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Inhibitory effect of luteolin on hepatocyte growth factor/scatter factor-induced HepG2 cell invasion involving both MAPK/ERKs and PI3K–Akt pathways

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Abstract

Hepatocyte growth factor (HGF), also known as scatter factor (SF), and its receptor, the c-Met tyrosine kinase, play roles in cancer invasion and metastasis in a wide variety of tumor cells. Clinical observations suggest that HGF can promote metastasis of hepatoma cells while stimulating tumor invasiveness. We use HGF as an invasive inducer of human hepatoma HepG2 cells to investigate the effect of flavonoids on anti-invasion. In our preliminary study, we investigated the effect of flavonoids including luteolin, quercetin, baicalein, genistein, taxifolin and catechin on HGF-mediated migration and invasion of HepG2 cells. We found that luteolin presented the most potent potential on anti-migration and anti-invasion by Boyden chamber assay. Furthermore, luteolin inhibited HGF-induced cell scattering and cytoskeleton change such as filopodia and lamellipodia was determined by both phase-contrast and fluorescence microscopy studies. In addition, Western blotting and immunoprecipitation were performed to confirm luteolin suppressed the phosphorylation of c-Met, the membrane receptor of HGF, as well as ERK1/2 and Akt, but not JNK1/2, which is activated by HGF. Our investigation demonstrated that luteolin similar to PD98059, which acts as a specific inhibitor of MEK, an up stream kinase regulating ERK1/2, and wortmannin, a PI3K inhibitor, inhibited the invasiveness induced by HGF. In conclusion, the luteolin inhibited HGF-induced HepG2 cell invasion involving both MAPK/ERKs and PI3K–Akt pathways.

Keywords: Luteolin; Invasion; HGF; HepG2; ERK; Akt

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors seen in the tropics and the Far East, including Taiwan. During the early stage of hepatocarcinogenesis, HCC is predominantly well differentiated, and the HCCs proliferate slowly [1]. However, HCC becomes progressively dedifferentiated with tumor enlargement, and most advanced HCCs have high proliferation activity. During this stage, the tumors progress to give rise to intrahepatic metastasis as well as extrahepatic metastasis [2]. Many factors have been reported to be involved in metastasis including hepatocyte growth factor (HGF). HGF is produced by nonparenchymal liver cells, and serum levels of HGF are elevated in patients

Abbreviations: ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; MAPK, mitogen activator protein kinase; HGF, hepatocyte growth factor

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with acute and chronic liver diseases such as hepatocellular carcinoma. HGF is a pleiotropic factor, inducing mitogenesis, morphogenesis and metastagenesis on a variety of epithelial cells [3]. These pleiotropic effects of HGF could contribute to tumor invasion and metastasis. The receptor for HGF is a receptor type tyrosine kinase encoded by the c-Met proto-oncogene. Increased c-Met and HGF expression by human tumor cells is often associated with high tumor grade and poor prognosis [4]. While normal HGF-Met signaling is involved in many aspects of embryogenesis, abnormal HGF-Met signaling has been shown to play a significant role in promoting tumor invasion, metastasis and angiogenesis. Cell invasion is a complicated process that involves partial detachment from intercellular adhesions, and from cell–ECM interaction mediated by integrins, reorganization of the actin cytoskeleton and movement through the ECM [5,6]. Cell scattering is an important component of several physiological and pathological processes such as embryonic morphogenesis, tissue regeneration and tumor invasion. In addition, directed cell migration, a fundamental characteristic of tumor cell invasion, involves protrusive activities at the leading edge of the cell driven by a regulated cycle of polymerization and depolymerization of actin filaments. The initial protrusive structure of filopodia and lamellipodia, for example, contains dense arrays of actin filaments.

Flavonoids are the most abundant polyphenols in our diet and are found in soybeans, tea, fruits and vegetables. They have been suggested to possess anticancer and chemopreventive property in numerous epidemiological studies and to inhibit the proliferation of tumor cells including breast, prostate and lung cancer cells in vitro [7,8]. However, their anti-metastasis or anti-invasiveness properties remain unclear. Luteolin (3′4′5′7′-tetrahydroxyflavone), an important member of the flavonoid family, is present in various fruits and vegetable. It exhibits a wide spectrum of pharmacological properties including anti-inflammatory and anti-allergic properties [9]. Much attention has been recently paid to its antioxidant properties and to its anti-proliferative effects [10,11]. It was reported that luteolin has potential for anticancer therapy through inhibiting DNA topoisom erase I and II [12,13]. Ko et al. reported that luteolin demonstrated inhibition of proliferation and induction of apoptosis in human myeloid leukemia cells [14]. In addition, our previous study showed that luteolin induced apoptosis via a mechanism involving mitochondria translocation of Bax/Bak and activation of JNK [15]. The aim of this study is to evaluate the anti-migration and anti-invasion potential of luteolin and to investigate the signal pathway involved.

2. Materials and methods

2.1. Materials

Luteolin and recombinant human HGF (rhHGF) (Sigma, St. Louis, MO, USA), Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY), Matrigel (Collaborative Biomedical Products, Bedford, MA), wortmannin (PI3K inhibitor; Tocris Cookson, Bristol, UK), PD98059 (MEK inhibitor; Promega, WI, USA), SP600125 (JNK inhibitor; Promega), anti-phospho-ERKs (Thr^{202}/Tyr^{204}), anti-phospho-JNKs (Thr^{183}/Tyr^{185}), anti-phospho-p38 (Thr^{180}/Tyr^{182}), anti-phospho-Akt (Ser^{473}) and anti-phospho-tyrosine antibodies were purchased from Cell Signaling Technology (Beverly, MA), ERK, JNK, p38, Akt and c-Met antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA) and anti-β-actin was obtained from Sigma.

