determine the ubiquity of these processes in normal and pathological processes.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2010.10.038.

References

[1] Thiery, J.P. (2002) Epithelial-mesenchymal transitions in tumor progression. Nat. Rev. Cancer 2, 442–454.
[2] Moustakas, A. and Heldin, C.H. (2007) Signaling networks guiding epithelial-mesenchymal transitions during embryogenesis and cancer progression. Cancer Sci. 98, 1512–1520.
[3] Massague, J. and Wotton, D. (2003) Transcriptional control by the TGF-beta/Smad signaling system. EMBO J. 19, 1745–1754.
[4] Ikushima, H. and Miyazono, K. (2010) TGF-beta signalling: a complex web in cancer progression. Nat. Rev. Cancer 10, 415–424.
[5] Nieto, M.A. (2002) The Snail superfamily of zinc-finger transcription factors. Nat. Rev. Mol. Cell Biol. 3, 155–166.
[6] Cano, A., Perez-Moreno, M.A., Rodrigo, I., Locascio, A., Blanco, M.J., del Barrio, M.G., Portillo, F. and Nieto, M.A. (2000) The transcription factor Snail controls

epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat. Cell Biol. 2, 76–83.
[7] Batlle, E., Sancho, E., Francí, C., Domínguez, D., Monfar, M., Baulida, J. and García De Herreros, A. (2000) The transcription factor Snail is a repressor of E-cadherin gene expression in epithelial tumor cells. Nat. Cell Biol. 2, 84–89.
[8] Peinado, H., Olmeda, D. and Cano, A. (2007) Snail, Zeb and bHLH factors in tumor progression: an alliance against the epithelial phenotype? Nat. Rev. Cancer 7, 415–428.
[9] Blanco, M.J., Moreno-Bueno, G., Sarrio, D., Locascio, A., Cano, A., Palacios, J. and Nieto, M.A. (2002) Correlation of Snail expression with histological grade and lymph node status in breast carcinomas. Oncogene 21, 3241–3246.
[10] Karin, M. (1998) New twists in gene regulation by glucocorticoid receptor: is DNA binding dispensable? Cell 93, 487–490.
[11] Evans, R.M. (1988) The steroid and thyroid hormone receptor superfamily. Science 240, 889–895.
[12] Beato, M., Herrlich, P. and Schütz, G. (1995) Steroid hormone receptors: many actors in search of a plot. Cell 83, 851–857.
[13] Mao, J.R., Regelson, W. and Kalimi, M. (1992) Molecular mechanism of RU486: a review. Mol. Cell Biochem. 109, 1–8.
[14] Su, S., Dehnade, F. and Zafarullah, M. (1996) Regulation of tissue inhibitor of metalloproteinases-3 gene expression by transforming growth factor-beta and dexamethasone in bovine and human articular chondrocytes. DNA Cell Biol. 15, 1039–1048.
[15] Slavin, J., Unemori, E., Hunt, T.K. and Amento, E. (1994) Transforming growth factor beta (TGF-beta) and dexamethasone have direct opposing effects on collagen metabolism in low passage human dermal fibroblasts in vitro. Growth Factors 11, 205–213.
[16] Guller, S., Wozniak, R., Kong, L. and Lockwood, C.J. (1995) Reduction of extracellular matrix protein expression in human amnion epithelial cells by glucocorticoids: a potential role in preterm rupture of the fetal membranes. J. Clin. Endocrinol. Metab. 80, 3273–3278.
[17] Meisler, N., Keefer, K.A., Ehrlich, H.P., Yager, D.R., Myers-Parrelli, J. and Cutroneo, K.R. (1997) Dexamethasone abrogates the fibrogenic effect of transforming growth factor-beta in rat granuloma and granulation tissue fibroblasts. J. Invest. Dermatol. 108, 285–289.
[18] Pierce, G.F., Mustoe, T.A., Lingelbach, J., Masakowski, V.R., Gramates, P. and Deuel, T.F. (1989) Transforming growth factor beta reverses the glucocorticoid-induced wound-healing deficit in rats: possible regulation in macrophages by platelet-derived growth factor. Proc. Natl. Acad. Sci. USA 86, 2229–2233.
