Abstract. Nasopharyngeal carcinoma (NPC) is a tumor located in the nasopharynx with highly invasive and metastatic properties. Metastasis is a primary cause of mortality in patients with NPC. The terpenoid polyphenol pinosylvin is a known functional compound of the *Pinus* species that exhibits anti-inflammatory effects; however, the effect of pinosylvin on human NPC cell migration and invasion is unclear. The present study aimed to investigate the functional role of pinosylvin in NPC cells (NPC-039, NPC-BM and RPMI 2650). Gap closure and Transwell assay indicated that pinosylvin at increasing concentrations inhibited migration and invasion of NPC-039 and NPC-BM cells. In addition to inhibiting the enzyme activity of MMP-2, pinosylvin also decreased the protein expression levels of MMP-2 and MMP-9. Pinosylvin decreased the expression of vimentin and N-cadherin and significantly increased the expression of zonula occludens-1 and E-cadherin in NPC cells. Additionally, pinosylvin suppressed the invasion and migration ability of NPC-039 and NPC-BM cells by mediating the p38, ERK1/2 and JNK1/2 pathways. The present results revealed that pinosylvin inhibited migration and invasion in NPC cells.

Introduction

The terpenoid polyphenol pinosylvin (trans-3,5-dihydroxystilbene) is a stilbene present in the heartwood of coniferous trees of the genus *Pinus* (1). Many studies have demonstrated that biological characteristics of pinosylvin include antibacterial and antifungal activity (2) and protection against oxidative stress in human cells (3). Pinosylvin regulates Src/ERK and GSK-3/β-catenin signaling to inhibit tumor cell growth (4). Pinosylvin has been shown to inhibit the expression of MMP-2 and MMP-9 in human fibrosarcoma HT1080 cells (5).

Nasopharyngeal carcinoma (NPC) is a tumor located in the nasopharynx and is caused by epithelial cells covering the nasopharyngeal surface. Unlike other head and neck epithelial cancers, NPC is highly invasive and metastatic (6). NPC is particularly prevalent in Southern China, Southeast Asia, North Africa and the Arctic region, which is a unique geographical distribution (7). Four primary causes of nasopharyngeal carcinoma have been identified, including Epstein-Barr and human papillomavirus infection, genetic susceptibility and consumption of salted fish (8). NPC occurs adjacent to cervical lymph nodes, which increases the risk of metastasis in other parts of the body, thereby causing difficulties in surgical treatment (8). Currently, chemotherapy and radiotherapy can improve the survival rate of patients with advanced NPC (9). Preventing distant metastasis is key to treatment, and more effective systemic drugs should be investigated (10).

Correspondence to: Dr Ming-Ju Hsieh, Oral Cancer Research Center, Changhua Christian Hospital, 135 Nanxiao Street, Changhua, Changhua 500, Taiwan, R.O.C. E-mail: 170780@cch.org.tw
Dr Jen-Tsun Lin, Division of Hematology and Oncology, Department of Medicine, Changhua Christian Hospital, 135 Nanxiao Street, Changhua, Changhua 500, Taiwan, R.O.C. E-mail: 111227@cch.org.tw

*Contributed equally

Abbreviation: NPC, nasopharyngeal carcinoma

Key words: pinosylvin, nasopharyngeal cancer, matrix metalloproteinase, epithelial-mesenchymal transition, MAPK
The metastasis of NPC occurs in two stages: Translocation to distant tissue and colonization (11). The initial step degrades and penetrates the extracellular matrix of surrounding tissue (12). Among the involved proteolytic enzymes, zinc-dependent MMPs contribute substantially to proteolytic degradation and intercellular interaction damage (13).

Research has indicated that MMP-2 and MMP-9 are key treatment targets for regulation of tumor metastasis in NPC (14), cervical cancer (15) and retinoblastoma (16). Lyu et al (17) reported that liposome-containing thermosensitive liposomes can deliver MMP inhibitors, decreasing the activity of MMP-2 and MMP-9 by 50 and 43%, respectively, to inhibit metastasis and angiogenesis. Huang et al (18) demonstrated that exosomes with low expression levels of microRNA-34c-3p affect expression of integrin α2β1 and promote the invasion and migration of non-small cell lung cancer cells.