2.2. Cell culture

Human hepatoma HepG2 cells were maintained in Dulbecco's modified Eagle's medium and in a humidified atmosphere of 5% CO_2/95% air at 37 °C. The medium was supplemented with 10% (v/v) fetal calf serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine. Before the addition of HGF, the cells were incubated overnight without serum.

2.3. Assessment of cell viability

The effect of flavonoids on cell viability was estimated by the MTT assay. In brief, the cells were seeded at 5×10^4 cells/ml density and incubated with flavonoids (40 μM) including luteolin, quercetin, baicalein, genistein, taxifolin and catechin for 24 h. Thereafter the medium was changed and incubated with MTT (0.5 mg/ml) for 4 h. The number of viable cells was directly proportional to the production of formazan, which was then solubilized with isopropanol, and measured spectrophotometrically at 563 nm.

2.4. Morphologic study

HepG2 cells were cultured at 2×10^4 cells/ml in 10% FBS–DMEM in a six-well plate for 12 h, followed by starvation overnight. After pretreatment with various concentrations of luteolin for 2 h, HGF (40 ng/ml) was added and the morphologic changes of HepG2 for 24 h were observed under a phase-contrast microscope. In addition, cells plated onto the six-well plate for 24 h with a density of 2×10^2 cells/ml were starved overnight.

After pretreatment with various concentrations of luteolin for 2 h, HGF (40 ng/ml) was applied for 4 h in 5% CO_2 37 °C incubator, fixed in 4% formaldehyde for 10 min and washed with PBS. The cells were stained with TRITC-phalloidin (500 ng/ml) for 1 h and washed with PBS. The actin stress fibers in the cells were observed under fluorescence microscopy.

2.5. In vitro assays of cell migration and invasion

Cell invasion assays were performed using Boyden chemotaxis chamber obtained from Neuro Probe Inc. The upper culture chamber consisted of an 8-μm pore size polycarbonate filter coated with a uniform layer of 40 μg/cm^2 of Matrigel basement membrane matrix and was placed on the top of the lower culture chamber. HepG2 cells (5×10^4) suspended in DMEM medium were placed in the upper compartment of the chemotaxis chamber in the presence of flavonoids (40 μM) or various concentrations of luteolin. In the lower chamber, serum-free DMEM medium containing 20 ng/ml HGF served as a source of chemoattractants. After incubation for 24 h, the cells on the upper surface of the filter were wiped with a cotton swab. The cells on the lower surface of the filters were fixed for 10 min with methanol and stained with Giemsa for 1 h, and the cells that invaded the lower surface of the filter were then counted under a microscope. For each replicate, the HepG2 cells in five randomly selected fields were determined, and the counts were averaged. Migration assays were done by the same procedure, except that the polycarbonate filters were not coated with Matrigel.

2.6. Preparation of total cell extracts and immunoblots analysis

To prepare the whole-cell extract, cells were washed with PBS plus zinc ion (1 mM) and suspended in a lysis buffer (50 mM Tris, 5 mM EDTA, 150 mM NaCl, 1% NP 40, 0.5% deoxycholic acid, 1 mM sodium orthovanadate, 81 μg/ml aprotinine, 170 μg/ml leupeptin, 100 μg/ml PMSF; pH 7.5). After mixing for 30 min at 4 °C, the mixtures were centrifuged (10,000 × *g*) for 10 min, and the supernatants were collected as whole-cell extracts. The protein content was determined with Bio-Rad protein assay reagent using bovine serum albumin as a standard. The ECL Western blotting was performed as follows. An equal protein content of total cell lysates from HGF-treated samples with pretreatment of luteolin (0–40 μM) was resolved on 10–12% SDS-PAGE gels along with prestained protein molecular weight standard (Bio-Rad). Proteins were then blotted onto NC membrane (Sartorious), and probed with ERKs, JNKs, p38 and Akt. The total and phosphorylated proteins and β-actin were detected with each specific antibody for 2 h, and then with an appropriate peroxidase-conjugated secondary antibody for 1 h. After binding, the bands were examined by enhanced chemiluminescence using the ECL commercial kit and relative photographic density was quantitated by scanning the photographic negatives on a gel documentation and analysis system (Alpha Imager 2000, Alpha Innotech Corporation). All the data are the representative of three independent experiments.

2.7. Immunoprecipitation assay

Approximately 0.5 mg of the lysate protein was immunoprecipitated using monoclonal antiserum of c-Met (1 μg) and 15 μl of protein A/G-agarose (Santa Cruz). Pellet beads were incubated at 4 °C for overnight. After centrifugation at 2500 rpm, the eluates were analyzed by immunoblotting against phospho-tyrosine antibodies.

2.8. Characterization of effect of luteolin on signal pathway mediated by HGF

First, the effect of HGF on MAPKs and PI3K–Akt pathways was investigated by Western blotting analysis. Then, HepG2 cells were pretreated with MEK inhibitor PD98059, JNK inhibitor SP600125 or PI3K inhibitor wortmannin for 2 h, followed by a treatment with 20 ng/ml of HGF for 30 min to analyze the p-ERKs, p-JNKs and p-Akt. In addition, to evaluate the effect of cell invasion, HepG2 cells were pretreated with MEK inhibitor PD98059, JNK inhibitor SP600125 or PI3K inhibitor wortmannin HGF and combination with 40 μM of luteolin for 2 h, followed by a treatment with 20 ng/ml of HGF for 24 h.

