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MART-10, a 1α,25(OH)$_2$D$_3$ Analog, Potently Represses Metastasis of ER$^+$ Breast Cancer Cells with VEGF-A Overexpression

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Abstract. *Background: Breast cancer ranks second in the list of cancer-related deaths for women. Even under multidisciplinary treatment, 25-50% of patients with breast cancer still ultimately develop metastasis, leading to poor prognosis. In addition to inducing angiogenesis, vascular endothelial growth factor-A (VEGF-A) is believed to directly increase cancer cell metastatic potential and overexpression of VEGF-A is associated with higher invasiveness of breast cancer. 1α,25(OH)$_2$D$_3$, the active form of vitamin D, and its analogs have been widely applied as anticancer agents in the past. Material and Methods: Western blot, migration and invasion assays, enzyme-linked immunosorbent assay, and immunofluorescent stain were applied in this study. Result: VEGF-A increased cell migration and invasion in estrogen receptor-positive (ER+) breast cancer MCF-7 cells. VEGF-A induced an autocrine loop in MCF-7 cells as VEGF-A treatment increased both VEGF-A expression and secretion. The expression of VEGF receptor type 2 (VEGFR2) and neuropilin 1 was also up-regulated by VEGF-A in MCF-7 cells. In addition, F-actin synthesis and LIM domain kinase 1 (LIMK-1) phosphorylation were increased by VEGF-A. VEGF-A also increased β-catenin expression and nuclear translocation of both β-catenin and nuclear factor-κB (NF-κB), indicating increased β-catenin and NF-κB activity. 1α,25(OH)$_2$D$_3$ and MART-10, an analog of 1α,25(OH)$_2$D$_3$, effectively repressed VEGF-A-induced MCF-7 cell migration and invasion and other VEGF-A-induced effects on MCF-7 cells, with MART-10 being more potent than 1α,25(OH)$_2$D$_3$. Conclusion: MART-10 can be deemed as a promising agent for prevention and treatment of metastasis of ER+ breast cancer with VEGF-A overexpression.*

Breast cancer is the most commonly found cancer and ranks second in cancer-related death in women (1). Due to the breakthrough in molecular understanding of breast cancer, the treatment and prognosis of breast cancer have recently taken steps forward. However, cancer metastasis is still the main cause of breast cancer-related death. A number of regimens have been developed to prevent or treat breast cancer metastasis. Even so, the rate of breast cancer metastasis is still 25-50% even after receiving state-of-the-art treatments (2, 3). Thus, more efforts are needed to address the issue of breast cancer metastasis.

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In addition to inducing angiogenesis, a process whereby endothelial cells form neo-vessels vital for cancer growth and metastasis, vascular endothelial growth factor-A (VEGF-A) also plays an important role in cancer cells. VEGF-A binds to receptors (VEGFRs) to exert most of its functions; among others, VEGFR2 is the most dominant, with the VEGRA–VEGFR2 axis being the most important signaling pathway in VEGF-A-induced physiological functions (4-6). The correlation of high expression of VEGF-A and VEGFR2 in human cancer with aggressive disease implies the crucial role of VEGRA–VEGFR2 axis for cancer (7, 8). For breast cancer, VEGF-A and VEGFR2 are more highly expressed in more invasive cancer types and are linked to worse outcome (9).

In the past decades, abundant studies have been published regarding vitamin D application in cancer treatment [reviewed in (10)] following the emergence of non-mineral functions of $1\alpha,25(OH)_2D_3$, the active form of vitamin D. Further modification of $1\alpha,25(OH)_2D_3$ to strengthen its anticancer effects and prevent hypercalcemia has led to creation of thousands of analogs. One of the 19-nor series $1\alpha,25(OH)_2D_3$ analogs with modifications of the C2 position, 19-nor-2α-(3-hydroxypropyl)-$1\alpha,25(OH)_2D_3$ (MART-10) (11), has been shown to be more active than $1\alpha,25(OH)_2D_3$ regarding repression of cancer cell growth *in vitro* and *in vivo* without obvious induction of side-effects (12-17). In terms of breast cancer, our group has demonstrated that MART-10 effectively repressed growth and metastatic potential of estrogen receptor-positive (ER+) breast cancer cells (18, 19).

In the current study, we aimed to investigate the effect of VEGF-A on the metastatic potential of ER+ breast cancer cell and related mechanisms, and evaluate the influence of MART-10 and $1\alpha,25(OH)_2D_3$ on ER+ breast cancer cells after VEGF-A stimulation, with the hope of providing a potential therapy against ER+ breast cancer with VEGF-A overexpression.

Materials and Methods

Vitamin D compounds. $1\alpha,25(OH)_2D_3$ was obtained from Sigma (St. Louis, MO, USA). MART-10 was synthesized by Kittaka *et al*. (11). VEGF-A was purchased from R&D Systems (Minneapolis, MN, USA).

