

Olive oil polyphenols suppress the TGF- α -induced migration of hepatocellular carcinoma cells

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Abstract. Oleuropein and 3-hydroxytyrosol (3-HT) are natural polyphenols present in olive oil that are known to exhibit potent anti-oxidant activities and exert protective effects against a number of human diseases. In the liver, olive oil polyphenols have been demonstrated to prevent hepatocellular carcinoma (HCC) cell growth. However, little is known about their effects against HCC cell migration. Therefore, the present study investigated whether or not oleuropein and 3-HT were involved in the suppression of transforming growth factor- α (TGF- α)-induced migration of human HCC cells using human HCC-derived HuH7 cells. The TGF- α -induced migration of HuH7 cells was significantly and dose-dependently suppressed by oleuropein and 3-HT. This study group demonstrated previously that the TGF- α -induced activation of AKT and stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) were involved in HuH7 cell migration. In addition to these protein kinases, the present study examined the involvement of TGF- α -induced activation of extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK) and Rho kinase in HuH7 cell migration. TGF- α -induced HuH7 cell migration was decreased by SB203580, a p38 MAPK inhibitor, and Y27632, a Rho kinase inhibitor. However, PD98059, an inhibitor of the upstream kinase activating ERK, did not suppress the TGF- α -induced migration of HuH7 cells. Although AKT, SAPK/JNK, p38 MAPK and Rho kinase pathways were suggested to be involved in the TGF- α -induced migration of HuH7 cells, 10-30 μ M 3-HT did not exhibit any suppressive effect on the TGF- α -stimulated activities of these kinases. The results of the present study suggest that olive oil polyphenols suppressed the TGF- α -induced migration of HCC cells.

Introduction

Olive oil is a key ingredient in the Mediterranean diet, and a decreased incidence of cardiovascular disease and several types of cancer in people living in the Mediterranean region has been observed through epidemiological studies (1-3). The regular intake of extra-virgin olive oil decreases the oxidant status in humans, largely due to the anti-oxidant activities of olive oil polyphenols (1-6).

Oleuropein and 3-hydroxytyrosol (3-HT) are natural polyphenols present in olive oil, particularly in extra-virgin olive oil. These polyphenols may be divided into simple phenols, secoiridoids and lignans (2). 3-HT is a simple phenol and formed from the hydrolysis of the secoiridoid oleuropein (2). During the storage of olive oil, hydrolysis of oleuropein results in the production of 3-HT (2). *In vivo*, oleuropein is also time-dependently hydrolyzed into 3-HT in the stomach following consumption (7). These olive oil polyphenols are absorbed in the small intestine and accumulate in the plasma, urine and liver (7). Oleuropein has demonstrated strong anti-angiogenic properties, and it inhibits platelet aggregation and macrophage-mediated low-density lipoproteins (LDL) oxidation (3). 3-HT also decreases LDL oxidation and stimulates mitochondrial biosynthesis to prevent diabetes mellitus (2,3). In addition, these olive oil polyphenols exert anti-cancer effects; oleuropein exhibits anti-cancer activities in breast adenocarcinoma, melanoma, urinary bladder carcinoma, colorectal adenocarcinoma, prostate cancer, lung carcinoma, glioblastoma, renal cell adenocarcinoma and glioma (3), while 3-HT significantly inhibits cell proliferation of colon adenocarcinoma and exhibits cytotoxicity in breast cancer cells (3). The anti-proliferative, pro-apoptotic, anti-mutagenic, anti-inflammatory and anti-angiogenic effects of olive oil polyphenols contribute to their anti-cancer activities (1-3).

In the liver, olive oil polyphenols have been demonstrated to inhibit inflammation by decreasing the production of tumor necrosis factor- α , a proinflammatory cytokine, thereby preventing the liver damage that leads to steatohepatitis and hepatocellular carcinoma (HCC) (7). Furthermore, 3-HT suppresses HCC cell proliferation and induces HCC cell apoptosis by inhibiting the activation of NF- κ B (7).

Liver cancer is the second-most common cause of cancer-associated mortalities in the world (8). Chronic hepatic inflammation and tissue damage induce liver cancer (7,8).

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HCC accounts for 85-90% of all cases of primary liver cancer (8). Frequent recurrence and metastasis in patients with HCC have resulted in a relatively low survival rate of patients with HCC (8), with circulating HCC tumor cells considered to be the leading factor in the metastatic process (9,10). A number of growth factor-growth factor receptor signaling pathways are known to be involved in HCC progression (11-13). Transforming growth factor- α (TGF- α), a ligand for epidermal growth factor receptor (EGFR), and EGFR signaling pathways, including mitogen-activated protein kinases (MAPKs) and AKT pathways, are also known to be involved in metastatic recurrence of patients with HCC (11-14).

