

## Luteolin inhibits invasion of prostate cancer PC3 cells through E-cadherin

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### Abstract

Luteolin, a common dietary flavonoid, has been found to have antitumor properties and therefore poses special interest for the development of preventive and/or therapeutic agent for cancers. E-cadherin, a marker of epithelial cells, mediates cell-cell adhesion. Decreased expression of E-cadherin results in a loss of cell-cell adhesion and an increased cell invasion. Many studies have shown the antiproliferative activities of luteolin on cancer cells. However, the effects of luteolin on invasion of cancer cells remain unclear. In this article, we show that luteolin inhibits invasion of prostate cancer PC3 cells through E-cadherin. We found that Luteolin induced expression of E-cadherin through mdm2. Overexpression of mdm2 or knockdown of E-cadherin could restore invasion of PC3 cells after luteolin treatment. Luteolin inhibits mdm2 through AKT and overexpression of active AKT attenuated luteolin-induced expression of E-cadherin, suggesting that luteolin regulates E-cadherin through AKT/mdm2 pathway. The *in vivo* experiments showed that luteolin inhibited spontaneous lung metastasis of PC3 cells implanted onto the nude mice. These findings provide a new sight into the mechanisms that luteolin is against cancer cells, and suggest that molecular targeting of E-cadherin by luteolin may be a useful strategy for treatment of invasive prostate cancers. [Mol Cancer Ther 2009;8(6):1684–91]

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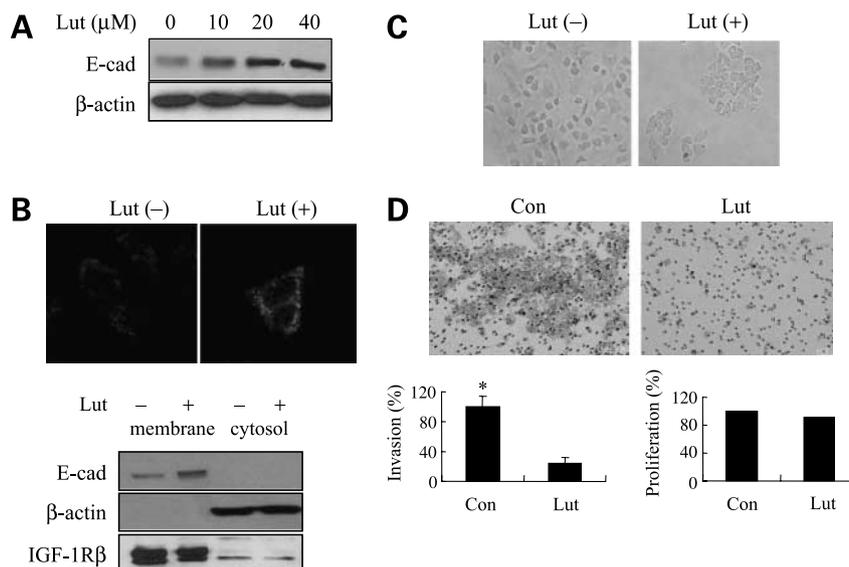
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### Introduction

Cadherins were originally identified as cell surface glycoproteins responsible for Ca<sup>2+</sup>-dependent homophilic cell-cell adhesion (1). There are three types of cadherins: classic cadherins, protocadherins, and atypical cadherins. Classic cadherins function in cell sorting via their differential adhesive strengths, and provide strong cell-cell adhesion to maintain the structural and functional integrity of tissues. Defective cadherin expression has been linked directly to a wide variety of diseases including metastatic cancer. Classic cadherins are subdivided into type I and type II. Type I classic cadherins mediate strong cell-cell adhesion. They include epithelial (E) and neuronal (N) cadherin. E-cadherin, the most commonly studied cadherin, forms the adherens junction in epithelial cells and facilitates the formation of the entire epithelial junctional complex (2). Decreased expression of E-cadherin results in a loss of cell-cell adhesion and increased cell migration and invasion (3). Cadherin-mediated cell-cell adhesion is modulated by changes in the level of cadherin on the cell surface (4, 5).

Expression of E-cadherin is regulated at several levels including transcriptional and posttranscriptional regulation (1). Transcription of E-cadherin is directly regulated by methylation and repression of promoter activity. During carcinogenesis, methylation of the E-cadherin promoter is associated with reduced E-cadherin expression, disease progression, and metastasis (6). Transcription of E-cadherin is also regulated by zinc finger proteins of the Slug/Snail family and Smad-Interacting Protein (7, 8). These proteins are repressors of E-cadherin transcription. Expression of E-cadherin can also be regulated at posttranscriptional level. E-cadherin can be endocytosed via clathrin-coated vesicles, leading to a rapid loss of cell-cell adhesion (9). Stability of E-cadherin is also regulated by p120 as loss of cadherin-p120 binding results in rapid endocytosis of the E-cadherin (10). It was reported recently that mdm2 is involved in regulation of E-cadherin (11). E-cadherin was found a substrate for the mdm2 E3 ubiquitin ligase.

