Original Article

Methyl vanillate for inhibiting the proliferation, migration, and epithelial-mesenchymal transition of ovarian cancer cells via the ZEB2/Snail signaling pathway

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Background: Globally, ovarian cancer is the leading cause of female reproductive-related death, with a 5-year survival rate below 50%. Conventional therapies, such as cancer cell reduction and paclitaxel chemotherapy, have strong toxicity and are prone to drug resistance. Thus, the development of alternatives for the treatment of ovarian cancer is urgently needed. Methyl vanillate is a principal component of *Hovenia dulcis* Thunberg. It is known that several cancer cells are inhibited by methyl vanillate; however, whether methyl vanillate can inhibit the proliferation and migration of ovarian cancer cells still needs to be further studied.

Methods: In this study, cell counting kit 8 (CCK8) was used to examine the effects of methyl vanillic acid on the proliferation of SKOV3 cell lines and human ovarian surface epithelial cell (HOSEpiC) lines. Wound healing and transwell assays were used to determine the effect of methyl vanillate on cell migration. The expression of epithelial-mesenchymal transition (EMT) marker proteins (E-cadherin and vimentin), transcription factors (Snail and ZEB2), and skeletal proteins (F-actin) were evaluated with Western blotting. F-actin was detected by immunofluorescence assay.

Results: The proliferation and migration of SKOV3 cells were dose-dependently inhibited by methyl vanillate, but HOSEpiC cells were not inhibited by low concentrations of methyl vanillate. Western blotting analyses revealed a significant decrease in the expression of vimentin and a significant increase in the expression of E-cadherin in SKOV3 cells treated with methyl vanillate. This finding indicated that EMT inhibition was induced by the vanillate. Furthermore, methyl vanillate inhibited the expression of transcription factors (Snail and ZEB2) in SKOV3 cells as well as cytoskeletal F-actin assembly.

Conclusions: Methyl vanillate plays an important role in inhibiting EMT and cell proliferation and the migration of ovarian cancer, likely via the inhibition of the ZEB2/Snail signaling pathway. Consequently, methyl vanillate may be a promising therapeutic drug for ovarian cancer.

Keywords: Epithelial-mesenchymal transition (EMT); methyl vanillate; ovarian cancer cells; migration

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Introduction

Ovarian cancer is the leading cause of reproductive-related death worldwide. According to estimates by the American Cancer Society, approximately 21,410 of women in the United States were diagnosed with ovarian cancer in 2021, with 13,770 dying due to this disease (1). Moreover, the 5-year survival rate after an ovarian cancer diagnosis is less than 50% (2). Based on the anatomic location, ovarian cancer mainly spreads in the peritoneum (3). It is a key site with recurrence in patients with ovarian cancer, leading to the death of patients from peritoneal complications (4). Conventional therapies, such as cancer cell reduction and paclitaxel chemotherapy, have strong toxicity and are prone to drug resistance. Thus, the development of effective alternative drugs for treating ovarian cancer is urgently needed.

In recent years, the pharmacological application of many traditional medicinal plants has been expanded. *Hovenia dulcis* Thunberg, a medicinal plant seed used in traditional Chinese medicine for detoxification after alcoholic poisoning, has been revealed to have further pharmaceutical applications based on its antitumor activity. Ammar *et al.* (5) found that the extract of *Hovenia dulcis* Thunb could reduce the level of oxidative stress and induce apoptosis in the leukemia cell line K562. Morales *et al.* (6) found that the extract of *Hovenia dulcis* Thunb had broad-spectrum antitumor activity against various cancer cell lines, such as MCF-7, HepG2, NCI-H460, and HCT15. In another study, the HT29 and HepG2 cell lines were significantly inhibited by *Hovenia dulcis* Thunb extract, with 80% inhibition at 100 g/mL (7).

To explain the pharmacological mechanism and enhance the pharmacological effect of *Hovenia dulcis* Thunb, one study isolated a single chemical component from *Hovenia dulcis* Thunb, and its biological function was investigated. Up to 8 phenolic compounds have been found in the stem bark of *Hovenia dulcis* Thunb, including phenolic acids (vanillate acid, ferulic acid, and trihydroxybenzoic acid), flavan-3-alcohol (catechin and Daphne odora), aromadendrin (dihydroflavonol), 3,5-dihydroxystilbene, and methyl vanillate (8). Among these compounds, methyl vanillate was revealed to inhibit the proliferation of human esophageal squamous carcinoma cells (oe21), human colon cancer cells (LoVo), mouse melanoma cells (B16F10), human esophageal adenocarcinoma cells (OE33), human prostate cancer cells (PC3), human glioblastoma (T98G), human glioma cells (u373), and human lung adenocarcinoma cells (A549) (9). However, whether methyl vanillate can inhibit the proliferation and migration of ovarian cancer cells still needs to be further studied.

