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Baicalin and baicalein inhibit transforming growth factor-β1-mediated epithelial-mesenchymal transition in human breast epithelial cells

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ABSTRACT

Since the epithelial–mesenchymal transition (EMT) is involved in many crucial functions of cancer cells, we set out to identify a natural compound capable of inhibiting EMT processes. TGF-β1 treatment induces EMT among normal mammary epithelial cells (MCF10A cells), as reflected by characteristic morphological changes into the fibroblastic phenotype, reduced expression of E-cadherin. Interestingly, butanol extracts of *Scutellaria baicalensis* Georgi significantly reduced the TGF-β1-mediated EMT of MCF10A cells. Further analysis revealed that baicalin and baicalein, the major flavones of these butanol extracts, inhibited TGF-β1-mediated EMT by reducing the expression level of the EMT-related transcription factor, Slug via the NF-κB pathway, and subsequently increased migration in MCF10A cells. Finally, both compounds reduced the TGF-β1-mediated EMT, anchorage-independent growth and cell migration of human breast cancer cells (MDA-MB-231 cells). Taken together, these results suggest that baicalin and baicalein of *Scutellaria baicalensis* Georgi may suppress the EMT of breast epithelial cells and the tumorigenic activity of breast cancer cells. Thus, these compounds could have potential as therapeutic or supplementary agents for the treatment of breast cancer.

1. Introduction

Breast cancer, which is one of the most malignant cancers in women, has a poor prognosis due to its high aggressiveness, detection difficulties and strong chemoresistance [1]. In large part, these characteristics are dependent on the epithelial–mesenchymal transition (EMT) of breast cancer cells. EMT is the acquisition of mesenchymal cell characteristics by epithelial cells; it is an early program during development, tissue reorganization and tumorigenesis. In many cases, EMT may be seen both during the initial steps of tumorigenesis and the later stage of metastasis. Indeed, a strong correlation exists between EMT processes and metastatic features in cancer cells [2,3]. During initial tumorigenesis, normal epithelial cells lose their epithelial characteristics and obtain mesenchymal characteristics in their morphology, migration, anti-apoptotic capacity and invasiveness. Many studies have examined EMT in the context of understanding cancer, and such results have contributed to the treatment of various cancers, including breast cancer.

Transforming growth factor-β (TGF-β) is a well-known inducer of EMT in various cancer cells [4]. It binds to its receptors (TβRI, TβRII and TβRIII) and activates various transcription factors for cadherin isoform switching [5–7]. During TGF-β1-induced EMT, epithelial cells undergo downregulation of E-cadherin, which is a major cell–cell adhesion molecule and tumor suppressor [8], and upregulation of N-cadherin [9]. TGF-β1 also induces Ras-MEK-ERK-mediated EMT in pancreatic cancer cell lines (PANC-1, COLO-357 and IMIM-PC1) [10]. In breast cancer cells, TGF-β1 stimulates Rho-p38 MAPK-mediated EMT [11]. In this process, TGF-β1 activates Snail (zinc finger protein Snail1), Slug (also called Snail2), Twist, ZEB1 (zinc finger E-box-binding homeobox1) and ZEB2, which repress E-cadherin and induce EMT [12].

Abbreviations: EMT, epithelial mesenchymal transition; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; TGF-β, transforming growth factor-β.

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Scutellaria baicalensis Georgi (also known as Baikal skullcap) is an herb that is widely used in the traditional medical systems of Asia. Numerous functional studies have examined various extracts and compounds from *Scutellaria baicalensis* Georgi, and have revealed that they have anti-cancer [13], anti-inflammatory [14], anti-viral [15], anti-fungal [16] and neuroprotective [17] effects. For example, oroxylin A stimulates the cell cycle arrest of breast cancer cells through the Chk2/P53/NF-κB pathway [18]; baicalein promotes apoptosis of esophageal squamous cell carcinoma cells through the PI3K/AKT pathway [13]; baicalin induces apoptosis of Burkitt lymphoma cells through the PI3K/AKT pathway [19]; and wogonin inhibits the invasiveness of gallbladder carcinoma cells through the ERK1/2 pathway [20]. Therefore, it is highly possible that extracts and compounds from *Scutellaria baicalensis* Georgi might regulate EMT.

In this study, we sought to identify anti-EMT compounds from *Scutellaria baicalensis* Georgi. We found that baicalin and baicalein inhibit TGF-β1-mediated EMT in breast epithelial cells and suppress the tumorigenic characteristics of breast cancer cells. This suggests that baicalin and baicalein may inhibit EMT in breast cancer, and could therefore have potential therapeutic relevance.

2. Materials and methods

2.1. Materials

The monoclonal antibody against E-cadherin was purchased from Abcam (Cambridge, MA, USA). The antibodies against Slug (monoclonal), cytokeratin-18 (monoclonal), N-cadherin (polyclonal), NF-κB (p65, polyclonal) and IκB-α (polyclonal) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antibodies against Snail (monoclonal) and phospho-Smad2/3 (polyclonal) were purchased from Cell Signaling (Danvers, MA, USA). Human TGF-β1 was purchased from R&D Systems (Minneapolis, MN, USA). Baicalin and baicalein were purchased from Sigma (St. Louis, MO, USA). Bay11-7085 was purchased from Santa Cruz Biotechnology.