Polyphenols, including resveratrol and curcumin, have been identified to suppress HCC invasion (15,16). Although the anti-proliferative effects of olive oil polyphenols on HCC cells have been demonstrated, their effects on the migration of HCC cells remain unclear. The aim of the present study was to clarify the effects of olive oil polyphenols on HCC cell migration. It was demonstrated that oleuropein and 3-HT, olive oil polyphenols, suppressed the TGF- α -induced migration of human HCC-derived HuH7 cells.

Materials and methods

Antibodies and chemicals. Recombinant human TGF- α was obtained from R&D Systems, Inc. Oleuropein and 3-HT were purchased from Sigma-Aldrich; Merck KGaA. SB203580 was purchased from EMD Millipore. PD98059 and Y27632 were purchased from Calbiochem; Merck KGaA. Phospho-specific p38 MAPK antibodies (cat. no., 4511), p38 MAPK antibodies (cat. no., 9212), phospho-myosin phosphatase targeting subunit 1 (MYPT-1) antibodies (cat. no., 4653), MYPT-1 antibodies (cat. no., 2634), phospho-specific AKT (T308) antibodies (cat. no., 9275), AKT antibodies (cat. no., 9272), phospho-specific stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) antibodies (cat. nos., 4668 and 9252) were purchased from Cell Signaling Technology, Inc. GAPDH antibodies (cat. no., sc47724) were purchased from Santa Cruz Biotechnology, Inc. An ECL Western blotting detection system was purchased from GE Healthcare Life Sciences. Paraformaldehyde was obtained from Alfa Aesar, Thermo Fisher Scientific, Inc. Other chemicals were purchased from FUJIFILM Wako Pure Chemical Co. All other materials were obtained from commercial sources. Oleuropein, 3-HT, PD98059, SB203580 and Y27632 were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich; Merck KGaA). The maximum concentration of DMSO was 0.1%, which did not affect cell viability and the phosphorylated protein levels as determined by the cell migration assay and western blot analyses, respectively.

Cell culture. Human HCC-derived HuH7 cells (17) were obtained from the Japanese Collection of Research Bioresources Cell Bank (JCRB0403). The cells were maintained in RPMI-1640 (Sigma-Aldrich; Merck KGaA) containing 10% fetal calf serum (FCS; Hyclone; GE Healthcare Life Sciences) at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. For western blot analyses, the cells were seeded into 100-mm diameter dishes (7x10⁵ cells/dish) in RPMI-1640 medium containing 10% FCS. After 3 days, the medium was

replaced with serum-free RPMI-1640 medium. The cells were then used for experiments after 24 h. For the cell migration assay, the cells were seeded into 100-mm diameter dishes (4x10⁵ cells/dish) in RPMI-1640 medium containing 10% FCS for 4 days and then used for experiments.

Cell migration assay. A Transwell cell migration assay was performed using a Boyden chamber (polycarbonate membrane with 8 μ m pores, Transwell; Costar; Corning, Inc.) as described previously (18,19). Briefly, the cultured cells were seeded (1x10⁵ cells/well) onto the upper chamber in serum-free RPMI-1640 medium. When required, the cells were pretreated with oleuropein (0, 10, 30 or 100 μ M), 3-HT (0, 1, 3 or 10 μ M), PD98059 (0 or 50 μ M), SB203580 (0 or 7 μ M) or Y27632 (0 or 3 μ M) in the upper chamber for 60 min at 37°C. To investigate whether or not 3-HT interacted physically with TGF- α , the medium including 3-HT was removed from the Boyden chamber following pretreatment. TGF- α was then added to the lower chamber for 23 h at 37°C. Subsequent to incubation, the cells on the upperside of the membrane were mechanically removed. The migrated cells adherent to the underside of the membrane were fixed with 4% paraformaldehyde (Alfa Aesar, Thermo Fisher Scientific, Inc.) for 20 min at room temperature, and stained with 1:500 DAPI solution for 10 min at room temperature. Images of the migrated cells were then captured and counted using fluorescence microscopy at magnification, x20, by counting the stained cells in 3 randomly chosen high power fields.