The separation of ovarian cancer cells from the primary tumor is the first step in the abdominal spread of ovarian cancer (3), also known as epithelial-mesenchymal transition (EMT) (10). Tumor cells lose epithelial features, such as cell polarity, intercellular interactions, and cube shape. Tumor cells dedifferentiating into mesenchymal stem cells gain a greater potential for medication resistance and a greater ability to move quickly to new locations (11). A decrease in the epithelial marker E-cadherin and a concomitant increase in mesenchymal markers contribute to the migration of ovarian cancer cells (12). As a result of the weakening of the tight cell-cell connection (13), ovarian cancer cells have the basic conditions for peritoneal spread.

EMT is one of the molecular targets of many traditional Chinese medicines and plays a crucial role in tumor proliferation and migration (14). Normal epithelial cells lose their cell polarity and cell-cell adhesion during carcinogenesis but gain the capacity to migrate and invade. The transcription factors responsible for inducing EMT include several protein families, including ZEB2 and Snail (15).

We demonstrated the impact of methyl vanillate on the proliferation and migration of ovarian cancer cells. Furthermore, our results indicated that methyl vanillate might exert antitumor effects by inhibiting EMT through the ZEB2/Snail pathway. Therefore, this clarified the

Highlight box

Key findings

- Methyl vanillate plays an important role in inhibiting EMT and the cell proliferation and migration of ovarian cancer, which may be caused by the inhibition of ZEB2/Snail signaling pathway.

What is known and what is new?

- Methyl vanillate, a main component of *Hovenia dulcis* Thunberg, has been revealed to have further pharmaceutical applications based on its antitumor activity. Several cancer cells are inhibited by methyl vanillate. However, whether methyl vanillate can inhibit the proliferation and migration of ovarian cancer cells still needs to be further studied.
- We found that Methyl vanillate could inhibit the proliferation and migration of the ovarian cancer cell line SKOV3. This finding was related to the inhibition of transcription factors ZEB2 and Snail, which in turn inhibited EMT.

What is the implication, and what should change now?

- Our current results contribute to an increasing amount of evidence supporting the use of methyl vanillate as a potential anticancer drug for ovarian cancer.

crucial roles and potential mechanisms of methyl vanillate in ovarian cancer treatment. We present the following article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2240/rc).

Methods

Cell culture

SKOV3 (cat no. HTB-77) and human ovarian surface epithelial cells (HOSEpiCs; cat no. XY-XB-2078) were obtained from the American Type Culture Collection. The cells were cultured in RPMI 1640 and Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS), and 1% penicillin and streptomycin with 5% CO_2 in a 37 ℃ humidified incubator. In vitro studies were conducted with methyl vanillate purchased from Med Chem Express and dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) according to the manufacturer's protocols. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Cell counting kit 8 (CCK8)

CCK8 (Absin) was used to determine cell viability. The cells were plated in 96-well microplates (Corning) with a seeding density of 5×10^3 per well and 100 µL of the medium. Cells were treated with methyl vanillate at different concentrations (100, 200, 400, 800, and 1,600 µmol/L) for 24 hours. CCK8 reagent (10 µL) was added to each well and then cultured for 2 hours. All experiments were performed 3 times. The absorbance was measured at 450 nm using a microplate reader with a blank well (SPECTROstar). The proliferation of cells was reflected in the absorbance.

Monolayer wound healing assay

The migration ability was measured using wound healing assays. SKOV3 cells and HOSEpiCs were grown in Corning 6-well plates. After the cells reached 80% density, a horizontal line was created through each well using a 100-µL pipette tip. Then, the cells were incubated for 24 hours in a serum-free medium containing methyl vanillate (0, 50, 100, and 200 µmol/L). Each experiment was repeated at least 3 times. ImageJ software (US National Institutes of Health) was used to analyze the images and calculate the proportion of relative wound closure.

Transwell migration assay

Cell migration was assessed with Transwell chambers (Corning, USA). The cells were seeded at a density of 1.5×10^4 cells/well in 100 µL of FBS-free medium containing methyl vanillate (0, 50, 100, 200, and 400 µmol/L) in the upper chamber. The lower chambers were filled with 600 L of medium containing 20% FBS. We removed nonmigrated cells after 24 hours of incubation, fixed migrated cells with 4% paraformaldehyde, and stained them with 0.1% crystal violet. Following this, the cells were observed and photographed under an inverted microscope (ECLIPSE Ti, Nikon). Cells were counted in at least 5 random regions of each chamber, and then the average was calculated.

