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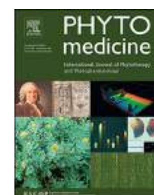
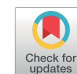


Original Article

Duchesnea indica extract attenuates oral cancer cells metastatic potential through the inhibition of the matrix metalloproteinase-2 activity by down-regulating the MEK/ERK pathway



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ABSTRACT

Background: *Duchesnea indica* (Andr.) Focke, an herb in folk medicine used extensively in traditional Chinese medicine, has cytostatic properties as well as antioxidant and antimetastasis activities in various cancer cells. However, the effects and underlying mechanisms of *Duchesnea indica* extracts (DIEs) on human oral squamous cell carcinoma (OSCC) metastases remain unclear.
Purpose: In this study, we posit the hypothesis that DIE possesses antimetastatic effects on human OSCC cells.
Methods: The effects of DIE on cell viability, motility, migration, and invasion were investigated. Gelatin zymography, Western blotting, migration and invasion assays were used to further study the underlying mechanisms involved in the antimetastatic effects of DIE in OSCC cells.
Results: The results from MTT assay revealed that DIE did not affect the cell viability of OSCC cells. Moreover, DIE significantly attenuated OSCC cells' motility, migration, and invasion by reducing the MMP-2 protein expression and MMP-2 activity in a dose-dependent manner. In addition, DIE reduced the phosphorylation of both ERK1/2 and its upstream kinase but had no effect on the phosphorylation of p38 and JNK.
Conclusion: DIE triggers the antimetastatic activity in OSCC cells by suppressing the MMP-2 activity via the MEK/ERK signaling pathways. Therefore, these findings are promising for the use of DIE antimetastatic activity in oral cancer metastasis treatment.

Introduction

Head and neck cancer is a growing worldwide malignancy that includes oral cancer, laryngeal cancer, nasopharyngeal carcinoma, oropharyngeal cancer, hypopharyngeal cancer, and salivary gland cancer. In this classification, nearly 40% of cases are diagnosed as oral cancer with 177,384 deaths per year (Bray et al., 2018). Over 90% of patients with oral cancer are diagnosed as oral squamous cell carcinoma (OSCC). The treatment for oral cancer mainly relies on surgery, followed by adjuvant chemotherapy or radiotherapy in case of advanced stage tumors (Bernier et al., 2004). Carcinogenesis is a multistep process that originates in cellular transformation, progresses to hyperproliferation, angiogenesis, and cell invasion, before culminating in the acquisition of metastatic lesions (Gupta et al., 2010). This type of malignancy is related to various environmental carcinogens, including alcohol consumption, betel nut chewing, and smoking (Shiu et al., 2000). The risk factor for oral cavity causes the formation of tumorigenesis. Moreover, the neck lymph node metastasis, which has incidence in oral cancer, becomes a key prognostic factor (Sacco and Cohen, 2015). The survival rates for oral cancer at 5 years and 10 years were 42% and 38%, respectively. The outcomes can still be improved by identifying a new diagnostic or prognostic molecule biomarkers

Abbreviations: DIE, *Duchesnea indica* extracts; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; OSCC, oral squamous cell carcinoma

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(Moro et al., 2018).

Several studies have documented that suppressing cancer cell metastasis is a key phase in the inhibition of cell progression. The metastasis of tumor progression consists of complex processes, including epithelial-mesenchymal transition (EMT) activation, basement membrane degradation, blood vessel intravasation or extravasation, and microenvironment establishment (Lu et al., 2011). Among the aforementioned processes, basement membrane degradation, which implies the deregulation of extracellular matrix (ECM) dynamics, is a major indicator of cancer initiation (Arvelo et al., 2016). The components of the ECM can be divided into proteins, glycoproteins, and proteoglycans. In ECM remodeling, metalloproteinases are special enzymes that contain a disintegrin and metalloproteinase from the thrombospondin motifs (ADAMTS) and matrix metalloproteinase (MMP) families. In these two proteinase families, MMPs crucially target a wide range of ECM components and other extracellular proteins (Cawston and Young, 2010). Some MMPs, namely MMP-2 and MMP-9, are involved in early cancer development, progression, and metastasis in oral cancer (Fang et al., 2018; Hsieh et al., 2018; Vayrynen et al., 2019). Clinical research also indicated that the expression of MMP-2 and MMP-9 in malignant tissues was higher than in adjust normal tissue; this can be used as the molecular target of metastasis in oral cancer (Patel et al., 2005, 2007). Lotfi et al. suggested that the serum levels of both MMP-2 and MMP-9 were elevated in patients with OSCC. However, to evaluate the tumor grade and lymph node involvement, MMP-2 is preferred to MMP-9 (Lotfi et al., 2015).

Chemoprevention designates the process of preventing the development of cancer by inhibiting one or more stages of the tumorigenesis using nontoxic, natural, or synthetic compounds. Natural compounds in plants, herbal formulas, or from dietary agents have been classified as chemopreventive agents have and have been increasingly used in recent years (CFG et al., 2019; Jayaprakasha et al., 2008; Tang et al., 2011; Vijayakurup et al., 2019). *Duchesnea indica* (Andr.) Focke, a member of the rose family, is distributed in Asian countries and a common herb used in folk medicine in China. Previous studies have mentioned that the extract of *D. indica* has a variety of biological activities associated with the antimutagenic, antioxidative (Gyu Kim et al., 2002), and anti-inflammatory activities (Zhao et al., 2008), as well as anticancer properties (Lee and Yang, 1994; Tsai et al., 2009). The methanolic extract from *D. indica* provided protection against H_2O_2-induced cytotoxicity in human skin fibroblast (Hu et al., 2011). Aqueous extracts of *D. indica* exhibited growth inhibitory activity of human cancer cell lines *in vitro* (Shoemaker et al., 2005). In addition, *D. indica* phenolic fraction exhibited cytotoxicity through a triggered apoptosis via the mitochondrial pathway and caused cell cycle arrest in the S phase in human ovarian SKOV-3 cancer cells. However, the effects of *D. indica* on the mechanisms of its anticancer activity remain unclear in OSCC. According to aforementioned, the present study focused on the anticancer properties and related mechanisms of *D. indica* in OSCC. In this study, we demonstrated that the suppression of metastatic ability by *D. indica* is produced by the downregulation of the MMP-2 expression. We intend to provide a potential therapeutic use in chemoprevention.

