Luteolin attenuates TGF-β1-induced epithelial–mesenchymal transition of lung cancer cells by interfering in the PI3K/Akt–NF-κB–Snail pathway

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Aims: Luteolin is a natural flavonoid that possesses a variety of pharmacological activities, such as anti-inflammatory and anti-cancer abilities. Whether luteolin regulates the transformation ability of lung cancer cells remains unclear. The current study aims to uncover the effects and underlying mechanisms of luteolin in regulation of and epithelial–mesenchymal transition of lung cancer cells. Main methods: The lung adenocarcinoma A549 cells were used in this experiment; the cells were pretreated with luteolin followed by administration with TGF-β1. The expression levels of various cadherin and related upstream regulatory modules were examined.

Key findings: Pretreatment of luteolin prevented the morphological change and downregulation of E-cadherin of A549 cells induced by TGF-β1. In addition, the activation of PI3K–Akt–κB–NF–Snail pathway which leads to the decline of E-cadherin induced by TGF-β1 was also attenuated under the pretreatment of luteolin.

Significance: We provide the mechanisms about how luteolin attenuated the epithelial–mesenchymal transition of A549 lung cancer cells induced by TGF-β1. This finding will strengthen the anti-cancer effects of flavonoid compounds via the regulation of migration/invasion and EMT ability of various cancer cells.

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Introduction

A critical step in the transformation from a benign to a malignant tumor is epithelial–mesenchymal transition (EMT), which also plays important roles in normal physiological functions such as embryogenesis, restitution, wound repair, and tissue remodeling (Yang and Weinberg, 2008; Thiery et al., 2009; Yilmaz and Christofori, 2009). EMT is considered a pathological process that promotes cancer progression, particularly invasion and metastasis (Kalluri and Weinberg, 2009). EMT is thought to be a consequence of the cadherin switch because the expression of different types of cadherins marked for epithelial or mesenchymal state is changed; for example, E-cadherin is known to be expressed only in epithelial cells while N-cadherin is mainly expressed in mesenchymal ones (Acloque et al., 2008). It has been reported that E-cadherin expression levels decreased in many invasive tumors, suggesting a high correlation between E-cadherin and poor prognosis (Onder et al., 2008). Many transcriptional factors have been reported to be involved in the regulation of E-cadherin expression via binding of the E-box on the E-cadherin promoter; or example, Snail, Snug, ZEB1, ZEB2, E47, and Twist, all inhibit the transcriptional activation of E-cadherin (Peinado et al., 2007; Barenwal and Alahari, 2009).

The most well-characterized factor responsible for induction of EMT by far is TGF-β, which is a multifunctional cytokine and is involved in many biological processes including cell proliferation, differentiation and migration (Lawrence, 1996; Zhang, 2011). Three members of TGF-β have been identified, namely TGF-β1, 2, and 3, and TGF-β1 is proved to be highly correlated with EMT (Dalal et al., 1993; Fong et al., 2009). Some molecules including Smad, RhoA, Rac1, Ras and MAPK were found to be involved in TGF-β1-triggered EMT (Janda et al., 2002; Huang et al., 2004; Zavadil et al., 2004; Willis and Borok, 2007). It has been reported that TGF-β1 signaling pathway-mediated EMT might yield beneficial effects in treating cancer patients with advanced metastasis.

It is worth noting that the use of medicinal plants or their active components is becoming an increasingly attractive approach for the treatment of cancer. Luteolin, the 3′,4′,5′,7-tetrahydroxyflavone, an active flavonoid compound from *Lonicera japonica* (Caprifoliaceae), is widely distributed in the plant kingdom and serves as a common

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**Main methods:** The lung adenocarcinoma A549 cells were used in this experiment; the cells were pretreated with luteolin followed by administration with TGF-β1. The expression levels of various cadherin and related upstream regulatory modules were examined.

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**Significance:** We provide the mechanisms about how luteolin attenuated the epithelial–mesenchymal transition of A549 lung cancer cells induced by TGF-β1. This finding will strengthen the anti-cancer effects of flavonoid compounds via the regulation of migration/invasion and EMT ability of various cancer cells.