2.9. Statistical analysis

Data were reported as the mean ± standard deviation of three independent experiments and evaluated by one-way ANOVA. Significant differences were established at $p < 0.05$.

3. Results

3.1. Cytotoxicity of flavonoids to HepG2 cells

Fig. 1 illustrates the results of MTT assay performed with the logarithmically growing HepG2 cells treated with 40 μM of various flavonoids including luteolin,

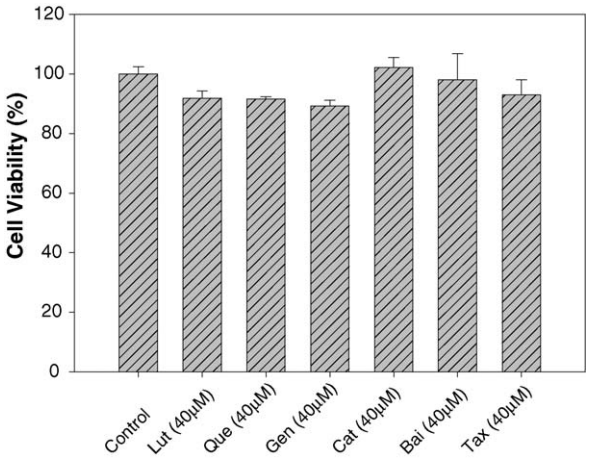

Fig. 1. Effect of flavonoids on viability of HepG2 cells. Cells were treated with 40 μM of flavonoids including luteolin (Lut), baicalein (Bai), quercetin (Que), genistein (Gen), catechin (Cat) and taxifolin (Tax) for 24 h. Viability of HepG2 cells was measured by MTT assays as described in Section 2. The results were presented as means ± S.D. of three independent experiments.

quercetin, baicalein, genistein, taxifolin and catechin. The result showed that there was no significant difference in cell viability. It was therefore clear that all the flavonoids, at a concentration ranging from 0 to 40 μM, pose no cytotoxicity to HepG2 cells.

3.2. Effect of flavonoids and luteolin on HGF-induced cell migration and invasion

By Boyden chamber assay, 20 ng/ml of HGF induced in vitro migration and invasion of HepG2 cells (Fig. 2A and B). The effect of flavonoids on HGF-induced migration and invasion was evaluated. It was found that all the flavonoids (40 μM) exhibit significant inhibitory effect on HGF-induced migration and invasion and that luteolin is the most potent. The effect of luteolin and mode of action involved were then investigated. Luteolin resulted in a dose-dependent inhibition of HGF-induced cell migration and invasion (Fig. 3A and B). Comparison with the effect of luteolin on cell viability at 40 μM within 24 h treatment (Fig. 1) shows that inhibitory effect of luteolin on cell migration and invasion is independent of its cellular cytotoxicity.

3.3. Effect of luteolin on HGF-induced cell scattering and cytoskeleton change

Cell scattering is an important component of several physiological and pathological processes such as embryonic morphogenesis, tissue regeneration and tumor invasion. As seen in Fig. 4, when the HepG2 cells were

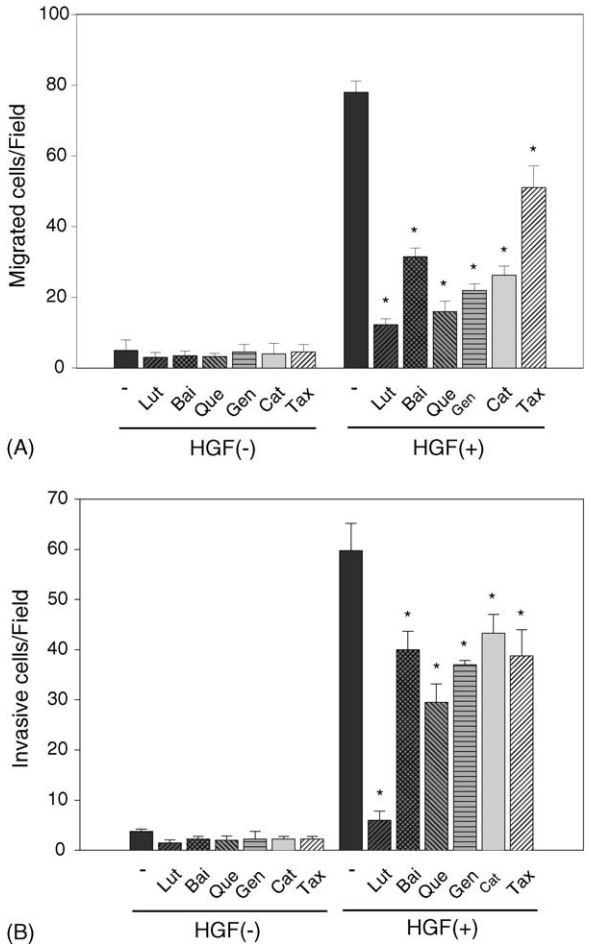

Fig. 2. Effect of flavonoids on HGF-induced HepG2 cell migration and invasion. The HGF (20 ng/ml) was applied to the lower chamber. HepG2 cells (5×10^4 cells/ml) were seeded onto the upper chamber consisting of 8 μm pore-size filters coated without (A) and with (B) Matrigel basement membrane matrix, then treated with 40 μM of luteolin (Lut), baicalein (Bai), quercetin (Que), genistein (Gen), catechin (Cat) and taxifolin (Tax) for 24 h and with or without 20 ng/ml HGF/SF as a chemoattractive agent in the lower chamber. Cells that migrated or invaded the filter were counted as described in Section 2. $^{*}p < 0.01$, compared with HGF treatment alone.