Materials and methods

DIE preparation

D. indica leaves were purchased from traditional Chinese herbal medicine pharmacy in Taichung, Taiwan. Condensation was used to extracts dried *D. indica* leaf peduncle, and the process of lyophilization afterwards (Lin et al., 2014). Briefly, air-dried *D. indica* leaf peduncles were boiled twice with 50% ethanol at 50 °C for 12 h, the percentage of *D. indica* leaves and ethanol is 1:5. Afterwards, removed the solvent, and the D. indica extract was lyophilized and stored at −20 °C. The components of *D. indica* leaves were analyzed by HPLC chromatography as previous described (Chen et al., 2017) (Supplemental Figure 1).

Cell culture and DIE treatment

SCC-9 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and maintained in DMEM/F12 supplemented with 10% FBS, 400 ng/ml hydrocortisone and 0.1 mM non-essential amino acids (NEAA; Life Technologies). SCC-14 cells were purchased from CLS Cell Line Service GmbH (Eppelheim, Germany) and maintained in DMEM/F12 supplemented with 5% FBS. The tumorigenic TW2.6 cells were obtained from Dr. Kuo's group where the cell line is originally established and authenticated (Kok et al., 2007). All the cells were cultured and maintained in a humidified atmosphere containing 95% air and 5% CO2 at 37 °C. For DIE treatment, DIE was dissolved in dimethylsulfoxide (DMSO) (Sigma, St. Louis, MO), and add to the culture medium to achieve the indicated concentrations (0, 10, 20, and 40 µg/ml) for 24 h incubation. The final concentration of DMSO in culture medium is less than 0.1%.

MTT assay for cell viability

MTT (3-(4, 5-dimethylthiazol-2-yl) − 2,5-diphenyl tetrazolium bromide) assay was performed to detect the cytotoxicity of DIE. Cells were seeded in 24-well plate for 24 h incubation. The following day, DIE was added with an indicated concentration (0, 10, 20, and 40 µg/ml) and 24 h later, culture media were removed and the cells were washed with PBS, followed by the addition of MTT (0.5 mg/ml) to the culture medium for 4 h at 37 °C. Subsequently, the amount of MTT formazan product was dissolved in isopropanol the absorbance values were measured by a microplate photometer at 563 nm.

Wound-healing assay

An *in vitro* wound healing assay was used to observe the motility of cells after DIE treatment. Cells were seeded in plated in 6 cm dish for 24 h until they visibly reached confluence. Before DIE treatment (0, 10, 20, and 40 µg/ml), a pipette tip was used to create a straight scratch on the plate to simulate a wound. The width of the remaining gap was imaged using phase-contrast microscopy (X100) at indicated time which depends on different cells.

Cell migration and invasion assays

Cell invasion and migration assays were performed according to the methods described by Yang et al. (2010). Cells were seeded in 6 cm dish for 24 h incubation. The following day, DIE was added with an indicated concentration (0, 10, 20, and 40 µg/ml) for 24 h. Otherwise, cell were pre-treated with an indicated concentration of specific inhibitors, 10 µM U0126 (MEK inhibitor), for 1 h followed by incubated with or without DIE (20 µg/ml) for an additional 24 h. The cells were harvested and seeded in a Boyden chamber (Neuro Probe, Cabin John, MD) in a serum-free medium and incubated for 24 h or 48 h. For invasion assay, 10 µl Matrigel (25 mg/50 ml; BD Biosciences, MA) was applied to polycarbonate membranes (8 µm pore-size), and the bottom chamber contained a standard culture medium. Afterwards, the membrane was fixed with methanol and stained with 10% Giemsa (Sigma Chemical Co, St. Louis, MO, USA). The image of cells migrated or invaded through the membrane was capture and counted under the light microscope (× 100, three random field per well).

Gelatin zymography

Cells were seeded in 24-well plate for 24 h incubation. The following day, DIE was added with an indicated concentration (0, 10, 20, and 40 µg/ml) for 24 h. Otherwise, cell were pre-treated with an indicated concentration of specific inhibitors, 10 µM U0126 (MEK inhibitor), for 1 h followed by incubated with or without DIE (20 µg/ml) for an additional 24 h. For MMP-2 activity detection, conditioned

medium from treated cells were prepared without boiling or reduction, and subjected to 0.1% gelatin-8% SDS-PAGE electrophoresis. After electrophoresis, the gels were washed twice in 2.5% Triton X-100 for 30 min to remove SDS and then incubated in a reaction buffer (40 mM Tris-HCl, pH 8.0, 10 mM $CaCl_2$, 0.02% NaN_3) at 37 °C for 18 h. Finally, the gel was stained with Coomassie brilliant blue R-250.

Western blotting analysis

Cells were seeded in 6 cm dish for 24 h incubation. The following day, DIE was added with an indicated concentration (0, 10, 20, and 40 µg/ml) for 6 or 24 h. Total cell lysates were collected using PRO-PREP protein extraction solution (iNtRON) containing protease inhibitor and then lysed by sonication using ultrasonic processor. Afterward, cells extracts were microcentrifuged at 13,000 rpm for 30 min at 4 °C and the supernatants were collected. After measured protein concentrations of cell lysate by Bradford assay, equal amount of proteins were separated by 10% polyacrylamide gel and transferred onto a nitrocellulose membrane (Life Science). Subsequently, the membrane was incubated with 5% non-fat milk in TBST buffer for 1 h blocking and then the indicated primary antibodies were added. After incubated for 18 h at 4 °C, secondary antibodies with horseradish peroxidase were used for the indirect detection specific primary antibody for 1 h at room temperature. Finally, the protein expression was detected by chemiluminescence with an ECL detection kit. The relative photographic density was quantified by Multi Gauge V2.2 software.