Western blot analysis. To examine the effect of time of TGF- α stimulation on p38 MAPK and Rho kinase, the cultured cells were stimulated with 10 ng/ml TGF- α for the indicated periods (0, 1, 3, 5, 10, 20 and 30 min). To examine the effect of olive oil polyphenols on the TGF- α -stimulated AKT, SAPK/JNK, p38 MAPK and Rho kinase pathways, the cultured cells were pretreated with the indicated doses of 3-HT (0, 1, 3 or 10 μ M) or oleuropein (0, 10, 30 or 100 μ M) for 60 min and then stimulated with 10 ng/ml TGF- α or vehicle for 3 min for AKT, 15 min for SAPK/JNK, 5 min for p38 MAPK and 1 min for MYPT-1 at 37°C. The cells in each dish were washed twice with PBS and then lysed and sonicated in 800 μ l lysis buffer [62.5 mM Tris-HCl (pH 6.8), 2% SDS, 50 mM dithiothreitol and 10% glycerol]. Proteins were separated via SDS-PAGE, as described by Laemmli (20). A total of 10 μ l lysates were applied per lane of all SDS-PAGE gels. Then, 10% SDS-PAGE gel was used for analyses of AKT, SAPK/JNK, p38 MAPK and GAPDH, and 7.5% gel was used to analyze MYPT-1. Proteins were then transferred to on polyvinylidene difluoride membranes (Bio-Rad Laboratories, Inc.). Blocking was performed prior to incubation with the primary antibodies using 5% skim milk for 1 h at room temperature. Western blot analyses were performed as described previously (18,19,21) using phospho-specific AKT (T308) (1:1,000), AKT (1:10,000), phospho-specific SAPK/JNK (1:1,000), SAPK/JNK (1:3,000), phospho-specific p38 MAPK (1:20,000), p38 MAPK (1:20,000), phospho-specific MYPT-1 (1:1,000), MYPT-1 (1:1,000) and GAPDH (1:1,000) antibodies with horseradish peroxidase (HRP)-labeled anti-rabbit IgG and anti-mouse IgG antibodies (1:1,000; cat. nos., 7074 and 7076, respectively, Cell Signaling Technology, Inc.) as secondary antibodies. Incubation time and temperature for primary antibodies and

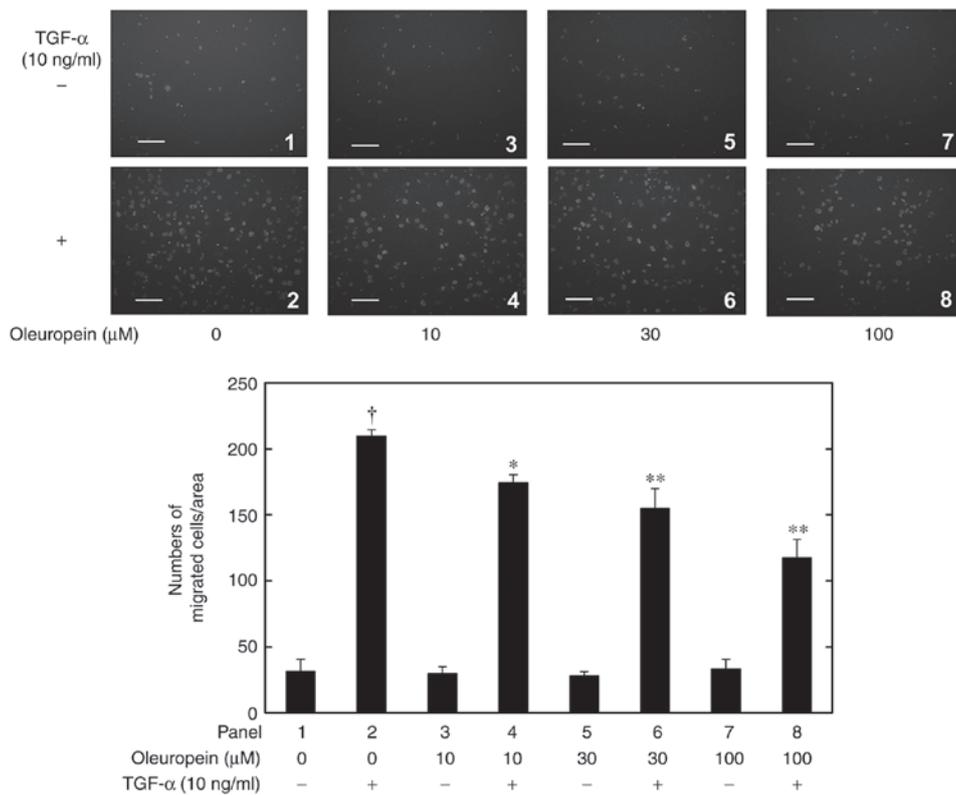


Figure 1. Effects of oleuropein on the TGF- α -induced cell migration of HuH7 cells. The cells were pretreated with the indicated concentrations of oleuropein for 60 min and then stimulated with 10 ng/ml of TGF- α or vehicle for 23 h. The migrated cells were fixed with paraformaldehyde and stained with DAPI for the nuclei. The cells were photographed by fluorescence microscopy at magnification, x20 (upper panel) and counted. Quantitative data are presented in the bar graph (lower panel). Each value represents the mean \pm standard deviation of data from 3 independent cell preparations performed in triplicate. †P<0.001 vs. control cells without TGF- α . *P<0.05 and **P<0.001 vs. cells with TGF- α alone. Scale bar=100 μ m. TGF- α , transforming growth factor- α .

secondary antibodies were 16-64 h at 4°C and 1 h at room temperature, respectively. The HRP activity was visualized on an X-ray film using an ECL Western blotting detection system (GE Healthcare Life Sciences). Each protein was detected on different gels. Densitometric analyses of the western blot analysis data were performed using a scanner and an image analysis software program (ImageJ v1.48; National Institutes of Health). The phosphorylated protein levels were calculated as follows: The background-subtracted signal intensity of each phosphorylation signal was normalized to the respective intensities of total protein and GAPDH, and then plotted as the fold increase in comparison with that of the control cells without stimulation.