cultured, they showed a cobblestone shape and tight junction of the cells (Fig. 4A). After treatment with HGF for 24 h, the cells showed a remarkable scattering (Fig. 4B and C), and pretreatment with 10 μM of luteolin was partially effective in blocking HGF-stimulated cell scattering of HepG2 (Fig. 4D). In addition, pretreatment with 20 and 40 μM luteolin increased the cobblestone shape cells of HepG2 (Fig. 4E and F). Because modulation of cytoskeleton proteins has been linked with cell growth and invasion, we proceeded to investigate the cytoskeleton change by staining with TRICT-conjugated phalloidin. In Fig. 5, to enhance the HGF-

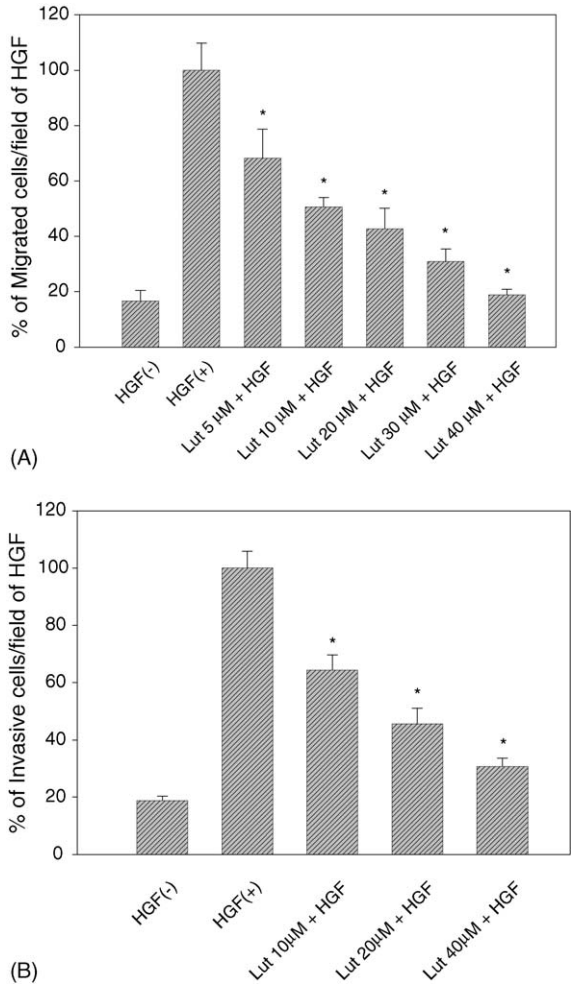

Fig. 3. Dose effect of luteolin on HGF-induced HepG2 cell migration and invasion. The HGF (20 ng/ml) was applied to the lower chamber. HepG2 cells (5×10^4 cells/ml) were seeded onto the upper chamber consisting of 8 μm pore-size filters coated without (A) and with (B) Matrigel basement membrane matrix, then treated with various concentrations of luteolin (Lut) for 24 h. Cells that migrated or invaded the filter were counted as described in Section 2. $^{*}p < 0.01$, compared with HGF treatment alone.

mediated cytoskeleton change in short-duration treatment, we used the concentration of 40 ng/ml. The results demonstrate that filamentous actin in serum-starved cells is mainly seen in the more peripheral regions. However, HGF-induced membrane protrusions such as lamellipodia (Fig. 5B) and filopodia (Fig. 5C), which provide attachment and movement of cytoplasmic components that are responsible for cell moving, were seen obviously in HGF-treated cells. With the pretreatment of luteolin HGF-induced cytoskeleton change was inhibited (Fig. 5D–F).

3.4. Effect of luteolin on HGF-mediated c-Met phosphorylation

c-Met is a member of the tyrosine kinase-type receptor family and binding of HGF induces autophosphorylation of tyrosine residues in c-Met [16]. HGF-induced c-Met tyrosine phosphorylation was detected by Western blot analysis with anti-phospho-tyrosine monoclonal antibody after immunoprecipitation with anti-c-Met antibody. The c-Met of HepG2 cells were strongly phosphorylated in response to stimulation with 20 ng/ml HGF for 30 min (Fig. 6). Pretreatment with luteolin inhibited the HGF-mediated c-Met phosphorylation.

3.5. Effect of luteolin on HGF-mediated signal activation

The activation of several signaling molecules has been observed in HGF-stimulated cells that may play a role in mediating cell motility and metastasis. HGF-induced activation of PI3K, which is often associated with the stimulation of cell motility, results in the production of inositol 3,4,5-triphospate, which activates multiple downstream targets including Akt [17,18]. HGF induces phosphorylation of ERKs, which are phosphorylated and activated by MEK involving cell scattering [19]. When HepG2 cells were stimulated with HGF, the phosphorylated forms of ERK, JNK/SAPK and Akt were increased. Western blot analysis showed that the activation of these signaling molecules occurred 30 min after treatment, whereas there was no change in total ERK, JNK/SAPK and Akt expression. However, p38 was not activated by treatment with HGF, although this kinase was present in the cells (Fig. 7A). We then evaluated the effect of luteolin on the phosphorylation of ERK1/2, JNK1/2 and Akt induced by HGF and compared it with the application of MEK inhibitor PD98059, JNK inhibitor SP600125 or PI3K inhibitor wortmannin. The results revealed that luteolin similar to PD98059 and wortmannin suppresses HGF-induced ERK1/2 and Akt activation (Fig. 7B).