Statistical analysis

Statistical analyses were performed using SigmaPlot, vers. 10.0 (Systat Software, Inc. SigmaPlot for Windows). Data comparisons between two independent groups were used with one-way ANOVA test. (Sigma-Stat 2.0; Jandel Scientific, San Rafael, CA). Differences were considered significant at 95% confidence level when $p < 0.05$. Data represent the mean (± standard deviation, SD) of at least three independent experiments.

Results

Human OSCC cell lines, SCC-9, SCC-14, and TW2.6, were incubated with *Duchesnea indica* extracts (DIEs) extracted from *D. indica* using 50% ethanol. A previous research reported that DIE used to treat human lung cancer A549 cells demonstrated significantly toxic effects at the concentration of 50 µg/ml (Chen et al., 2017). In cervical cancer cells, Duchesnea phenolic fraction exhibited an inhibitory effect of cell growth in HeLa cells at concentrations of more than 40 µg/ml (Peng et al., 2009). DIE concentrations of less than 40 µg/ml were selected in this study. As illustrated in Fig. 1A, after a 24-hour treatment with various concentrations (0–40 µg/ml) of DIE, the cell viability was not significantly different from the control in the MTT assay. A DIE concentration ranging from 10 to 40 µg/ml was used in all subsequent experiments to investigate its antimetastatic properties.

A wound-healing assay was used to investigate the motility of OSCC cells treated with DIE. After a 24-hour treatment of DIE concentrations ranging from 0 to 40 µg/ml, the images of cells that directionally migrated toward the wound were captured at the indicated incubation time depending on the cell lines (0, 12, and 24 h for SCC-9 cells; 0, 24, and 48 h for SCC-14 cells; 0, 4, and 6 h for TW2.6 cells observation). The results revealed that DIE significantly attenuated the human OSCC cells motility of SCC-9, SCC-14, and TW2.6 cells in a dose-dependent manner (Fig. 1B–D).

To examine whether DIE also suppresses the cellular abilities in the migration and invasion of SCC-9, SCC-14, and TW2.6 cells, the Boyden chamber assay and the Transwell migration and invasion assay, with or without Matrigel, were subsequently tested. The results indicated that the migration activity and invasive potential of SCC-9 (Fig. 2A and B), SCC-14 (Fig. 2C and D), and TW2.6 (Fig. 2E and F) cells were reduced after incubation with various concentrations (0, 10, 20, and 40 µg/ml) of DIE for 24 h in a concentration-dependent manner. Therefore, DIE could be considered to decrease the activity of metastasis in OSCC cell lines.

Matrix metalloproteinases have been proven to be correlated with tumor invasion and metastasis (Chien et al., 2013; Su et al., 2017; Yang et al., 2016). To understand the role of MMP-2 in SCC-9, SCC-14, and TW2.6 cells motility reduced by DIE, gelatin zymography was used to analyze the activity of the secreted MMP-2. As illustrated in Fig. 3A, the secreted MMP-2 activity was significantly inhibited by DIE in a dose-dependent manner. The protein level of the MMP-2 expression was indicated through Western blotting (Fig. 3B). However, the protein expression of MMP-9, E-cadherin and Vimentin in the SCC-9 cells was not altered after DIE treatment (Supplemental Figure 2).

We have further tested the effects of MMP-2 activity and cell migration assay by TNF-α in SCC-9 cells. Our data showed that TNF-α significantly increased MMP-2 activity and the cell migratory abilities of SCC-9 cells and also reversed the DIE-mediated suppression of MMP-2 activity and the migratory ability in SCC-9 cells (Fig. 3C and D). According to the aforementioned results, DIE may inhibit the secretion and expression of MMP-2.

After having demonstrated that the treatment of OSCC cells with DIE inhibited the cell migration, cell invasion, and MMP-2 secretion, the molecular mechanisms of DIE on cell migration were further investigated. Western blotting analysis was used to detect the target proteins that were reportedly involved in cell migration. First, we evaluated the expression of the mitogen-activated protein kinase (MAPK) pathway, including p-ERK1/2, p-JNK1/2, and p-p38 in SCC-9, SCC-14, and TW2.6 cells to elucidate the antimetastatic and anti-invasive mechanisms of DIE. The results demonstrated that DIE reduced the phosphorylation of ERK1/2 in a dose-dependent manner, whereas the phosphorylation of the JNK1/2 and p38 protein kinases was not significantly inhibited (Fig. 4A). Moreover, the upstream signaling pathway of ERK1/2 was detected through the Western blotting analysis. We observed that the phosphorylation of FAK Y397, Src, c-Raf, and MEK1/2 was dose-dependently reduced by the DIE-treated OSCC cells (Fig. 4B). Therefore, these results suggested that the inactivation of the MAPK/ERK signaling pathway is required for the DIE to suppress the protein expression of MMP-2.

To further investigate whether the suppression of the MMP-2 expression by DIE was mainly caused by the inhibition of the MEK/ERK1/2 signaling pathway, the MEK inhibitor, U0126, was used to confirm the mechanism according to which DIE reduces the MMP-2 activity through the downregulation of the ERK1/2 expression in SCC-9, SCC-14, and TW2.6 cells. The MMP-2 activity was suppressed when the cells were treated with DIE only (Fig. 5B). The combination treatment of U0126 (10 µM) and DIE (20 µg/ml) resulted in the intensive inhibition of the MMP-2 activity in OSCC cells (Fig. 5B). The cell cytotoxicity test also confirmed that all conditions did not affect the cell growth (Fig. 5A). In addition, the DIE or ERK1/2 inhibitor treatment suppressed the migration of cells, and the combination of DIE and U0126 exhibited stronger inhibition of the cell migration (Fig. 5C). We have further tested the effects of cell migration assay by tBHQ (ERK1/2 activator) in SCC-9 cells. Our data showed that tBHQ significantly increased the cell migratory abilities of SCC-9 cells and also reversed the DIE-mediated suppression of the migratory ability in SCC-9 cells (Fig. 5D).

Therefore, the inhibition of the ERK1/2 signaling pathway may result in a reduced activity of MMP-2 and an attenuated OSCC cell migration. Taken together, our results suggested that DIE inhibited the OSCC cells migration through the suppression of the MMP-2 expression by down-regulating the MAPK/ERK signaling pathway (Fig. 6).