Epithelial-mesenchymal transition (EMT) is a key process involved in tumor metastasis and recurrence (19,20). Research has indicated that the expression of mesenchymal markers, such as vimentin and N-cadherin, increases during EMT, whereas epithelial marker E-cadherin, a powerful tumor cell invasion inhibitor, is downregulated (21,22). The MAPK pathway is an important intracellular signal transduction pathway that serves a key role in regulating tumor metastasis, as well as regulating cell proliferation, differentiation, apoptosis and angiogenesis (23). The ERK subfamily (typical ERK 1/2/5 and atypical ERK 3/4/7/8) of proteins is known for its contributions to EMT (23,24). PI3K/AKT and MAPK pathways contribute to TGF-β2-induced upregulation of Jagged-1, which mimics TGF-β2-induced EMT in retinal pigment epithelium cells (25). TGF-β, in addition to its role in cell differentiation, migration and adhesion, also induces EMT via both Smad and MAPK pathways (26). A previous study indicated that pinosylvin exerts antimetastatic effects on human oral cancer cells (27). However, the antiinflammatory effect of pinosylvin on NPC cells remains unknown. Therefore, the present study investigated the effect of pinosylvin on NPC cell metastasis and regulation of its signaling.

Materials and methods

Chemicals. Pinosylvin (≥97% purity) was purchased from ChemFaces. DMSO was used to prepare 100 mM storage solution of pinosylvin, which was stored at -20°C. The maximum concentration of DMSO used for treatment in medium was <0.2%. MTT, ERK1/2, p38 and JNK1/2 specific inhibitors (U0126, SB203580 and SP600125) were obtained from Sigma-Aldrich (Merck KGaA).

Cell culture. Nasal cavity cancer cells (RPMI 2650) were obtained from Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). Human nasopharyngeal cancer cell lines (NPC-039 and NPC-BM) were provided by Dr Jen-Tsun Lin, Department of Hematology and Oncology, Changhua Christian Hospital (Changhua, Taiwan). RPMI-2650 cells were cultured in Eagle’s Minimum Essential Medium (Gibco; Thermo Fisher Scientific, Inc.); NPC cell lines were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.). All culture media were supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.). 1 mM glutamine, 1% penicillin/streptomycin (10,000 U/ml penicillin and 10 mg/ml streptomycin), 1.5 g/l sodium bicarbonate and 1 mM sodium pyruvate. All cell cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂.

In vitro cytotoxicity assay (MTT assay). Cytotoxicity was assessed via MTT (0.1%) assay. All cells were cultured in 96-well plates (1x10⁴/well) at 37°C in 5% CO₂ overnight. Subsequently, supernatant was removed and cultures were treated with different concentrations of pinosylvin (0, 20, 40 and 80 µM) at 37°C for 24 h. Following treatment, the medium containing pinosylvin was removed and MTT reagent (1 mg/ml) was added to each well at 37°C in 5% CO₂. After 4 h, the supernatant containing MTT reagent was removed and DMSO was added to dissolve the formed blue formazan crystals. Absorbance was measured at 595 nm using spectrophotometry. A total of three independent experimental replicates was performed.

Gap closure assay. Gap closure assay was used to measure migration of NPC-039 and NPC-BM cells over a certain distance. NPC-039 and NPC-BM cells (3x10⁵) were grown onto each side of a culture insert (Ibidi GmbH) at 37°C overnight. After reaching 90% confluence, culture inserts were removed and gap closure assay was performed. Cultures were treated with pinosylvin (0, 20, 40 and 80 µM) in serum-free RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc.) at 37°C for 24 h. The cell migration distance was observed and photographed after 0, 3 and 6 h. Migration was measured using ImageJ 1.47 version software (National Institutes of Health) and expressed as a percentage using the following formula: (Initial gap width of the experimental group-remaining width of the experimental group)/(initial gap width of the control group-remaining width of the untreated control group) x100. Images were captured under a light microscope (Leica GmbH). The entire procedure was repeated three times and the values are indicated as mean ± SD.