2.2. Cell culture and transfection

The MCF10A human breast epithelial cell line was maintained in Dulbecco's-modified Eagle's medium/F12 (DMEM/F12; WelGene, Daegu, Korea) supplemented with 5% (v/v) horse serum (Gibco, Grand Island, NY, USA), L-glutamine (2 mM; Hyclone, Logan, UT, USA), EGF (20 ng/ml; R&D Systems), hydrocortisone (0.5 μg/ml; Sigma), insulin (10 μg/ml; Sigma), cholera toxin (0.1 μg/ml; Sigma) and gentamicin (50 μg/ml; Sigma), at 37 °C in a humidified 5% CO_2 atmosphere. The MDA-MB-231 human breast cancer cell lines was maintained in RPMI 1640 medium (WelGene) supplemented with 10% (v/v) fetal bovine serum (FBS; Hyclone) and gentamicin (50 μg/ml), at 37 °C in a humidified 5% CO_2 atmosphere. A construct of human E-cadherin was kindly provided by Dr S.G. Kim (Seoul National University, Seoul, Korea). Cells were transfected using Viva-Magic™ transfection reagent according to the manufacturer's instructions (VIVAGEN Co., Gyeonggi-Do, Korea).

2.3. Immunoblotting

The cultures were washed twice with phosphate-buffered saline (PBS) and the cells were lysed in RIPA buffer (50 mM Tris, pH 8.0,

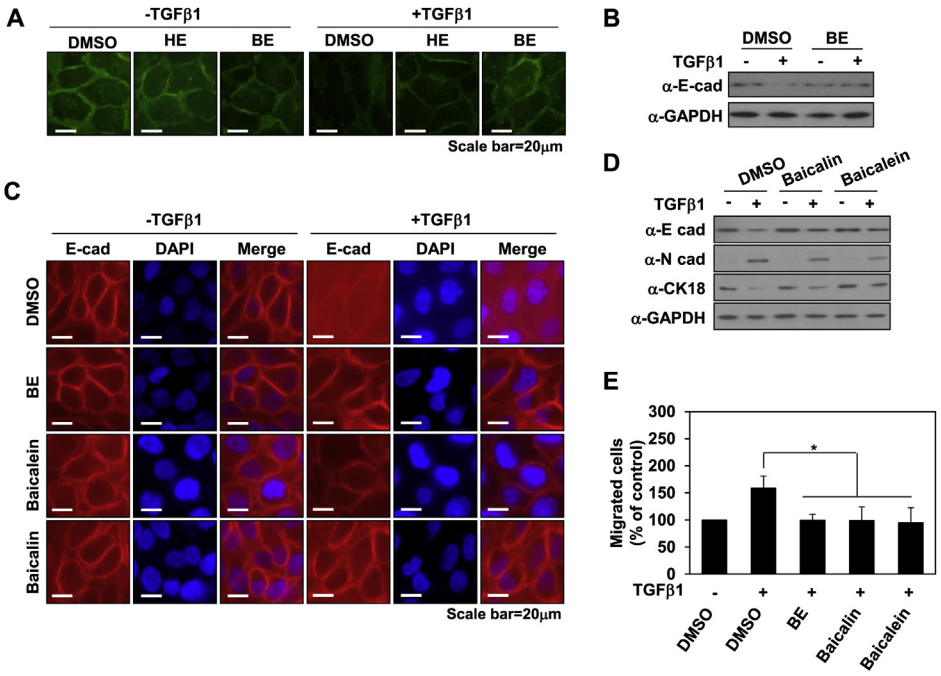

Fig. 1. Baicalin and Biacalein from the butanol fraction of *Scutellaria baicalensis* Georgi inhibits TGF-β1-mediated EMT in breast epithelial cells. (A) MCF10A cells (8×10^4 cells/well in a 12-well) were treated with control (DMSO), butanol extract (BE) or hexane extract (HE, 20 μg/ml final) with/without TGF-β1 (2.5 ng/ml; to induce EMT). After 48 h, cells were immunostained with an anti-E-cadherin antibody. The results were visualized with a FITC-conjugated goat anti-mouse antibody (green). (B) MCF10A cells (2.5×10^5 cells/well in a 6-well) were co-treated as indicated. After 48 h, Immunoblotting was performed with the indicated antibodies. An anti-GAPDH antibody was used as a loading control. (C) MCF10A cells (8×10^4 cells/well in a 12-well) were co-treated with the butanol extract (20 μg/ml), baicalin or baicalein (2 μM), plus TGF-β1 (2.5 ng/ml). After 48 h, the cells were immunostained with anti-E-cadherin antibody (E-cad). The results were visualized with a Texas red-conjugated goat anti-mouse antibody (red), and DAPI was used to counterstain nuclei (blue). (D) MCF10A cells were treated either DMSO (control), baicalin or baicalein (2 μM) either in the presence or absence of TGF-β1 (2.5 ng/ml). After 48 h, total cell lysates were analyzed by Immunoblotting with the indicated antibodies. An anti-GAPDH antibody was used as a loading control. (E) MCF10A cells were treated described in (D). After 48 h, cells (7.5×10^4 cells/well in a Transwell apparatus) were subjected to a Transwell migration assay. After 6 h, migrated cells were stained with H&E, and counted. Error bars indicate mean ± S.D. *, $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

150 mM NaCl, 1% Nonidet P-40, 10 mM NaF, and 2 mM Na_3VO_4) containing protease inhibitors (1 μg/ml aprotinin, 1 μg/ml antipain, 5 μg/ml leupeptin, 1 μg/ml pepstatin A, and 20 μg/ml phenylmethylsulfonyl fluoride). The lysates were clarified by centrifugation at 13,000 × g for 15 min at 4 °C, boiled with SDS sample buffer, and analyzed by SDS-PAGE. The resolved proteins were transferred to polyvinylidenedifluoride membranes (Millipore, Billerica, MA, USA) and probed with the appropriate antibodies. The signals were detected by enhanced chemiluminescence (AbClon, Seoul, Korea).