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dietary additive (Lin et al., 2008). Preclinical studies have shown that luteolin possesses multiple pharmacological activities such as anti-inflammatory, -oxidant, -microbial and -tumor abilities (Lin et al., 2008; Seelinger et al., 2008a, 2008b). Luteolin shows a strong anti-proliferative activity against different human cancer cell lines including lung cancer cells (Seelinger et al., 2008a, 2008b). The role of luteolin in the inhibition of invasion/metastasis of lung cancer cells, however, remains unclear. In this study, we demonstrated that luteolin inhibits TGF-β1-induced EMT of human lung cancer cells. Our data show that the PI3K/Akt-NF-κB-Snail-E-cadherin signaling pathway is involved in the reversion of EMT primed by TGF-β1 in A549 cells when engaged with luteolin.

**Material and methods**

**Reagents**

Luteolin (Fig. 1, adopted from http://www.ncbi.nlm.nih.gov/pccompound) was purchased from Extrasynthese (Genay Cedex, France). Luteolin was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 40 mM and stored at −20 °C. Anti-phospho-PI3K (P85), anti-phospho-Akt Ser473, anti-vimentin, anti-phospho-mTOR, anti-Snail, anti-phospho Smad2, and anti-phospho Smad3 antibodies, and LY294002 (inhibitor of PI3K/Akt) were purchased with luteolin.

Cell culture, viability assay, and morphological observation

The human adenocarcinoma cell line A549 was purchased from the American Type Culture Collection. The A549-p53shRNA cell lines were constructed in our previous work (Wu et al., 2011). The H460, CL-1-0 and CL1-5 cells were kindly provided by Dr. Jinghua Tsai Chang. The cells were cultured in RPMI 1640 medium (Invitrogen, CA, USA) supplemented with 5% fetal bovine serum (FBS), 1 mM sodium pyruvate, 100 U/ml penicillin along with 100 μg/ml streptomycin, and 2 mM glutamine at 37 °C in a humidified atmosphere comprising 95% air and 5% CO2. The culture medium was changed every 2 days. A549 cells were seeded at a density of 6 × 104 cells/2 ml medium in 12-well dishes. Various doses of TGF-β1 or luteolin were added into cells for different time periods. The morphological changes in the cells were observed under a Nikon phase-contrast light microscope. The number of viable cells was scored by the Trypan blue dye exclusion method.

**Fig. 1.** The chemical structure of luteolin.

**RNA extraction and RT-PCR**

Total cellular RNA was extracted using the RNA-Beem™ RNA Isolation Kit (Tel-Test, Friendswood, TX) in accordance with the manufacturer’s instructions. One microgram of total RNA was reverse-transcribed using Advantage RT for the PCR Kit (Clontech, Mountain View, CA) at 42 uC for 1 h, as described in the manufacturer’s protocol. The PCR primers for E-cadherin were as follows: forward: ATTCTTGTGTCTACCGTGGGACT and reverse: GTGGGTGTACCTCTCATACAGAG. For vimentin they were as follows: forward: CTAAGGGCCAGGGCAAC and reverse: TGCCATCTGTCGACT. For N-cadherin they were as follows: forward: GAGAATTTGGACCATCCTG and reverse: CTTCCACATACATGCAG. For Snail they were as follows: forward: CCAATGTTCATCCTGGACT and reverse: TGACATCTGAGGGTCTGG.

**Gel mobility shift assay**

The nuclear lysates were extracted as described previously (Wu et al., 2011). The DNA sequence on the Snail promoter for the binding of NF-κB was designed as (GAGGAAAATTTCGCC)x3. Next, 5 pmol of the biotinylated DNA probe was then incubated with 5 μg of nuclear lysates in 20 μl of the reaction buffer (containing 2 μl of buffer D; 20 mM HEPES/pH 7.9, 20% glycerol, 100 mM KCl, 0.5 mM EDTA/pH 8.0, 0.25% NP-40, 2 mM DTT, 0.1% PMSF, and 4 μl of buffer F: 20% Ficoll 400, 100 mM HEPES/pH 7.9, 300 mM KCl, 10 mM DTT, and 0.1% PMSF) at 30 °C for 30 min with gentle shaking every 10 min. The reaction mixtures were then separated using native PAGE, transferred to a nitrocellulose paper, and subjected to Western blot analysis using streptavidin horseradish peroxidase (Jackson ImmunoResearch Laboratories, Inc., PA, USA).