Cell migration and invasion assay. NPC-039 and NPC-BM cells migration and invasion assays were performed as described by Yang et al (28). Briefly, NPC cells (3x10⁵) were placed on the upper well of a Transwell insert (Greiner Bio-One International GmbH) with serum-free medium (RPMI-1640) and 10% FBS-containing medium (RPMI-1640 medium) (600 µl) was added to the lower chamber for 24 h at 37°C. For the invasion assay, Matrigel (25 mg/50 ml; 60 µl; BD Biosciences) was coated on the upper Transwell at 37°C, overnight. Migrated or invaded cells were fixed with 99% methanol at room temperature for 15 min and stained with Giemsa (1X) at room temperature for 2 h. Images were captured and number of cells was counted under an optical light microscope (Leica Germany) at 100x magnification using ImageJ 1.47 version cell count software (National Institutes of Health). A total three fields of view was randomly selected for each concentration. Data are presented as the mean ± SD (n=3).

Gelatin zymography. Enzyme activity of MMP-2 was analyzed via gelatin zymography. Briefly, after plating NPC-039 and NPC-BM cells (5x10⁴ cells/well) in 24-well plates at 37°C for 16 h, cells were treated with different concentrations
Western blot analysis. Following treatment with different concentrations of pinosylvin, cells were lysed with 1X RIPA buffer (EMD Millipore) containing protease and phosphatase inhibitor cocktails and subjected to BCA (Thermo Fisher Scientific, Inc.) protein concentration assay. All samples were separated using 10.0 or 12.5% SDS-PAGE and proteins were transferred onto a PVDF membrane (EMD Millipore). Membranes were blocked with 5% non-fat milk in TBST (0.05% Tween-20) at room temperature for 1 h. Detection was performed with a primary antibody overnight at 4˚C followed by a horseradish peroxidase (HRP)-conjugated secondary antibody (Anti‑rabbit IgG, #7074, 1:3,000; Anti‑mouse IgG, #7076, 1:3,000, Cell Signaling Technology, Inc.) at room temperature for 1 h. The following antibodies (all 1:1,000; all Cell Signaling Technology, Inc. unless otherwise indicated) were used: Anti‑ERK1/2 (cat. no. #4695; 42, 44 kDa), anti‑JNK1/2 (cat. no. #9252; 46, 54 kDa), anti‑p38 (cat. no. #8690; 40 kDa), anti‑phosphorylated (phospho‑) ERK1/2 (cat. no. #4695; 42, 44 kDa), anti‑phospho‑JNK1/2 (cat. no. #4668; 46, 54 kDa), anti‑phospho‑p38 (cat. no. #4511; 43kDa), anti‑MMP‑2 (cat. no. #87809; 64 kDa), anti‑N‑cadherin (cat. no. #13116; 140 kDa), anti‑E‑cadherin (cat. no. #3195; 135 kDa), anti‑zonula occludens (ZO)‑1 (cat. no. #8193; 220 kDa), anti‑vimentin (cat. no. #5741; 57 kDa), anti‑MMP‑9 (cat. no. #AB19016; 92 kDa; EMD Millipore) and anti‑b‑actin (1:5,000; cat. no. NB600‑501; 42 kDa; Novus Biologicals). Immunoblotting was observed using HRP chemiluminescent substrate (EMD Millipore). Images were captured using ImageQuant LAS 4000 mini (GE Healthcare) and relative density was quantitated by ImageJ 1.47 version software (National Institutes of Health).