Luteolin, the 3',4',5,7-Tetrahydroxyflavone, is a common dietary flavonoid and has been found to have antitumor properties. Luteolin was found to inhibit proliferation (12–14) and induce apoptosis of cancer cells (13, 15). It may function to sensitize tumor necrosis factor  $\alpha$ - and cisplatin-induced apoptosis of human tumor cells (16–18). However, whether luteolin can inhibit invasion of cancer cells remains unknown. Prostate cancer is the second most frequently diagnosed cancer and the second leading cause of cancer death in the Western male population. Androgen ablation therapy is an effective treatment for hormone-dependent prostate cancer; however, a subset of patients ultimately develops hormone-refractory disease. There is a bunch of evidence that during carcinogenesis, developing



**Figure 1.** Luteolin induces expression of E-cadherin and inhibits invasion of PC3 cells. **A**, luteolin induces expression of E-cadherin. PC3 cells were treated with luteolin for 24 h. The cells were harvested, lysed, and protein extracts were subjected to immunoblotting analysis. **B**, luteolin increases E-cadherin protein levels in cell membrane. Immunofluorescence staining of PC3 cells with E-cadherin antibody was done as described under Materials and Methods. PC3 cells were treated with luteolin (20 μmol/L) for 24 h. After treatment, the proteins from cell membrane and cytosol were isolated and subjected to immunoblotting. **C**, luteolin influences morphology of PC3 cells. PC3 cells were incubated with luteolin (20 μmol/L) for 24 h. The morphology of the cells was photographed by a phase contrast microscopy. **D**, luteolin inhibits invasion of PC3 cells. PC3 cells were pretreated with luteolin (20 μmol/L) for 24 h, the cells were trypsinized and suspended in serum-free medium. The cells ( $2 \times 10^5$ ) suspended in 200 μL of serum-free medium were placed in the upper compartment of the chamber of *trans*-well. In the lower chamber, complete medium served as a source of chemo-attractants. Invasion assay was determined as described in Materials and Methods. For each replicate, the cells in five randomly selected fields were determined. At the same time,  $5 \times 10^4$  of the cells suspended in serum-free medium were seeded in a 24-well plate and incubated. In 24 h, the cell proliferation was determined by counting cell numbers. \*,  $P < 0.05$  versus control. Lut, luteolin; E-cad, E-cadherin.

prostate cancer cells acquire mesenchymal characteristics and migratory features concomitant with a loss of epithelial characteristics such as E-cadherin expression (19, 20). Molecular targeting of E-cadherin may be a useful and novel strategy for treatment of invasive prostate cancers. Herein, we show that luteolin inhibits invasion of prostate cancer PC3 cells through up-regulating expression of E-cadherin.

## Materials and Methods

### Reagents

The antibody against E-cadherin was from BD Biosciences. Antibodies against pAKT and AKT were purchased from Cell Signaling. The antibodies against mdm2 and insulin-like growth factor receptor  $\beta$  subunit (IGF-1R $\beta$ ) were from Santa Cruz Biotechnology. Luteolin and antibody against  $\beta$ -actin were purchased from Sigma.

### Cell Culture

The prostate cancer PC3 cells were grown in RPMI 1640 (Invitrogen) with 10% heat-inactivated fetal bovine serum (Life Technologies), 100 units/mL penicillin, and 100 μg/mL streptomycin, in 5% CO<sub>2</sub> incubator at 37°C. Human prostate cancer DU145 cells, ovarian cancer A2780 cells, lung cancer A549 cells, cervical cancer HeLa cells, liver cancer HepG2 cells, and breast cancer MCF-7 cells were grown under the same conditions.

### Reverse-transcription PCR

Cells were treated with luteolin. Total RNAs were extracted and used for cDNA synthesis by reverse transcrip-

tion. The primers used for E-cadherin are 5'-GACAACAAGCCCGAATT-3' (upstream) and 5'-GGAAACTCTCTCGGTCCA-3' (downstream). The primers for glyceraldehyde-3-phosphate dehydrogenase are 5'-CCACCCATGGCAAATTCATGGCA-3' (upstream) and 5'-TCTAGACGGCAGGTCAGTCCACC-3' (downstream).

### Construction of Vectors

The cDNAs encoding human E-cadherin and mdm2 were constructed using reverse transcription-PCR. The primers for cloning E-cadherin are as follows: 5'-AAAAAGCT-TATGGGCCCTTGGAGCCGCAG-3' (sense), 5'-TTTCTCGAGCTAGTCGTCCTCGCCGCCTC-3' (anti-sense).

The primers for cloning mdm2 are as follows: 5'-AAAG-GATCCATGTGCAATACCAACATGTC-3' (sense) and 5'-TTTGAATTCCTAGGGGAAATAAGTTAGCA-3' (anti-sense).

The cDNA fragment was ligated into a pcDNA3.1 vector. The plasmid encoding myr-AKT was described previously (21).