Statistical analyses. The data were analyzed by using SPSS software (v. 24.0, IBM Corp.). The data are expressed as the mean \pm standard deviation of data from 3 independent cell preparations performed in triplicate. The statistical significance of the data from the cell culture experiments was analyzed by two-way analysis of variance followed by Tukey post-hoc test for multiple comparisons between pairs. P<0.05 was considered to indicate a statistically significant difference.

Results

Effects of oleuropein and 3-HT on the TGF- α -induced migration of HuH7 cells. Whether or not olive oil polyphenols affected the TGF- α -induced HuH7 cell migration

was first examined. As demonstrated in Fig. 1, oleuropein significantly and dose-dependently suppressed the 10 ng/ml TGF- α -induced migration of human HCC-derived HuH7 cells at concentrations of 10-100 μ M. Oleuropein alone did not affect the cell migration, even at 100 μ M. Similarly, 3-HT, which also did not affect the cell migration alone, significantly inhibited the 10 ng/ml TGF- α -induced HuH7 cell migration in a dose-dependent manner at concentrations of 1-10 μ M (Fig. 2A). In addition, whether or not 3-HT interacted physically with TGF- α and inhibited its migratory activity was investigated. Following pretreatment of HuH7 cells by 10 μ M 3-HT for 60 min, the medium including 3-HT was washed from the Boyden chamber, and then the cells were stimulated with 10 ng/ml TGF- α . As indicated in Fig. 2B, the TGF- α -stimulated migration of HuH7 cells with 3-HT-pretreatment was significantly decreased compared with the control cells.

Effects of PD98059, SB203580 or Y27632 on TGF- α -induced migration of HuH7 cells. Regarding the intracellular signaling system of TGF- α , this study group demonstrated previously that TGF- α induced the activation of the extracellular signal-regulated kinase (ERK), SAPK/JNK and AKT pathways and promotes the cell proliferation of human HCC-derived HuH7 cells (21). In addition to these pathways, it is generally recognized that the p38 MAPK (22) and Rho kinase (23) pathways are also involved in HCC cell functions. Therefore, the present study examined whether or not TGF- α