2.4. Immunofluorescence analysis

Cells were plated to 12-well plates containing coverslips, and fixed with 3.5% paraformaldehyde for 10 min. The cells were then washed with PBS, blocked with 0.5% BSA and incubated overnight with the anti-E-cadherin antibody at 4 °C. For detecting intracellular proteins, cells were permeablized by 0.5% triton X-100 (in PBS) for 10 min before blocking. After a further wash with PBS, the cells were incubated with fluorescent dye conjugated secondary antibodies (Invitrogen, Carlsbad, CA, USA) for 1 h at 25 °C. The coverslips were mounted on glass slides with mounting solution containing 4′,6-diamidino-2-phenylindole (DAPI), and the results were observed by fluorescence microscopy.

2.5. Transwell migration assay

Gelatin B (10 μg/ml) was added to each well of a Transwell plate (8-μm pore size; Corning Costar, Tewksbury, MA, USA), and the membranes were allowed to dry at 25 °C for 1 h. The Transwell units were assembled in a 24-well plate, and the lower chambers were filled with fresh medium containing FGF-2 (100 ng/ml). Cells were added to each upper chamber, and the plates were incubated at 37 °C in 5% CO_2. The cells that had migrated to the lower surface of the filters were stained with 0.6% hematoxylin and 0.5% eosin (H&E), and counted.

2.6. Cell proliferation assay

Cell proliferation was measured by a colorimetric assay using MTT [3-(4,5-dimethythiazol-2-yl) 2,5-diphenyltetrazolium bromide; Amresco, Solon, OH, USA]. In brief, MDA-MB-231 cells were harvested with 0.05% trypsin/EDTA and seeded to a 96-well plate at 1.0×10^4 cells/well. After incubation for the indicated time periods, 100 μl of medium containing 0.5 mg/ml MTT was added to each well, and the plate was incubated for 1 h. The medium was then removed and 100 μl of acidic isopropanol (90% isopropanol, 0.5% SDS, 25 mM NaCl) was added to each well. The mean absorbance at 570 nm in each set of samples was measured using a 96-well plate reader (Dynatech, Chantilly, VA, USA).

2.7. Colony formation assay

Each well of a 6-well culture dish was coated with 3 ml of bottom layer mixture (RPMI 1640 containing 10% FBS and 0.6% agar). After the bottom layer solidified, we added 1 ml of top agar mixture (RPMI 1640 containing 10% FBS and 0.3% agar) containing MDA-MB-231 cells (5×10^4 cells/well) pretreated with either TGF-β1 (10 ng/ml) or one of the test compounds (5 μM) and incubated the plate at 37 °C in a 5% CO_2 atmosphere. After 16 days, the colonies were stained with 0.005% crystal violet and photographed with a digital camera.