Statistical analysis. The experimental data are expressed as the mean ± SD (n≥3). Comparisons between >2 groups were analyzed by one‑way ANOVA followed by post hoc Tukey's test. Paired student's t‑test was used to analyze differences between two groups. All statistical analyses were performed using GraphPad Prism Software Version 5.0 (GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

Pinosylvin does not induce cytotoxicity in three cell lines. The cytotoxic effects of various concentrations of pinosylvin (0, 20, 40 and 80 µM) on cell lines were assessed using MTT assay for 24 h (Fig. 1A‑C). Pinosylvin did not exert significant cytotoxic effects on the viability of NPC‑039, NPC‑BM and RPMI‑2650 cell lines. All subsequent experiments examined antimetastatic properties of pinosylvin at non‑cytotoxic concentrations.

Pinosylvin inhibits migration and invasion in NPC cell lines. Gap closure assay was performed to assess the effect of pinosylvin on the mobility of NPC cells treated with 0‑80 µM pinosylvin for 0, 3 and 6 h (Fig. 2). Compared with the control group, the migrated distance of the cell monolayers was significantly decreased at high concentrations (80 µM) of pinosylvin. In addition, the effect of pinosylvin on the migration and invasion ability in NPC cells was assessed by Transwell assay (Fig. 3A‑D); pinosylvin significantly decreased the migration and invasion abilities of two NPC cell lines.

Pinosylvin changes migration of NPC cell line and inhibits MMP‑2 activity. According to the results of Proteome Profiler Human Protease Array (Fig. S1), to lack of observed differences. MMPs regulate cancer cell migration and invasion (13). In order to determine whether MMP-2 and MMP-9

(0, 20, 40 and 80 µM) of pinosylvin at 37°C for 24 h. Culture medium was collected and subjected to 8% SDS‑PAGE with 0.1% gelatin as described previously (29).
are regulated by pinosylvin in two NPC cell lines, zymography and western blotting were performed to analyze enzyme activity and protein concentration. Pinosylvin at the highest concentration significantly decreased enzymatic activity of MMP-2 in two NPC cell lines (Fig. 4A-D). Following 24 h treatment, a high pinosylvin concentration (80 µM) decreased expression levels of MMP-2 and MMP-9 to 54 and 66 in NPC-039 and 52 and 41% in NPC-BM cells, respectively (Fig. 4E-H).

**Pinosylvin affects EMT-associated protein expression in NPC cell lines.** When wound healing occurs, organ fibrosis and the initiation of metastasis in cancer progression prompt EMT (30). Analysis of expression levels of EMT-specific proteins (Fig. 5A-D) demonstrated that pinosylvin at a high concentration significantly decreased expression of vimentin and N-cadherin to 58.5 and 62.5 in NPC-039 and 55.0 and 58.5% in NPC-BM, respectively, and significantly increased expression of ZO-1 and E-cadherin to 80.0 and 101.5 in NPC-039 and 90.0 and 123.0% in NPC-BM, respectively.

**Pinosylvin decreases invasion and migration ability of NPC cell lines via MAPK pathways.** Western blotting was performed to detect changes in the molecular mechanisms of MAPK pathways in response to treatment with pinosylvin (Fig. 6A-D). As the concentration of pinosylvin increased, phosphorylation of ERK1/2 and p38 decreased significantly. According to ImageJ analysis of blots, treatment with 80 µM

---

Figure 2. Effect of pinosylvin on gap closure. Cell motility was determined by gap closure assay from 0 and 6 h in (A) NPC-039 and (B) NPC-BM. Migration of (C) NPC-039 and (D) NPC-BM cells was quantified. Data are presented as the mean ± SD (n=3). *P<0.05 vs. control. Scale bar, 100 µm. NPC, nasopharyngeal carcinoma.
Pinosylvin decreased the phosphorylation of ERK1/2 and p38 to 63 and 51 in NPC-039 cells and 59 and 46% in NPC-BM cells, respectively, at 24 h compared with untreated controls. By contrast, phosphorylation of JNK1/2 was significantly increased in the two NPC cell lines. In order to confirm the molecular mechanism underlying pinosylvin-induced inhibition of NPC cell migration, cells were pre-treated with specific inhibitors of ERK1/2, p38 and JNK1/2; following pre-treatment with specific inhibitors, pinosylvin-inhibited cell migration and invasion ability were significantly improved (Fig. 7A-D). Taken together, these findings indicate that pinosylvin exerted anti-metastatic effects via p38, ERK1/2 and JNK1/2 signaling pathways in human NPC cells.