Western blotting

The total protein in the treated cells was extracted with radioimmunoprecipitation assay (RIPA) buffer containing 1 mmol/L of phenylmethylsulphonyl fluoride (PMSF; Beyotime) and quantified with a BCA Protein Assay Kit (Beyotime). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted to separated the protein bands, which were then transferred to a polyvinylidene difluoride (PVDF) membrane (MilliporeSigma). The PVDF membranes were blocked in phosphate-buffered saline (PBS) for 2 hours with 5% skimmed milk powder (MilliporeSigma). Primary antibodies against E-cadherin (rabbit monoclonal antibody, Cell Signaling Technology; cat. no. 3195S), vimentin (rabbit monoclonal antibody, Cell Signaling Technology; cat. no. 3879T), Snail (rabbit monoclonal antibody, Cell Signaling Technology; cat. no. 5741T), ZEB2 (rabbit monoclonal antibody, Cell Signaling Technology; cat. no. 97885S), and GAPDH (rabbit polyclonal antibody; Signalway Antibody; cat. no. 21612) were incubated at 4 ℃ overnight on PVDF membranes. The membrane was then washed 3 times with horseradish peroxidase (HRP)-conjugated secondary antibodies (goat anti-rabbit immunoglobin G secondary antibody; Signalway Antibody; cat. no. L3012) for 1 hour at room temperature. Finally, the blots were visualized using an enhanced chemiluminescence kit (Beyotime). ImageJ software (version 1.46) was used to measure the intensity of the Western bands.

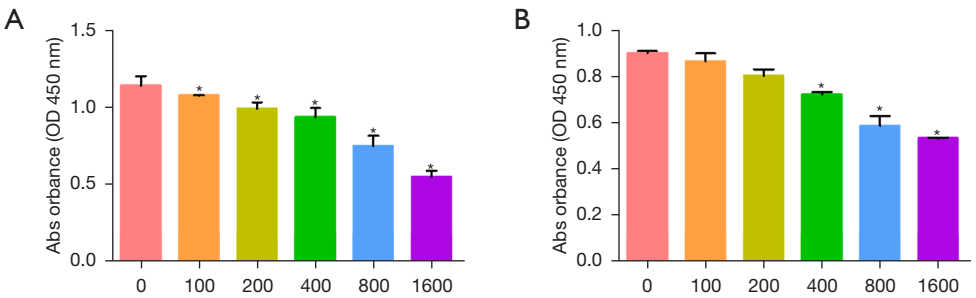

Figure 1 Impact of MV at different concentrations on proliferation of SKOV3 and HOSEpiC detected by the CCK8 assay. Statistical results of CCK8 analysis of SKOV3 cells (A) and HOSEpiC cells (B) treated with MV at 100, 200, 400, 800, 1,600 μmol/L for 24 h, respectively. Values represented the mean ± SD from three independent experiments. *, P<0.05 *vs.* 0 μmol/L. OD, optical density; MV, methyl vanillate; HOSEpiC, human ovarian surface epithelial cell; CCK8, cell counting kit 8.

Immunofluorescence staining

Indirect immunofluorescence staining was used to determine the cellular localization of F-actin. SKOV3 cells and HOSEpiCs were plated on polylysine-coated chamber slides. Cells were fixed for 20 minutes at 24 ℃ in 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. F-actin was stained with SF488-phalloidin (Solarbio; cat no. CA1640). We counterstained the cell nuclei with DAPI. Images were taken with a fluorescence microscope (ECLIPSE Ti, Nikon).

Statistical analysis

Each experiment was repeated at least 3 times. The results are presented as the mean ± SD. Data were assessed using SPSS 19.0 (IBM Corp.). The statistical significance of comparisons among 2 or 3 groups was determined with the Student *t*-test and 1-way analysis of variance, respectively. P values of less than 0.05 were considered statistically significant.

Results

Methyl vanillate inhibited SKOV3 cell and HOSEpiC proliferation

CCK8 assay was used to examine the effects of methyl vanillate on the proliferation of SKOV3 cells and HOSEpiCs. We found that methyl vanillate dose-dependently inhibited SKOV3 cell proliferation (*Figure 1A*). Interestingly, there was no statistically significant difference in the inhibitory effect of low concentrations of methyl vanillate (0–200 μmol/L) on HOSEpiC proliferation. Conversely, high concentrations of methyl vanillate (400–1,600 μmol/L) had a similar inhibitory effect on HOSEpiC proliferation as they did on SKOV3 cell proliferation (*Figure 1B*). These results suggest that low-dose methyl vanillate (0–200 μmol/L) can effectively inhibit the proliferation of SKOV3 cells and slightly decrease the proliferation of HOSEpiCs.

