Biochemical and Biophysical Research Communications 437 (2013) 550–556

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Celastrol inhibits TGF-β1-induced epithelial–mesenchymal transition by inhibiting Snail and regulating E-cadherin expression

Hyereen Kang [a,1], Minjae Lee [a,1], Sung-Wuk Jang [a,b,*]

[a] Department of Biomedical Sciences, University of Ulsan College of Medicine, Seoul 138-736, Republic of Korea
[b] Department of Biochemistry and Molecular Biology, University of Ulsan College of Medicine, Seoul 138-736, Republic of Korea

ARTICLE INFO

Article history:
Received 27 June 2013
Available online 10 July 2013

Keywords:
Celastrol
EMT
E-cadherin
Snail
Invasion

ABSTRACT

The epithelial–mesenchymal transition (EMT) is a pivotal event in the invasive and metastatic potentials of cancer progression. Celastrol inhibits the proliferation of a variety of tumor cells including leukemia, glioma, prostate, and breast cancer; however, the possible role of celastrol in the EMT is unclear. We investigated the effect of celastrol on the EMT. Transforming growth factor-beta 1 (TGF-β1) induced EMT-like morphologic changes and upregulation of Snail expression. The downregulation of E-cadherin expression and upregulation of Snail in Madin–Darby Canine Kidney (MDCK) and A549 cell lines show that TGF-β1-mediated the EMT in epithelial cells; however, celastrol markedly inhibited TGF-β1-induced morphologic changes, Snail upregulation, and E-cadherin expression. Migration and invasion assays revealed that celastrol completely inhibited TGF-β1-mediated cellular migration in both cell lines. These findings indicate that celastrol downregulates Snail expression, thereby inhibiting TGF-β1-induced EMT in MDCK and A549 cells. Thus, our findings provide new evidence that celastrol suppresses lung cancer invasion and migration by inhibiting TGF-β1-induced EMT.

1. Introduction

The epithelial–mesenchymal transition (EMT), an important morphological event where polarized epithelial cells convert to contractile and motile mesenchymal cells, is recognized as an important process during embryonic development and tissue organization. EMT also plays a critical role in cancer invasion and metastasis. EMT induction is characterized by cell–cell junction dissolution, cytoskeletal rearrangement, increased cell motility, and synthesis of extracellular matrix [1]. One protein prominently associated with EMT is the epithelial cell adhesion molecule E-cadherin. E-cadherin is a cell–cell adhesion molecule and the loss of its expression is a hallmark of EMT. Reduction of E-cadherin increased cell mobility and promoted tumor cell invasion. Several transcriptional repressors of E-cadherin have been identified, including the zinc finger factors Snail and Slug, and the two-handed zinc factor ZEB1 [2,3]. Snail and Slug, a related Snail superfamily member, mediate E-cadherin repression and are overexpressed in epithelial cell lines during EMT [4–6]. Correlative studies have demonstrated an inverse relationship between E-cadherin and Snail expression in human samples [7].

Transforming growth factor-beta 1 (TGF-β1) is a multifunctional cytokine that regulates a wide range of cellular functions, including tissue morphogenesis, differentiation, and extracellular matrix remodeling [8]. TGF-β1-stimulated cells become spindle-shaped and undergo morphological changes, such as a decrease in cell–cell adhesion and loss of structural polarity [9]. Recent studies have revealed that TGF-β1 functions as a pro-oncogenic factor through induction of EMT; TGF-β1-induced EMT in a variety of cells is mediated by the Snail signaling pathway. Therefore, regulation of Snail expression plays a crucial role in EMT induction via TGF-β1 signaling [10].

Celastrol was identified from the traditional Chinese medicine "God of Thunder Vine" or *Tripterygium wilfordii* Hook F. almost 3 decades ago and has been used to treat cancer and other inflammatory diseases [11]. Various studies have indicated that celastrol exhibits anticancer potential and eradicates leukemia stem cells [12]. It suppresses the production of inflammatory cytokines such as interleukin-1 (IL-1), TNF-α, IL-6, and IL-8, induces the heat shock response, and disrupts heat shock protein 90 (Hsp90), possibly through its interaction with cdc37 and co-chaperone p23 [13–15]. Celastrol also inhibits NF-κB activation and arrests the cell cycle [15–18]. The molecular mechanism underlying the invasion effects of celastrol is not fully understood in TGF-β1-activated epithelial cells.

In this study, we investigated the effects of celastrol on TGF-β1-induced Snail expression in epithelial cells and explored the underlying downstream signaling mechanism. We found that celastrol

* Corresponding author at: Department of Biomedical Sciences, University of Ulsan College of Medicine, Seoul 138-736, Republic of Korea. Fax: +82 2 3010 2098.
E-mail address: swjang@amc.seoul.kr (S.-W. Jang).
[1] These authors are contributed equally to this work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.06.113

reduced invasion of epithelial cells by inhibiting EMT through the suppression of Snail and E-cadherin expression in TGF-β1-activated MDCK and A549 cells.

2. Materials and methods

2.1. Cells and reagents

MDCK and A549 cells were maintained in DMEM supplemented with 10% heat-inactivated FBS. Lipofectamine 2000 reagent was purchased from Invitrogen (Carlsbad, CA). Anti-E-cadherin, and anti-β-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Snail antibody was from Cell signaling (Beverly, MA). TGF-β1 was purchased from Calbiochem (San Diego, CA). All the chemicals not included above were from Sigma.