**Western blot analysis**

A549 cells were seeded into a 10-cm Petri dish at a density of 80% confluence and cultured under normal conditions. The cells were then untreated or pretreated with luteolin for 0.5 h under 0.1% serum-cultured conditions followed by the treatment with TGF-β1 for the indicated number of times. After treatment, both adherent and floating cells were harvested, washed twice with ice-cold PBS, and lysed in ice-cold modified RIPA buffer. After 30 min of incubation on ice, the cells were centrifuged at 100,000 × g for 30 min at 4 °C and the supernatants were collected. Protein concentration was determined using the Bradford method. For Western blot analysis, equal amounts of total proteins were loaded onto SDS-polyacrylamide gels and the proteins were electrophoretically transferred onto a PVDF membrane (Millipore, Bedford, MA). Immunoblots were analyzed using the indicated primary antibodies. After exposure to horseradish peroxidase-conjugated secondary antibody for 1 h, proteins were visualized using the enhanced chemiluminescence detection kit (ECL Kits; Amersham Life Science).

**Chromatin immunoprecipitation**

The chromatin immunoprecipitation assay was performed as described by the manufacturer (Exacta ChIPTM-Human Snail Chromatin Immunoprecipitation Kit, R&D Systems, Inc., USA & Canada or Europe). In brief, A549 cells were treated with 1% formaldehyde for 15 min to cross-link proteins with DNA followed by the addition of 1.5 M glycine to stop the reaction. The cross-linked chromatin was then prepared and sonicated to an average size of 400–600 bp. The DNA fragments with proteins were immunoprecipitated overnight with anti-Snail antibody at 4 °C. After reversal of cross-linking, the genomic region of the Snail promoter on the E-cadherin promoter was amplified using
PCR with the primers provided by the kit. The PCR products were subjected to electrophoresis on a 1.5% agarose gel, and the bands were visualized using a gel documentation system (Alpha Innotech).

**Indirect immunofluorescence**

A549 cells were seeded onto coverslips and maintained under 0.1% serum-containing medium. After reaching 75% confluence, the cells were treated with various doses of luteolin for 30 min followed by treatment with TGF-β1 for another 1 h. The cells were washed with PBS twice and then fixed with methanol at −20 °C for 20 min. After air drying, the cells were incubated with polyclonal antibodies against NF-κB (p65) at room temperature for 2 h. The coverslips were washed three times with PBS and incubated with goat anti-rabbit FITC or Texas Red (Jackson, PA, USA) and 1 mg/ml DAPI (Sigma, MO, USA). After washing with PBS containing 0.1% Tween 20, the samples were mounted. The fluorescence images on the coverslips were analyzed using a Zeiss microscope.

**Statistical analysis**

All data are presented as the mean ± S.D. of at least nine replicates from three separate experiments. Statistical differences were evaluated using the Student’s t-test and considered significant at *P < 0.05, **P < 0.01, or ***P < 0.001. All the figures shown in this article have been obtained from at least three independent experiments with similar results.

![Image of the experiment with TGF-β1 and luteolin](image-url)

**Fig. 2.** Luteolin affected TGF-β1-induced morphological change in A549 cells. (A) A549 cells maintained in 0.1% FBS medium were treated with or without 1 ng/ml TGF-β1 for 48 h followed by the observation under phase-contrast microscopy, scale bar = 100 μM. (B) A549 cells maintained under 0.1% FBS medium were pretreated with different doses of luteolin (0, 10, and 40 μM) for 0.5 h followed by treatment with 1 ng/ml TGF-β1 for 48 h, then followed by the observation under phase-contrast microscopy, scale bar = 100 μM. (C) A549 cells maintained under 0.1% FBS medium were treated with different doses of luteolin (0, 10, and 40 μM) alone or along with 1 ng/ml TGF-β1. The treated cells were collected, and the cell growth ratio was determined by the Trypan blue exclusion method. (D) A549 cells maintained under 0.1% FBS medium were pretreated with different doses of luteolin (0, 10, and 40 μM) for 0.5 h followed by treatment with 1 ng/ml TGF-β1 for 48 h. The treated cells were collected, fixed, stained with annexin-V and PI followed by subjecting to flow cytometry assay.
Results