Methyl vanillate attenuated the migration of SKOV3 cells and HOSEpiCs

Wound healing and transwell assays were used to determine the effect of methyl vanillate on cell migration. As shown in *Figures 2,3*, when SKOV3 cells were treated with methyl vanillate (100 μmol/L), the wound healing percentage and the number of migrating cells were reduced. In contrast, the migration of HOSEpiC cells was not affected by methyl vanillate, which suggested that methyl vanillate could attenuate the migration of SKOV3 cells.

Methyl vanillate inhibited EMT activation by decreasing ZEB2 and Snail expression in SKOV3 cells and HOSEpiCs

Western blotting was performed to investigate the influence of different concentrations of methyl vanillate on EMT activation in SKOV3 cells. As shown in *Figure 4A*, compared with the control, methyl vanillate dose-dependently increased E-cadherin levels and decreased vimentin levels. In contrast, as shown in *Figure 4B*, the methyl vanillate-treated HOSEpiC group showed no significant difference compared with the control group.

To obtain further insights into the EMT-inducing transcription factor capability of methyl vanillate in ovarian

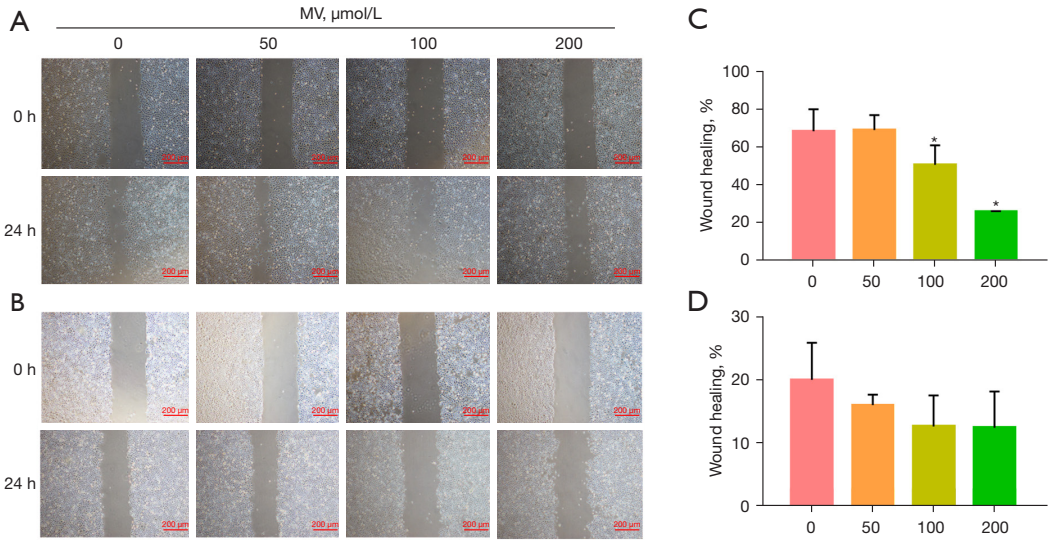

Figure 2 Impact of MV at different concentrations on the migration ability of SKOV3 and HOSEpiC illustrated by the monolayer wound healing assay. Typical optical images illustrating the scratch injury wound of SKOV3 (A) and HOSEpiC (B) at 24 h by the MV (50, 100 and 200 μmol/L). (C) Statistical results of the wound healing percentage of SKOV3 at 24 h based on the scratch injury wound. (D) Statistical results of the wound healing percentage of HOSEpiC at 24 h based on the scratch injury wound. Values represented the mean ± SD from three independent experiments. *, P<0.05 *vs*. 0 μmol/L. MV, methyl vanillate; HOSEpiC, human ovarian surface epithelial cell.