[19] Beck, L.S., DeGuzman, L., Lee, W.P., Xu, Y., Siegel, M.W. and Amento, E.P. (1993) One systemic administration of transforming growth factor-beta 1 reverses age- or glucocorticoid-impaired wound healing. J. Clin. Invest. 92, 2841–2849.
[20] Chang, D.J., Ji, C., Kim, K.K., Casinghino, S., McCarthy, T.L. and Centrella, M. (1998) Reduction in transforming growth factor beta receptor I expression and transcription factor CBFa1 on bone cells by glucocorticoid. J. Biol. Chem. 273, 4892–4896.
[21] Batuman, O.A., Ferrero, A.P., Diaz, A. and Jimenez, S.A. (1991) Regulation of transforming growth factor-beta 1 gene expression by glucocorticoids in normal human T lymphocytes. J. Clin. Invest. 88, 1574–1580.
[22] Rowley, D.R. (1992) Glucocorticoid regulation of transforming growth factor-beta activation in urogenital sinus mesenchymal cells. Endocrinology 131, 471–478.
[23] Oursler, M.J., Riggs, B.L. and Spelsberg, T.C. (1993) Glucocorticoid-induced activation of latent transforming growth factor-beta by normal human osteoblast-like cells. Endocrinology 133, 2187–2196.
[24] Watanabe, H., Kawabe, T., Yodoi, J., Tanaka, M., Kim, K.M., Nambu, M., Tsuruta, S., Morita, M., Yorifuji, T., Mayumi, M. and Mikawa, H. (1990) Transforming growth factor beta and dexamethasone suppress the expression of Fc epsilon receptor 2 (CD23) on a human eosinophilic cell line EoL-3. Immunol. Lett. 25, 313–318.
[25] Chaffer, C.L., Thompson, E.W. and Williams, E.D. (2007) Mesenchymal to epithelial transition in development and disease. Cells Tissues Organs 185, 7–19.
[26] Bakin, A.V., Rinehart, C., Tomlinson, A.K. and Arteaga, C.L. (2002) P38 mitogen-activated protein kinase is required for TGF-beta-mediated fibroblastic transdifferentiation and cell migration. J. Cell Sci. 115, 3193–3206.
[27] Bakin, A.V., Tomlinson, A.K., Bhowmick, N.A., Moses, H.L. and Arteaga, C.L. (2000) Phosphatidylinositol 3-kinase function is required for transforming growth factor beta-mediated epithelial to mesenchymal transition and cell migration. J. Biol. Chem. 275, 36803–36810.
[28] Lei, W.W., Zhang, K.H., Pan, X.C., Hu, Y., Wang, X.D., Yuan, X.W., Shu, G.W. and Song, J.G. (2010) Histone deacetylase 1 is required for transforming growth factor-beta1-induced epithelial–mesenchymal transition. Int. J. Biochem. Cell Biol. 42, 1489–1497.
[29] Wang, X.D., Pan, X.C. and Song, J.G. (2010) AMP-activated protein kinase is required for induction of apoptosis and epithelial-to-mesenchymal transition. Cell. Signal. 22, 1790–1797.
[30] Godoy, P., Lakkapamu, S., Schug, M., Bauer, A., Stewart, J.D., Bedawi, E., Hammad, S., Amin, J., Marchan, R., Schormann, W., Maccoux, L., von Recklinghausen, I., Reif, R. and Hengstler, J.G. (2010) Dexamethasone-dependent versus -independent markers of epithelial to mesenchymal transition in primary hepatocytes. Biol. Chem. 391, 73–83.
[31] Klaunig, J.E., Kamendulis, L.M. and Hocevar, B.A. (2010) Oxidative stress and oxidative damage in carcinogenesis. Toxicol. Pathol. 38, 96–109.
[32] Liu, R.M. and Pravia, K.A.G. (2010) Oxidative stress and glutathione in TGF-β-mediated fibrogenesis. Free Radic. Biol. Med. 48, 1–15.
[33] Rhyu, D.Y., Yang, Y., Ha, H., Lee, G.T., Song, J.S., Uh, S.T. and Lee, H.B. (2005) Role of reactive oxygen species in TGF-beta1-induced mitogen-activated protein kinase activation and epithelial–mesenchymal transition in renal tubular epithelial cells. J. Am. Soc. Nephrol. 16, 667–675.