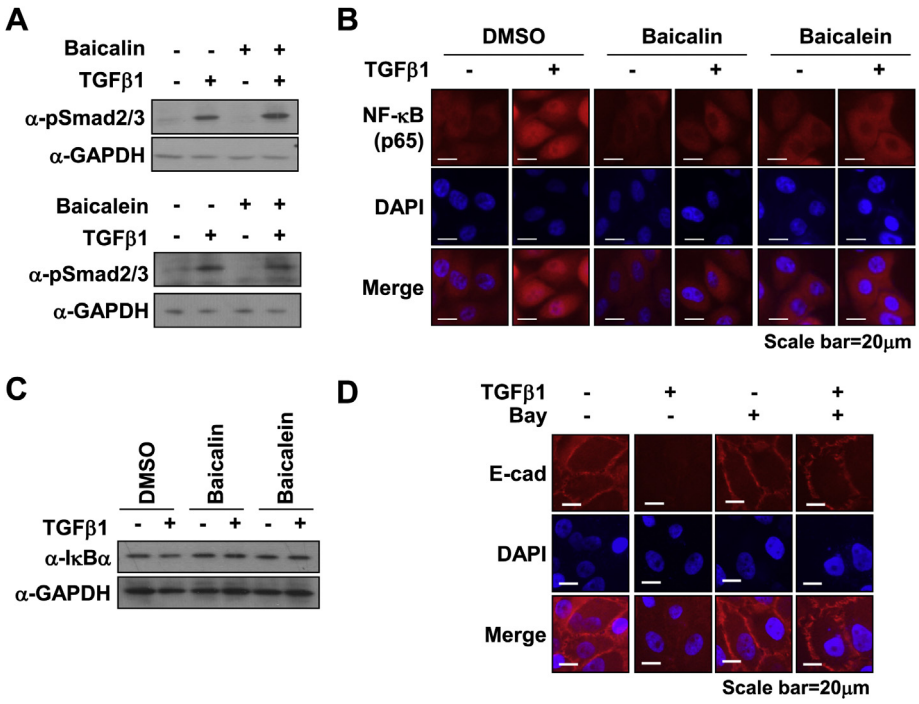

Fig. 2. Baicalin and Baicalein inhibit TGF-β1-mediated EMT processes by reducing NF-κB activation. (A) MCF10A cells were treated with compounds (2 μM) and TGF-β1 (2.5 ng/ml) for 24 h, and total cell lysates were analyzed by Immunoblotting with anti-phospho-Smad2/3 antibody. An anti-GAPDH antibody was used as a loading control. (B) MCF10A cells were treated with TGF-β1 (2.5 ng/ml) and compounds (2 μM). After 30 min, cells were the cells were immunostained with anti-NF-κB (p65) antibody. The results were visualized with a Texas red-conjugated goat anti-rabbit antibody (red), and DAPI was used to counterstain nuclei (blue). (C) MCF10A cells were treated with TGF-β1 (2.5 ng/ml) and indicated compounds (2 μM) for 30 min. IκB-α level was analyzed by Immunoblotting. An anti-GAPDH antibody was used as a loading control. (D) MCF10A cells were pre-treated with Bay11-7085 (10 μM). After 30 min, cells were treated with TGF-β1 (2.5 ng/ml) for additional 48 h. E-cadherin level was visualized by Immunofluorescence analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.8. Statistical analysis

Data are represented as the mean ± S.D. from each independent experiment. Statistical analysis was performed using student's t-test of variance. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Scutellaria baicalensis Georgi inhibits TGF-β1-mediated EMT in breast epithelial cells

In an effort to identify new EMT inhibitors, we screened natural extracts from *Scutellaria baicalensis* Georgi for the ability to alter the fibroblastic morphological changes induced by TGF-β1 treatment of MCF10A breast epithelial cells. To begin narrowing in on the active factor(s), we obtained *Scutellaria baicalensis* Georgi extracts using three solvents: hexane, butanol and ethylacetate. We found that TGF-β1 treatment induced the expected morphological changes of MCF10A cells toward the fibroblastic phenotype; this was first seen at 24 h post-treatment and was evident at 48 h post-treatment. When cells were co-treated with extracts from *Scutellaria baicalensis* Georgi and TGF-β1, the butanol fraction (butanol extract), but not the other fractions, inhibited the TGF-β1-induced morphological changes (data not shown). Since E-cadherin is expressed at much higher levels in epithelial cells than mesenchymal cells, whereas mesenchymal cells express higher levels of N-cadherin, cadherin levels are considered to be strong markers of EMT [21]. Consistent with the observed morphological changes, TGF-β1 treatment reduced E-cadherin expressions in MCF10A cells, and this effect could be rescued by co-treatment with the butanol extract from *Scutellaria baicalensis* Georgi (Fig. 1A and B). Together, these data suggest that compounds in the butanol fraction of *Scutellaria baicalensis* Georgi may inhibit EMT processes in breast epithelial cells.

3.2. Baicalin and baicalein inhibit TGF-β1-mediated EMT

To date, 295 compounds have been isolated from *Scutellaria baicalensis* Georgi, including various flavonoids, phenylethanoidglycosides, iridoid glycosides, diterpenes, triterpenoids, alkaloids, phytosterols and polysaccharides [22]. The butanol fraction of *Scutellaria baicalensis* Georgi is known to contain baicalein (14.24%), baicalin (baicalein-7-glucuronide, 19.58%) and wogonin (3.61%) together with other compounds [23]. Because baicalin and baicalein are the major compounds in the butanol fraction, we examined whether these compounds could inhibit TGF-β1-mediated EMT. Indeed, E-cadherin expression was reduced by TGF-β1 treatment of MCF10A cells, but this effect was inhibited by both baicalin and baicalein (Fig. 1C and D). In contrast, the levels of N-cadherin remained unchanged (Fig. 1D). In addition, the TGF-β1-induced migration of MCF10A cells was suppressed by co-treatment with baicalin or baicalein (Fig. 1E). These results suggest that baicalin and baicalein in the butanol fraction of *Scutellaria baicalensis* Georgi can inhibit TGF-β1-mediated EMT in breast epithelial cells.

3.3. Baicalin and baicalein suppress EMT by reducing NF-kB signaling

Various signaling pathways contribute to inducing EMT. Among them, the Smad signaling pathway is a well-known TGF-β1-mediated pathway [24]. However, we found that neither baicalin nor baicalein affected the phosphorylation of Smad2/3 (Fig. 2A).