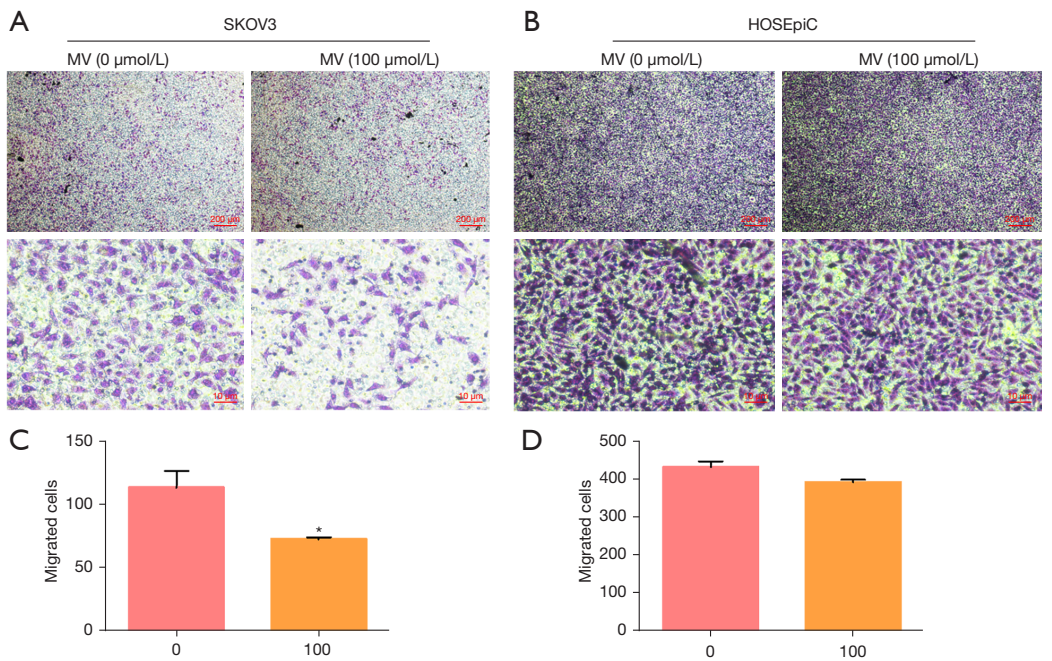

Figure 3 The effects of MV on the migration ability of SKOV3 and HOSEpiC illustrated by the Transwell assay. Typical optical images of SKOV3 (A) and HOSEpiC (B) illustrated cell migration at 24 h. The cells crossed through the pores of Transwell chamber were stained by crystal violet. (C) Statistical results of the migrated SKOV3 cell number based on the Transwell assay. (D) Statistical results of the migrated A2780 cell number based on the Transwell assay. Values represented the mean ± SD from three independent experiments. *, P<0.05 *vs*. MV (0 μmol/L). MV, methyl vanillate; HOSEpiC, human ovarian surface epithelial cell.