3.6. Inhibitory effect of luteolin on HGF-induced invasion involving ERKs and Akt

To determine whether the inhibition of HGF-induced cell invasion by luteolin involved mainly the ERKs and Akt signal pathways, we examined the effect of these signal inhibitors on in vitro invasion assay. It showed that treatment with PD98059 or wortmannin similar to luteolin decreases the number of invasive cells stimulated by HGF. Combination treatment of luteolin with PD98059 or wortmannin promoted the inhibitory effect (Fig. 8A

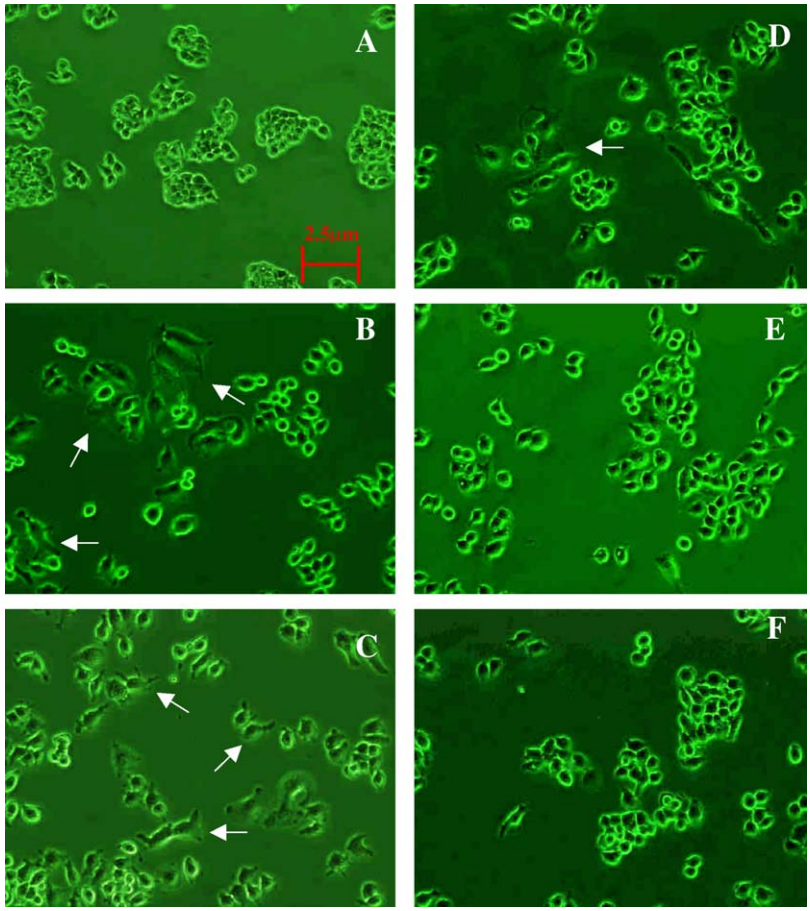

Fig. 4. Effect of luteolin on HGF-induced morphologic change of HepG2 cell. HepG2 cells (2×10^4 cells/ml) were seeded on six-well plate in serum-free medium overnight and then cultured in medium containing without (A) or with HGF (B and C) for 24 h. In addition, cells pretreated with various concentrations of luteolin: (D) 10 μM, (E) 20 μM and (F) 40 μM for 2 h, and then treated with HGF for 24 h. The cells were observed under phase contrast microscope 100× and arrow bar indicated cell scattering.

and B). Therefore, we suggest that both MAPK/ERKs and PI3K–Akt signaling pathways were involved in the anti-invasion effect of luteolin induced by HGF in HepG2 cells.

4. Discussion

It is suggested that HGF is a multifunctional modulator of biological activities in a variety of cell types and it influences the growth motility, differentiation and morphogenesis through the c-Met tyrosine kinase receptor of its target cells [20]. Following ligand binding and autophosphorylation, Met transmits intercellular signals using a unique multisubstrate docking site present within the c-terminal end of the receptor. The multisubstrate docking site mediates the binding of several adapter proteins such as Grb2, Srk, Crk/CRKL, and the large adapter protein Gab1. These adapter proteins in turn recruit several signal transducing proteins to form an intricate signaling complex which leads to more substantial models of HGF-Met signal transduction that mediates tumor cell invasion and metastasis [21]. It has been reported that divergent signaling pathways initiated by PI3K, Ras and Rac are involved in scattering, morphologic changes and migration induced by HGF in different cell types [22,23] and MAPK pathway plays some other roles in cell scattering [24]. In addition, the ETS1 transcription factor is activated by HGF through RAS–RAF–MEK–ERK signaling pathway [25] and that promotes the expression of matrix metalloproteinases, which can facilitate cell motility [26]. In primary cultures of rat hepatocytes it has been reported that JNK is activated by HGF and that it plays a role in mediating their proliferation [27]. In addition, it was found that the oncogenic form of c-Met, TPR-MET, activates JNK in FR3T3 fibroblast cells, an activation that seemed to be required for their transfor-