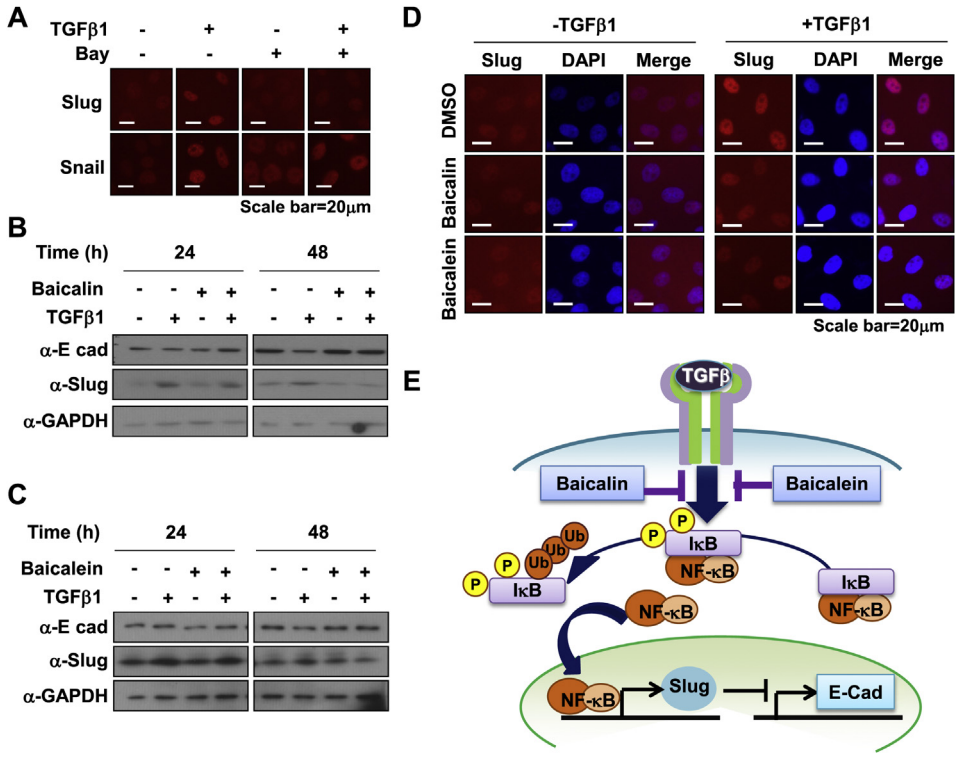

Fig. 3. Baicalin and baicalein negatively regulate Slug expression in breast epithelial cells. (A) MCF10A cells were treated with Bay11-7085 and TGF-β1 as described in Fig. 2D. The cells were immunostained with either anti-Snail or anti-Slug antibodies. (B, C) MCF10A cells were treated with each compound (2 μM) and TGF-β1 (2.5 ng/ml) for the indicated periods, and total cell lysates were analyzed by Immunoblotting with the indicated antibodies. An anti-GAPDH antibody was used as a loading control. (D) MCF10A cells were co-treated with baicalin or baicalein (2 μM) plus TGF-β1 (2.5 ng/ml). After 48 h, the cells were immunostained with anti-Slug antibody. The results were visualized with a Texas red-conjugated goat anti-mouse antibody (red), and DAPI was used to counterstain nuclei (blue). (E) Schematic representation of the downregulation of E-cadherin, with Slug indicated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a crucial transcription factor known to stimulate EMT in cancer cells [25–27], and both baicalin and baicalein are known to inhibit NF-κB activation during anti-inflammatory responses [28,29]. Therefore, we investigated whether these compounds could inhibit EMT by inactivating NF-κB. As expected, both baicalin and baicalein prevented TGF-β1 from triggering the activation and nuclear localization of NF-κB in MCF10A cells (Fig. 2B). In addition, both baicalin and baicalein inhibited TGF-β1-mediated IκB-α degradation (Fig. 2C), and the TGF-β1-induced downregulation of E-cadherin expression was recovered by the NF-κB inhibitor, Bay11-7085 (Fig. 2D). Together, these data suggest that baicalin and baicalein suppress EMT by inhibiting the activation of NF-κB.

3.4. Baicalin and baicalein negatively regulate Slug expression in breast epithelial cells

E-cadherin expression is repressed by various transcription factors, including Snail (also called Snail1), Slug (also called Snail2) and ZEB1/2 [30]. Thus, we investigated whether these transcription factors could be involved in the effects of baicalin and baicalein on the TGF-β1-induced EMT of MCF10A cells. Interestingly, the TGF-β1-mediated nuclear localization of Slug, but not Snail, was reduced by the NF-κB inhibitor (Fig. 3A). Similarly, both compounds downregulated the TGFβ1-mediated induction of Slug, in parallel with increased E-cadherin expression (Fig. 3B and C). Our immunofluorescence analyses showed that Slug was highly localized to the cell nucleus following TGF-β1 treatment, whereas the nuclear levels of Slug were decreased in cells co-treated with baicalin and baicalein (Fig. 3D). These results suggest that baicalin and baicalein negatively regulate EMT by inhibiting the expression of Slug that is responsible for repressing E-cadherin expression, and that this process is governed by TGF-β1-mediated NF-κB activation (Fig. 3E).