Discussion

In an analysis of metastasis patterns of 629 patients with NPC, Huang et al (31) found that 95% of distant metastases occurred <3 years after completion of radiotherapy. Hence, determining effective methods of suppressing distant metastasis is important in the treatment of NPC. Plant polyphenols are important plant secondary metabolites with biological functions (such as countering infection by pathogens or mitigating environmental stresses), as well as antioxidant, anticancer and anti-inflammatory properties (1,3,5,7). Studying compounds with such biochemical activity is beneficial for drug development in the pharmaceutical industry (32). Pinosylvin and resveratrol are terpenoid polyphenols with similar structures (2). Research has indicated that pinosylvin inhibits growth of human colorectal cancer cells (4), suppresses MMP-2 and MMP-9 activity in HT1080 cells (5) and suppresses migration and invasion in SCC-9, SAS and HSC-3 cell lines (27). In the present study, pinosylvin did not decrease the viability of the two NPC cell lines or a nasal cavity cancer cell line (RPMI 2650), however, high concentrations of pinosylvin inhibited the migration and invasion of NPC-039 and NPC-BM cells. Furthermore, the present results indicated that pinosylvin inhibited NPC cell metastatic effects by downregulating MMP-2/MMP-9 expression levels and modifying the regulation of EMT markers.

MMPs serve important roles in mediating cancer cell growth, differentiation, apoptosis, migration, invasion and angiogenesis (33). Di Carlo et al (34) performed zymography analysis and demonstrated that the ratio of MMP-9/MMP-2 in patients with cancer was increased compared with that in patients with benign disease and healthy individuals. High expression of MMP-2 and MMP-9 is significantly correlated with local and distant metastatic tumor recurrence and poor prognosis in head and neck squamous cell carcinoma (35-37). In the present study, gelatin zymography and western blotting were performed to analyze the effects of pinosylvin on MMPs in two NPC cell lines; pinosylvin significantly inhibited expression of MMP-2 and MMP-9 as well as MMP-2 activity. Tissue inhibitors of metalloproteinase (TIMPs) control proteolytic activity and are a specific endogenous inhibitor of MMPs (38). Western blotting here showed that pinosylvin did not increase TIMP-1 or -2 protein levels in the two NPC cell lines (data not shown). This indicated that pinosylvin decreased MMP-2 protein expression levels and activity, via regulated the activation of zymogen at the post-transcriptional level.
EMT is a key step in tumor cell migration and invasion in various types of human cancer (39-41). Upregulation of N-cadherin induces EMT (40); another regulator of EMT is E-cadherin, which inhibits the occurrence of EMT and serves as a tumor suppressor (41). Vimentin is the primary cytoskeletal component of mesenchymal cells (42). ZO-1 and ZO-2 are required for tight junction formation and function (43,44); mutations in ZO-1 and claudin-1 induce EMT (45). In the present study, pinosylvin-treated NPC-BM and NPC-039 cells exhibited significantly induced E-cadherin and ZO-1 expression, but decreased expression of N-cadherin and vimentin. These findings suggest that pinosylvin inhibited EMT at the initiation step of tumor metastasis.