Cell culture. Human breast cancer cell line, MCF-7, was purchased from the ATCC (Manassas, VA, USA). After 2-3 passages, MCF-7 cells were grown to 95% confluence in Dulbecco's modified Eagle's medium (DMEM) (Sigma) supplemented with 5% fetal bovine serum (FBS). Culture medium was changed three times per week.

Trans-well filter migration assay. MCF-7 cells, which were treated with or without 20 ng/ml VEGF-A for 1 day and with or without 10^{-7} or 10^{-8} M of either MART-10 or $1\alpha,25(OH)_2D_3$ for 24 h, were seeded on trans-well filters (Costar, Cambridge, MA, USA). The experiment was conducted as previously described (19). The cells migrating to the lower surface of the filter were counted under four random high-power microscopic fields (HPF;100×) per filter, and the mean number of cells that migrated through the filter was calculated.

Matrigel invasion assay. MCF-7 cells were pretreated with or without 20 ng/ml VEGF-A and with or without 10^{-7} or 10^{-8} M of $1\alpha,25(OH)_2D_3$ or MART-10 for1 day. The assay was performed as previously described (19). The number of invading cells was counted under a microscope (IX71; Olympus, Tokyo, Japan).

RT/real-time polymerase chain reaction (PCR). RT/real-time PCR procedures were performed as described elsewhere (20). Human sequence-specific primers were as follows: *VEGF-A*, forward 5'-ATTATGCGGATCAAACCT-3' and reverse, 5'-TTCTTGTCTTG CTCTATCTT-3'. Taqman PDAR eukaryotic *18S* rRNA (Applied Biosystems, MDBio, Inc., Taiwan, ROC) was used as the endogenous control for normalizing the amount of cDNA sample. Relative quantitation of gene expression was calculated using the ΔΔCt method (Applied Biosystems).

VEGF-A enzyme-linked immunosorbent assay (ELISA). Conditioned media collected from MCF-7 cell cultures after 18 h of different treatments (with or without 10^{-7} or 10^{-8} M MART-10 or $1\alpha,25(OH)_2D_3$) were measured by VEGF-A ELISA, that was conducted according to the manufacturer's instructions (DY293B; R&D Systems, Inc., Minneapolis, MN, USA).

Western blot. The western blot procedures were conducted as previously described (19). The antibodies used in this experiment were monoclonal antibodies against neuropilin 1 (1:1,000, ab81321; Abcam, Cambridge, MA, USA), VEGFR2 (1:500, sc-6251; Santa Cruz Biotechnology, Santa Cruz, CA, USA), F-actin (1:500, ab205; Abcam), LIM domain kinase 1 (LIMK-1; 1:1,000, #3842; Cell Signaling Technology, Irvine, CA, USA), p-LIMK-1 (1:1000, PA5-37629; Thermo Fisher Scientific, Waltham, MA, USA), β-catenin (1:200, sc-7199; Santa Cruz), nuclear factor κB (NF-κB; 1:200, sc-8008; Santa Cruz), and pNF-κB (1:1,000, #9936; Cell Signaling Technology). The secondary antibodies used in this experiment were horseradish peroxidase (HRP)-linked antibody anti-rabbit IgG (1:5,000, #7074; Cell Signaling Technology) or HRP-linked antibody anti-mouse IgG (1:5,000, #7076; Cell Signaling Technology) or HRP-conjugated goat anti-mouse IgM antibody (1:5,000, A90-101P; Leinco Technologies, Montgomery, TX, USA).

F-actin, β-catenin, and NF-κB staining. After 1 day of with or without 10^{-7} or 10^{-8} M MART-10 or $1\alpha,25(OH)_2D_3$ treatment, MCF-7 cells were seeded onto glass bottom culture dishes (MatTek, Ashland, MD, USA) precoated with 50 μl fibronectin and allowed to attach overnight. Cells were then fixed with 3.7% formaldehyde, permeabilized with 0.1% Triton X-100, and blocked in 1% bovine serum albumin at room temperature. F-Actin, β-catenin, and NF-κB protein expressions were revealed by incubation with fluorescein isothiocyanate-labeled phalloidin (1:800, P5282; Sigma). Nuclei were revealed by incubation with DAPI. Immunofluorescence was examined using confocal microscopy (LSM510 Meta; Zeiss, Oberkochen, Germany).

Statistical analysis. The data from each group were compared by Student *t*-test with *p*-values of less than 0.05 being considered as a significant difference. The program of Excel 2010 was employed to conduct the statistics.