3.5. Baicalin and baicalein inhibit the tumorigenic activities of breast cancer cells

Since the EMT process is a critical event in cancer progression, we investigated whether baicalin and/or baicalein could inhibit the tumor-related activities (e.g., overgrowth, mobility and anchorage-independent growth) of human breast cancer (MDA-MB-231) cells. In these cells, as in MCF10A cells, both compounds inhibited TGFβ1-mediated EMT by reducing NF-κB activity without affecting Smad 2/3 activation (Fig. 4A and B), and by downregulating Slug (Fig. 4C). Furthermore, baicalin and baicalein suppressed the TGF-β1-mediated upregulations of Slug and

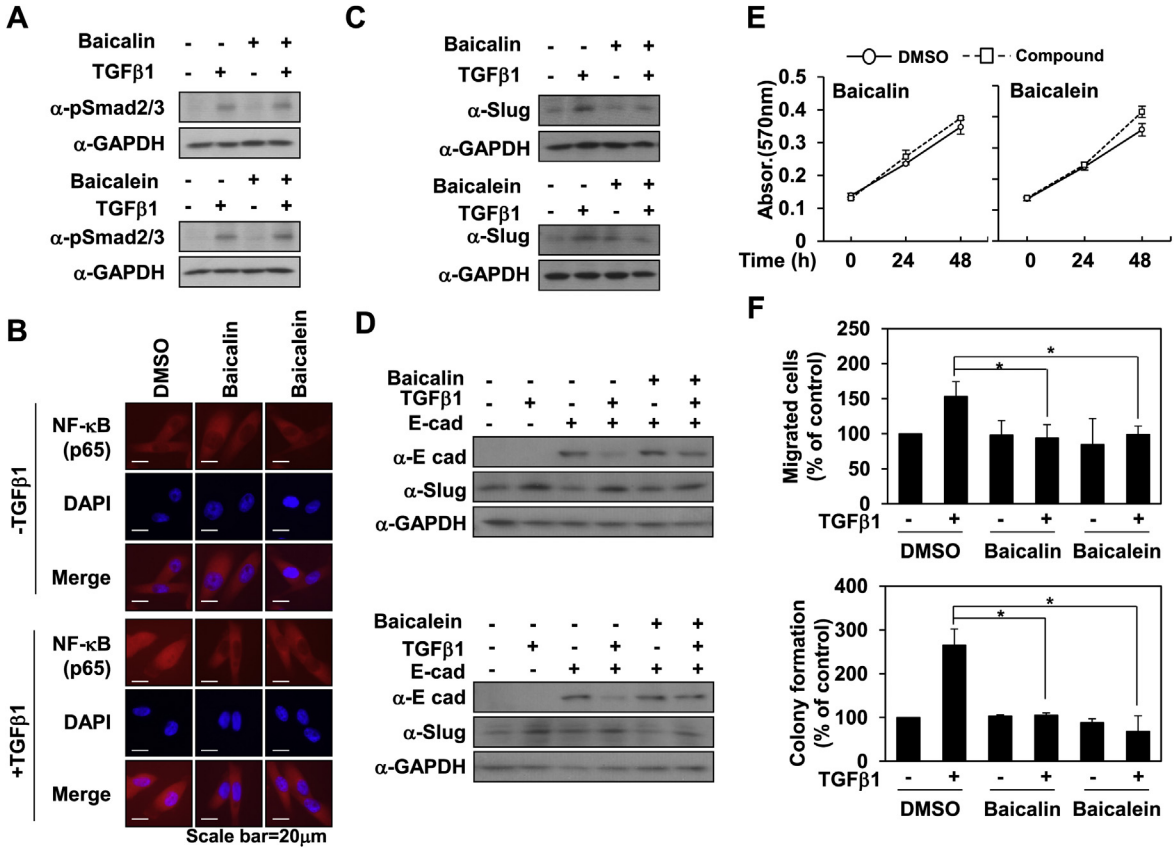

Fig. 4. Baicalin and baicalein inhibit the tumorigenic activity of breast cancer cells. (A) MDA-MB-231 cells were treated with indicated compounds (5 μM) and TGF-β1 (10 ng/ml) for 24 h. Total cell lysates were analyzed by Immunoblotting with anti-phospho Samd2/3 antibody. (B) MDA-MB-231 cells (1.5×10^5 cells/well in a 12well) were treated with indicated compounds (5 μM) and TGF-β1 (10 ng/ml). After 30 min, the cells were immunostained with anti-NF-κB (p65) antibody. The results were visualized with a Texas red-conjugated goat anti-rabbit antibody (red), and DAPI was used to counterstain nuclei (blue). (C) MDA-MB-231 cells were treated as described as (A). After 48 h, total cell lysates were analyzed by Immunoblotting with indicated antibodies. (D) MDA-MB-231 cells, transfected with human E-cadherin were treated with described as (A). After 48 h, total cell lysates were analyzed by Immunoblotting with indicated antibodies. (E) MDA-MB-231 cells (1.0×10^4 cells/well in a 96-well) were treated with the indicated compounds (5 μM). After incubation, MTT assays were performed, and the mean absorbance was measured at 570 nm. (F) MDA-MB-231 cells were transfected human E-cadherin and these cells were treated with TGF-β1 (10 ng/ml) and/or indicated compounds (5 μM). After 48 h cells (1.0×10^5 cells/well in a Transwell apparatus) were seeded to the upper chamber. After 24 h, migrated cells were stained with H&E, and counted (upper panel). MDA-MB-231 cells overexpressing E-cadherin were treated with TGF-β1 (10 ng/ml) and/or the indicated compound (5 μM) and seeded on soft agar. After 16 days, colonies were stained with 0.005% crystal violet and counted (bottom panel). Error bars indicate mean ± S.D. *, $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

rescued the TGF-β1-induced downregulation of E-cadherin exogenously expressed in MDA-MB-231 cells (Fig. 4D). Therefore, it seems that baicalin and baicalein regulate TGF-β1-mediated EMT not only in normal breast epithelial cells, but also in breast cancer cells. Under our experimental conditions, neither baicalin nor baicalein showed overgrowth effect on MDA-MB-231 cells (Fig. 4E). In contrast, however, both compounds reduced the TGF-β1-induced migration and colony formation (Fig. 4F) of MDA-MB-231 cells, suggesting that both compounds have anti-tumorigenic activity. Collectively, our results strongly suggest that baicalin and baicalein may inhibit EMT processes, thereby suppressing tumorigenic activity in breast cancer cells.