Compared with other intracellular signal transduction pathways (23), the MAPK pathway serves a more important role in cell proliferation, differentiation, apoptosis, angiogenesis and tumor metastasis (23,24). A study indicated that TBL-12, a sea cucumber extract, inhibits migration and invasion of human PCa cells by inhibiting MMP-2 and MMP-9 via decreased phosphorylation of p38 (46). Additionally, 18β-glycyrrhetinic acid inhibits migration and invasion of gastric cancer cells via the reactive oxygen species/protein kinase C-α/ERK signaling pathway (47). Therefore, the present study investigated whether the MAPK pathway is altered by pinosylvin treatment. Western blot analysis revealed that pinosylvin suppressed ERK1/2 and p38 protein phosphorylation but induced JNK protein phosphorylation in both NPC cell lines. This result is consistent with previously reported inhibition of Huh7 cell proliferation and metastasis by cucurbitacin E via suppression of MAPKs (48). A previous study showed that pinosylvin inhibits the growth of human colorectal cancer cells via suppression of Src/ERK and GSK-3/β-catenin signaling (4). In our previous research, pinosylvin inhibited migration and invasion of oral cancer cells by suppressing the expression and activity of MMP-2 and ERK1/2 signaling (27). The present results suggest that pinosylvin was involved in MMP-2/MMP-9 regulation in NPC cells and that the MAPK pathway may serve a key role.
Figure 5. Pinosylvin affects mesenchymal marker protein expression in NPC cell lines. Western blotting was used to measure the expression of mesenchymal marker proteins following 24 h pinosylvin treatment in (A and B) NPC-039 and (C and D) NPC-BM cell lines. ImageJ software was used for quantitative analysis of protein. Data are presented as the mean ± SD (n=3). *P<0.05 vs. control. NPC, nasopharyngeal carcinoma; ZO-1, zonula occludens-1.

Figure 6. Pinosylvin affects MAPK pathways in NPC cell lines. Western blotting was used to measure expression levels changes in phospho-p38, -ERK1/2 and -JNK1/2 following 24 h pinosylvin treatment in (A and B) NPC-039 and (C and D) NPC-BM cell lines. Data are presented as the mean ± SD (n=3). *P<0.05 vs. control. NPC, nasopharyngeal carcinoma; phospho-, phosphorylated.
Identifying effective methods for treating distant metastases resulting from NPC is crucial. In summary, the present results demonstrated that pinosylvin decreased activity of MMP-2 and expression of MMP-2/MMP-9 in both NPC-BM and NPC-039 cell lines. Pinosylvin significantly inhibited both cell migration and invasion. The expression levels of epithelial markers increased, while those of mesenchymal markers decreased following treatment with pinosylvin. Following pre-treatment with specific inhibitors of ERK1/2, p38 and JNK1/2, pinosylvin-inhibited cell migration and invasion significantly improved. However, the lack of activator experiments is a potential limitation to the present study. A recent study suggested that pinosylvin is mostly metabolized in vivo and may provide a material basis for studying the pharmacological action of pinosylvin, thus providing information for the clinical treatment of chronic gastritis and gastric ulcers using Radix Linderae Reflexae (49). The short half-life and limited systemic exposure of pinosylvin prompt caution in its therapeutic application (50). However, the lack of in vivo experiments is a potential limitation to the present study. The present results suggested that pinosylvin may be useful in the development of drugs for treating NPC and preventing migration and invasion of NPC cells.

Acknowledgements

Not applicable.

Funding

The present study was supported by Changhua Christian Hospital (grant no. 109-CCH-IRP-013).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors’ contributions

MCH, MJH and JTL conceptualized and designed the study. CCL, YCC, YSL and HYH received and interpreted data. MCH, YCC and MJH drafted and revised the manuscript.
17. Lyu Y, Xiao Q, Yin L, Yang L and He W: Potent delivery of an MMP inhibitor to the tumor microenvironment with thermo-responsive liposomes for the suppression of metastasis and angiogenesis. Signal Transd Ther 4: 26, 2019.

18. Huang W, Yan Y, Liu Y, Lin M, Ma J, Zhang W, Dai J, Li J, Guo Q, Chen H, et al: Exosomes with low miR-34c-3p expression promote invasion and migration of non-small cell lung cancer by upregulating integrin α2β1. Signal Transd Target Ther 5: 39, 2020.

19. Floor S, van Staveren WC, Larsimont D, Dumont JE and Maenhaut C: Cancer cells in epithelial-to-mesenchymal transition and tumor-propagating-cancer stem cells: Distinct, overlapping or same populations. Oncogene 30: 4609-4621, 2021.

20. Wan FZ, Chen KH, Sun YC, Chen XC, Liang RB, Chen L and Zhu XD: Exosomes overexpressing miR-34c inhibit malignant behavior and reverse the radioresistance of nasopharyngeal carcinoma. J Transl Med 18: 12, 2020.