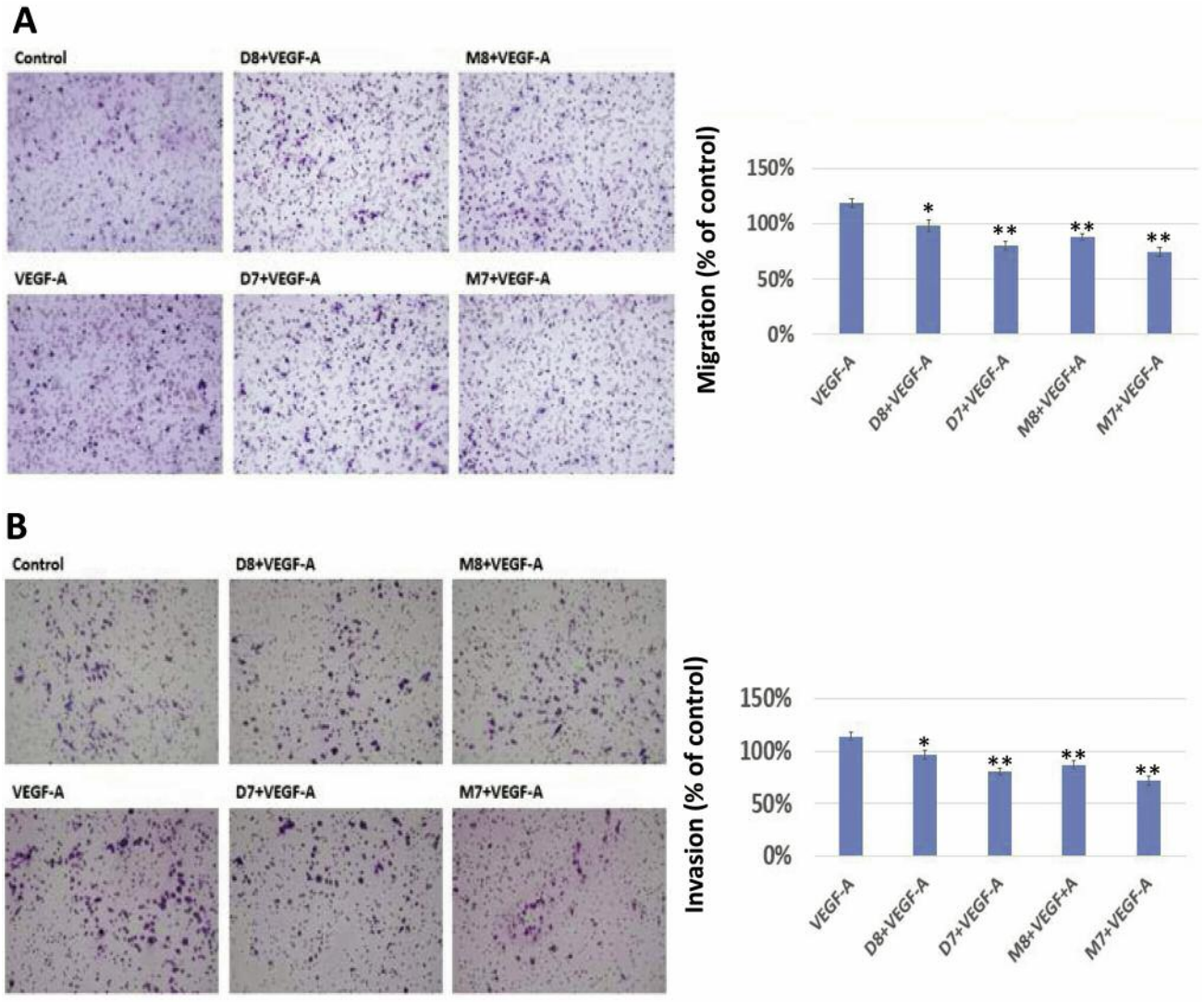

Figure 1. *The effect of 1α,25(OH)$_2$D$_3$ and MART-10 on migration (A) and invasion (B) of VEGF-A-stimulated MCF-7 cells. MCF-7 cells were treated with 20 ng/ml VEGF-A with and without 10^{-7} or 10^{-8} M of 1α,25(OH)$_2$D$_3$ (D7/D8) or MART-10 (M7/M8) for 24 h before the experiment. After 4 and 24 h, the numbers of migrating and invading cells, respectively, were counted under a microscope and cells were digitally photographed. Data are presented as the mean percentage (compared to the control) ±SD. Experiments were performed in triplicate and repeated at least three times. Significantly different from VEGF-A-treated cells at *p<0.05 and **p<0.01.*

Results

Evaluation of the effects of VEGF-A, 1α,25(OH)$_2$D$_3$ and MART-10 on migration and invasion of MCF-7 cells. The migration and invasion of MCF-7 cells were significantly increased by 18±4 and 14±3.7%, respectively, when treated with VEGF-A (20 ng/ml) for 1 day. Both 1α,25(OH)$_2$D$_3$ and MART-10 at the dose of 10^{-7} and 10^{-8} M significantly repressed VEGF-A-induced migration and invasion of MCF-7 cells in a dose-dependent manner, with MART-10 being significantly more potent than 1α,25(OH)$_2$D$_3$ (Figure 1). Our result indicated that VEGF-A was a stimulator of MCF-7 cell metastasis and 1α,25(OH)$_2$D$_3$ and MART-10 were able to repress the effect of VEGF-A on MCF-7 cells.