### Short Interfering RNA

The vector pSilencer 2.1 (Ambion, Austin, TX) was used to construct mdm2 short interfering RNA (siRNA) according to the instruction of the manufacture. The target sequences for mdm2 are 5'-GAAGCAGTAGCAGTGAATC-3' (1), 5'-GCCTGG-CTCTGTGTGTAAT-3' (2), and 5'-GCCCTG-CCCAGTATGTAGA-3' (3). The sequence of control siRNA is 5'-ACTACCGTTGTTATAGGTGT-3'. All sequences were

submitted for BLAST search to ensure that only the *mdm2* gene was targeted. The control sequence was not homologous to any known human genes. siRNA oligonucleotides against E-cadherin were purchased from GenePharma. The sequences of the sense strands were as follows: CAUGGAUAACCAGAAUAAATT (siE-cad-1), GCUCGU-GUUUGACUAUGAATT (siE-cad-2), 5'-UUCUCCGAAC-GUGUCACGUTT-3' (scramble).

#### Isolation of Cell Membrane Proteins

The cells suspended in buffer A [1 mmol/L KCl, 5 mmol/L NaCl, 3 mmol/L MgCl<sub>2</sub>, 50 mmol/L Hepes, 1 mmol/L DTT, 0.5 μg/mL Leupeptin, 20 μmol/L phenylmethylsulfonyl fluoride (pH 7.4)] were frozen and thawed in liquid nitrogen twice. The cells were spun at 2,500 g for 10 min (4°C) to remove the unbroken cells and nucleus. The supernatants were harvested and spin at 16,000 g for 10 min (4°C). The precipitates were resuspended in buffer B (1 mmol/L KCl, 5 mmol/L NaCl, 3 mmol/L MgCl<sub>2</sub>, 50 mmol/L Hepes, 1 mmol/L DTT, 1 mmol/L EGTA 0.5 μg/mL Leupeptin, 20 μmol/L phenylmethylsulfonyl fluoride (pH 7.4)] and spin at 16,000 g for 10 min as above. The precipitates were resolved in buffer C [0.5 μg/mL Leupeptin, 20 μmol/L phenylmethylsulfonyl fluoride, 50 mmol/L Tris-Cl (pH 7.0)] and used for immunoblotting.

#### Immunoblotting

Immunoblotting was done as described previously (21).

#### Immunofluorescence Staining

PC-3 cells were seeded onto cover slides in a 24-well plate ( $1.2 \times 10^4$  cells per well). The next day, luteolin (20 μmol/L) was added and the incubation continued. After 24 h, the plate was placed on ice for 5 min and the cells were washed with cold PBS twice. The cells were then fixed with 4% formaldehyde for 20 min and permeabilized with 1% Triton X-100 for 4 min at room temperature. The antibody against E-cadherin was added and the cells were incubated at 4°C overnight. The cells were washed with PBS for thrice, followed by incubation with FITC-labeled second antibody (Alexa Fluor 488 goat anti-mouse IgG) for 30 min at 37°C. After washing with PBS for thrice, the cover slides were mounted with GEL/MOUNT (Biomed). The cells were photographed under a confocal microscope.

#### Cell Invasion Assay

Cell invasion assay was done using transwells as described (22). For the Matrigel invasion assay, the upper chambers of the Transwell plates were precoated for 4 h at 37°C with Matrigel (1 mg/mL in serum-free RPMI 1640). The cells pretreated with luteolin or DMSO were trypsinized, washed, resuspended in serum-free medium, and placed in the upper compartment of the chamber. In the lower chamber, complete medium served as a source of chemo-attractants. These cells were incubated for 24 h. The cells on the upper surface of the filter were wiped with a cotton swab and the cells invaded onto the lower surface of the filter were fixed and stained using 3-step stain set (Richard-Allan Scientific). The filters were mounted onto a slide and the cells that invaded onto the lower surface of the filter were counted under a microscope. For each replicate, the cells in five randomly selected fields were determined.

#### Transient Transfection

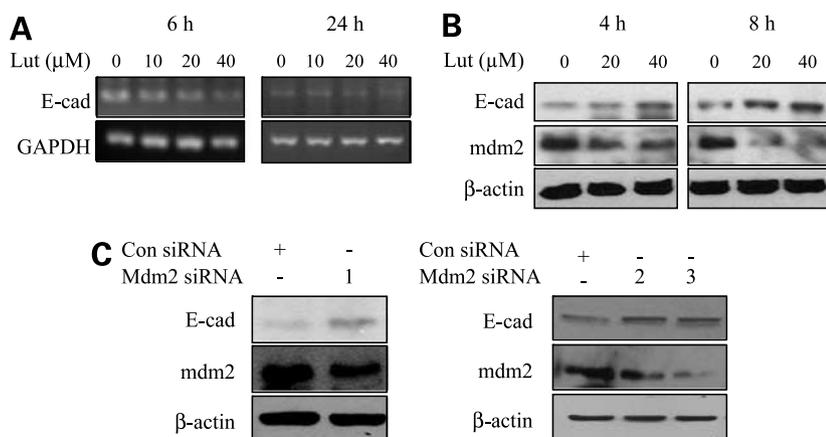
PC3 cells at 60% to 70% confluence were transfected with plasmids using the Cell Line Nucleofector kit V from Amaxa as per the manufacture's instruction. The transfection efficiency was ~80%.