4. Discussion

Since EMT is involved in many crucial cancer cell functions, including cancer recurrence, targeting EMT represents an important therapeutic strategy for cancer treatment. Here, we sought to identify a natural compound capable of inhibiting EMT processes. Using human MCF10A breast epithelial cells, we directly evaluated the impact of a *Scutellaria baicalensis* Georgi extract on several TGF-β1-mediated EMT parameters, including cell morphology and the expression levels of epithelial and mesenchymal markers. Our data revealed that the butanol extract from *Scutellaria baicalensis* Georgi effectively reduced TGF-β1-mediated EMT (Fig. 1A and B). Further experiments showed that baicalin and baicalein, the major compounds in this butanol fraction [23], effectively reduced EMT in MCF10A cells (Fig. 1C and D). Consistent with their ability to inhibit the TGF-β1-induced acquisition of the mesenchymal phenotype, baicalin and baicalein significantly rescued the TGF-β1-mediated downregulation of E-cadherin.

NF-κB signaling is also reportedly required to stimulate EMT processes in various cancers. For example, NF-κB acts as a transcription factor to induce both EMT stimulators (e.g., ZEB-1, ZEB-2, Snail, Slug and Twist) and mesenchymal markers (e.g., fibronectin, vimentin and MMPs) [31]. In addition, NF-κB activation is involved in the cancer stem cell (CSC) formation of breast cancer cells. Because there are close correlations between CSC formation and EMT processes [32], these reports may be taken as further suggesting that NF-κB could be a key transcription factor in EMT. Here, we report that the Bay 11-7085-induced blockade of NF-κB reduces the expression level of Slug and rescues E-cadherin expression in MCF10A cells (Figs. 2D and 3A), and that baicalin and baicalein inhibit EMT by reducing NF-κB (Fig. 2). Both compounds appear to inhibit TGF-β1-mediated EMT by reducing the expression level of the EMT-related transcription factor, Slug via the NF-κB pathway (Fig. 3). Consistent with the above-described results, baicalin and baicalein also appeared to have inhibitory effects on oncogenic EMT, suppressing TGF-β1-induced migration and colony formation (Fig. 4F) in MDA-MB-231 breast cancer cells, suggesting that the baicalin- or baicalein-mediated inhibition of EMT may inhibit the tumorigenic activities of breast cancer cells.

In summary, we herein report for the first time that baicalin and baicalein effectively inhibit EMT processes in breast epithelial cells, and that this inhibitory activity effectively inhibits tumorigenic activity in breast cancer cells. Although future work will be needed to examine the underlying mechanisms, these results suggest that baicalin and baicalein could potentially be developed into anticancer agents aimed at treating breast cancer.

Conflict of interest

The authors state no conflicts of interest.

Acknowledgments

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.032.