VEGF-A treatment increased VEGF-A, VEGFR2 and neuropilin 1 expression in MCF-7 cells. To investigate whether the autocrine phenomenon of VEGF-A exists or not in MCF-7 cells, after 18 h of VEGF-A treatment, the mRNA of VEGF-A was measured by RT-qPCR. As shown in Figure 2A, VEGF-A treatment induced an significant increase of VEGF-A mRNA expression compared to the control MCF-7

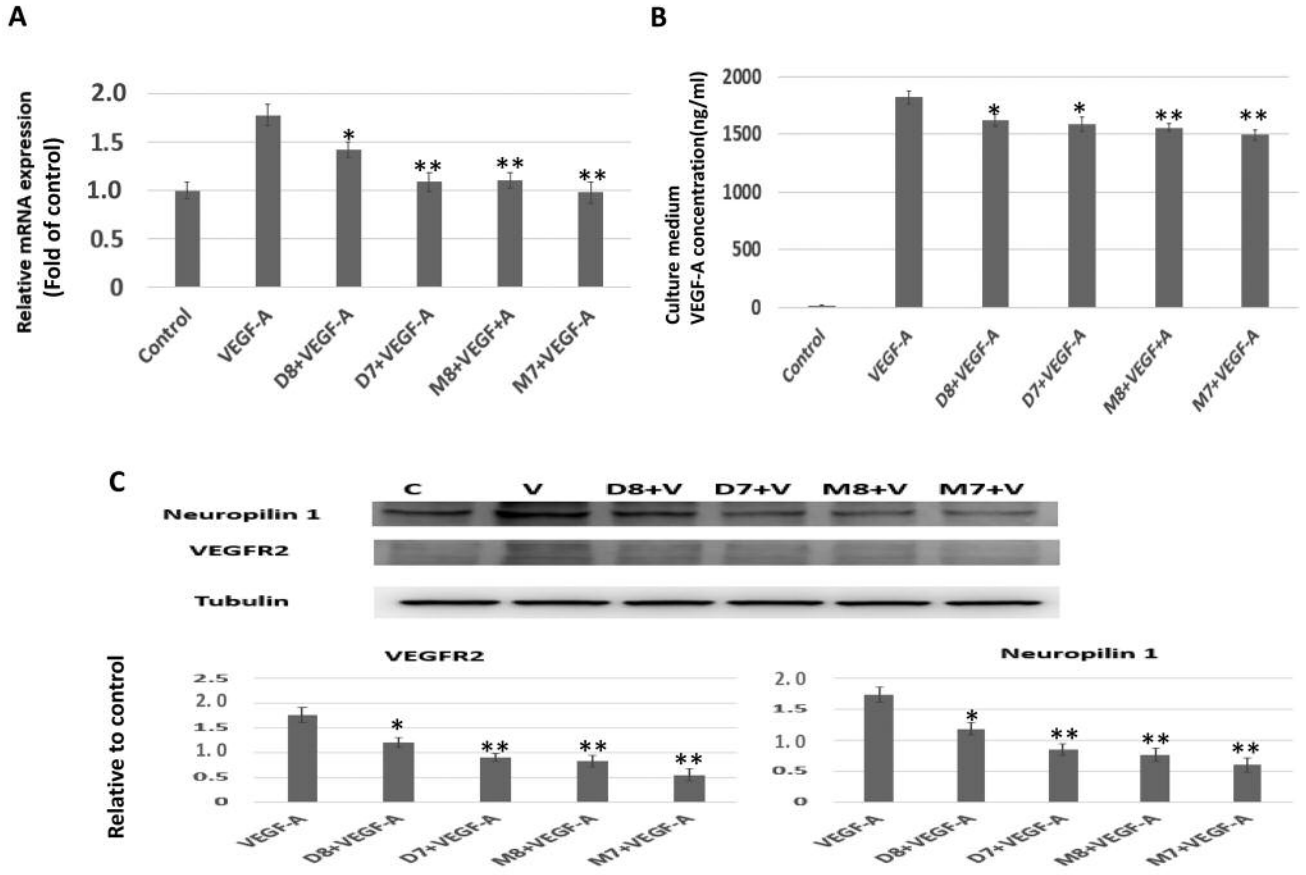

Figure 2. *The effects of 1α,25(OH)$_2$D$_3$ and MART-10 on VEGF-A-induced VEGF-A expression and secretion, VEGFR2 and neuropilin 1 expression in MCF-7 cells. A: VEGF-A mRNA expression of MCF-7 cells was analyzed by RT-qPCR after VEGF-A stimulation for 18 h with and without 10^{-7} or 10^{-8} M of 1α,25(OH)$_2$D$_3$ (D7/D8) or MART-10 (M7/M8). B: The amount of VEGF-A secreted by MCF-7 cells in culture was determined by ELISA. MCF-7 cells were treated with VEGF-A for 18 hours with and without 25(OH)$_2$D$_3$ or MART-10. C: Upper panel: A representative western blot showing the expression of VEGFR2 and neuropilin 1 in MCF-7 cells after VEGF-A (V) stimulation with or without MART-10 or 1α,25(OH)$_2$D$_3$ for 24 h. Lower panel: The quantitative result of the western blot. Data are presented as the mean percentage (as compared to the control, C) ± SD. Experiments were performed in triplicate and repeated at least three times. Significantly different from VEGF-A-treated cells at *p<0.05 and **p<0.01.*