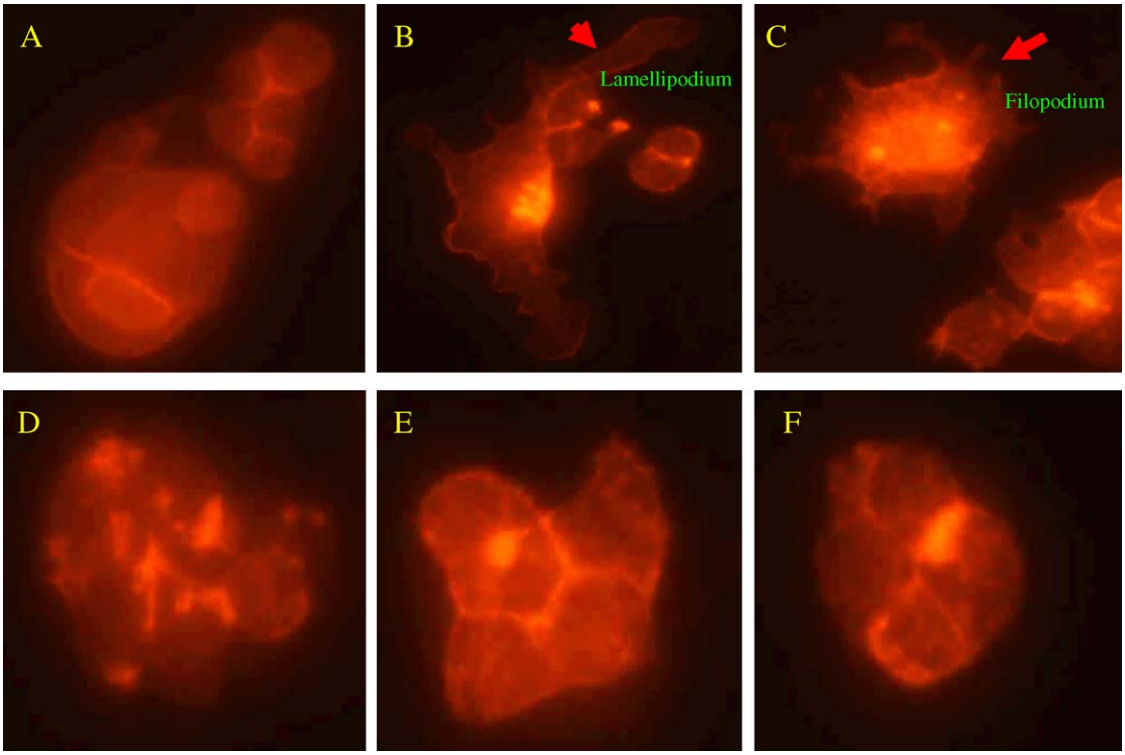

Fig. 5. Effect of luteolin on HGF-induced cytoskeleton change of HepG2 cell. HepG2 cells were treated without (A) or with HGF (40 ng/ml) (B and C) or in the presence of luteolin: (D) 10 μM, (E) 20 μM and (F) 40 μM for 4 h, and F-actin polymerization was visualized by TRITC-conjugated phalloidin and observed under fluorescence microscope (200×).

mation [28]. From Figs. 6 and 7, we find that luteolin inhibits the HGF-activated c-Met and MAPK/ERKs and PI3K–Akt signaling pathways, but not JNK.

The lethality of malignant tumors is attributable largely to the metastasis of neoplastic cells [29]. Several agents, including flavonoids, have been reported to inhibit invasion and metastasis [30–34]. Up to now, no established anti-metastatic agents are available for clinical use. Therefore, there is an urgent need for compounds capable of interfering successfully with one or more steps of the metastatic process [35]. c-Met and HGF are dysregulated in human cancer and are also believed to contribute to dysregulation of cell growth and tumor invasion during disease progression and metastasis. c-Met and HGF are highly expressed relative to the surrounding tissue in numerous cancers, and their expression correlates with poor patient prognosis [20]. Therefore, c-Met and HGF may be attractive candidates for targeted chemoprevention or cancer therapy. Selective small molecular inhibitor of c-Met kinase has recently been developed [36]. In this study, luteolin presents inhibitory effect on HGF–c-Met signaling pathway resulting in blocking cell invasion. However, metastasis involves matrix remoulding of organ tissues, which thus allow cells to migrate away from its origin and through circulation to a distant organ.

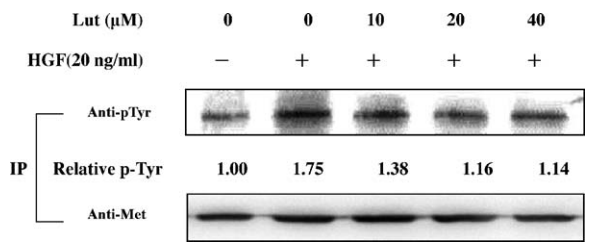

Fig. 6. Effect of luteolin on HGF-induced c-Met phosphorylation. Cells were cultured in medium containing HGF (20 ng/ml) with or without luteolin (10, 20 and 40 μM) for 4 h, and treatment cells were then harvested. Met was immunoprecipitated (IP) from HepG2 cell lysates with a specific anti-human Met polyclonal antibody, and the resulting immune complexes were separated by SDS-8% polyacrylamide gel electrophoresis. The blots were probed sequentially with antibodies against the phospho-tyrosine and Met β subunit.