Luteolin inhibited TGF-β1-induced EMT of A549 cells

In this experiment, we used low serum culture conditions (0.1% serum) to avoid the interference of the cell growth factor presented in the serum (Kasai et al., 2005). Using different doses of TGF-β1 (1, 2, and 5 ng/ml), the EMT phenomenon of A549 cells was observed under treatment with 1 ng/ml of TGF-β1 at 24 h (Fig. 2A). To analyze the effects of luteolin on TGF-β1 functionality, A549 cells were pretreated with 10 or 40 μM luteolin, which showed no significant growth inhibition or apoptosis in cells (Fig. 2C and D), for 30 min followed by the addition of TGF-β1 for another 48 h. The results showed that treatment with luteolin did not induce morphological changes in A549 cells; however, pretreatment with luteolin significantly prevented the fibroblast-like morphology of A549 cells induced by TGF-β1 into a rounded epithelial cell type in a dose-dependent manner (Fig. 2B), indicating that luteolin was able to block TGF-β1-induced EMT of A549 cells. To validate these observations, we applied p53-knockdown A549 (Wu et al., 2011), H460, CL1-0 and CL1-5 lung cancer cells to test the ability of luteolin in blocking TGF-β1 induced morphological change. Surprisingly, the results showed that only A549-p53shRNA cells are resistant to luteolin-induced mortality, as A549 cells do (Supplementary Figs. 1A and 2). The viability of CL 1-0 and CL1-5 cells decreased dramatically at low concentration of luteolin (10μM), while nearly perished at higher concentration (40μM) of luteolin for 48 h (Supplementary Figs. 1A and 2).

The treatment of luteolin indeed prevented the morphological change induced by TGF-β1 in A549-p53shRNA cells (Supplementary Fig. 1A). In addition, the pretreatment of relative low concentration of luteolin (10μM) also prevented the morphological change of H460 cells induced by TGF-β1, while higher concentration of luteolin also eventually eliminated most of H460 cells at 48 h (Supplementary Figs. 1B and 2). These results indicate that luteolin shows different biological effects at various concentrations and might be able to act as an anti-TGF-β1 functionality compound at non-lethal dosage in different types of lung cancer cells.

Luteolin prevented TGF-β1-induced EMT-related gene expression

Since luteolin was able to inhibit TGF-β1-induced EMT of A549 cells, we further dissected the expression variations of EMT-related molecules during this process via Western blotting. The results showed that addition of 1 ng/ml TGF-β1 reduced E-cadherin expression while it increased vimentin and N-cadherin expression. Meanwhile, pretreatment with 10μM luteolin effectively prevented these expression variations in A549 cells (Fig. 3A). Furthermore, results from RT-PCR showed that luteolin also affected mRNA expression of these genes (Fig. 3B). The reduction in E-cadherin mRNA level induced by TGF-β1 was partially inhibited by pretreatment with luteolin; on the contrary, N-cadherin, whose expression was induced by TGF-β1, showed a complete reduction upon pretreatment with luteolin. These results suggested that the
interfering effects of luteolin on TGF-β1-induced EMT-related gene expression occurred mainly via transcriptional-dependent pathways.

**Luteolin prevented TGF-β1-induced E-cadherin downregulation via Snail**

In the following experiment, the expression of molecules upstream of E-cadherin was examined. The results show that treatment of A549 cells with TGF-β1 induced Smad phosphorylation (Fig. 4A); however, pretreatment with different doses of luteolin failed to block such phosphorylation (Fig. 4A), suggesting that Smad was not one of the targets regulated by luteolin. In contrast, the results from chromatin immunoprecipitation showed that addition of TGF-β1 increased the interaction between Snail and the E-cadherin promoter, and pretreatment with luteolin blocked this interaction (Fig. 4B), indicating that luteolin blocked TGF-β1-induced E-cadherin downregulation via the Snail pathway. To further characterize the underlying mechanisms, we examined Snail expression levels during treatment with TGF-β1 alone or along with luteolin. The results showed increased protein and mRNA patterns of Snail after the addition of TGF-β1. However, pretreatment with luteolin attenuated such induction of Snail levels (Fig. 4C and D), suggesting that the ability of luteolin to reduce the interaction between Snail and the E-cadherin promoter in the engagement with TGF-β1 might simply be because of the downregulation of Snail expression by transcriptional manner.
Luteolin inhibited TGF-β1-induced Snail upregulation via the PI3K/Akt–NF-κB pathway

Snail expression is known to be regulated by NF-κB (Julien et al., 2007). In the next experiment, we performed a chromatin immunoprecipitation assay to verify the functional correlations between NF-κB and the Snail promoter. The result showed that TGF-β1 effectively induced the binding of NF-κB to the Snail promoter region, while pretreatment with luteolin significantly blocked TGF-β1-induced interaction in A549 cells (Fig. 5A). The function of NF-κB was found to be inhibited by binding with l-κB-α; our results showed that TGF-β1-induced decrease in l-κB-α is significantly reversed by pretreatment with luteolin (Fig. 5B), and the immunostaining also showed that luteolin blocked the translocation of NF-κB induced by TGF-β1 (Fig. 5C). To confirm the connections between NF-κB and E-cadherin, PDTC or Bay 11-7082, both of which are inhibitors of NF-κB (Mori et al., 2002), were introduced into A549 cells followed by treatment with TGF-β1. The results showed that addition of PDTC significantly prevented TGF-β1-induced reduction of E-cadherin at relatively low dosages (5 and 10 μM) (Fig. 5D); meanwhile, addition of Bay 11–7082 also exhibited similar effects. Furthermore, inhibition of NF-κB also reduced the binding of Snail to the E-cadherin promoter.