cells. A dose-dependent inhibition of VEGF-A mRNA expression was observed in VEGF-A-stimulated MCF-7 cells when treated by 10^{-7} or 10^{-8} M 1α,25(OH)$_2$D$_3$ or MART-10. VEGF-A treatment also significantly increased the amount of VEGF-A secreted in MCF-7 cells. Figure 2B shows that VEGF-A treatment increased the VEGF-A concentration in the medium to 1,823±56 ng/ml, which was repressed by 10^{-7} or 10^{-8} M 1α,25(OH)$_2$D$_3$ or MART-10. We next evaluated expression of VEGFR2 and neuropilin 1, two important proteins involved in VEGF signaling, in MCF-7 cells after VEGF-A treatment. Figure 2C shows that VEGFR2 and neuropilin 1 expression significantly increased after VEGF-A treatment. The VEGF-A-increased expression of both proteins was significantly down-regulated dose-dependently by 1α,25(OH)$_2$D$_3$ and MART-10, with MART-10 being much more potent than 1α,25(OH)$_2$D$_3$. Our data demonstrate that an autocrine loop exists in MCF-7 cells treated by VEGF-A. 1α,25(OH)$_2$D$_3$ and MART-10 blocked the above-mentioned VEGF-A effects on MCF-7 cells.

Evaluation of F-actin, LIMK-1 and pLIMK-1 expression after VEGF-A with and without 1α,25(OH)$_2$D$_3$ or MART-10 treatment in MCF-7 cells. Since F-actin plays a vital role in cell migration, we evaluated the effect of VEGF-A on F-actin in MCF-7 cells. Figure 3A demonstrates that F-actin expression of MCF-7 cells was increased significantly compared to the control cells were treated with VEGF-A. 1α,25(OH)$_2$D$_3$ significantly repressed the increase of F-actin expression as did MART-10. This result was further supported by the immunofluorescent staining shown in Figure 3B. The change of green color intensity (F-actin) was shown to have the same trend as that shown by western blot. LIMK-1 and