#### Spontaneous Metastasis Assay

Spontaneous metastasis assay was done as described (23). To examine the effects of luteolin on metastatic ability of cells in animals, PC3 cells ( $5 \times 10^6$ ) were injected onto dorsal flank of 4-wk-old male nude mice. The nude mice [BALB/cA-nu (nu/nu)] were obtained from Shanghai Experimental Animal Center (Shanghai, China) and the mice were maintained in pathogen-free conditions. Treatment was started 5 d after implantation. The mice of luteolin-treated group were given i.p., and the mice of control group received solvent at equal volume. The mice were treated thrice a week. In 1 mo, the mice were sacrificed and metastatic nodules on the lungs were counted macroscopically.

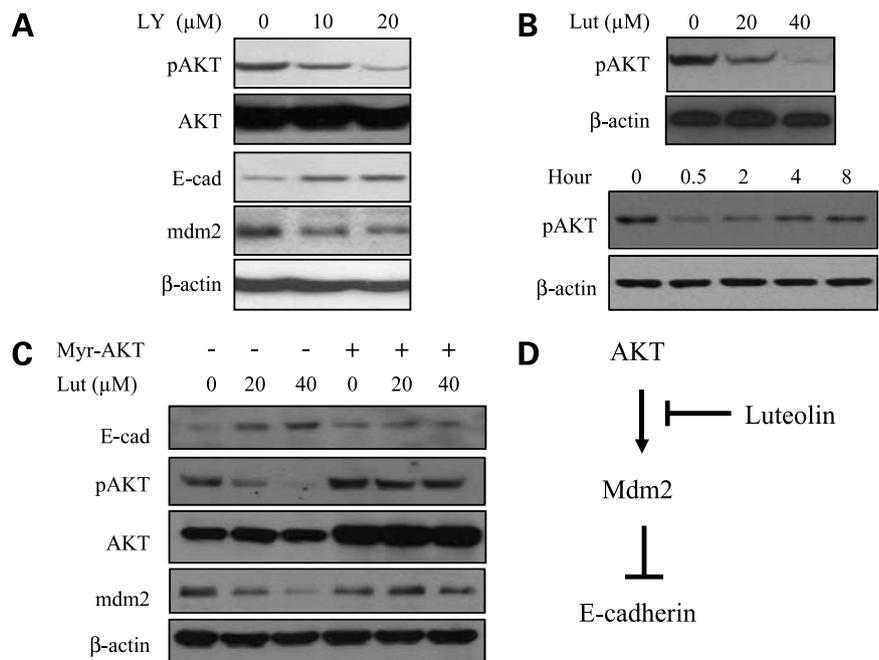
#### Statistical Analysis

The data represent mean  $\pm$  SD from three independent experiments except where indicated. Statistical analysis was done by Student's *t* test at a significance level of  $P < 0.05$ .



**Figure 2.** Luteolin induces E-cadherin through *mdm2*. **A**, luteolin has little effects on E-cadherin mRNA levels. PC3 cells were treated with luteolin for 6 and 24 h, respectively. RT-PCR was done as described in the Materials and Methods. **B**, luteolin inhibited expression of *mdm2*. PC3 cells were treated with luteolin for different times as indicated. Cellular proteins were extracted and subjected to immunoblotting. **C**, knockdown of *mdm2* induced expression of E-cadherin. The cells were transfected with *mdm2* siRNA plasmid or control vector. In 24 h, the total cellular proteins were extracted for immunoblotting.

**Figure 3.** Luteolin inhibits mdm2 and induces E-cadherin through AKT. **A**, inhibition of AKT phosphorylation attenuated expression of mdm2 and enhanced that of E-cadherin. PC3 cells were incubated with LY294002 (0, 10, 20  $\mu\text{mol/L}$ ) for 12 h. **B**, luteolin inhibited AKT phosphorylation at a dose- and time-dependent manner. PC3 cells were incubated with luteolin (0, 20, and 40  $\mu\text{mol/L}$ ) for 2 h. Cellular proteins were prepared for Western blot. PC3 cells were incubated with luteolin (40  $\mu\text{mol/L}$ ) for different times as indicated. **C**, overexpression of myr-AKT blocked luteolin-induced expression of E-cadherin. PC3 cells were transfected with myr-AKT or control vector. The cells were incubated overnight and treated with luteolin (0, 20, and 40  $\mu\text{mol/L}$ ) for 24 h. LY, LY294002. **D**, a model that luteolin inhibits expression of E-cadherin.