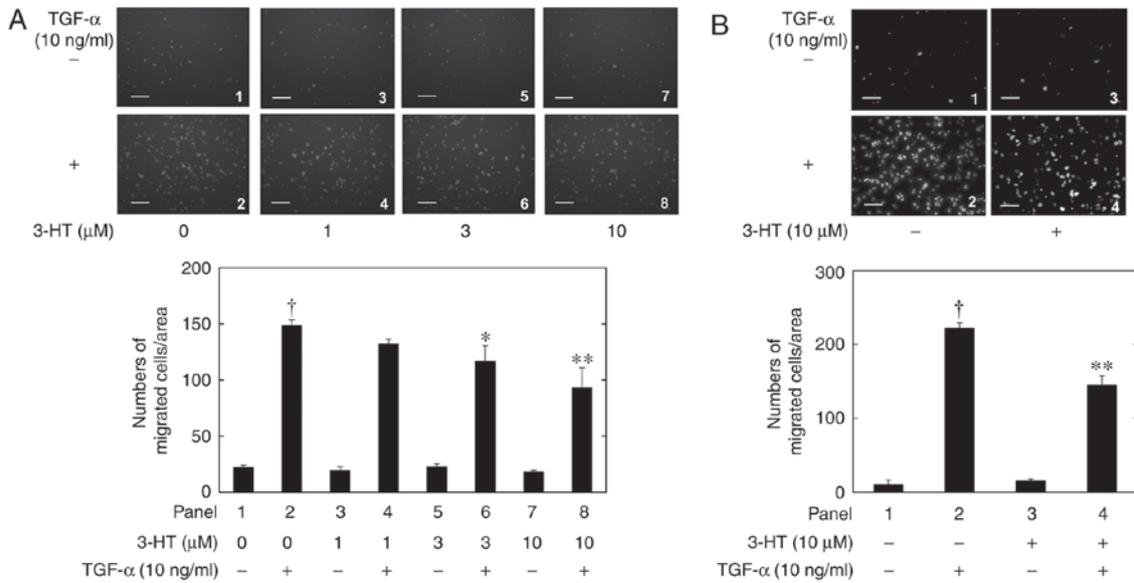


Figure 2. Effect of 3-HT on the TGF- α -induced cell migration of HuH7 cells. (A) The cells were pretreated with the indicated concentrations of 3-HT for 60 min and then stimulated with 10 ng/ml of TGF- α or vehicle for 23 h. (B) The cells were pretreated with 10 μ M of 3-HT for 60 min. Following pretreatment, the medium including 3-HT was washed from the Boyden chamber, and then the cells were stimulated with 10 ng/ml of TGF- α or vehicle for 23 h. The migrated cells were fixed with paraformaldehyde and stained with DAPI for the nuclei. Images of the cells were captured by fluorescent microscopy at magnification, x20 (upper panel) and quantitative data are presented in the bar graphs (lower panels). Each value represents the mean \pm standard deviation of data from 3 independent cell preparations performed in triplicate. [†]P<0.001 vs. control cells without TGF- α . ^{*}P<0.05 and ^{**}P<0.001 vs. cells with TGF- α alone. Scale bar=100 μ m. 3-HT, 3-hydroxytyrosol; TGF- α , transforming growth factor- α .

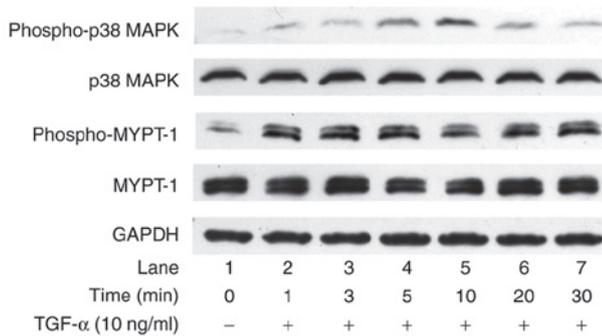


Figure 3. Effects of TGF- α on the phosphorylation of p38 MAPK and MYPT-1 in HuH7 cells. The cultured cells were stimulated with 10 ng/ml TGF- α for the indicated time periods. The extracts of cells were then subjected to SDS-PAGE with subsequent western blot analyses using antibodies against phospho-specific p38 MAPK, p38 MAPK, phospho-specific MYPT-1, MYPT-1 or GAPDH. TGF- α , transforming growth factor- α ; phospho, phosphorylated; p38 MAPK, p38 mitogen-activated protein kinase; MYPT-1, myosin phosphatase targeting subunit 1.

induced the phosphorylation of p38 MAPK and MYPT-1, a downstream substrate of Rho kinase, in HuH7 cells. As revealed in Fig. 3, TGF- α enhanced the phosphorylation of p38 MAPK and MYPT-1 in HuH7 cells. These results suggested that TGF- α activated the p38 MAPK and Rho kinase pathways, in addition to the ERK, SAPK/JNK and AKT pathways, in HuH7 cells.

This study group recently demonstrated that TGF- α induced the migration of HuH7 cells via the SAPK/JNK (18) and AKT (19) pathways. Therefore, the present study examined whether or not the ERK, p38 MAPK and Rho kinase pathways were also involved in the TGF- α -induced HuH7 cell migration. PD98059, an inhibitor of the upstream kinase

of ERK (24), did not exhibit a suppressive effect on the TGF- α -induced HuH7 cell migration (Fig. 4A). By contrast, the p38 MAPK inhibitor SB203580 (25) (Fig. 4B) and the Rho kinase inhibitor Y27632 (26) (Fig. 4C) significantly suppressed the TGF- α -induced HuH7 cell migration. This suggested that the p38 MAPK and Rho kinase pathways, but not the ERK pathway, are involved in the TGF- α -induced HuH7 cell migration.

Effects of olive oil polyphenols on the TGF- α -stimulated phosphorylation of AKT, SAPK/JNK, p38 MAPK and MYPT-1 in HuH7 cells. Whether or not 3-HT affected the TGF- α -activated AKT, SAPK/JNK, p38 MAPK and Rho kinase pathways was examined. As indicated in Fig. 5, the TGF- α -stimulated phosphorylation of AKT, SAPK/JNK, p38 MAPK and MYPT-1 was not suppressed by 3-HT concentrations \leq 30 μ M (Fig. 5A-D). In addition, 3-HT conversely enhanced the TGF- α -induced activation of p38 MAPK at 20 and 30 μ M (Fig. 5C). Therefore, whether or not oleuropein activated p38 MAPK activity as 3-HT in HuH7 cells was investigated. As demonstrated in Fig. 5E, oleuropein did not affect the TGF- α -induced p38 MAPK phosphorylation.