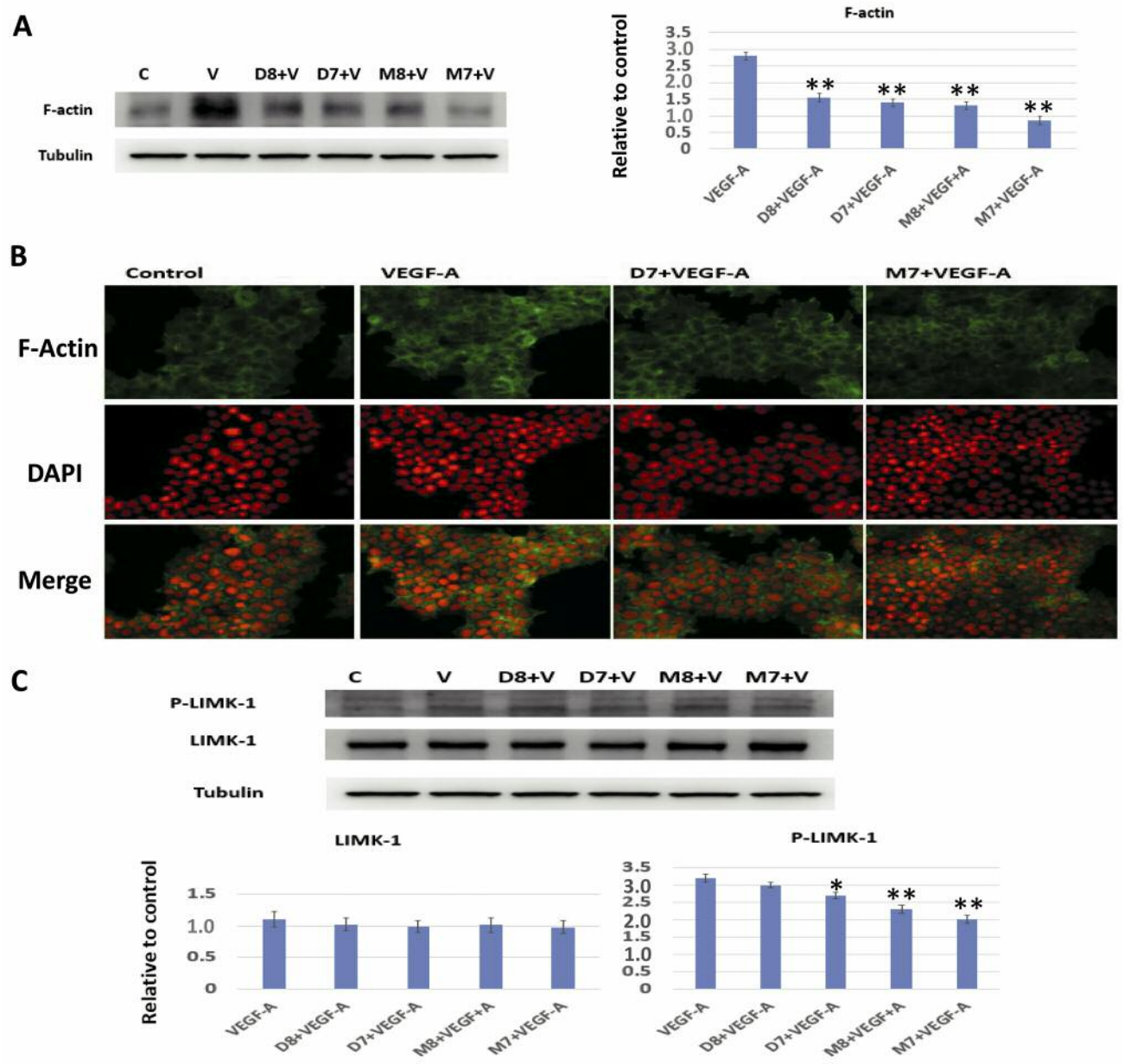

Figure 3. *The effect of 1α,25(OH)$_2$D$_3$ and MART-10 on VEGF-A-induced F-actin, LIMK-1, and p-LIMK-1 expression in MCF-7 cells. A: Upper panel: A representative western blot showing the actin expression in MCF-7 cells after VEGF-A stimulation with and without 10^{-7} or 10^{-8} M of 1α,25(OH)$_2$D$_3$ (D7/D8) or MART-10 (M7/M8) treatment for 24 h. Lower panel: The quantitative result of the western blot. B: Immunofluorescence staining of F-actin (green) expression in MCF-7 cells after VEGF-A (V) stimulation with and without 1α,25(OH)$_2$D$_3$ or MART-10 treatment for 24 h. DAPI (red) was applied for nuclear staining. C: Upper panel: A representative western blot showing LIMK-1 and p-LIMK-1 expression in MCF-7 cells after VEGF-A stimulation with and without MART-10 or 1α,25(OH)$_2$D$_3$ for 24 h. Lower panel: The quantitative result of the western blot. Data are presented as the mean percentage (compared to the control, C) ±SD. Experiments were performed in triplicate and repeated at least three times. Significantly different from VEGF-A-treated cells at *p<0.05 and **p<0.01.*

pLIMK-1 (the active form of LIMK-1) are two important proteins regulating F-actin synthesis. As shown in Figure 3C, VEGF-A significantly increased pLIMK-1 but not LIMK-1 in MCF-7 cells relative to the control. 1α,25(OH)$_2$D$_3$ and MART-10 effectively attenuated this effect dose-dependently.

Evaluation of the effects VEGF-A, 1α,25(OH)$_2$D$_3$, and MART-10 on β-catenin expression and its nuclear translocation. Figure 4A shows that VEGF-A increased β-catenin expression significantly relative to the control, with 10^{-7} or 10^{-8} M 1α,25(OH)$_2$D$_3$ or MART-10 effectively