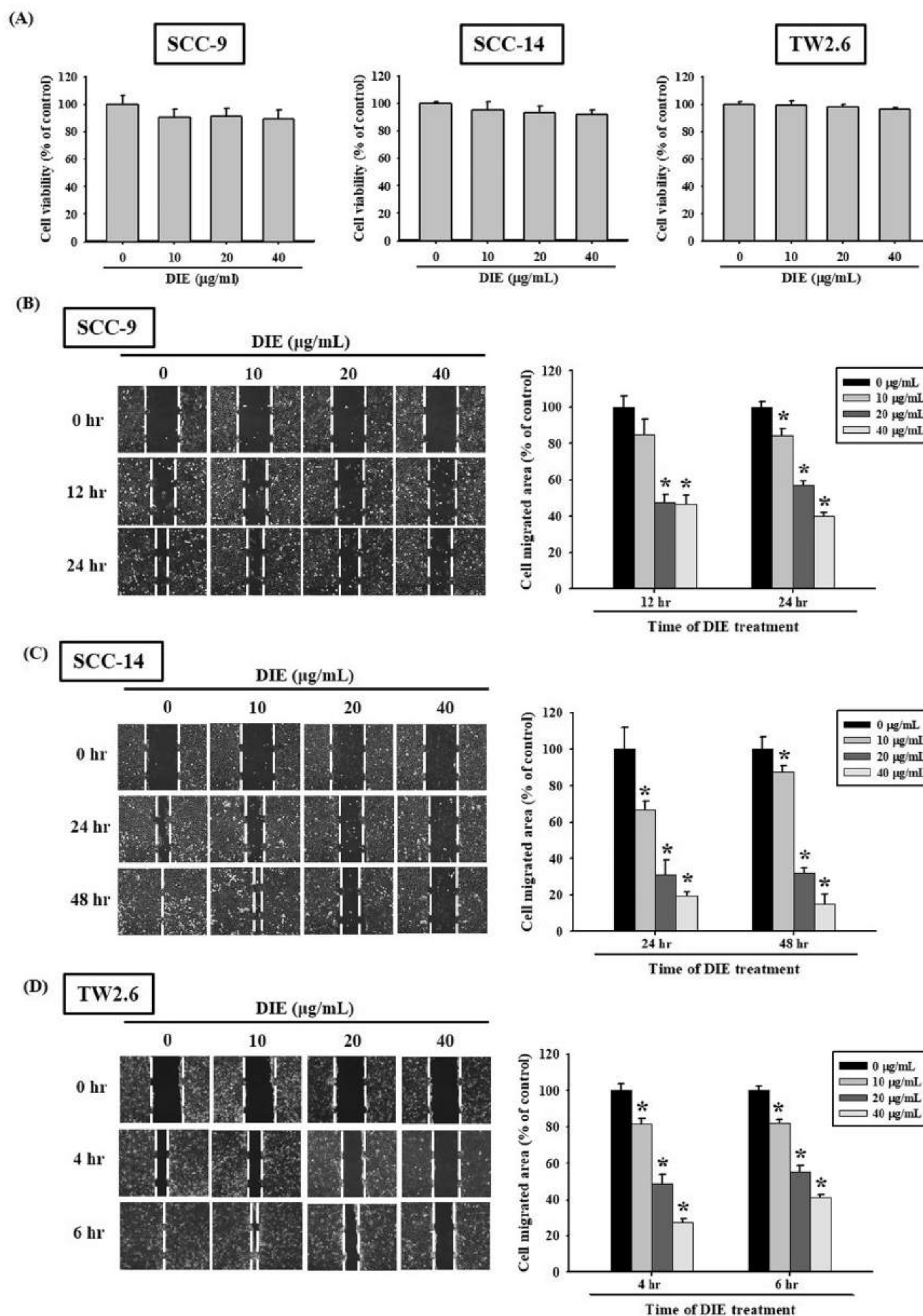

Fig. 1. Effects of DIE on cell viability and cell mobility. Human OSCC cell lines, SCC-9, SCC-14 and TW2.6 cells, were treated with DIE (0, 10, 20, and 40 μg/ml) for 24 h and then subjected to MTT assay for cell viability analysis (A). Cell mobility of SCC-9, SCC-14 and TW2.6 cells were evaluated using the wound healing assay. Furthermore, cells were wounded and then treated with DIE (0, 10, 20, and 40 μg/ml) for 24 h in a serum-containing medium. At different time point (0, 12, and 24 h for SCC-9; 0, 24, and 48 h for SCC-14; 0, 4, and 6 h for TW2.6 observation), phase-contrast photographs of the wounds at four locations were taken and analyzed. (B-D) The values represented the means ± SD of at least 3 independent experiments. *$p < 0.05$, compared with the vehicle group.