[34] Zhang, K.H., Tian, H.Y., Gao, X., Ying, H., Wang, D.M., Pan, X.C., Yu, M.L., Xu, G.J., Zhao, F.K. and Song, J.G. (2009) Ferritin heavy chain-mediated iron homeostasis and subsequent increased reactive oxygen species production are essential for epithelial–mesenchymal transition. Cancer Res. 69, 5340–5348.
[35] Radisky, D.C., Levy, D.D., Littlepage, L.E., Liu, H., Nelson, C.M., Fata, J.E., Leake, D., Godden, E.L., Albertson, D.G., Nieto, M.A., Werb, Z. and Bissell, M.J. (2005) Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 436, 123–127.
[36] Zhou, G., Dada, L.A., Wu, M., Kelly, A., Trejo, H., Zhou, Q., Varga, J. and Sznajder, J.I. (2009) Hypoxia-induced alveolar epithelial-mesenchymal transition requires mitochondrial ROS and hypoxia-inducible factor 1. Am. J. Physiol. Lung Cell Mol. Physiol. 297, L1120–L1130.
[37] Zhang, A., Jia, Z., Guo, X. and Yang, T. (2007) Aldosterone induces epithelial–mesenchymal transition via ROS of mitochondrial origin. Am. J. Physiol. Renal Physiol. 29, F723–F731.
[38] Felton, V.M., Borok, Z. and Willis, B.C. (2009) N-Acetylcysteine inhibits alveolar epithelial-mesenchymal transition. Am. J. Physiol. Lung Cell Mol. Physiol. 297, L805–L812.
[39] Auersperg, N., Pan, J., Grove, B.D., Peterson, T., Fisher, J., Maines-Bandiera, S., Somasiri, A. and Roskelley, C.D. (1999) E-cadherin induces mesenchymal-to-epithelial transition in human ovarian surface epithelium. Proc. Natl. Acad. Sci. USA 96, 6249–6254.
[40] Das, S., Becker, B.N., Hoffmann, F.M. and Mertz, J.E. (2009) Complete reversal of epithelial to mesenchymal transition requires inhibition of both ZEB expression and the Rho pathway. BMC Cell Biol. 10, 94–111.
[41] Carayol, N., Vachier, I., Campbell, A., Crampette, L., Bousquet, J., Godard, P. and Chanez, P. (2000) Regulation of E-cadherin expression by dexamethasone and tumour necrosis factor-α in nasal epithelium. Eur. Respir. J. 20, 1430–1436.
[42] Lecanda, F., Cheng, S.L., Shin, C.S., Davidson, M.K., Warlow, P., Avioli, L.V. and Civitelli, R. (2000) Differential regulation of cadherins by dexamethasone in human osteoblastic cells. J. Cell. Biochem. 77, 499–506.
[43] Kang, P. and Svoboda, K.K.H. (2005) Epithelial-mesenchymal transformation during craniofacial development. J. Dent. Res. 84, 678–690.
[44] Andrew, F.D. and Zimmerman, E.F. (1971) Glucocorticoid induction of cleft palate in mice; no correlation with inhibition of mucopolysaccharide synthesis. Teratology 4, 31–37.
[45] Yoneda, T. and Pratt, R.M. (1982) Glucocorticoid receptors in palatal mesenchymal cells from the human embryo: relevance to human cleft palate formation. J. Craniofac. Genet. Dev. Biol. 1, 411–423.
[46] Salomon, D.S. and Pratt, R.M. (1978) Inhibition of growth in vitro by glucocorticoids in mouse embryonic facial mesenchyme cells. J. Cell Physiol. 97, 315–327.
[47] Shah, R.M. and Kilistoff, A. (1976) Cleft palate induction in hamster fetuses by glucocorticoid hormones and their synthetic analogues. J. Embryol. Exp. Morphol. 36, 101–108.
[48] Doernerand, A.M. and Zuraw, B.L. (2009) TGF-β1 induced epithelial to mesenchymal transition (EMT) in human bronchial epithelial cells is enhanced by IL-1β but not abrogated by corticosteroids. Respir. Res. 10, 100.