Discussion

The beneficial effects of olive oil polyphenols on human health, in particular their anti-oxidant effects, have been evaluated by a number of studies (1-3). In addition to their anti-oxidant activities, oleuropein and 3-HT are also known to exhibit anti-cancer properties (1-3). For example, 3-HT and other extra-virgin olive oil polyphenols were demonstrated to induce apoptosis of HCC cells and inhibit their proliferation (7,27-29). However, while there are a number of previous

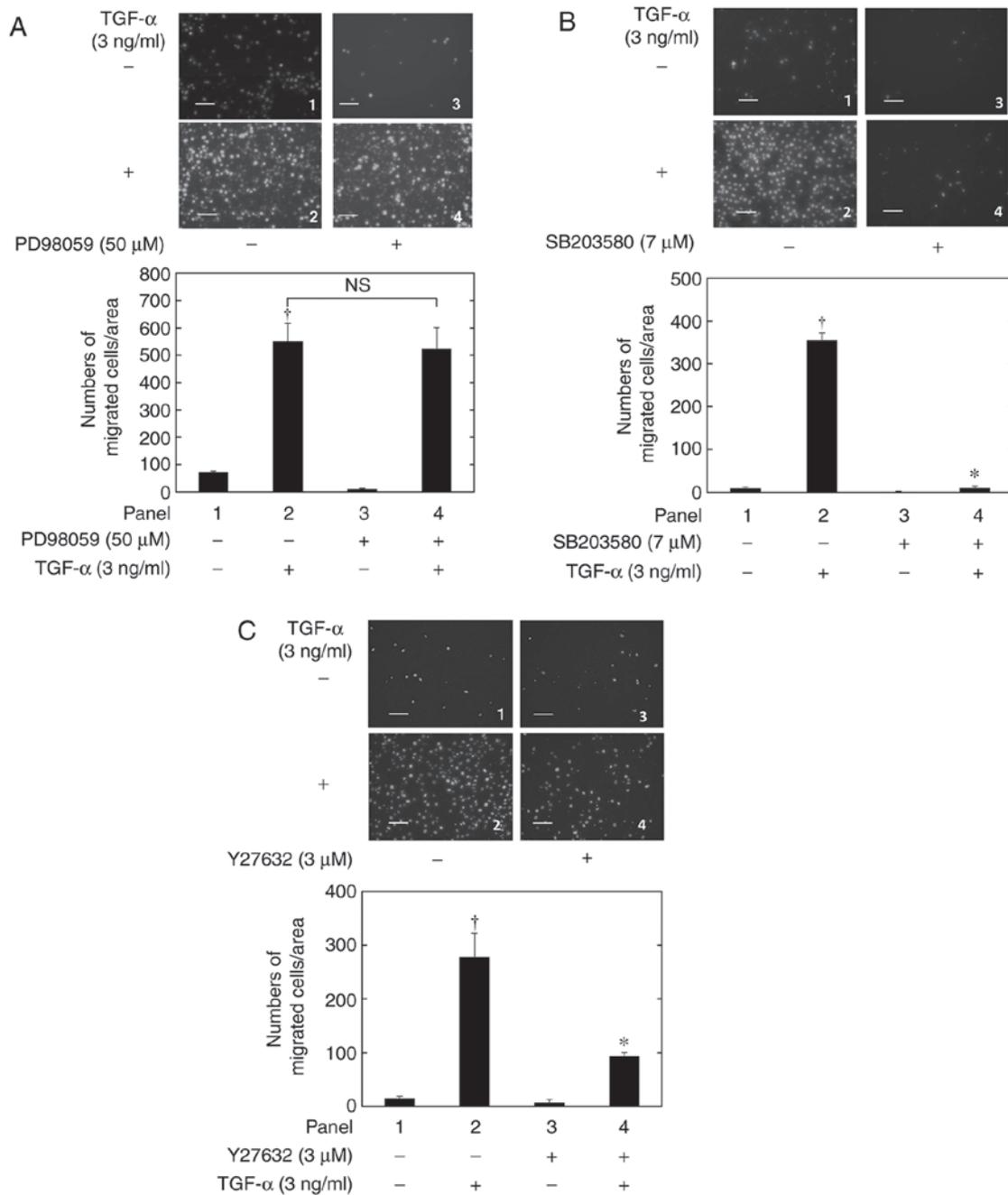


Figure 4. Effects of PD98059, SB203580 or Y27632 on the TGF- α -induced migration of HuH7 cells. The cells were pretreated with the indicated doses of (A) PD98059, (B) SB203580 and (C) Y27632 for 60 min, and then stimulated with 3 ng/ml TGF- α or vehicle for 23 h. The migrated cells were fixed with paraformaldehyde and stained with DAPI to visualize the nuclei. Images of the cells were captured by fluorescence microscopy at magnification, x20 (upper panels) and quantitative data are presented in the bar graphs (lower panels). Each value represents the mean \pm standard deviation of data from 3 independent cell preparations performed in triplicate. †P<0.001 vs. control cells without TGF- α . *P<0.001 vs. cells with TGF- α alone. Scale bar=100 μ m. TGF- α , transforming growth factor- α ; N.S., no significant difference between the indicated pairs.

studies concerning the protective effects of olive oil polyphenols on the liver (7), to the best of our knowledge, none have described the effects of olive oil polyphenols on HCC cell migration.