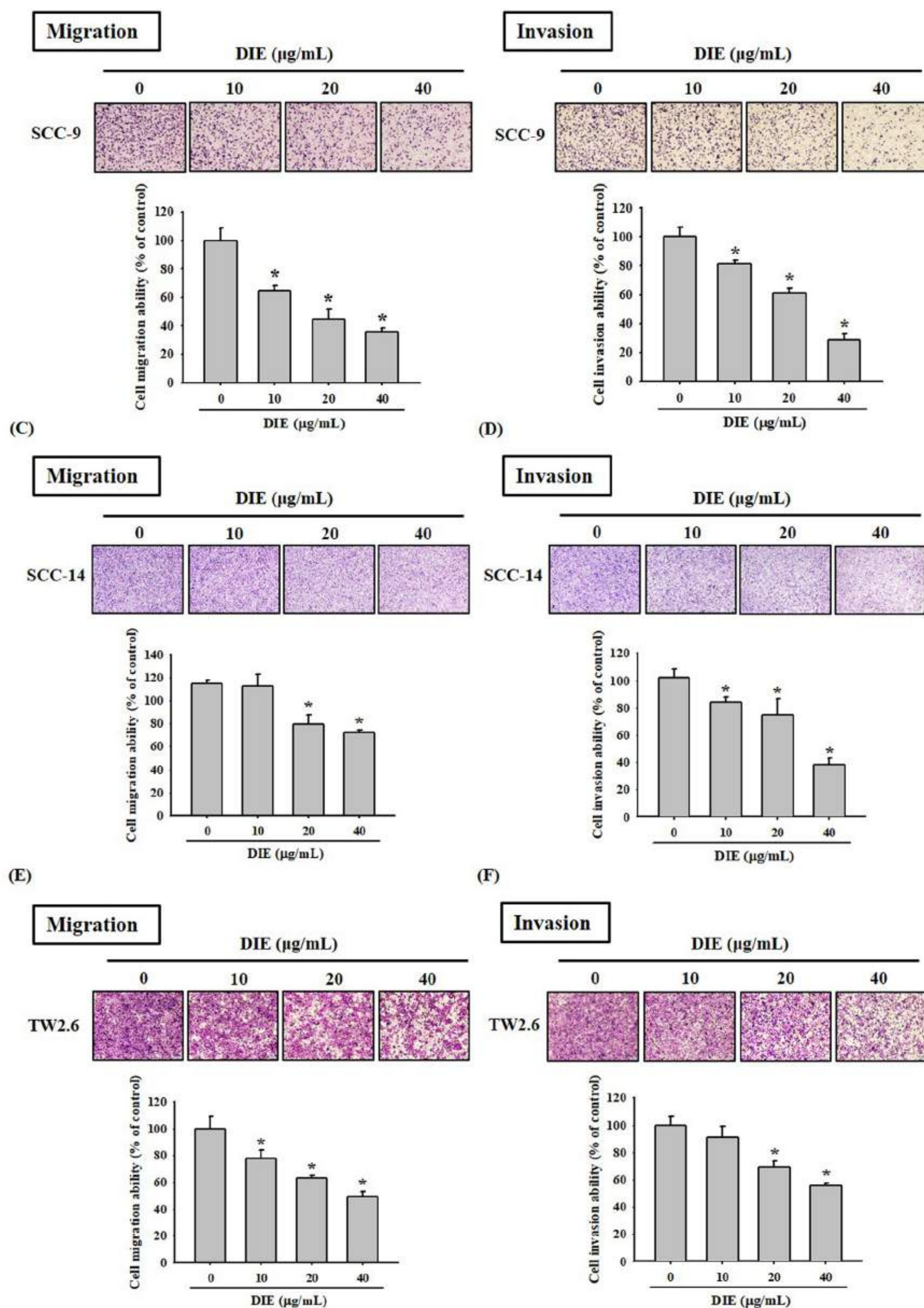

Fig. 2. Effects of DIE on cell migration and invasion. Human OSCC cell lines, SCC-9, SCC-14 and TW2.6 cells, were pre-treated with DIE (0, 10, 20, and 40 μg/ml) for 24 h and subjected to cell migration and invasion assay. The cell migration (A, C, and E) and invasion (B, D, and F) were analyzed using a Boyden chamber (for SCC-9 cells) or transwell (for SCC-14 and TW2.6 cells) with or without matrigel for 24 h and 48 h with polycarbonate filters. The migration and invasion ability of SCC-9 (A and B), SCC-14 (C and D) and TW2.6 cells (E and F) were quantified by counting migrated or invaded cells to the underside of polycarbonate filters. The values represented the means ± SD of at least 3 independent experiments. $^{*}p < 0.05$, compared with the vehicle group.