2.2. Cell proliferation and viability assay

All proliferation and viability assays were based on the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Cells were seeded in a 96-well plate at a density of 1×10^4 cells/well. The cells were treated with various concentration of celastrol and allowed to grow for 48 h. At the end of the experiment, the media was removed and DMSO was added with MTT solubilization solution. Absorbance was measured at 550 nm.

2.3. Western blot analysis

Cell lysates were separated on 10% SDS–polyacrylamide gels and transferred to nitrocellulose membranes. The blots were incubated with anti-E-cadherin and anti-Snail antibodies and developed with the enhanced chemiluminescence detection system (Amersham Pharmacia Biotech., Piscataway, NJ). The same blot was stripped and reprobed with anti- β-actin antibody for use as an internal control.

2.4. RNA extraction and semi-quantitative RT-PCR

Total RNA was extracted from cells with Trizol (Invitrogen) according to the manufacturer's protocol. Approximately 1 μg of total RNA was used to prepare cDNA using the Superscript First Strand cDNA synthesis Kit (Bioneer, Daejeon, South Korea). The following primers were used in this study: 5′-AACATCCTAGCCAAGATCC-3′ and 5′-GCACCTGACCCTTGTACGTG-3′ for E-cadherin; 5′-TCTAGGCCCTGGCTGCTACAA-3′ and 5′-ACATCTGAGTGGG TCTGGAGGTG-3′ for Snail; 5′-CCATCACCATCTTCCAGGAG-3′ and 5′-CCTGCTTCACCACGTTCTTG-3′ for GAPDH. PCR was performed with Platinum Taq polymerase (Invitrogen) under the following conditions: 30 cycles of 96 °C for 40 s, 55 °C (E-cadherin and Snail) or 60 °C (GAPDH) for 40 s, and 72 °C for 1 min followed by 10 min at 72 °C. All the PCR reactions were repeated at least three times. GAPDH was amplified as an internal control. The intensity of each band amplified by RT-PCR was analyzed using MultiImageTM Light Cabinet (version 5.5, Alpha Innotech Corp., San Leandro, CA).

2.5. Immunofluorescent staining

MDCK or A549 cells were treated with or without 1 μM celastrol for 30 min and then incubated with TGF for 72 h. Cells were fixed with 4% paraformaldehyde in PBS at room temperature for 10 min. Nonspecific sites were blocked by incubating with 200 μl of 1% BSA in PBS at 37 °C for 15 min. A rabbit polyclonal antibody against E-cadherin was diluted 1:200 in PBS containing 1% BSA and incubated with the coverslips at 37 °C for 1 h. Cells were then washed with 1% BSA/PBS for 10 min at room temperature before incubating with a 1:200 dilution of FITC-labeled goat anti-rabbit IgG antibody at room temperature for 45 min, and then the coverslips were rinsed with a 1% BSA/PBS solution for 10 min. Then the cells were stained with 4,6-diamidino-2-phenylindole (DAPI) for another 1 min at room temperature. The coverslips containing the cells were then mounted with AquaMount (Lerner Laboratories, New Haven, CT) containing 0.01% 1,4-diazobicy-

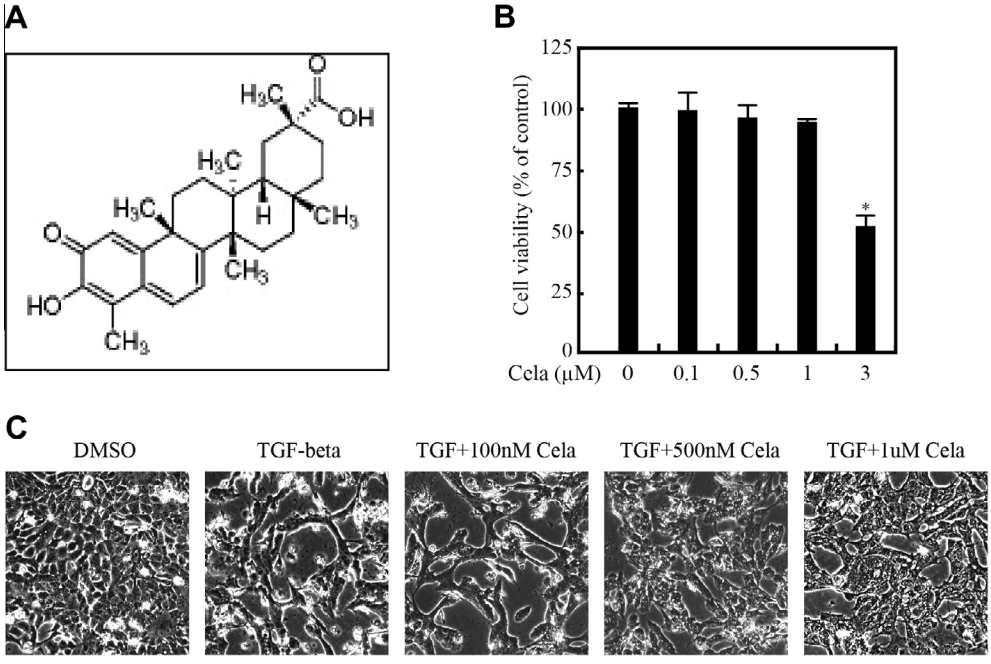

Fig. 1. Celastrol inhibits TGF-β1-induced morphological changes in MDCK cells. (A) Chemical structure of celastrol. (B) MDCK cells were treated with 0–3 μM celastrol for 48 h and cell viability was measured by MMT assay; $^*P < 0.05$ versus vehicle. (C) MDCK cells were pretreated with the indicated concentration of celastrol for 30 min and then stimulated with 5 ng/ml TGF-β1 for 72 h. TGF-β1 treatment induces cell elongation and increases scattering, while celastrol inhibits the activation of these processes in a dose-dependent manner. Scale bar 20 μm, magnification 40×.