## Results

### Luteolin Induces Expression of E-Cadherin and Inhibits Invasion of PC3 Cells

We first determined whether luteolin influenced the expression of E-cadherin. As shown in Fig. 1A, luteolin induced expression of E-cadherin in PC3 cells. We did immunofluorescence staining of PC3 cells with antibody against E-cadherin. We found that luteolin increased E-cadherin protein levels in cell membrane, which was confirmed by western-blot (Fig. 1B). The E-cadherin protein was localized in cell membrane. The IGF-IR $\beta$  was used as loading control of membrane proteins because its expression was not affected by luteolin (24). The membrane localization of E-cadherin has been indicated as activation of this protein (25). Epithelial to mesenchymal transition is one of the major events during the acquisition of the invasive phenotype in tumors of epithelial origin (26). This process is often accompanied by loss of epithelial markers, especially E-cadherin (26, 27). We found that the morphology of luteolin-treated PC3 cells changed from a more elongated fibroblast-like to a round and packed appearance of epithelial cells (Fig. 1C).

Finally, we determined the effects of luteolin on invasion of PC3 cells. As shown in Fig. 1D, luteolin inhibited invasion of PC3 cells significantly. To exclude the possibility that the inhibition of cell invasion was due to suppression of cell proliferation, we determined the proliferation of PC3 cells under the same experimental conditions. Luteolin at 20  $\mu\text{mol/L}$  had no significant inhibitory effects on proliferation of PC3 cells (Fig. 1D). Moreover, there was no increased cell death (determined by trypan blue staining) of luteolin-exposed cells under the same conditions (data not shown). Because luteolin at 20  $\mu\text{mol/L}$  inhibited invasion of PC3 cells significantly and had little effects on cell death,

we selected this concentration in our following experiments unless where indicated.

We also determined the effects of luteolin on E-cadherin expression in other cancer cells. We found that luteolin induced expression of E-cadherin in another prostate cancer DU145 cells, ovarian cancer A2780 cells, lung cancer A549 cells, and cervical cancer HeLa cells but had little effects on expression of E-cadherin in breast cancer MCF-7 and liver cancer HepG2 cells (data not shown).

### Luteolin Induces E-cadherin through mdm2

We next determined the possible mechanisms that luteolin regulated expression of E-cadherin. We first determined the mRNA levels of E-cadherin after luteolin treatment. As shown in Fig. 2A, luteolin did not increase mRNA levels of E-cadherin, suggesting that luteolin induces E-cadherin at a posttranscriptional level.

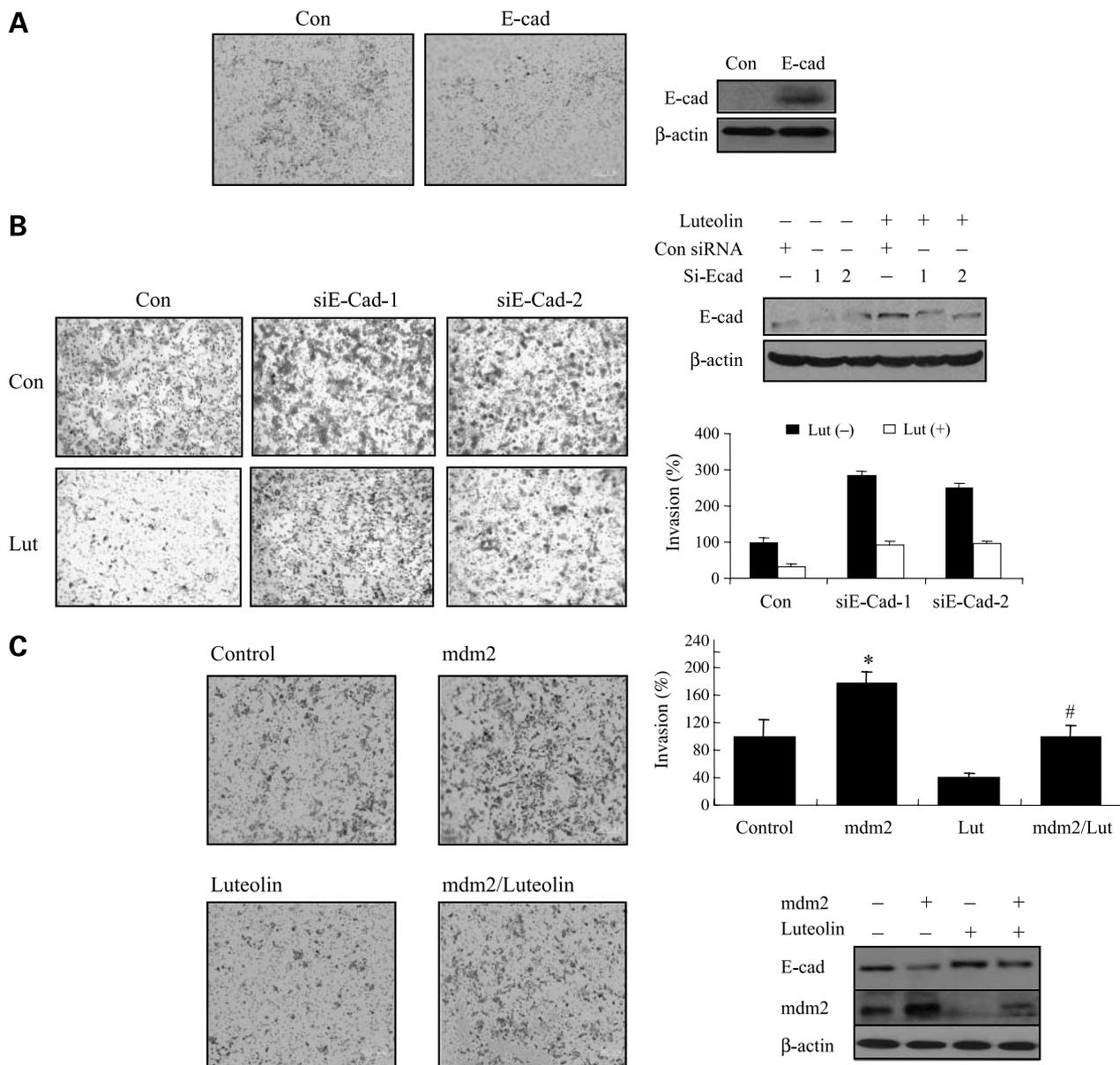
It was reported recently that mdm2 is involved in regulation of E-cadherin (11). So, we determined whether mdm2 mediated luteolin-induced expression of E-cadherin. We found that luteolin inhibited expression of mdm2 in PC3 cells, and the down-regulation of mdm2 was accompanied with up-regulation of E-cadherin (Fig. 2B). The data suggest that luteolin induces E-cadherin through mdm2. To confirm this, we inhibited expression of mdm2 and determined E-cadherin protein levels. As shown in Fig. 2C, knockdown of mdm2 enhanced protein levels of E-cadherin. Taken together, these results suggest that luteolin regulate E-cadherin through mdm2.