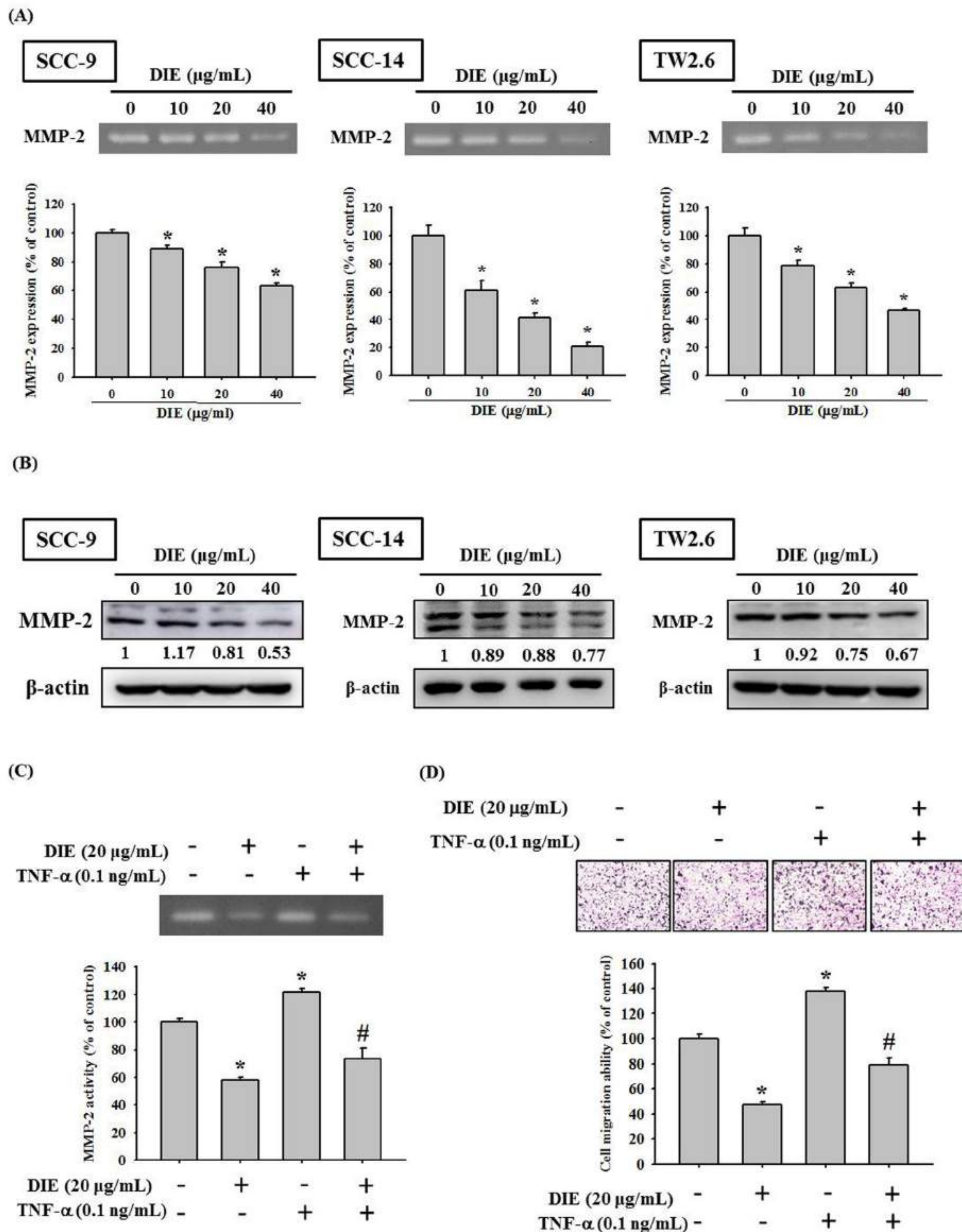

Fig. 3. Effects of DIE on the expression and secretion of MMP-2. (A) Human OSCC cell lines, SCC-9, SCC-14 and TW2.6 cells, were pre-treated with DIE (0, 10, 20, and 40 µg/ml) for 24 h in a serum-free medium and then subjected to gelatin zymography to analyze the activity of MMP-2. (B) Western blotting was used to analyze the protein levels of MMP-2 after DIE (0, 10, 20, and 40 µg/ml) treatment for 24 h in SCC-9, SCC-14 and TW2.6 cells. β-actin was used as the internal control of Western blotting. (C-D) Human SCC-9 cell lines were pre-treated with DIE (20 µg/ml) for 1 h and then incubated in the presence or absence of TNF-α (0.1 ng/ml) for 24 h. The culture media were collected to analyze the activity of MMP-2 by gelatin zymography, and the migration ability of cells was observed by Boyden chamber migration assay. The density of each band was subsequently quantified by ImageJ software. The values represented the means ± SD of at least 3 independent experiments. $^{*}p < 0.05$, compared with the vehicle group. $^{\#}p < 0.05$, compared with the TNF-α treated group.

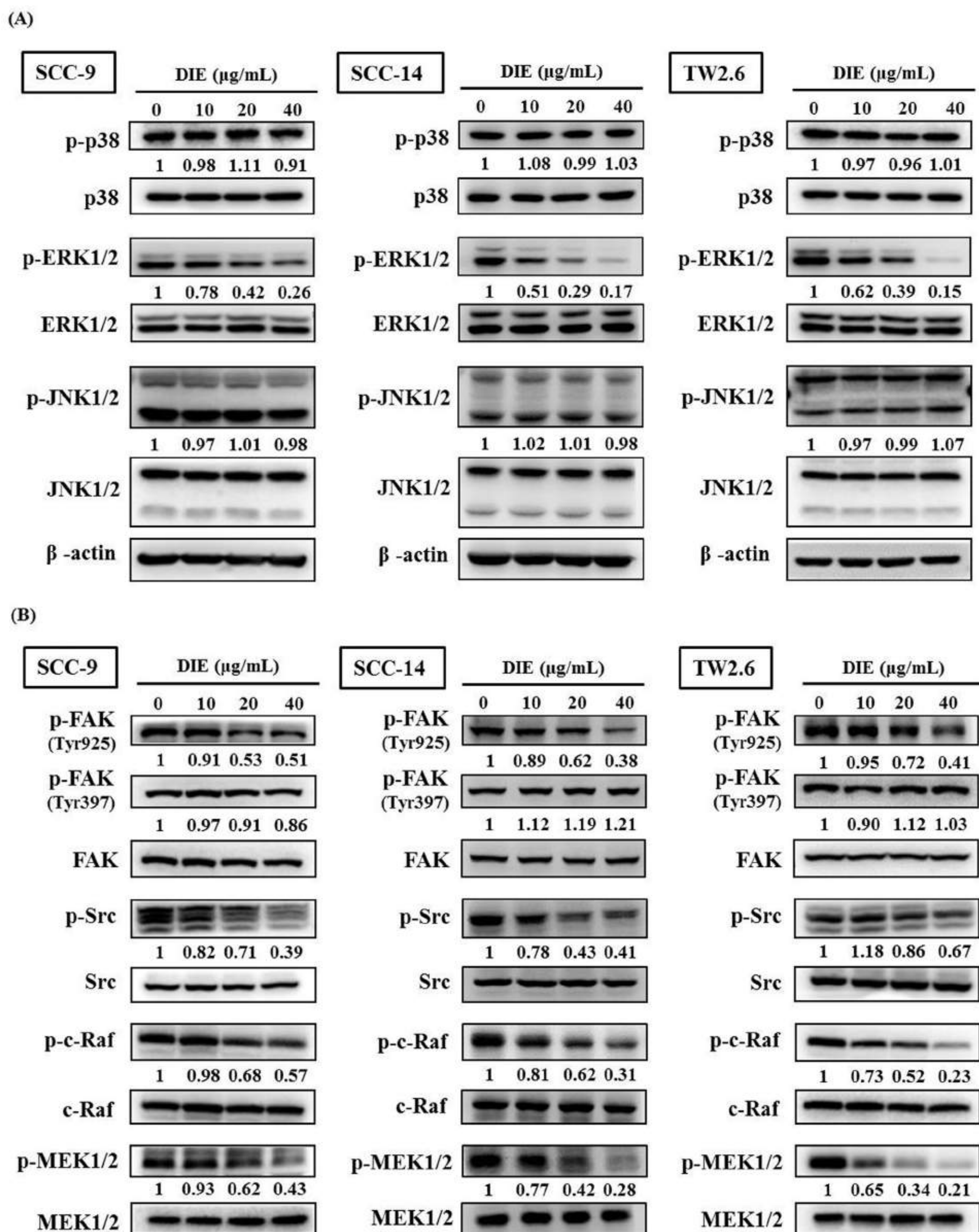

Fig. 4. Effect of DIE on the FAK, Src, MEK, MAPK pathway. (A) Human OSCC cell lines, SCC-9, SCC-14 and TW2.6 cells, were pre-treated with DIE (0, 10, 20, and 40 μg/ml) for 6 h and the total cell lysates were then subjected to Western blotting to analyze the indicated total and phosphorylation of (A) ERK 1/2, JNK 1/2, and p38, (B) FAK, Src, Raf, and MEK.