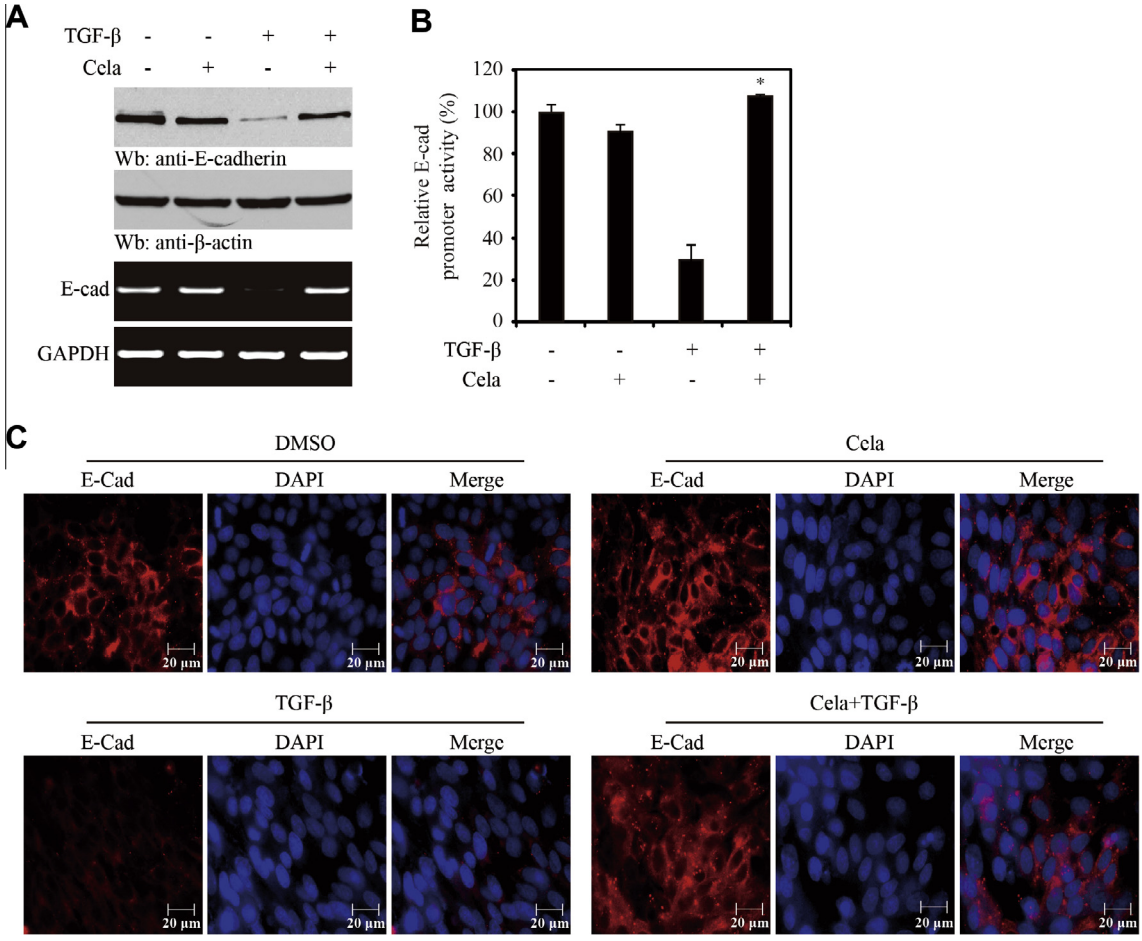

Fig. 2. Effect of celastrol on E-cadherin expression in TGF-β1-mediated MDCK cells. MDCK cells were incubated with 1 μM celastrol for 30 min followed by treatment with TGF-β1 for 72 h. (A) Western blotting and RT-PCR analyses for E-cadherin expression in MDCK cells. (B) The pE-cadherin-Luc plasmid (0.2 μg) was co-transfected into MDCK cells, along with the pRL-null vector (50 ng). At 24 h post-transfection, cells were treated with 1 μM celastrol for 30 min followed by treatment with TGF-β1 for 24 h. Firefly luciferase activity was normalized to Renilla activity, and the luciferase activity in untreated cells was designated as a relative activity of one. The data shown represent the means ± S.D. of 3 independent experiments performed in triplicate. The statistical significance of the assay was evaluated using Student's *t*-test (*$P < 0.05$ compare with TGF-β1 alone). (C) MDCK cells were incubated with 1 μM celastrol for 30 min followed by treatment with TGF-β1 for 72 h. After 72 h of culture, the cells were immunostained with anti-E-cadherin antibody and nuclei were counterstained with DAPI. Fluorescence imaging was performed on an OLYMPUS IX71 fluorescence microscope. Scale bar 20 μm, magnification 40×.

clo(2,2,2)octane. Fluorescent images were taken by OLYMPUS IX71 fluorescence microscope.