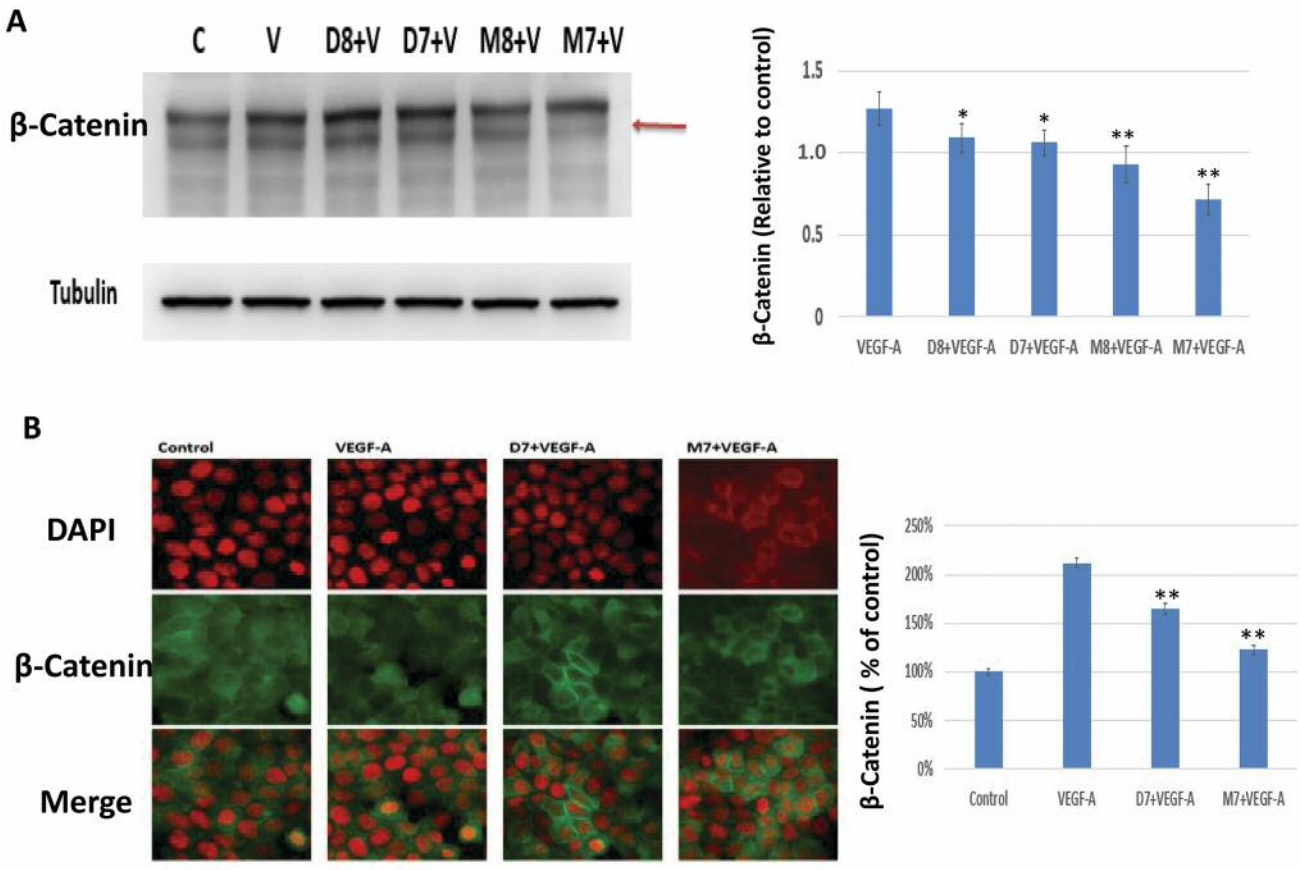

Figure 4. *The effect of 1α,25(OH)$_2$D$_3$ and MART-10 on VEGF-A-induced β-catenin expression and nuclear translocation in MCF-7 cells. A: Upper panel: A representative western blot showing β-catenin expression in MCF-7 cells after VEGF-A (V) stimulation without 10^{-7} or 10^{-8} M of 1α,25(OH)$_2$D$_3$ (D7/D8) or MART-10 (M7/M8) for 24 h. Lower panel: The quantitative result of the western blot. B: Upper panel: Immunofluorescence staining of β-catenin (green) expression in MCF-7 cells after VEGF-A stimulation with or without 1α,25(OH)$_2$D$_3$ or MART-10 treatment for 24 h. DAPI (red) was applied for nuclear staining. Lower panel: The quantitative result of nuclear translocation of β-catenin, present as orange color in the nucleus. Data are presented as mean percentage (as compared to the control, C)± SD. Experiments were performed in triplicate and repeated at least three times. Significantly different from VEGF-A-treated cells at *p<0.05 and **p<0.01.*

attenuating this effect. Since β-catenin needs to be translocated into the nucleus to exert its function, intra-nuclear β-catenin was assessed by immunofluorescent staining. Figure 4B shows that VEGF-A significantly increased nuclear β-catenin (present as orange nuclei) compared with the control and 10^{-7} M 1α,25(OH)$_2$D$_3$ and MART-10 effectively blocked this effect.

Evaluation of the effects of VEGF-A, 1α,25(OH)$_2$D$_3$, and MART-10 on NF-κB pathway activity. Figure 5A shows that pNF-κB expression was significantly increased by VEGF-A in MCF-7 cells. 10^{-7} or 10^{-8} M 1α,25(OH)$_2$D$_3$ and MART-10 treatment inhibited VEGF-A-stimulated pNF-κB expression. Since NF-κB also needs to enter the nucleus to become active, the intracellular NF-κB expression was then analyzed by immunofluorescent staining. Figure 5B shows that nuclear NF-κB expression (present as orange nuclei) was significantly increased by VEGF-A in MCF-7 cells, which was inhibited by 10^{-7} M 1α,25(OH)$_2$D$_3$ and MART-10.

Discussion

In this study, we demonstrated that VEGF-A increased MCF-7 cell migration and invasion (Figure 1). An autocrine loop was observed as VEGF-A treatment induced both an increase of VEGF-A expression and secretion in MCF-7 cells (Figure 2A and 2B). The expressions of VEGFR2 and neuropilin 1 were also up-regulated by VEGF-A in MCF-7 cells (Figure 2C). Furthermore, the F-actin and pLIMK-1 were found to be up-regulated by VEGF-A in MCF-7 cells (Figure 3). The expression and nuclear translocation of β-catenin and NF-κB were also increased by VEGF-A in MCF-7 cells (Figure 4 and