Currently, matrix metalloproteinases (MMPs) are able to degrade basement membranes and the stromal connective tissue [37], that are believed to play an important role in cancer metastasis. Therefore, MMPs have been implicated in processes leading to cancer invasion and metastasis [38], and may also play a major

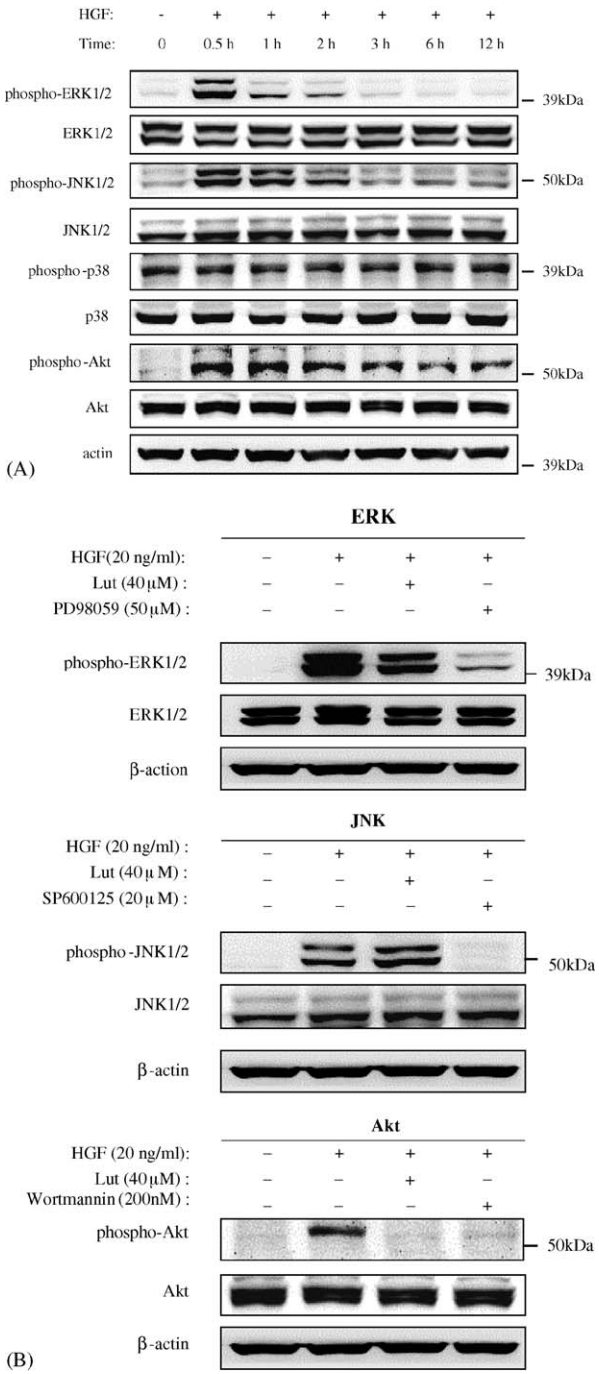

Fig. 7. Effect of luteolin on HGF-mediated activation of MAPKs and PI3K/Akt pathways. (A) The serum-starved HepG2 cells were stimulated with 20 ng/ml of HGF for the indicated time. The cell lysates were subjected to the Western blot analysis using either specific antibodies against the active phosphorylated forms of ERK, JNK/SAPK, p38 and Akt, or ERK, JNK, p38 and Akt, and actin used for equal loading as control. (B) The serum-starved HepG2 was pretreated with luteolin (40 μM) and the specific MEK inhibitor PD98059 (50 μM), JNK inhibitor SP600125 (20 μM) and PI3K inhibitor wortmanin (200 nM) for 2 h, and then incubated in the absence or presence of HGF (20 ng/ml) for an additional 0.5 h. The status of active form of ERK, JNK and Akt was determined by Western blot analysis: 50 μg of protein extract from each condition was electrophoresed in each lane of 10% SDS-PAGE and using the specific antibody against phospho-ERK1/2, phospho-JNK1/2 and Akt and ERK1/2, JNK1/2 and Akt antibody or actin used for equal loading.

role in tumor angiogenesis [39]. It has been reported that flavonoids including genistein, apigenin and 3-hydroxyflavone inhibit VEGF/bFGF-induced angiogenesis [40], in part via preventing the VEGF/bFGF-induced MMP-1, MT1-MMP and uPA expression and the activation of pro-MMP-2. Our study also demonstrates the inhibitory activity of flavonoids on HGF-induced invasion in HepG2 cells. Though luteolin, the most potent anti-invasion potential of flavonoids in our study, is shown to suppress phosphorylation of c-Met, ERK and Akt that involved in HGF-induced invasion, the effects on MMPs need further investigation.

Flavonoids, which are benzo-γ-pyrone (phenylchromone) derivatives, comprise a very large class of naturally occurring, low-molecular-weight polyphenol plant compounds. Previous studies on melanoma lines using several flavonoids including luteolin and quercetin of a *Citrus* origin showed that the presence of the C2–C3 double bond on the C ring [41], conjugated with the 4-oxo function [42], was critical for this biological activity. From a structural point of view, the double bond between C2 and C3 results in ring B and ring C being on the same plane, which might be critical for access to the kinase substrate binding site [43], and the two adjacent polar OH groups on C3 and C4 of ring B are required for suppressing kinase activity [41]. Consisting with our result it demonstrates that flavones such as luteolin exhibit potent anti-invasion bioactivity. However, a more systematic study, employing X-ray crystallography of flavonoids and known kinase data, is required to elucidate the detailed structure–function relationship involved in the anti-invasion biological activity of flavonoids.