2.6. Transient transfection and luciferase reporter assay

Transcriptional activities of E-cadherin and Snail were measured by the luciferase reporter assay using the pE-cadherin-Luc and pSnail-Luc reporter plasmids. MDCK or A549 cells were seeded into 6-well plates. Cells at 70–80% confluency were co-transfected with 0.2 μg of E-cadherin or Snail reporter constructs and 0.2 μg of pRL-null vector for 24 h. The luciferase activities were assayed according to the manufacture's protocol (Promega), using a Luminometer 20/20n (Turner BioSystems, Sunnyvale, CA).

2.7. In vitro invasion assay

The 8-μm pore size polycarbonate nucleopore filter inserts in a 24-well transwell chamber were coated with 30 μg/well Matrigel (Sigma). Celastrol treated-MDCD and A549 cells were seeded into the upper part of the matrigel-coated filter, and serum-free DMEM with or without TGF was added to the lower part. After 36 h, the cells that had migrated through the Matrigel and the 8-μm pore-size membranes were fixed, stained, and counted under a light microscope.

2.8. Statistics analysis

Data are presented as the mean ± S.D. Statistical evaluation was carried out by the Student's *t*-test. Data were considered statistically significant when $P < 0.05$. All statistical analysis was performed by the computer program Prism (GraphPad Software, La Jolla, CA).

3. Results

3.1. Celastrol suppresses TGF-β1-induced EMT in MDCK cells

TGF-β1 modulates cell morphology changes and the EMT in many cell types [19,20]. We examined the effects of celastrol on cell viability with the MTT assay. MDCK cells were treated with various concentrations of celastrol for 72 h. Celastrol (1 μM) slightly reduced cell viability, but 3 μM celastrol significantly suppressed cell viability by approximately 2-fold (Fig. 1B). To determine the regulatory effect of celastrol during TGF-β1-induced EMT, we analyzed morphological changes in TGF-β1-treated and