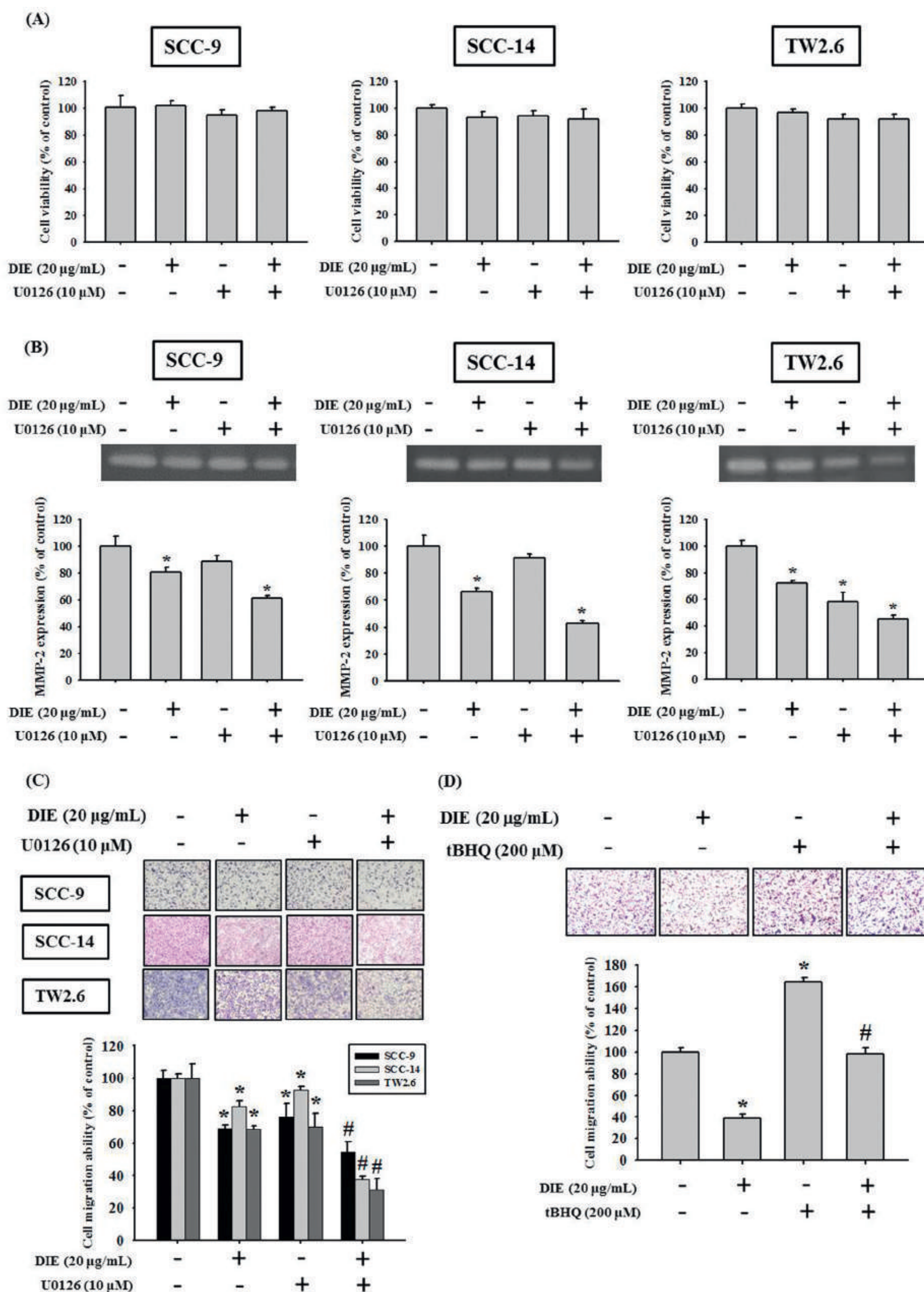

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Fig. 5. Inhibitory effect of MEK inhibitor (U0126) on MMP-2 activities and cell migration. Human OSCC cell lines, SCC-9, SCC-14 and TW2.6 cells, were pre-treated with U0126 (10 μM) for 1 h and then incubated in the presence or absence of DIE (20 μg/ml) for 24 h. (A) The cytotoxicity of SCC-9, SCC-14 and TW2.6 cells were evaluated using the MTT assay. (B) The culture media were collected to analyze the activity of MMP-2 by gelatin zymography, and the migration ability of cells were observed by Boyden chamber or transwell migration assay (C). (D) Human SCC-9 cell lines were pre-treated with DIE (20 μg/ml) for 1 h and then incubated in the presence or absence of tBHQ (200 μM) for 24 h. The migration ability of cells was observed by Boyden chamber migration assay. $^{*}p < 0.05$, compared with the vehicle group. $^{\#}p < 0.05$, compared with the tBHQ treated group.