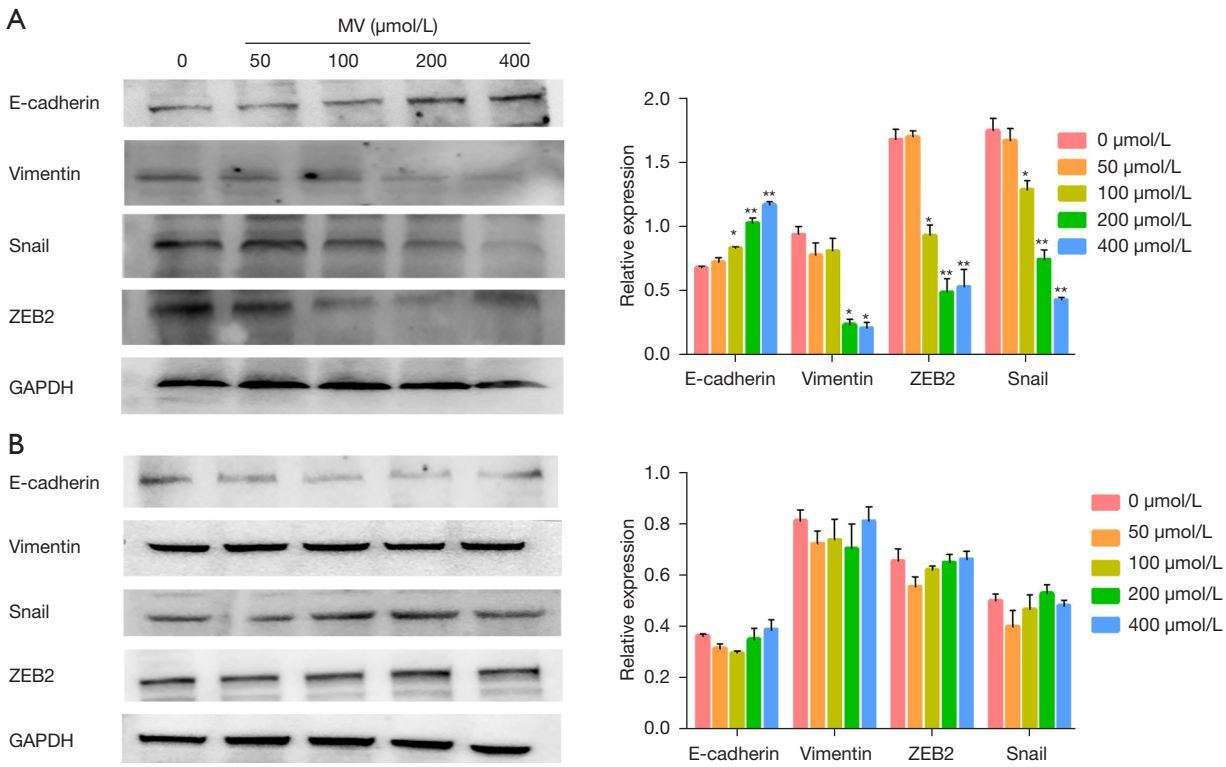

Figure 4 MV inhibited EMT process in SKOV3 by targeting ZEB2 and Snail. (A) The expression levels of EMT biomarkers, snail and ZEB2 in SKOV3 cells were quantified by image analysis of the Western blot bands. The expression of GAPDH in each group was taken as intrinsic controls, and relative expressions of each protein were calculated. (B) The expression levels of EMT biomarkers, snail and ZEB2 in HOSEpiC cells were quantified by image analysis of the Western blot bands. Values represented the mean ± SD from three independent experiments. *, P<0.05, **P<0.01 *vs.* MV (0 μmol/L). MV, methyl vanillate; EMT, epithelial-mesenchymal transition; HOSEpiC, human ovarian surface epithelial cell.

cancer, ZEB2 and Snail were detected using Western blotting. *Figure 4A* shows that the ZEB2 and Snail levels in the methyl vanillate-treated group were markedly lower than those in the control group. However, the methyl vanillate-treated HOSEpiC group showed no significant difference compared with the control group. The results revealed that treatment with methyl vanillate led to decreased EMT activity in SKOV3 cells by inhibiting ZEB2 and Snail levels. In addition, methyl vanillate had no effect on EMT or EMT-inducing transcription factor activity in HOSEpiCs.

The influence of methyl vanillate on the cytoskeleton of SKOV3 cells and HOSEpiCs

F-actin was detected using an immunofluorescence assay to determine whether methyl vanillate affected the cytoskeleton of SKOV3 cells and HOSEpiCs. Under methyl vanillate stimulation, SKOV3 cells formed fewer protrusions, while HOSEpiCs did not (*Figure 5*). Based on these results, methyl vanillate inhibited cytoskeletal F-actin assembly, attenuated the formation of cellular filopodia, and ultimately retarded cellular motility.

Discussion

One of the main components of *Hovenia dulcis* Thunb, methyl vanillate, is known to exert antitumor activity by suppressing cell proliferation and migration. Furthermore, methyl vanillate has been reported to suppress cell proliferation in diverse tumor cells (9). However, whether methyl vanillate acts on ovarian cancer cell proliferation remains unclear.