References

[1] S.R. Kim, S. Paik, Genomics of adjuvant therapy for breast cancer, Cancer J. 17 (2011) 500–504.
[2] M. Iwatsuki, K. Mimori, T. Yokobori, et al., Epithelial-mesenchymal transition in cancer development and its clinical significance, Cancer Sci. 101 (2010) 293–299.
[3] J.P. Thiery, Epithelial-mesenchymal transitions in tumour progression, Nat. Rev. Cancer 2 (2002) 442–454.
[4] J. Zavadil, E.P. Böttinger, TGF-beta and epithelial-to-mesenchymal transitions, Oncogene 24 (2005) 5764–5774.
[5] E. Piek, A. Moustakas, A. Kurisaki, et al., TGF-(beta) type I receptor/ALK-5 and Smad proteins mediate epithelial to mesenchymal transdifferentiation in NMuMG breast epithelial cells, J. Cell. Sci. 112 (1999) 4557–4568.
[6] A.V. Bakin, C. Rinehart, A.K. Tomlinson, et al., p38 mitogen-activated protein kinase is required for TGFbeta-mediated fibroblastic transdifferentiation and cell migration, J. Cell. Sci. 115 (2002) 3193–3206.
[7] C.B. Brown, A.S. Boyer, R.B. Runyan, et al., Requirement of type III TGF-beta receptor for endocardial cell transformation in the heart, Science 283 (1999) 2080–2082.
[8] H. Chen, N.E. Paradies, M. Fedor-Chaiken, et al., E-cadherin mediates adhesion and suppresses cell motility via distinct mechanisms, J. Cell. Sci. 110 (Pt3) (1997) 345–356.
[9] K. Gravdal, O.J. Halvorsen, S.A. Haukaas, et al., A switch from E-cadherin to N-cadherin expression indicates epithelial to mesenchymal transition and is of strong and independent importance for the progress of prostate cancer, Clin. Cancer Res. 13 (2007) 7003–7011.
[10] V. Ellenrieder, S.F. Hendler, W. Boeck, et al., Transforming growth factor beta1 treatment leads to an epithelial-mesenchymal transdifferentiation of pancreatic cancer cells requiring extracellular signal-regulated kinase 2 activation, Cancer Res. 61 (2001) 4222–4228.
[11] A.K. Kamaraju, A.B. Roberts, Role of Rho/ROCK and p38 MAP kinase pathways in transforming growth factor-beta-mediated Smad-dependent growth inhibition of human breast carcinoma cells in vivo, J. Biol. Chem. 280 (2005) 1024–1036.
[12] J.M. Lee, S. Dedhar, R. Kalluri, et al., The epithelial-mesenchymal transition: new insights in signaling, development, and disease, J. Cell. Biol. 72 (2006) 973–981.
[13] H.B. Zhang, P. Lu, Q.Y. Guo, et al., Baicalein induces apoptosis in esophageal squamous cell carcinoma cells through modulation of the PI3K/Akt pathway, Oncol. Lett. 5 (2013) 722–728.
[14] Y.M. Lee, P.Y. Cheng, S.Y. Chen, et al., Wogonin suppresses arrhythmias, inflammatory responses, and apoptosis induced by myocardial ischemia/reperfusion in rats, J. Cardiovasc. Pharmacol. 58 (2011) 133–142.
[15] L. Chen, J. Dou, Z. Su, et al., Synergistic activity of baicalein with ribavirin against influenza A (H1N1) virus infections in cell culture and in mice, Antivir. Res. 91 (2011) 314–320.
[16] R. Serpa, E.J. França, L. Furlaneto-Maia, et al., In vitro antifungal activity of the flavonoid baicalein against Candida species, J. Med. Microbiol. 61 (Pt 12) (2012) 1704–1708.
[17] Y.F. Liu, F. Gao, X.W. Li, et al., The anticonvulsant and neuroprotective effects of baicalin on pilocarpine-induced epileptic model in rats, Neurochem. Res. 37 (2012) 1670–1680.
[18] L. Zhu, L. Zhao, H. Wang, et al., Oroxylin A reverses P-glycoprotein-mediated multidrug resistance of MCF7/ADR cells by G2/M arrest, Toxicol. Lett. 219 (2013) 107–115.
[19] Y. Huang, J. Hu, J. Zheng, et al., Down-regulation of the PI3K/Akt signaling pathway and induction of apoptosis in CA46 Burkitt lymphoma cells by baicalin, J. Exp. Clin. Cancer Res. 20 (2012) 31–48.
[20] P. Dong, Y. Zhang, J. Gu, et al., Wogonin, an active ingredient of Chinese herb medicine Scutellaria baicalensis, inhibits the mobility and invasion of human gallbladder carcinoma GBC-SD cells by inducing the expression of maspin, J. Ethnopharmacol. 137 (2011) 1373–1380.
[21] R.B. Hazan, R. Qiao, R. Keren, et al., Cadherin switch in tumor progression, Ann. N. Y. Acad. Sci. 1014 (2004) 155–163.
[22] C. Li, G. Lin, Z. Zuo, Pharmacological effects and pharmacokinetics properties of Radix Scutellariae and its bioactive flavones, Biopharm. Drug Dispos. 32 (2011) 427–445.

[23] C.Z. Wang, X.L. Li, Q.F. Wang, et al., Selective fraction of Scutellaria baicalensis and its chemopreventive effects on MCF-7 human breast cancer cells, Phytomedicine 17 (2010) 63–68.
[24] S. Lamouille, J. Xu, R. Derynck, Molecular mechanisms of epithelial-mesenchymal transition, Nat. Rev. Mol. Cell. Biol. 15 (2014) 178–196.
[25] S. Baranwal, S.K. Alahari, Molecular mechanisms controlling E-cadherin expression in breast cancer, Biochem. Biophys. Res. Commun. 384 (2009) 6–11.
[26] Q. Zhang, B.T. Helfand, T.L. Jang, et al., Nuclear factor-kappaB-mediated transforming growth factor-beta-induced expression of vimentin is an independent predictor of biochemical recurrence after radical prostatectomy, Clin. Cancer Res. 15 (2009) 3557–3567.
[27] H.J. Maier, U. Schmidt-Strassburger, M.A. Huber, et al., NF-kappaB promotes epithelial-mesenchymal transition, migration and invasion of pancreatic carcinoma cells, Cancer Lett. 295 (2010) 214–228.
[28] H.A. Lim, E.K. Lee, J.M. Kim, et al., PPARγ activation by baicalin suppresses NF-κB-mediated inflammation in aged rat kidney, Biogerontology 13 (2012) 133–145.
[29] E.K. Lee, J.M. Kim, J. Choi, et al., Modulation of NF-κB and FOXOs by baicalein attenuates the radiation-induced inflammatory process in mouse kidney, Free Radic. Res. 45 (2011) 507–517.
[30] H. Peinado, F. Portillo, A. Cano, Transcriptional regulation of cadherins during development and carcinogenesis, Int. J. Dev. Biol. 48 (2004) 365–375.
[31] C. Min, S.F. Eddy, D.H. Sherr, et al., NF-kappaB and epithelial to mesenchymal transition of cancer, J. Cell. Biochem. 104 (2008) 733–744.
[32] S.A. Mani, W. Guo, M.J. Liao, et al., The epithelial-mesenchymal transition generates cells with properties of stem cells, Cell 133 (2008) 704–715.