### AKT Signaling Is Involved in E-Cadherin Regulation by Luteolin

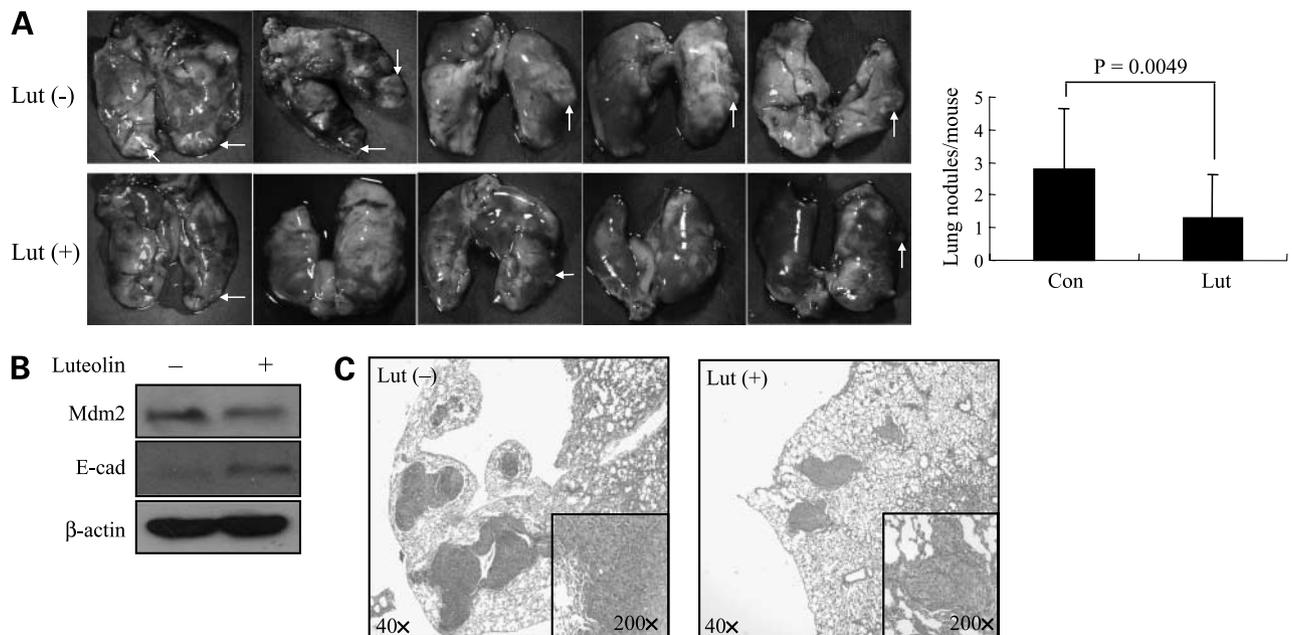
It has been reported that Epression of mdm2 is regulated by AKT (28) and luteolin is an inhibitor of AKT (24). Therefore, we asked whether the AKT was involved in

luteolin-induced expression of E-cadherin. LY294002, the specific inhibitor of AKT, inhibited expression of mdm2 and induced expression of E-cadherin (Fig. 3A). Luteolin inhibited phosphorylation of AKT at a dose- and time-dependent manner (Fig. 3B). These results suggest that luteolin regulates E-cadherin expression through AKT/

mdm2 pathway. To further confirm this, we overexpressed active myr-AKT in PC3 cells and treated the cells with luteolin. Luteolin inhibited expression of mdm2 and induced expression of E-cadherin in control cells (Fig. 3C). Overexpression of myr-AKT restored mdm2 and blocked the induction of E-cadherin (Fig. 3C). A model that how