This study identified the effect of methyl vanillate on

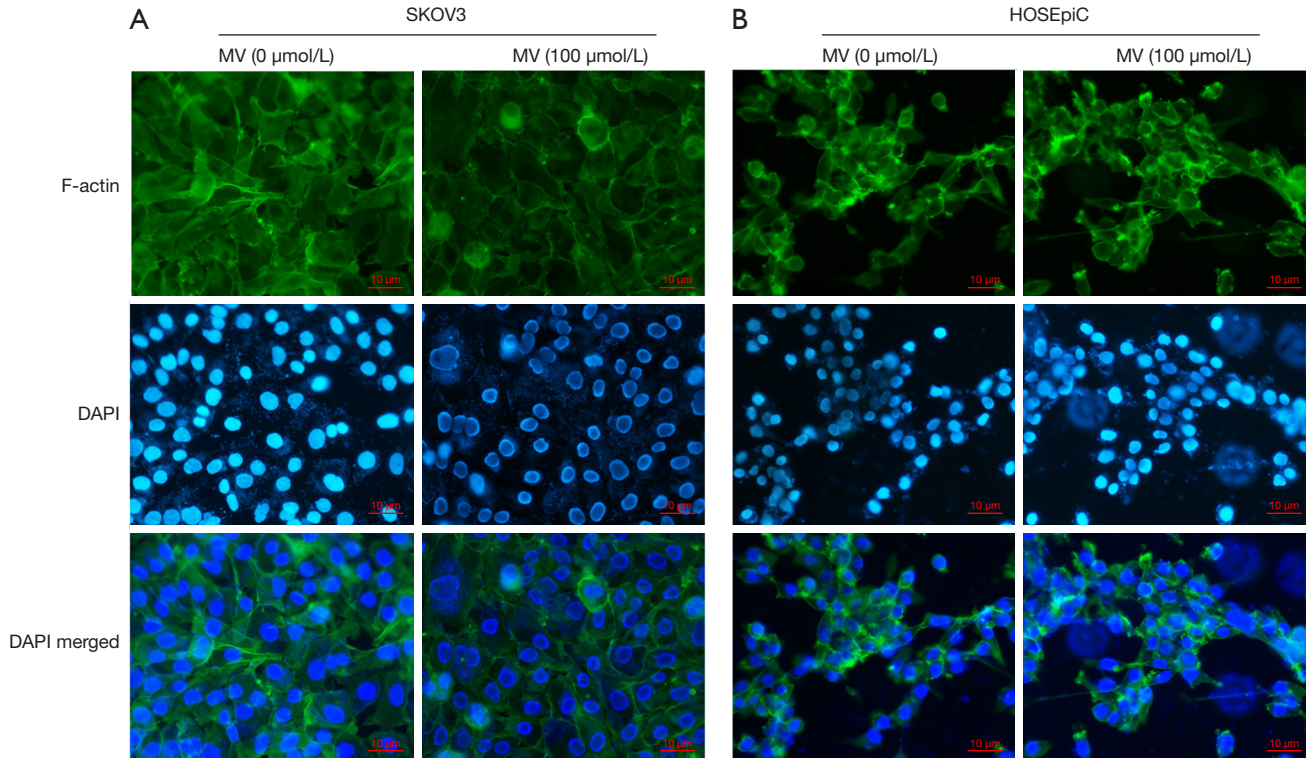

Figure 5 MV inhibits the formation of invadopodia in SKOV3 cells. (A) Representative immunofluorescence images show localization of DAPI (blue) and F-actin (green) as well as an overlay of both channels (merge) in SKOV3 cells. (B) Representative immunofluorescence images show localization of DAPI (blue) and F-actin (green) as well as an overlay of both channels (merge) in HOSEpiC cells. MV, methyl vanillate; HOSEpiC, human ovarian surface epithelial cell.

the proliferation and migration of ovarian cancer cells, which provides novel evidence for the antiproliferative activity of methyl vanillate. We found that methyl vanillate dramatically inhibited the proliferation and migration of SKOV3 cells in a dose-dependent manner while causing no toxicity in HOSEpiCs, the question remains as to the manner by which methyl vanillate inhibits ovarian cancer proliferation and migration. Under certain physiological conditions, EMT is a key mechanism required for malignancy, in which cancer cells express more mesenchymal markers than epithelial markers (16). Inhibition of E-cadherin expression and promotion of vimentin expression may enhance the invasive and metastatic abilities of ovarian cancer cells (17,18). In this study, we found that methyl vanillate led to a decreased vimentin protein level and an increased E-cadherin protein level. Therefore, methyl vanillate may weaken ovarian cancer EMT to inhibit ovarian cancer migration.

Furthermore, we investigated the possible mechanisms by which methyl vanillate inhibited ovarian cancer cells. Several transcription factors, including Snail and ZEB2, are known to downregulate E-cadherin expression to regulate EMT (19-21). ZEB2, the zinc finger EMT transcription factor, is highly expressed in metastatic ovarian cancer cells and is essential for ovarian cancer peritoneal metastasis (22-24). Snail is another pivotal transcription factor that induces EMT. Knockdown of Snail reverses stemness and inhibits tumor growth in ovarian cancer (25,26). However, it is not clear whether methyl vanillate affects ZEB2 and Snail. In our study, methyl vanillate significantly inhibited ZEB2 and Snail in SKOV3 cells. Thus, from our perspective, methyl vanillate downregulated ZEB2 and Snail expression, which weakened the EMT process and inhibited the proliferation and migration of ovarian cancer cells.

Conclusions

Our results constitute strong support for the anticancer effect of methyl vanilla. Methyl vanillate plays an important