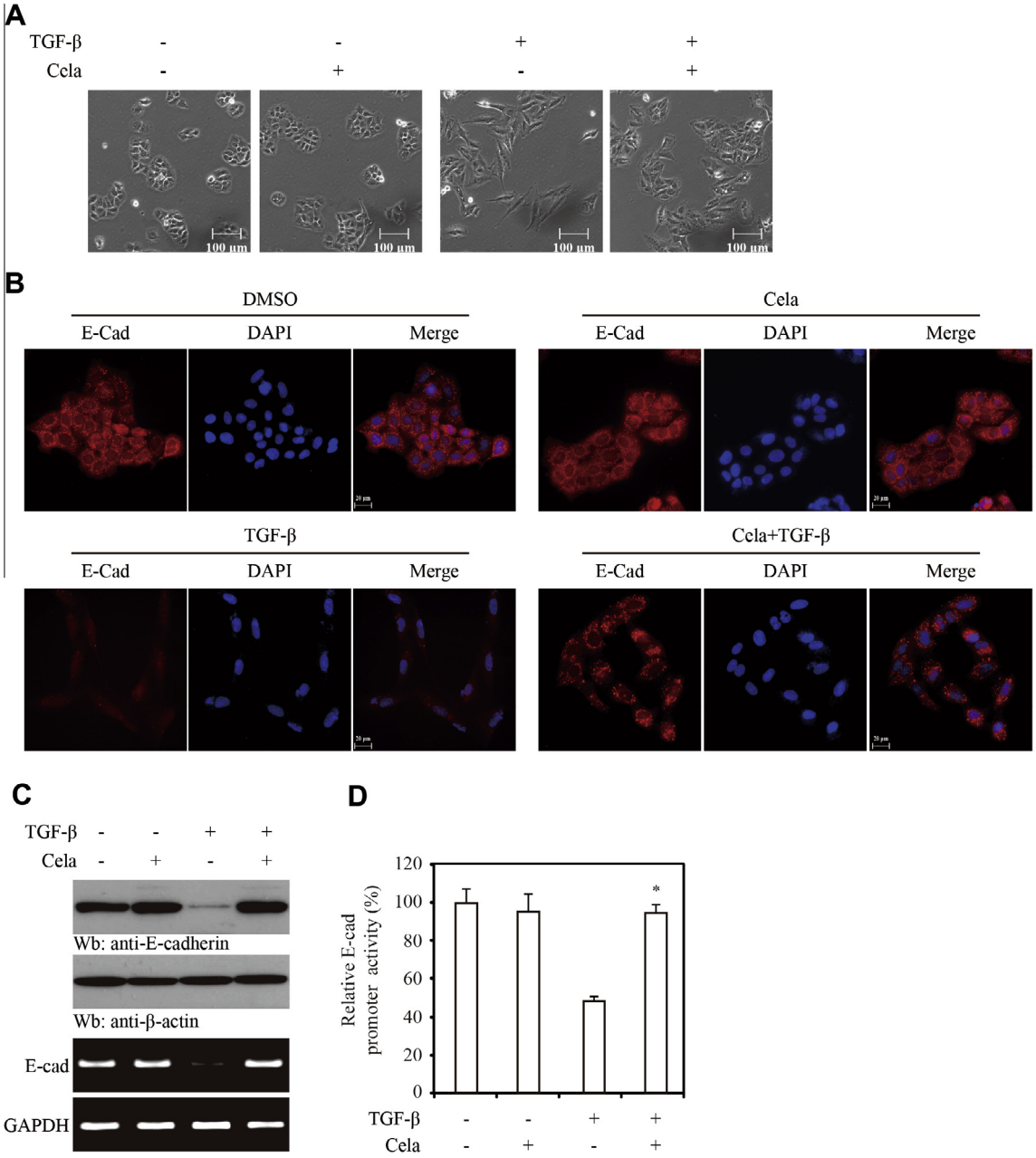

Fig. 3. Effects of celastrol on morphology and the expression of E-cadherin in TGF-β1-induced A549 cells. A549 cells were incubated with 1 μM celastrol for 30 min followed by treatment with TGF-β1 for 72 h. (A) Effects of 1 μM celastrol on morphology of A549 cells. (B) A549 cells were incubated with 1 μM celastrol for 30 min followed by treatment with TGF-β1 for 72 h. After 72 h of culture, the cells were fixed with paraformaldehyde, incubated with polyclonal anti-E-cadherin antibody for 1 h, and incubated with TRITC-conjugated anti-rabbit antibody for 45 min; nuclei were counterstained with DAPI. After mounting, fluorescence images were collected on the OLYMPUS IX71. Scale bar 20 μm, magnification 40×. (C) Western blotting and RT-PCR analyses of E-cadherin expression in A549 cells. (D) Cells were co-transfected with pE-cadherin-Luc and pRL-null vector. At 24 h after transfection, cells were incubated with 1 μM celastrol for 30 min followed by treatment with TGF-β1 for 24 h. The firefly luciferase activity was normalized to Renilla activity, and luciferase activity in untreated cells was designated as a relative activity of one. The data represent the means ± S.D. of 3 independent experiments performed in triplicate. The statistical significance of the assay was evaluated using Student's *t*-test (*$P < 0.05$ versus TGF-β1 alone).

control cells preincubated with celastrol. Treatment with TGF-β1 induced prominent morphological changes in MDCK cells, including elongated and spindle-like shapes, which were noticeably suppressed by pretreatment with celastrol (Fig. 1C).

TGF-β1 acts by directly reducing E-cadherin expression [20]. We examined the effect of celastrol on expression of epithelial marker E-cadherin in TGF-β1-induced MDCK cells by Western blotting and RT-PCR. As shown in Fig. 2A, E-cadherin expression was significantly reduced in TGF-β1 treated cells. In contrast, celastrol treatment rescued E-cadherin expression. To verify these findings, we examined TGF-β1-induced E-cadherin transcriptional activity using an E-cadherin-specific promoter assay. MDCK cells transfected with an E-cadherin-luciferase reporter plasmid were stimulated with TGF-β1 in the presence and absence of celastrol and assessed for luciferase activity in cell lysates. After 6 h TGF-β1 stimulation, as expected, E-cadherin luciferase reporter activity was significantly reduced. In contrast, celastrol treatment restored E-cadherin promoter activity in TGF-β1-treated cells (Fig. 2B). We performed immunofluorescence staining to investigate the effects of celastrol on the distribution of E-cadherin in MDCK cells. As shown in Fig. 2C, E-cadherin expression was completely lost at the membrane of TGF-β1-stimulated MDCK cells. In contrast,