Fig. 4. Luteolin interfered the Snail–E-cadherin but not Smad–E-cadherin signaling primed by TGF-β1. (A) A549 cells maintained in 0.1% FBS medium were pretreated with 0, 10, or 40 μM luteolin for 0.5 h with or without the addition of 1 ng/ml TGF-β1 for another 0.5 h. The cells were subjected to Western blot analysis to verify the phosphorylation patterns of Smad2 and 3. (B) A549 cells maintained in 0.1% FBS medium were pretreated with 0, 10, or 40 μM luteolin for 0.5 h with or without the addition of 1 ng/ml TGF-β1 for another 18 h. The cells were then collected and chromatin immunoprecipitation was evaluated as described in Material and methods section. (C) and (D) A549 cells maintained in 0.1% FBS medium were pretreated with 0, 10, or 40 μM luteolin for 0.5 h with or without the addition of 1 ng/ml TGF-β1 for another 2 h. The cells were either subjected to (C) Western blot or (D) RT-PCR analysis to verify Snail expression. Densitometry shows data from three experiments; the values from each experimental set were normalized with control as related percentage (*P < 0.05, **P < 0.01, or ***P < 0.001 versus TGF-β1 treatment alone).
These results demonstrated that luteolin might recover the reduction of E-cadherin under treatment with TGF-β1, via the regulation of the NF-κB–Snail pathway. Previous reports have indicated that PI3K/Akt is able to regulate the function of NF-κB (Burow et al., 2000); therefore, to evaluate the upstream signal of NF-κB regulated by luteolin, we first analyzed the activation status of PI3K/Akt under treatment with TGF-β1 alone or along with luteolin. The results showed an increased phosphorylated pattern of both PI3K and Akt under treatment with TGF-β1 (Fig. 6A). Furthermore, mTOR phosphorylation was observed, which was also recognized as an activation of Akt (Sarbassov et al., 2005), indicating that the activation of the PI3K/Akt pathway was induced by TGF-β1.
However, luteolin pretreatment significantly blocked such activation in a dose-dependent manner (Fig. 6A). To establish the associations among PI3K/Akt, NF-κB activation, and E-cadherin expression, A549 cells were treated with LY294002, an inhibitor of PI3K/Akt for 0.5 h with or without the addition of 1 ng/ml TGF-β1 for another 8 h. The cells were then collected and (B) E-cadherin expression and phosphorylation patterns of Snail or (C) IκB-α were examined via Western blot. Densitometry shows data from three experiments; the values from each experimental set were normalized with control as related percentage (*P < 0.05, **P < 0.01, or ***P < 0.001 versus TGF-β1 treatment alone). Results clearly indicate that luteolin might block TGF-β1-induced reduction of E-cadherin via the PI3K/Akt–NF-κB–Snail pathway (Fig. 7).

Discussion

The tumor microenvironment is the key factor that induces the evolution of metastatic cancer cells from benign ones based on previous reports (Rofstad, 2000; Cretu and Brooks, 2007). TGF-β1, a promoter molecule in middle-to-late stage of tumors, is known to be involved in...
such induction. The myeloid, mesenchymal, and tumor cells engaged in the microenvironment are all able to produce and secrete TGF-β1 to overcome hypoxia and/or inflammation, the constitutive action of TGF-β1; however, eventually it can cause EMT and aggressive transformation of benign tumors to malignant ones. Here we focus on the effects of luteolin, an active ingredient of *L. japonica*, in the regulation of the TGF-β1 function in A549 lung adenocarcinoma cells. Previous reports mentioned that the addition of TGF-β1 downregulates E-cadherin expression, which is represented as a critical event of EMT phenomena, by interfering in the Smad pathway in A549 cells. Our findings, however, indicate that the PI3K/Akt–NF-κB–Snail signaling pathway also participated in TGF-β1-induced reduction of E-cadherin. Furthermore; addition of luteolin significantly restores E-cadherin expression by blocking the function of the PI3K/Akt–NF-κB–Snail signaling pathway under treatment with TGF-β1 of A549 cells. Despite the fact that we do not perform the animal experiment to verify the in vivo effects of luteolin in lung cancer cells yet; we have proved the ability of luteolin in inhibition of the fibroblast properties and prevent the lung fibrosis in vivo in the earlier experiments (Chen et al., 2010). Besides, other reports also mentioned the ability of luteolin in inhibiting the invasion of prostate and epitheloid cancer cells (Lin et al., 2008; Attoub et al., 2011). Thus, it is reasonable to propose that luteolin might show some degree of inhibitory effects in regulating the invasion ability of lung cancer cells and might be worthy to elucidate in the future investigation.