role in inhibiting EMT, cell proliferation, and migration in ovarian cancer, possibly due to the inhibition of the ZEB2/Snail signaling pathway. Our findings contribute the growing body of evidence confirming the use of methyl vanilla as a potential anticancer drug for ovarian cancer.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2240/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2240/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-2240/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2022. CA Cancer J Clin 2022;72:7-33.
2. Moufarrij S, Dandapani M, Arthofer E, et al. Epigenetic therapy for ovarian cancer: promise and progress. Clin Epigenetics 2019;11:7.
3. Tan DS, Agarwal R, Kaye SB. Mechanisms of transcoelomic metastasis in ovarian cancer. Lancet Oncol 2006;7:925-34.
4. Kurnit KC, Fleming GF, Lengyel E. Updates and New Options in Advanced Epithelial Ovarian Cancer Treatment. Obstet Gynecol 2021;137:108-21.
5. Ammar RB, Neffati A, Skandrani I, et al. Anti-lipid peroxidation and induction of apoptosis in the erythroleukaemic cell line K562 by extracts from (Tunisian) Rhamnus alaternus L. (Rhamnaceae). Nat Prod Res 2011;25:1047-58.
6. Morales P, Maieves HA, Dias MI, et al. Hovenia dulcis Thunb. pseudofruits as functional foods: Phytochemicals and bioactive properties in different maturity stages. J Funct Foods 2017;29:37-45.
7. Park SH, Chang EY. Antimutagenic and Cytotoxic Effects of Hovenia dulcis Thumb Leaves Extracts. Korean J Soc Food Sci Nutr 2007;36:1371-6.
8. Li G, Min BS, Zheng C, et al. Neuroprotective and free radical scavenging activities of phenolic compounds from Hovenia dulcis. Arch Pharm Res 2005;28:804-9.
9. Lamoral-Theys D, Pottier L, Kerff F, et al. Simple di- and trivanillates exhibit cytostatic properties toward cancer cells resistant to pro-apoptotic stimuli. Bioorg Med Chem 2010;18:3823-33.
10. Yousefi M, Dehghani S, Nosrati R, et al. Current insights into the metastasis of epithelial ovarian cancer - hopes and hurdles. Cell Oncol (Dordr) 2020;43:515-38.
11. Heerboth S, Housman G, Leary M, et al. EMT and tumor metastasis. Clin Transl Med 2015;4:6.
12. Zhang S, Balch C, Chan MW, et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Res 2008;68:4311-20.
13. Vergara D, Merlot B, Lucot JP, et al. Epithelial-mesenchymal transition in ovarian cancer. Cancer Lett 2010;291:59-66.
14. Ge H, Xu C, Chen H, et al. Traditional Chinese Medicines as Effective Reversals of Epithelial-Mesenchymal Transition Induced-Metastasis of Colorectal Cancer:

Molecular Targets and Mechanisms. Front Pharmacol 2022;13:842295.
15. Brabletz S, Schuhwerk H, Brabletz T, et al. Dynamic EMT: a multi-tool for tumor progression. EMBO J 2021;40:e108647.
16. Matějka VM, Fínek J, Králíčková M. Epithelial-mesenchymal Transition in Tumor Tissue and Its Role for Metastatic Spread of Cancer. Klin Onkol 2017;30:20-7.
17. Gao M, Wu RC, Herlinger AL, et al. Identification of the NAC1-regulated genes in ovarian cancer. Am J Pathol 2014;184:133-40.
18. Arnouk H, Yum G, Shah D. Cripto-1 as a Key Factor in Tumor Progression, Epithelial to Mesenchymal Transition and Cancer Stem Cells. Int J Mol Sci 2021;22:9280.
19. Denecker G, Vandamme N, Akay O, et al. Identification of a ZEB2-MITF-ZEB1 transcriptional network that controls melanogenesis and melanoma progression. Cell Death Differ 2014;21:1250-61.
20. Drasin DJ, Robin TP, Ford HL. Breast cancer epithelial-to-mesenchymal transition: examining the functional consequences of plasticity. Breast Cancer Res 2011;13:226.
21. Peng Z, Wang CX, Fang EH, et al. Role of epithelial-mesenchymal transition in gastric cancer initiation and progression. World J Gastroenterol 2014;20:5403-10.
22. Liu C, Yang F. Akt2/ZEB2 may be a biomarker for exfoliant cells in ascitic fluid in advanced grades of serous ovarian carcinoma. Tumour Biol 2015;36:7213-9.
23. Scott CL, T'Jonck W, Martens L, et al. The Transcription Factor ZEB2 Is Required to Maintain the Tissue-Specific Identities of Macrophages. Immunity 2018;49:312-325.e5.
24. Song N, Liu H, Ma X, et al. Placental Growth Factor Promotes Ovarian Cancer Cell Invasion via ZEB2. Cell Physiol Biochem 2016;38:351-8.
25. Hojo N, Huisken AL, Wang H, et al. Snail knockdown reverses stemness and inhibits tumour growth in ovarian cancer. Sci Rep 2018;8:8704.
26. Taki M, Abiko K, Baba T, et al. Snail promotes ovarian cancer progression by recruiting myeloid-derived suppressor cells via CXCR2 ligand upregulation. Nat Commun 2018;9:1685.

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