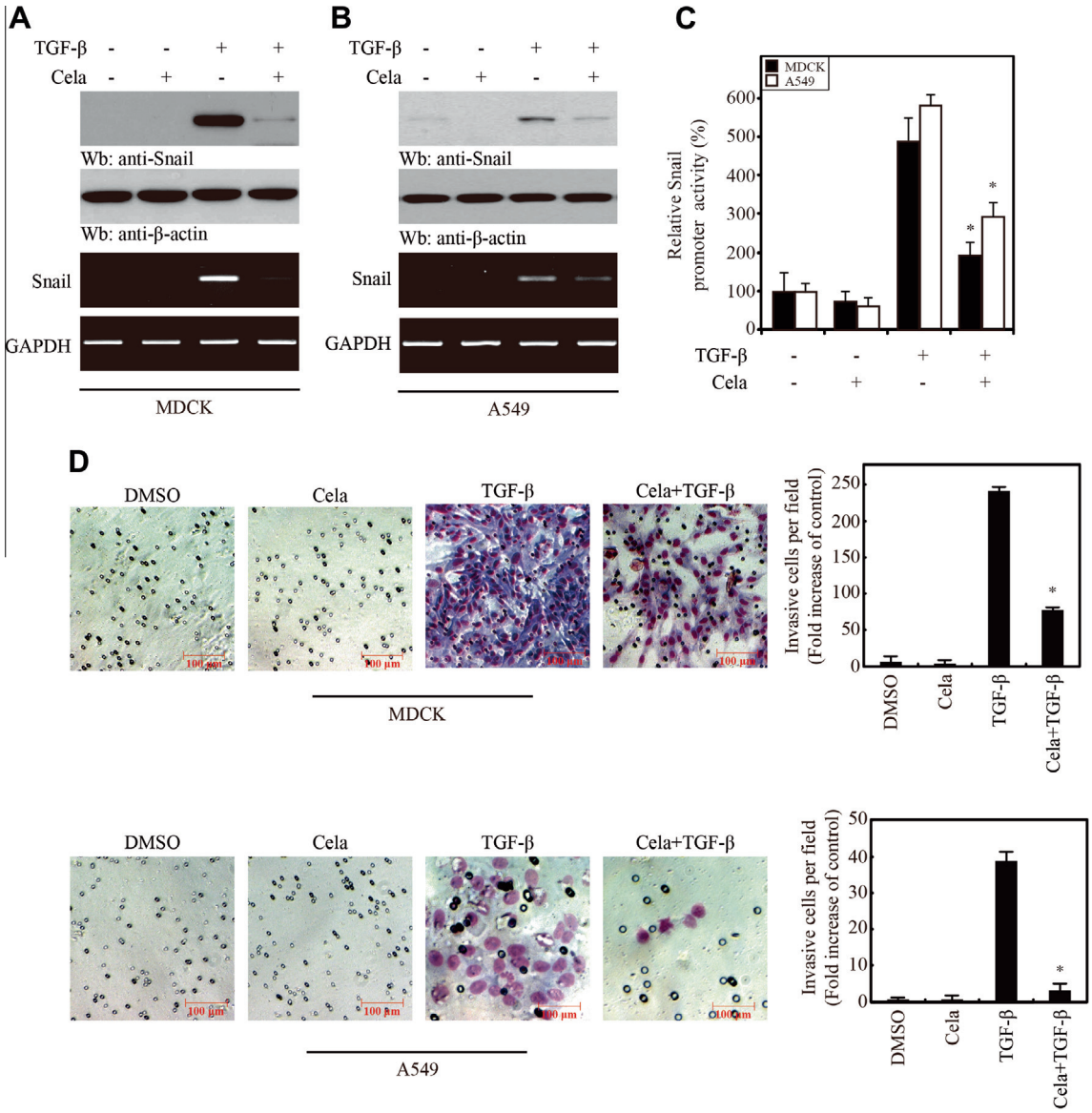

Fig. 4. Celastrol suppresses TGF-β1-induced Snail expression and cellular invasion. MDCK and A549 cells were incubated with 1 μM celastrol for 30 min followed by treatment with TGF-β1 for 24 h. (A and B) Western blotting and RT-PCR analyses of Snail expression in MDCK and A549 cells. (C) MDCK (black bars) and A549 (white bars) cells were co-transfected with pSnail-Luc and pRL-null vector. At 24 h after transfection, cells were incubated with 1 μM celastrol for 30 min followed by treatment with TGF-β1 for 24 h. Firefly luciferase activity was normalized to Renilla activity, and luciferase activity in the untreated cells was designated as a relative activity of one. The data represent the means ± S.D. of 3 independent experiments performed in triplicate. The statistical significance of the assay was evaluated using Student's *t*-test (**P* < 0.05 versus TGF-β1 alone). (D) Celastrol suppresses TGF-β1-induced MDCK and A549 cell invasion. MDCK and A549 cells were incubated with 1 μM celastrol for 30 min followed by treatment with TGF-β1 for 36 h. Invasion was determined using a Matrigel invasion assay. Migrated cells were stained with Diff-Quik and counted for quantitative analysis. Original magnification was 200×. Scale bars, 100 μm. Data represent the mean of 3 independent experiments. **P* < 0.05 versus TGF-β1 alone.

E-cadherin expression was restored in cells treated with TGF-β1 and celastrol (Fig. 2C). These findings showed that celastrol inhibited the effects of TGF-β1-induced EMT in MDCK cells.

3.2. Celastrol suppresses TGF-β1-induced EMT in A549 cells

We used a well-validated model of EMT in the A549 human alveolar cell line in which a suite of morphological, phenotypic, and functional markers and outcomes have been well characterized [8,21]. As shown in Fig. 3A and B, TGF-β1 induced EMT in A549 cells, as evidenced by mild morphologic alteration and loss of E-cadherin. In contrast, celastrol-treated cells failed to TGF-β1-induced EMT. To verify the ability of celastrol to inhibit TGF-β1-induced EMT, we cultured A549 cells in the presence of TGF-β1 with or without celastrol. Western blotting and RT-PCR showed that incubation of A549 cells with TGF-β1 strongly reduced E-cadherin expression. In contrast, when cells were incubated with TGF-β1 and then with celastrol, E-cadherin expression returned to control levels (Fig. 3C). We also examined the effect of celastrol on TGF-β1-induced E-cadherin transcriptional activity by an E-cadherin-specific promoter assay. As shown in Fig. 3D, A549 cells were transfected with an E-cadherin-luc construct and treated with celastrol in the presence of TGF-β1. E-cadherin promoter activity significantly decreased in TGF-β1-treated vs. control cells; however, celastrol rescued TGF-β1-mediated E-cadherin promoter activity. These results were consistent with the results in MDCK cells.

3.3. Celastrol inhibits TGF-β1-induced Snail expression and cellular invasion

Snail transcription factors are induced by TGF-β1 and repress E-cadherin expression [6]. To determine whether celastrol inhibits

Snail induction by TGF-β1, we used Western blotting and RT-PCR. As shown in Fig. 4A and B, celastrol inhibited the expression of TGF-β1-induced Snail in MDCKs and A549, indicating that celastrol regulates TGF-β1-mediated E-cadherin expression through Snail. Snail promoter activity was enhanced by TGF-β1 and this effect was strongly inhibited by celastrol, indicating that inhibition of Snail by celastrol may be important for rescue of TGF-β1-induced repression of E-cadherin expression in MDCK and A549 cells (Fig. 4C). We next examined whether celastrol inhibits TGF-β1-induced invasion in MDCK and A549 cells. MDCK and A549 cells treated with TGF-β1 caused about 20-fold increase in invasion of both cell lines, however, TGF-induced cell invasion was inhibited by celastrol (Fig. 4D). These results indicate that celastrol is an effective inhibitor of cell invasion in TGF-β1-induced MDCK and A549 cells.