The different time intervals of KOH–AL1 plus TGF-β1 treatment in A549 cells are aimed to establish the time-axis between TGF-β1 induced signaling transduction and morphological change. For example, the activation of PI3K/Akt, the translocation of NF-κB, the expression of Snail, the binding of Snail onto E-cadherin promoter, the reduction of E-cadherin levels and finally the EMT. These results might help to rule out the possibility that the activation of these molecules might not be correlated to each other under the treatment of TGF-β1.

In addition to the E-cadherin downregulation, the markers of mesenchymal cells such as N-cadherin, vimentin, MMP-2, and MMP-9 were also upregulated in A549 cells under treatment with TGF-β1 (Fig. 3), suggesting that the variation of these genes during EMT might be a ubiquitous phenomenon. The upstream transcriptional regulators of E-cadherin include Slug, Snail, Twist, and ZEB (Baramwal and Alahari, 2009). Our results showed that Snail was significantly upregulated by the addition of TGF-β1 (Fig. 4), which was in accordance with a previous report (Cho et al., 2007). The expression of Snail is known to be regulated by MAPK and GSK-3β signaling pathways (Zhou et al., 2004; Horiguchi et al., 2009), while our results suggested another potential pathway composed of PI3K/Akt–NF-κB–Snail in lung cancer cells. These results indicated that the signaling module underlying the EMT phenomena induced by TGF-β1 might be more complicated than expected.

Recently, a report mentioned the ability of luteolin in blocking hypoxia-induced EMT partly by inhibiting β3integrin–FAK signaling pathways (Ruan et al., 2012). It is proposed that hypoxia might reduce the expression of E-cadherin via the activation of β3integrin–FAK–RhoB–HIF-1α–Snail pathway (Skuli et al., 2009; Luo et al., 2011; Jing et al., 2013). Interestingly, the administration of TGF-β1 also induces the upregulation of β3 integrin which in turn activates FAK and subsequent downstream signaling transducers such as Ras, Src or PI3K/Akt pathways (Hecker et al., 2004; Mitra and Schlampfer, 2006; Pechkovsky et al., 2008). These results indicate that integrin is an important signaling transducer of both hypoxia and TGF-β1-induced EMT. Despite the fact that the roles of integrin in luteolin-inhibiting TGF-β1-induced EMT have not been investigated yet, it is very possible that integrin is also an early target gene affected by luteolin in blocking TGF-β1-primed malignancy of cancer cells.

The pursuit of novel compounds able to block or reverse EMT has become an emerging issue. Luteolin, a well-known flavonoid compound, has been reported to inhibit the migration/invasion and prevent the EMT phenomenon of some malignant cancers such as PC-3 (prostate cancer) and A431 (skin cancer) (Lee et al., 2008; Zhou et al., 2009). Other flavonoid compounds including quercetin, epigallocatechin-3-gallate, apigenin, theaflavin, and baicalein exhibit similar inhibitory effects of migration/invasion and EMT in skin, melanoma, breast, and HCC (hepatocellular carcinoma) cells (Noh et al., 2010; Sil et al., 2010; Wang et al., 2010). Although some small molecules that act as targets for TGF-β1 have been developed for in vitro tests, the safety of these compounds has not been confirmed yet (Yingling et al., 2004). Luteolin is usually considered as non-toxic and non-mutagenic; the administration of high dose of luteolin showed no acute toxicity in various animals (Yilmaz and Christofori, 2009). This study might provide potential candidate for treating of EMT phenomena induced by abnormal expression of TGF-β1 in lung cancer cells.

**Conclusion**

This is the first study to report about the ability of luteolin to interfere in TGF-β1-induced EMT in lung cancer cells. Besides, our
results also reveal relative underlying mechanisms. In combination with previous reports, our discovery might provide the anti-cancer effects of flavonoid compounds via the regulation of migration/invasion and EMT ability of various cancer cells.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jfs.2013.10.004.

Conflict of interest statement
None.

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