**Figure 4.** Overexpression of mdm2 restores invasion of PC3 cells inhibited by luteolin. **A**, overexpression of E-cadherin inhibited invasion of PC3 cells. PC-3 cells were transfected with empty vector or vector encoding E-cadherin. In 24 h, the cells were trypsinized and washed with serum-free medium twice. The cells ( $5 \times 10^4$ ) were resuspended in 200  $\mu$ L of serum-free medium and placed in the upper compartment of the chamber of *trans*-well. In the lower chamber, complete medium served as a source of chemo-attractants. In 24 h, cell invasion was determined. **B**, knockdown of E-cadherin restored cell invasion after luteolin treatment. PC3 cells were transfected with siRNA oligoes or control oligoes. In 24 h, cell invasion was determined as described in Fig. 1D. **C**, overexpression of mdm2 restored invasion of PC3 cells inhibited by luteolin. PC-3 cells were transfected with control vector or vector encoding mdm2 and incubated overnight. The cells were then treated with luteolin (20  $\mu$ mol/L) or solvent reagent DMSO. In 24 h, the cells were trypsinized and washed with serum-free medium twice. Cells ( $5 \times 10^4$ ) were placed in the upper compartment of the chamber. Cell invasion assay was done as described above. For each replicate, the cells in five randomly selected fields were determined, and the counts were averaged. \*,  $P < 0.05$  versus control. #,  $P < 0.05$  versus luteolin.



**Figure 5.** Luteolin inhibited spontaneous metastasis of PC3 cells *in vivo*. PC3 cells were implanted on the mice as described in Materials and Methods. In 5 d, the treatment of the mice with luteolin was initiated. Luteolin was injected i.p. to the mice at 5 mg/kg thrice a week. The mice of control group received solvent at equal volume. Each group had 10 mice. In 1 mo, the mice were sacrificed. The lungs were fixed, paraffin embedded, sectioned, and H&E stained conventionally. The experiment was repeated twice. **A**, representative of lungs from control and luteolin groups. The nodules on the lungs were counted macroscopically. The lung nodules per mouse were determined as an index of metastasis of PC3 cells. **B**, the tumors on the dorsal flank were excised and immunoblotting of the tumor extracts was done as described previously (24). **C**, H&E staining of lung samples.

pAKT, mdm2, and E-cadherin is affected after luteolin treatment is proposed (Fig. 3D).

#### Both E-cadherin and mdm2 are Important for Invasion of PC3 Cells

The role of E-cadherin on invasion of PC3 cells was determined. Overexpression of E-cadherin inhibited invasion of PC3 cells (Fig. 4A), indicating that E-cadherin plays a role in invasion of PC3 cells. To know whether the knockdown of E-cadherin could restore cell invasion after luteolin treatment, PC3 cells were transfected with E-cadherin siRNA oligos and treated with luteolin. Knockdown of E-cadherin not only increased invasion of PC3 cells without luteolin treatment but also restored cell invasion after luteolin treatment (Fig. 4B). We next determined the effects of mdm2 on invasion of PC3 cells. Overexpression of mdm2 promoted invasion of PC3 cells (Fig. 4C). Overexpression of mdm2 restored the invasion of PC3 cells inhibited by luteolin (Fig. 4C). We found that overexpression of mdm2 attenuated the expression of E-cadherin in both control and luteolin-treated cells (Fig. 4C).

#### Luteolin Inhibits Spontaneous Lung Metastasis of PC3 Cells *In vivo*

We finally determined the effects of luteolin on spontaneous lung metastasis of PC3 cells *in vivo*. The injection of PC3 cells into the dorsal flank of nude mice induced primary tumor formation and lung metastasis. Figure 5A shows the representatives of lungs of mice. Treatment of the mice with luteolin reduced the number of nodules per mouse. The tumors on the dorsal flank were excised and immunoblotting

of the tumor extracts showed that luteolin decreased expression of mdm2 and increased that of E-cadherin (Fig. 5B). H&E sections of the lung revealed cells with prominent and irregular nuclei (Fig. 5C).

#### Discussion

Luteolin is a dietary flavonoid with low toxicity. Numerous studies have shown the antiproliferation activity and possible mechanisms of luteolin in many types of human cancers. Luteolin was found to inhibit epidermal growth factor receptor tyrosine kinase activity (29), to promote degradation of signal transducer and activator of transcription 3 (15), to up-regulate expression of death receptor 5 (30), to inactivate IGF-I/IGF-IR signaling (24), and to inhibit expression of androgen receptor (31). However, the effect of luteolin on invasion of cancer cells remains unknown. In this article, we show that luteolin inhibits invasion of prostate cancer PC3 cells through E-cadherin.