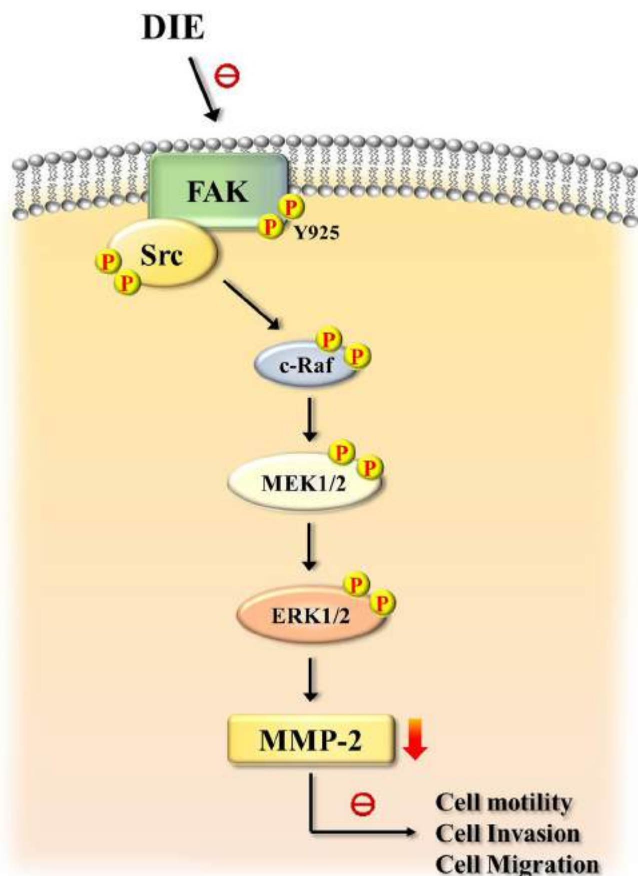

Fig. 6. Schematic diagram for proposed signaling pathways in the inhibitory mechanisms of DIE on cell migration in human OSCC cells. DIE suppresses the level of phosphorylate-FAK, Src, Raf/MEK/ERK signaling pathway, and furthermore inhibits MMP-2, leading to reduced OSCC cell motility, invasion and migration.

Discussion

The purpose of this study was to investigate the antimetastatic effects of DIE on human OSCC cell lines, SCC-9, SCC-14, and TW2.6 cells, because more than 90% of all cancers in the oral region were constituted by squamous cells. We provided clear evidence that DIE, at concentrations of 0–40 μg/ml without cytotoxicity, inhibited the cell motility, invasiveness, and migratory potential in SCC-9, SCC-14, and TW2.6 cells.

Herbal medicine, also called botanical medicine, using seeds, roots, berries, leaves, flowers, or bark, has been used since the prehistoric times to control and prevent a variety of diseases (Mustafa et al., 2017). The use of natural products as medication has been justified by ancient written documents originated from India, North Africa, and China. Although there are over 400,000 species of plants on earth containing unique bioactive compounds, only a small percentage has been mentioned in research studies (Shoemaker et al., 2005). In recent years, numerous bioactive compounds from plants have been discovered as a type of chemopreventive agents due to their antimicrobial activities (Benkeblia, 2004; Gou et al., 2011), antifungal activities (De Lucca and Walsh, 1999), antioxidative activities (Campbell et al., 2017; Kawanishi et al., 2005), anti-inflammatory activities (Nonn et al., 2007), and anticancer activities (Kim et al., 2012). Among the anticancer properties, several reports have demonstrated that chemopreventive agents can suppress multiple pathways through a specific molecular target, such as the inhibition of NF-kB (Bharti and Aggarwal, 2002), AP-1 (Manna et al., 2000), COX2 (Muller-Decker et al., 2002; Subbaramaiah et al., 1998), and JAK-STAT (Bharti et al., 2003) activation pathways, as inhibitors of cell proliferation (Surh, 2003), cell cycling (Mukhopadhyay et al., 2002), angiogenesis (Chen et al., 2004), and multidrug resistance (Anuchapreeda et al., 2002; Hong et al., 2003).

In various cancers, different stimuli induce the MMP-2 expression through the activation of the p38/MAPK, MEK/ERK, or PI3K-Akt signaling pathways (Chao et al., 2019; Huang et al., 2005; Jung et al., 2013), which subsequently activates AP-1 and NF-κB (Hsieh et al., 2007; Weng et al., 2008). During Western blotting analysis, the downregulation of the ERK phosphorylation was suspected to be involved in the DIE-mediated suppression of cell motility, migration, and invasion, as well as the MMP-2 expression of OSCC cells. The non-receptor protein tyrosine kinase Src and the FAK signaling downstream of cell − ECM interactions enhance the strength of cell − cell adhesions and modulate the cell migration and invasion through their interaction with integrins and regulators of the Rho-GTPases family (Weng et al., 2008). Our data indicated that the DIE-induced antimetastatic ability and suppression of MMP-2 in OSCC cells is associated with FAK/Src-mediated phosphorylation.

The leaf peduncles of *D. indica* were extracted with 50% ethanol. The epicatechin gallate (ECG) is considered a major bioactive component of DIE (Chen et al., 2017). Previous studies have addressed the role of the MAPK pathway in regulating the MMP-2 expression (Lin et al., 2013). Chen et al. indicated that DIE can downregulate ERK1/2 to decrease the activity of MMP-2, inhibit the FAK pathway to enhance the expression of E-cadherin, and reduce the expression of vimentin and fibronectin leading to an inhibition of the cell invasion, motility, and adhesion in human lung cancer cells (Chen et al., 2017). Our previous study also demonstrated that silibinin and kaempferol inhibit the migration of oral cancer cells by suppressing the activation of the ERK1/2 and MMP-2 expression (Hsieh et al., 2007; Lin et al., 2013). Furthermore, Chang et al. demonstrated that epigallocatechingallate (EGCG) downregulated MMP-2 in human uveal melanoma cells through the ERK1/2 signal pathway (Chang et al., 2014). In other studies, the JNK 1/2 pathway modulated the MMP-2 production through EGCG treatment in lung cancer cells (Deng and Lin, 2011), and genistein inhibited the MMP-2 and p38/MAPK pathway, which resulted in the suppression of cell invasion in human prostate epithelial cells (Huang et al., 2005). However, the present study revealed that DIE only inhibited the ERK phosphorylation, and no significant effects were detected on the JNK and p38 signaling pathways. In addition, our study was limited in interpreting the effects of DIE on oral cancer metastases due to the lack of an *in vivo* animal study. Thus, the *in vivo* effects of DIE on oral cancer metastases would be worth investigating, which will be included in our future work.

In conclusion, this is the first study to demonstrate that DIE inhibits OSCC cells' migration potential and invasive activity and represses the MMP-2 expression by down-regulating the p-FAK, p-Src, and Raf/MEK/ERK signaling pathways. Our work undeniably reinforces the idea that DIE may have great potential in the development of chemopreventive agents for the antimetastatic treatment of oral cancer.

Acknowledgments

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Competing interests

The authors have declared that no competing interests exist.

Supplementary materials

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