TGF- α , a ligand for EGFR, has demonstrated high expression levels in human metastatic liver tumors (11). Furthermore, the activation of the EGFR signaling pathway is known to enhance HCC cell movement (13), and this study group previously demonstrated that TGF- α induced the migration of human HCC-derived HuH7 cells (18,19). Therefore, in

the present study, whether or not olive oil polyphenols were involved in the TGF- α -induced migration of HuH7 cells was examined.

It was demonstrated that the TGF- α -induced migration of HuH7 cells was significantly and dose-dependently suppressed by oleuropein and 3-HT. To the best of our knowledge, this is the first study investigating the inhibitory effect of olive oil polyphenols on HCC cell migration. The data suggested that oleuropein and 3-HT at 10 and 1 μ M, respectively, significantly inhibited the TGF- α -induced migration of HuH7 cells, after

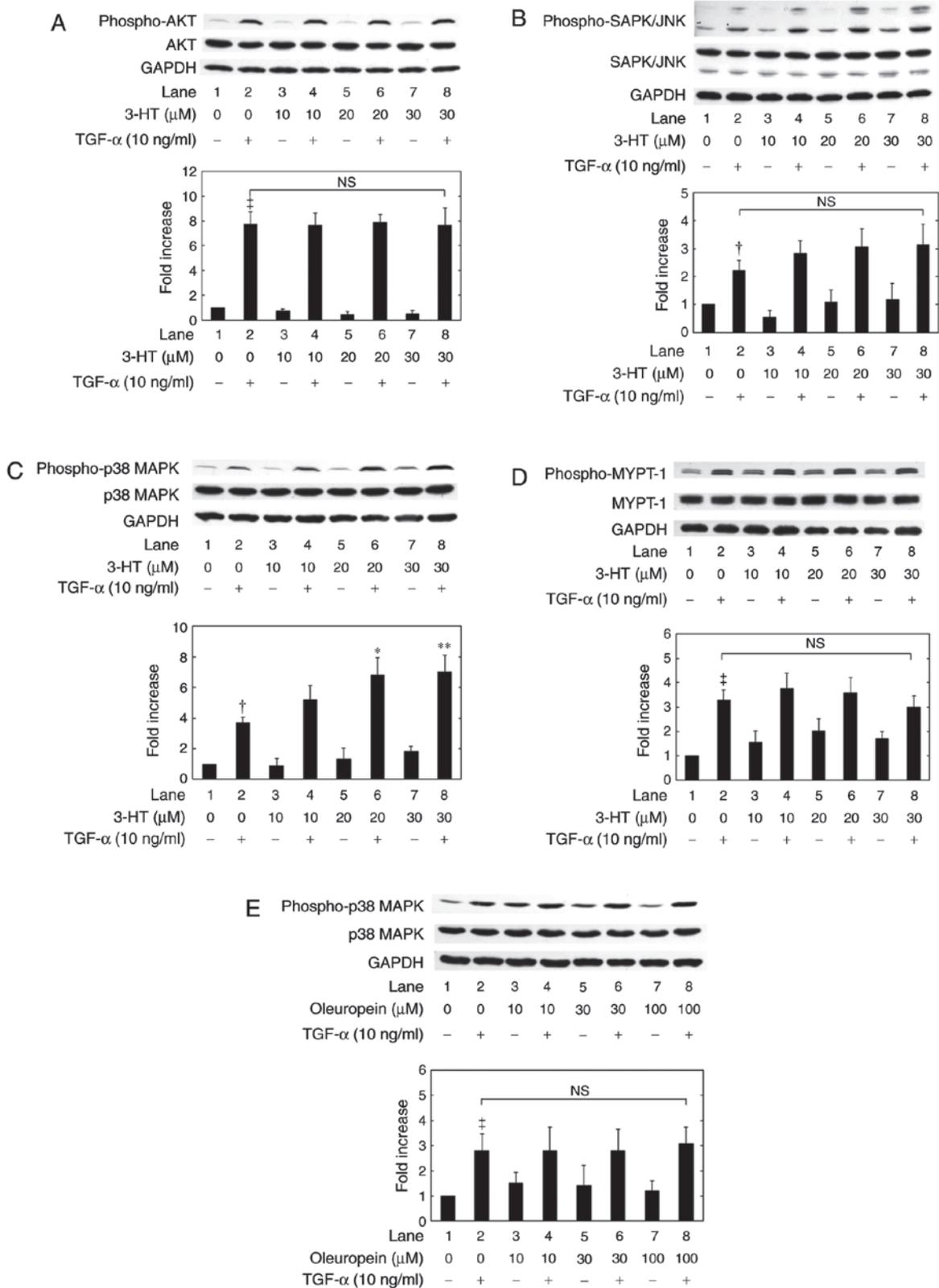


Figure 5. Effects of 3-HT and oleuropein on TGF-α-induced phosphorylation. The levels of (A) AKT, (B) SAPK/JNK, (C) p38 MAPK and (D) MYPT-1 phosphorylation following 3-HT treatment were measured using western blot analysis. (E) Effects of oleuropein treatment on the TGF-α-induced phosphorylation of p38 MAPK in HuH7 cells. The cells were pretreated with the indicated doses of 3-HT or oleuropein for 60 min and then stimulated by 10 ng/ml TGF-α or vehicle for (A) 3 min, (B) 15 min, (C) 5 min, (D) 1 min or (E) 5 min. The extracts of cells were then subjected to SDS-PAGE with subsequent western blot analyses using antibodies against phospho-specific AKT, AKT, phospho-specific SAPK/JNK, SAPK/JNK, phospho-specific p38 MAPK, p38 MAPK, phospho-specific MYPT-1, MYPT-1 or GAPDH. The histograms represent the quantification of the levels of TGF-α-induced phosphorylation obtained from laser densitometric analysis of 3 independent experiments. The phosphorylation levels were corrected by the total protein levels and the levels of GAPDH, and then expressed as the fold increase compared with the basal levels presented in lane 1. Each value represents the mean ± standard deviation of data from 3 independent cell preparations performed in triplicate. †P<0.05 and ‡P<0.001 vs. control cells without TGF-α. *P<0.05 and **P<0.01 vs. cells with TGF-α alone. 3-HT, 3-hydroxytyrosol; TGF-α, transforming growth factor-α; p38 MAPK, p38 mitogen-activated protein kinase; MYPT-1, myosin phosphatase targeting subunit 1; SAPK/JNK, stress-activated protein kinase/c-Jun N-terminal kinase; N.S., no significant difference between the indicated pairs.

24 h of treatment. Although oleuropein and 3-HT induced the apoptosis of HCC cells, 24 h treatment with 20 μ M oleuropein, and 48 h treatment with 100 μ M 3-HT did not decrease the viability of HuH7 cells (27,28). The concentration and treatment period of oleuropein and 3-HT required to suppress the TGF- α -induced migration of HuH7 cells were much lower compared with those required to induce apoptosis of HCC cells (27,28).