21. Lin K, Baritaki S, Miliotis L, Malaponte G, Bevelacqua Y and Bonavida B: The role of B-RAF mutations in melanoma and the induction of EMT via dysregulation of the NF-kappaB/Snail/RKIP/PTEN Circuit. Genes Cancer 1: 409-420, 2010.

22. Nakamura M and Tokura Y: Epithelial-mesenchymal transition in the skin. J Dermatol Sci 61: 7-13, 2011.

23. Guo YJ, Pan WW, Liu SB, Shen ZF, Xu Y and Hu LL: TGF-β/β1-MAPK signalling pathway and tumorigenesis. Exp Ther Med 19: 1997-2007, 2020.

24. Olea-Flores M, Zuñiga-Eulogio MD, Mendoza-Catalán MA, Rodríguez-Ruiz HA, Castaña-Saucedo E, Ortúñov-Pineda C, Padilla-Benívades T and Navarro-Tito NE: Extracellular-signal-regulated kinase pathway: A central molecule driving epithelial-mesenchymal transition in cancer. Int J Mol Sci 20: 2885, 2019.

25. Chen X, Xiao W, Liu X, Zeng M, Luo L, Wu M, Ye S and Liu Y: Blockade of Jagged/Notch pathway abrogates transforming growth factor β2-induced epithelial-mesenchymal transition in human retinal pigment epithelial cells. Curr Mol Med 14: 523-534, 2014.

26. Balogh P, Katz S and Kiss AL: The role of endocytic pathways in TGF-β1 signaling. Pathol Oncol Res 19: 141-148, 2013.

27. Chen MK, Liu YT, Lin JT, Lin CC, Chuang YC, Lo YS, His YT and Hsieh MJ: Pinosylvin reduced migration and invasion of oral cancer carcinoma by regulating matrix metalloproteinase-2 expression and extracellular signal-regulated kinase pathway. Biomed Pharmacother 117: 109160, 2019.

28. Yang SF, Chu SC, Liu SJ, Chen YC, Chang YZ and Hsieh YS: Antimetastatic activities of Selaginella tamariscina (Beauv.) on lung cancer cells in vitro and in vivo. J Ethnopharmacol 110: 483-489, 2007.

29. Ho HY, Lin CW, Chien MH, Reiter RJ, Su SC, Hsieh YH and Yang SF: Melatonin suppresses TPA-induced metastasis by downregulating matrix metalloproteinase-9 expression through JNK/SAPK and NF-κB pathways. Neuroendocrinology 89: 479-497, 2014.

30. Nieto MA: Epithelial-mesenchymal transitions in development and disease: Old views and new perspectives. Int J Dev Biol 53: 1541-1547, 2009.

31. Huang CJ, Leung SW, Lian SL, Wang CJ, Fang FM and Ho YH: Patterns of distant metastases in nasopharyngeal carcinoma. Kaohsiung J Med Sci 12: 229-234, 1996.

32. Marienhagen J and Bott M: Metabolic engineering of microorganisms for the synthesis of plant natural products. J Biotechnol 163: 166-178, 2013.

33. Rahimzadeh, Z., Zari and Rahimi Z: Matrix metalloproteinase-9-1562T allele and its combination with MMP-2-735 C allele are risk factors for breast cancer. Asian Pac J Cancer Prev 16: 1175-1179, 2015.

34. Di Carlo A, Terracciano D, Mariano A and Macchia V: Matrix metalloproteinase-2 and matrix metalloproteinase-9 type IV collagenases in serum of patients with pleural effusions. Int J Oncol 26: 1363-1368, 2005.

35. Yoshizaki T, Maruyama Y, Sato H and Furukawa M: Expression of tissue inhibitor of matrix metalloproteinase-2 correlates with activation of matrix metalloproteinase-2 and predicts poor prognosis in tongue squamous cell carcinoma. Int J Cancer 95: 44-50, 2001.