4. Discussion

Celastrol derived from *T. wilfordii* HooK F., a traditional Chinese medicinal plant, is known for its anti-cancer activities through modulation of proteasome activity, heat shock response, and the NF-κB pathway [14–16]. Although several reports have suggested the involvement of celastrol in tumor metastasis [22,23], its role in EMT has not been examined. We investigated the inhibitory effects of celastrol on TGF-β1-induced EMT in MDCK and A549 cells. We demonstrated that celastrol (1) regulated TGF-β1-induced morphological changes and E-cadherin expression, (2) inhibited TGF-β1-induced Snail expression, and (3) strongly suppressed TGF-β1-induced invasion in MDCK and A549 cells. Thus, celastrol inhibits TGF-β1-induced EMT via suppression of Snail expression in epithelial cancer cells.

The EMT process is a morphological event crucial to tumor progression in physiological and metastasis development. EMT is defined by the loss of cell–cell adhesion, the modification of cell morphology, and the gain of cellular migration activity [24]. EMT involves the redistribution and loss of E-cadherin expression [1]. E-cadherin is an epithelial cell transmembrane protein with conserved cadherin repeats in the extracellular domain. In the presence of Ca^{2+}, the extracellular domain binds to that of E-cadherin on an adjacent epithelial cell to form tight cell–cell adhesion and to suppress dissociation of epithelial cells from their location [2,4]. TGF-β1 causes a loss of epithelial cell polarity, adherens junctions, and tight junctions, thus leading to rearrangement of F-actin stress fibers and the development of filopodia and lamellipodia [25]. Several *in vitro* studies have demonstrated that addition of TGF-β1 to cultured human epithelial cells downregulated E-cadherin expression and caused the cells to become mesenchymal cells that resembled myofibroblasts, via EMT [26,27]. Indeed, we found that TGF-β1 dramatically reduced the expression of E-cadherin and involved the morphological changes in MDCK (Figs. 1 and 2) and A549 cells (Fig. 3).

During EMT, epithelial cells lose their characteristic marker E-cadherin and gain a mesenchymal phenotype [26]. In this study, when TGF-β1-induced EMT was inhibited by celastrol in MDCK and A549 cells, E-cadherin expression was rescued and the mesenchymal phenotype was strongly inhibited.

The Snail family of transcription factors is one of the key regulators of EMT in normal development and during tumor progression. Correlative studies have shown a direct relationship between Snail expression and an invasive phenotype in a variety of cancers [7]. One regulator of Snail expression is TGF-β1 [5]. TGF-β1 treatment of MDCK and A539 cells triggers the EMT by enhancing Snail expression [4,5]. In this study, TGF-β1 also induced Snail expression in MDCK and A549 cells (Fig. 4). Using Western blotting, RT-PCR, and luciferase reporter assays, we showed that celastrol inhibited TGF-β1-induced Snail expression (Fig. 4). Presumably, decreased Snail expression is involved with rescued E-cadherin expression in celastrol-treated MDCK and A549 cells. These results are consistent with previous evidence of an inverse relationship between E-cadherin and Snail expression in human samples [28]. Moreover, our data show that TGF-β1-mediated invasion and migration was blocked by celastrol (Fig. 4).

Our findings suggest celastrol inhibits E-cadherin expression by decreasing Snail expression, leading to suppression of TGF-β1-induced EMT and invasion in MDCK and A549 cells. Therefore, celastrol is a potential chemotherapeutic candidate with a broad spectrum of anticancer activities.

Acknowledgments

This research was supported by basic science research program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2010-0024893) and Asan Institute for Life Sciences, Seoul, Korea (2013-529).