The initial steps of the invasion-metastasis cascade include the loss of cell-to-cell adhesion, invasion into the local microenvironment, intravasation into the blood and lymphatic vasculature, and extravasation into the parenchyma of distant tissues. This process is controlled by epithelial-mesenchymal transition (EMT), through which epithelial cells lose their epithelial traits and acquire many of the attributes of mesenchymal cells, including a loss of association with epithelial cell sheets and acquisition of cell motility, invasiveness, and resistance to apoptosis (32). The

EMT program involves the down-regulation of epithelial protein expression, notably E-cadherin. E-cadherin is the downstream effector of epithelial phenotype and a target of repression by EMT-inducing factors. When E-cadherin is lost, many of the changes associated with an EMT are triggered (25). EMT may only involve a small subset of carcinoma cells—the minority that reside at the interface between epithelium and stroma within a carcinoma (32, 33). Progression of solid tumors involves spatial and temporal occurrences of EMT, whereby tumor cells acquire a more invasive and metastatic phenotype. Down-regulation of E-cadherin is one of the most frequently reported phenomena in metastatic cancers (3, 25). During carcinogenesis, developing prostate cancer cells acquire mesenchymal characteristics and migratory features concomitant with a loss of epithelial characteristics such as E-cadherin expression (19, 20). We found that luteolin-induced expression of E-cadherin in PC3 cells and the luteolin-induced expression of E-cadherin resulted in inhibition of PC3 cells invasion (Fig. 1). Thus, luteolin may function to attenuate the first step of cancer cells metastasis. The luteolin-induced expression of E-cadherin was also observed in DU145, A2780, A549, and HeLa cells but not in MCF-7 and HepG2 cells (data not shown). The reason might be (a) different cell lines have different response to luteolin treatment; (b) regulation of E-cadherin expression in these cell lines might be different. The data suggest that induction of E-cadherin by luteolin is cell type dependent.

The precise mechanisms responsible for E-cadherin inactivation in cancer cells are not very clear. Alterations at transcriptional level seem to be one of the mechanisms responsible for decreased expression of E-cadherin in several cancer types (34–36). Our results suggest that luteolin induce expression of E-cadherin at a posttranscriptional level. We found that luteolin enhanced protein level of E-cadherin through decreasing that of mdm2 (Fig. 2). A few of flavonoids were found to inhibit expression of mdm2 (21, 37). Mdm2 can be phosphorylated by AKT (28, 38), which increases stability of mdm2. Luteolin inhibited pAKT and overexpression of active AKT blocked luteolin-induced expression of E-cadherin (Fig. 3B and C). Taken together, these results suggest that luteolin induce E-cadherin through AKT/mdm2 pathway.

Overexpression of E-cadherin inhibited invasion of PC3 cells (Fig. 4A) and knockdown of E-cadherin induced invasion of PC3 cells (Fig. 4B), suggesting that E-cadherin play an important role in invasion of PC3 cells. Furthermore, we found that knockdown of E-cadherin restored invasion of PC3 cells after luteolin treatment (Fig. 4B). The data suggest that luteolin suppress invasion of PC3 cells via E-cadherin. Overexpression of mdm2 promoted invasion of PC3 cells (Fig. 4C). Several studies have shown that amplification of the mdm2 gene occurs more frequently in metastatic and recurrent cancers than in primary tumors (39). Overexpression of mdm2 was detected in metastatic endometrial tumors (40). It was reported recently that mdm2 promotes cell motility and invasiveness by regulating E-cadherin degradation (11). Thus, mdm2 seems to correlate with the risk

of distant metastases. Our results suggest that inhibition of mdm2 contributes to the anti-invasive effects of luteolin.

Plants consumed by humans contain many kinds of flavonoids. The effects of dietary flavonoids are of great interest due to their potential in cancer prevention (41, 42). For example, in a 25-year follow-up study on 9959 Finnish men, dietary intake of flavonoids was inversely associated with the incidence of cancer at all sites combined (43). In a population-based case-control study in Hawaii involving 582 lung cancer patients, foods rich in certain flavonoids may protect against certain forms of lung cancer (44). Another case-control study in Scotland showed that there is a strong and linear inverse associations of flavonoids intake with colorectal cancer risk (45). A recent study showed that there was a significant decrease in incidence of ovarian cancer for the highest versus lowest quintile of luteolin intake (46).

Luteolin is a nontoxic dietary flavonoid and has been shown to possess antitumor properties and therefore poses special interest for the development of a chemopreventive and/or chemotherapeutic agent for cancer. Many patients died of cancer due to metastasis. Therefore, inhibition of cancer cells metastasis is a very important aspect for cancer prevention. We found that luteolin induced expression of E-cadherin in prostate cancer PC3 cells, leading to inhibition of invasion of PC3 cells. Thus, luteolin might be a potent agent for treatment of invasive prostate cancers. A bunch of studies have shown that luteolin possesses properties of inhibiting proliferation and inducing apoptosis of cancer cells. Herein, we showed that luteolin is able to inhibit invasion of PC3 cells. Our results provide a new sight into the mechanisms that luteolin is against cancers, and suggest that luteolin may be a potential agent for treatment of cancers.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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