36. Riedel F, Gottke K, Schwabl J, Bergler W and Hornmann K: Expression of 92-kDa type IV collagenase correlates with angiogenic markers and poor survival in head and neck squamous cell carcinoma. Int J Oncol 17: 1099-1105, 2000.

37. Huang CJ, Leung SW, Lian SL, Wang CJ, Fang FM and Ho YH: Patterns of distant metastases in nasopharyngeal carcinoma. Kaohsiung J Med Sci 12: 229-234, 1996.

38. Marienhagen J and Bott M: Metabolic engineering of microorganisms for the synthesis of plant natural products. J Biotechnol 163: 166-178, 2013.

39. Rahimzadeh, Z., Zari and Rahimi Z: Matrix metalloproteinase-9-1562T allele and its combination with MMP-2-735 C allele are risk factors for breast cancer. Asian Pac J Cancer Prev 16: 1175-1179, 2015.
38. Shrestha B, Bajracharya D, Byatnal AA, Kamath A and Radhakrishnan R: May High MMP-2 and TIMP-2 expressions increase or decrease the aggressivity of oral cancer? Pathol Oncol Res 23: 197-206, 2017.
39. Bagheri M, Kazli M, Saeednia S, Gholami Kharanagh M and Ahmadiankia N: Sulforaphane modulates cell migration and expression of β-catenin and epithelial mesenchymal transition markers in breast cancer cells. Iran J Public Health 49: 77-85, 2020.
40. Lopez-Novoa JM and Nieto MA: Inflammation and EMT: An alliance towards organ fibrosis and cancer progression. EMBO Mol Med 1: 303-314, 2009.
41. Techasen A, Loilome W, Namwat N, Khuntikeo N, Puapairoj A, Jearanaikoon P, Saya H and Yongvanit P: Loss of E-cadherin promotes migration and invasion of cholangiocarcinoma cells and serves as a potential marker of metastasis. Tumour Biol 35: 8645-8652, 2014.
42. Chernovishlyenkov IS, Minin AA and Minin AA: Role of vimentin in cell migration. Ontogenes 44: 186-202, 2013 (In Russian).
43. Helfand BT, Chang L and Goldman RD: Intermediate filaments are dynamic and motile elements of cellular architecture. J Cell Sci 117: 133-141, 2004.
44. Shin K, Fogg VC and Margolis B: Tight junctions and cell polarity. Annu Rev Cell Dev Biol 22: 207-235, 2006.
45. Oliveira SS and Morgado-Diaz JA: Claudins: Multifunctional players in epithelial tight junctions and their role in cancer. Cell Mol Life Sci 64: 17-28, 2007.
46. Yuan L, Huang X, Zhou K, Zhu X, Huang B, Qiu S, Cao K and Xu L: Sea cucumber extract TBL-12 inhibits the proliferation, migration, and invasion of human prostate cancer cells through the p38 mitogen-activated protein kinase and intrinsic caspase apoptosis pathway. Prostate 79: 826-839, 2019.
47. Cai H, Chen X, Zhang J and Wang J: 18β-glycyrrhetinic acid inhibits migration and invasion of human gastric cancer cells via the ROS/PKC-α/ERK pathway. J Nat Med 72: 252-259, 2018.
48. Liu Y, Yang H, Guo Q, Liu T, Jiang Y, Zhao M, Zeng K and Tu P: Cucurbitacin E inhibits Huh7 hepatoma carcinoma cell proliferation and metastasis via suppressing MAPKs and JAK/STAT3 pathways. Molecules 25: 560, 2020.
49. Fu Y, Sun X, Wang L and Chen S: Pharmacokinetics and tissue distribution study of pinosylvin in rats by Ultra-High-Performance liquid chromatography coupled with linear trap quadrupole orbitrap mass spectrometry. Evid Based Complement Alternat Med 2018: 4181084, 2018.
50. Yeo SC, Luo W, Wu J, Ho PC and Lin HS: Quantification of pinosylvin in rat plasma by liquid chromatography-tandem mass spectrometry: Application to a pre-clinical pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci 931: 68-74, 2013.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.