References

[1] A.M. Arias, Epithelial–mesenchymal interactions in cancer and development, Cell 105 (2001) 425–431.
[2] K. Vleminckx, L. Vakaet Jr., M. Mareel, W. Fiers, F. van Roy, Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role, Cell 66 (1991) 107–119.
[3] W. Birchmeier, J. Behrens, Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness, Biochim. Biophys. Acta 1198 (1994) 11–26.
[4] E. Batlle, E. Sancho, C. Franci, D. Dominguez, M. Monfar, J. Baulida, A. Garcia De Herreros, The transcription factor Snail is a repressor of E-cadherin gene expression in epithelial tumour cells, Nat. Cell Biol. 2 (2000) 84–89.
[5] A. Cano, M.A. Perez-Moreno, I. Rodrigo, A. Locascio, M.J. Blanco, M.G. del Barrio, F. Portillo, M.A. Nieto, The transcription factor Snail controls epithelial–mesenchymal transitions by repressing E-cadherin expression, Nat. Cell Biol. 2 (2000) 76–83.
[6] D. Medici, E.D. Hay, B.R. Olsen, Snail and Slug promote epithelial–mesenchymal transition through beta-catenin-T-cell factor-4-dependent expression of transforming growth factor-beta3, Mol. Biol. Cell 19 (2008) 4875–4887.
[7] C. Come, F. Magnino, F. Bibeau, P. De Santa Barbara, K.F. Becker, C. Theillet, P. Savagner, Snail and Slug play distinct roles during breast carcinoma progression, Clin. Cancer Res. 12 (2006) 5395–5402.
[8] H. Kasai, J.T. Allen, R.M. Mason, T. Kamimura, Z. Zhang, TGF-beta1 induces human alveolar epithelial to mesenchymal cell transition (EMT), Respir. Res. 6 (2005) 56.
[9] J. Zavadil, E.P. Bottinger, TGF-beta and epithelial-to-mesenchymal transitions, Oncogene 24 (2005) 5764–5774.
[10] H. Ikushima, K. Miyazono, TGFbeta signalling: a complex web in cancer progression, Nat. Rev. Cancer 10 (2010) 415–424.
[11] J.B. Calixto, M.M. Campos, M.F. Otuki, A.R. Santos, Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules, Planta Med. 70 (2004) 93–103.
[12] H. Yang, D. Chen, Q.C. Cui, X. Yuan, Q.P. Dou, Celastrol, a triterpene extracted from the Chinese "Thunder of God Vine", is a potent proteasome inhibitor and suppresses human prostate cancer growth in nude mice, Cancer Res. 66 (2006) 4758–4765.
[13] A. Chadli, S.J. Felts, Q. Wang, W.P. Sullivan, M.V. Botuyan, A. Fauq, M. Ramirez-Alvarado, G. Mer, Celastrol inhibits Hsp90 chaperoning of steroid receptors by inducing fibrillization of the co-chaperone p23, J. Biol. Chem. 285 (2010) 4224–4231.
[14] T. Zhang, Y. Li, Y. Yu, P. Zou, Y. Jiang, D. Sun, Characterization of celastrol to inhibit hsp90 and cdc37 interaction, J. Biol. Chem. 284 (2009) 35381–35389.
[15] S.D. Westerheide, J.D. Bosman, B.N. Mbadugha, T.L. Kawahara, G. Matsumoto, S. Kim, W. Gu, J.P. Devlin, R.B. Silverman, R.I. Morimoto, Celastrols as inducers of the heat shock response and cytoprotection, J. Biol. Chem. 279 (2004) 56053–56060.
[16] J.H. Lee, T.H. Koo, H. Yoon, H.S. Jung, H.Z. Jin, K. Lee, Y.S. Hong, J.J. Lee, Inhibition of NF-kappa B activation through targeting I kappa B kinase by celastrol, a quinone methide triterpenoid, Biochem. Pharmacol. 72 (2006) 1311–1321.
[17] P. Ge, X. Ji, Y. Ding, X. Wang, S. Fu, F. Meng, X. Jin, F. Ling, Y. Luo, Celastrol causes apoptosis and cell cycle arrest in rat glioma cells, Neurol. Res. 32 (2010) 94–100.
[18] D. Zhang, L. Xu, F. Cao, T. Wei, C. Yang, G. Uzan, B. Peng, Celastrol regulates multiple nuclear transcription factors belonging to HSP90's clients in a dose- and cell type-dependent way, Cell Stress Chaperones 15 (2010) 939–946.
[19] A.N. Kim, W.K. Jeon, K.H. Lim, H.Y. Lee, W.J. Kim, B.C. Kim, Fyn mediates transforming growth factor-beta1-induced down-regulation of E-cadherin in

human A549 lung cancer cells, Biochem. Biophys. Res. Commun. 407 (2011) 181–184.
[20] K.A. Brown, M.E. Aakre, A.E. Gorska, J.O. Price, S.E. Eltom, J.A. Pietenpol, H.L. Moses, Induction by transforming growth factor-beta1 of epithelial to mesenchymal transition is a rare event *in vitro*, Breast Cancer Res. 6 (2004) R215–231.
[21] X. Tan, H. Dagher, C.A. Hutton, J.E. Bourke, Effects of PPAR gamma ligands on TGF-beta1-induced epithelial–mesenchymal transition in alveolar epithelial cells, Respir. Res. 11 (2010) 21.
[22] V.R. Yadav, B. Sung, S. Prasad, R. Kannappan, S.G. Cho, M. Liu, M.M. Chaturvedi, B.B. Aggarwal, Celastrol suppresses invasion of colon and pancreatic cancer cells through the downregulation of expression of CXCR4 chemokine receptor, J. Mol. Med. (Berl) 88 (2010) 1243–1253.
[23] X. Pang, Z. Yi, J. Zhang, B. Lu, B. Sung, W. Qu, B.B. Aggarwal, M. Liu, Celastrol suppresses angiogenesis-mediated tumor growth through inhibition of AKT/mammalian target of rapamycin pathway, Cancer Res. 70 (2010) 1951–1959.
[24] J.P. Thiery, Epithelial–mesenchymal transitions in tumour progression, Nat. Rev. Cancer 2 (2002) 442–454.
[25] Y. Liu, Epithelial to mesenchymal transition in renal fibrogenesis: pathologic significance, molecular mechanism, and therapeutic intervention, J. Am. Soc. Nephrol. 15 (2004) 1–12.
[26] P.J. Miettinen, R. Ebner, A.R. Lopez, R. Derynck, TGF-beta induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors, J. Cell Biol. 127 (1994) 2021–2036.
[27] N.A. Bhowmick, M. Ghiassi, A. Bakin, M. Aakre, C.A. Lundquist, M.E. Engel, C.L. Arteaga, H.L. Moses, Transforming growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism, Mol. Biol. Cell 12 (2001) 27–36.
[28] J.P. Thiery, H. Acloque, R.Y. Huang, M.A. Nieto, Epithelial–mesenchymal transitions in development and disease, Cell 139 (2009) 871–890.