This study group previously demonstrated that TGF- α induced the migration of HuH7 cells via the JNK (18) and AKT (19) pathways. In the present study, it was revealed that the p38 MAPK and Rho kinase pathways were also involved in the TGF- α -induced migration of HuH7 cells, while the ERK pathway was not involved. Although the activation of the ERK pathway is generally considered to induce HCC progression, it may not be involved in TGF- α -induced HCC cell migration. Therefore, the present study examined whether or not 3-HT decreased the TGF- α -induced activation of the AKT, SAPK/JNK, p38 MAPK and Rho kinase activities. Unexpectedly, the TGF- α -stimulated activations of AKT, SAPK/JNK, P38 MAPK and Rho kinase were not suppressed by 3-HT up to 30 μ M. Treatment with 100 μ M 3-HT for 48 h was demonstrated to suppress AKT activity in HuH7 cells (27). However, in the present study, 3-HT exerted a suppressive effect on the TGF- α -induced migration of HuH7 cells, even at 1 μ M for 24 h. Therefore, the suppressive effect on AKT activity by the high doses and long treatment periods of 3-HT may not be associated with the effect of 3-HT on the TGF- α -induced migration of HuH7 cells.

Although both 3-HT and oleuropein suppressed the TGF- α -stimulated migration of HuH7 cells, and p38 MAPK served as a positive regulator in the cell migration, these olive oil polyphenols did not inhibit TGF- α -induced p38 MAPK activation. On the contrary, 3-HT activated p38 MAPK in HuH7 cells in the present study. While, oleuropein failed to affect the TGF- α -induced p38 MAPK phosphorylation. These observations suggest that the activation of p38 MAPK by 3-HT is not a common effect of olive oil polyphenols. The activation of p38 MAPK by 3-HT may be independent of the inhibitory effect on the migration. In addition, we proposed that 3-HT decreased the level of TGF- α -stimulated migration of HuH7 cells, even when removed from the cell culture before TGF- α stimulation. Thus, there was no plausible direct interaction between 3-HT and TGF- α . Therefore, the suppressive effect of olive oil polyphenols on the TGF- α -induced migration of HuH7 cells may be mediated through a pathway other than the AKT, SAPK/JNK, p38 MAPK and Rho kinase pathways, or it may occur at a point downstream of these kinases. Additional studies are required to clarify the exact roles of olive oil polyphenols in HCC.

In conclusion, the results from the present study suggest that the olive oil polyphenols oleuropein and 3-HT suppressed TGF- α -induced HCC cell migration.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NY, RMN and OK conceived and designed the experiments. NY, RMN, AM and KT performed experiments. NY, RMN and OK analyzed the data. NY, RMN and OK wrote the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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