Cancer chemoprevention is the use of agents to slow the progression of carcinogenesis, reverse or inhibit it, with the aim of lowering the risk of developing invasion or clinically significant disease. Flavonoids have been suggested to possess chemopreventive property in numerous studies [44]. This paper demonstrates that flavonoids possess anti-invasion potential that stimulated by HGF and luteolin exhibits the most potent potential. Furthermore, luteolin inhibited HGF-induced HepG2 cell invasion involving both MAPK/ERKs and PI3K–Akt signaling pathways. The results shed light

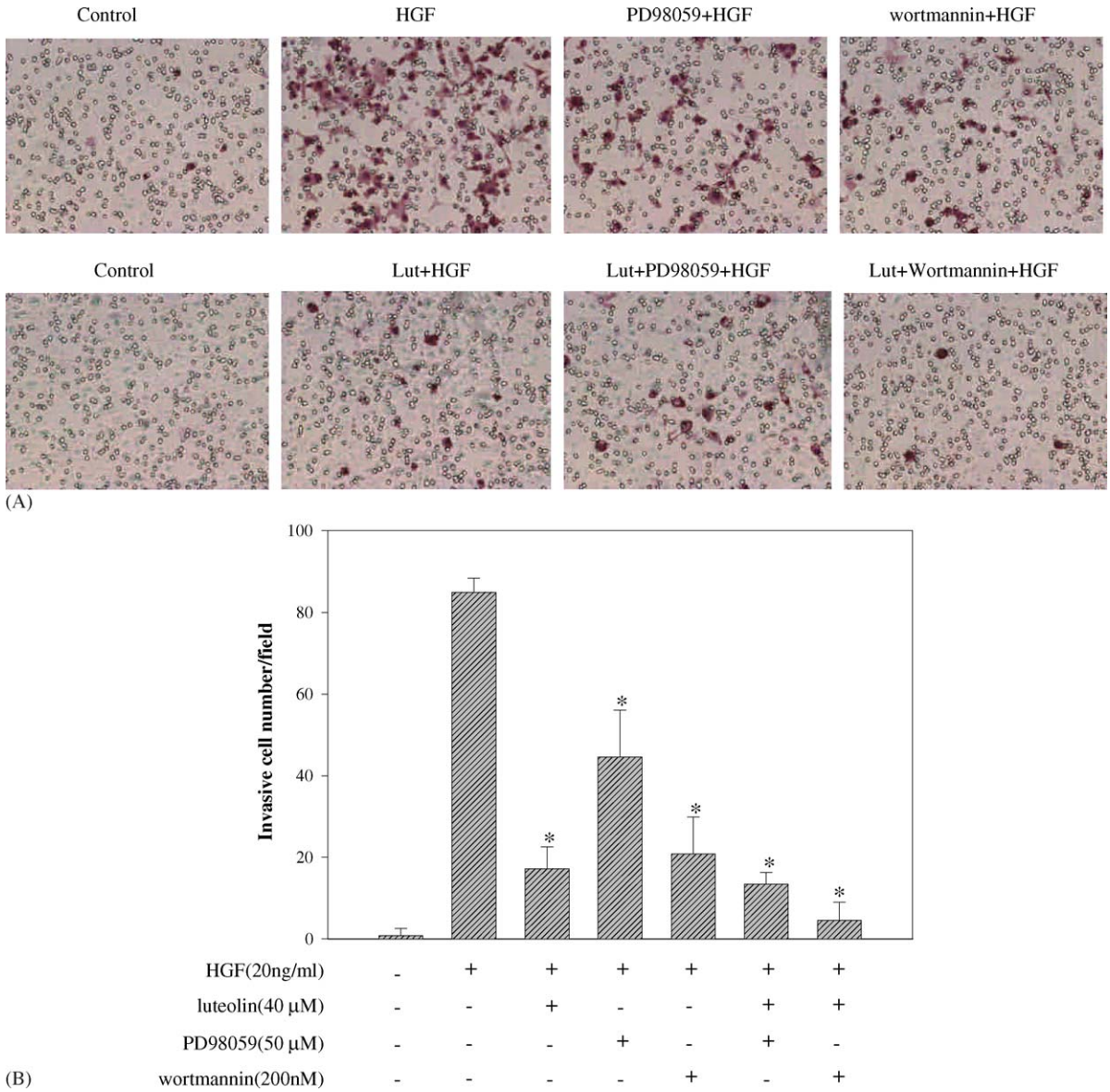

Fig. 8. Effect of luteolin with the combination of PD98059 and wortmannin on HGF-stimulated cell invasion. HepG2 cells (5×10^4 ml^{-1}) were seeded onto transwell plates and then incubated with 40 μM luteolin, 50 μM PD98059 and 200 nM wortmannin alone or in different combinations for 24 h. The lower surfaces of the membranes from the transwell units were fixed with 100% methanol and stained with Giemsa solution. (A) Photographs of HepG2 cells after invasion. (B) Cells (in red color) that had invaded to the lower surface of the membranes were counted in triplicate wells and in three identical experiments under a light microscope, and the data represent mean ± S.D. $^{*}p < 0.01$, compared with the group treated with HGF alone.

on the mechanism of action of phytochemicals, such as flavonoids, which might explain the protective action of plant-based diets on the progression of cancer. Moreover, it might provide the basis of development of more potent synthetic analogs for the inhibition of HGF-induced cell invasion.

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