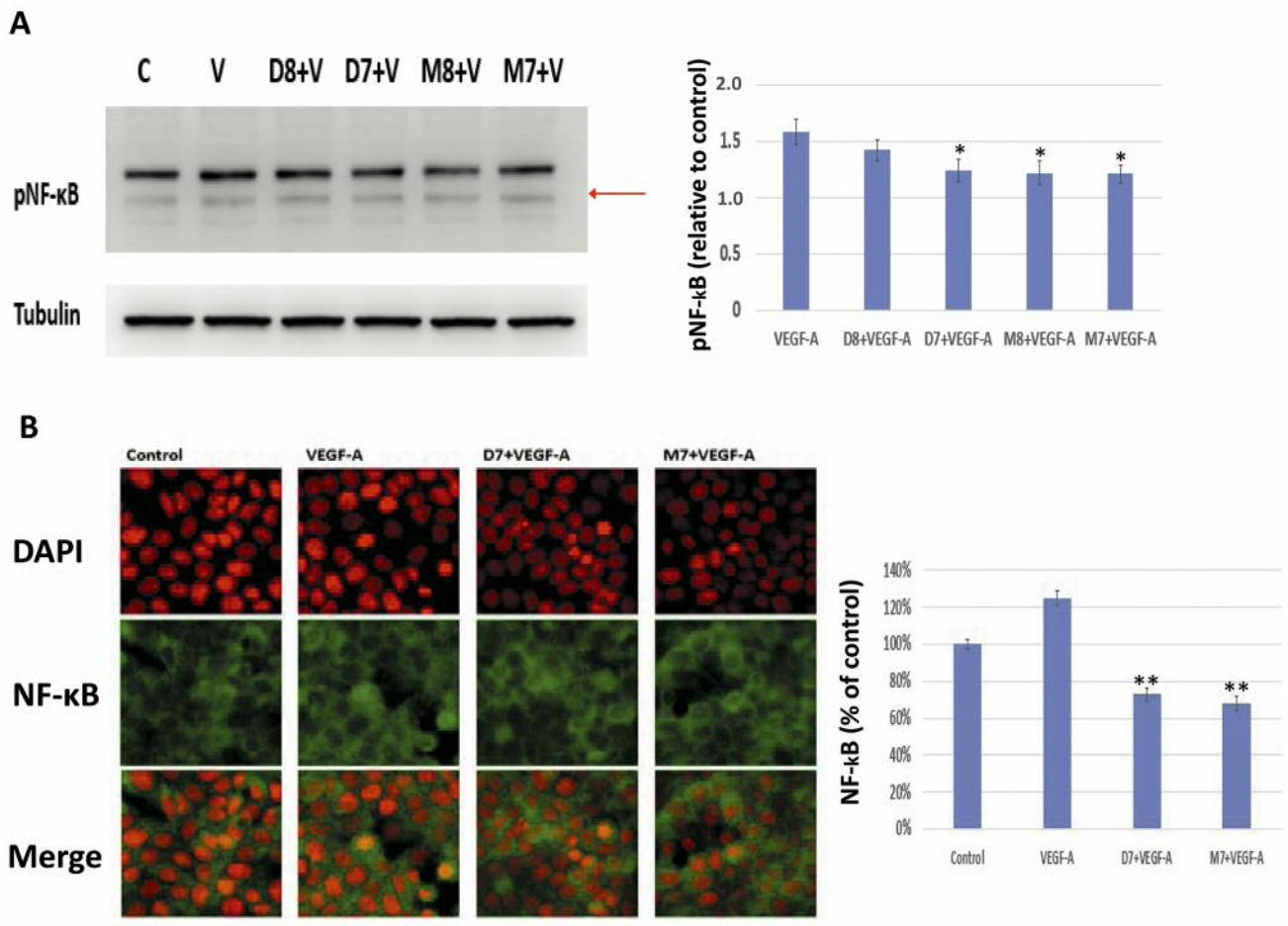

Figure 5. *The effect of 1α,25(OH)$_2$D$_3$ and MART-10 on VEGF-A-induced activation of NF-κB signaling pathway in MCF-7 cells. A Upper panel: A representative western blot depicting pNF-κB expression in MCF-7 cells after VEGF-A (V) stimulation with and without MART-10 or 1α,25(OH)$_2$D$_3$ treatment for 24 h. Lower panel: The quantitative result of the western blot. B: Upper panel: Immunofluorescence staining of NF-κB (green) expression in MCF-7 cells after VEGF-A stimulation with and without 1α,25(OH)$_2$D$_3$ or MART-10 treatment for 24 h. DAPI (red) was applied for nuclear staining. Lower panel: The quantitative result of nuclear translocation of NF-κB, present as orange color in the nucleus. Data are presented as the mean percentage (as compared to the control, C) ±SD. Experiments were performed in triplicate and repeated at least three times. Significantly different from VEGF-A-treated cells at *p<0.05 and **p<0.01.*

5). All the above-mentioned VEGF-A-induced effects in MCF-7 cells were attenuated by 1α,25(OH)$_2$D$_3$ and MART-10, with MART-10 being much more potent than 1α,25(OH)$_2$D$_3$. Thus, we concluded that VEGF-A is a strong inducer of MCF-7 cell metastatic ability. 1α,25(OH)$_2$D$_3$ and MART-10 were able to block these effects and significantly repress VEGF-A-induced MCF-7 migration and invasion. Collectively, MART-10 could be deemed as a promising agent for preventing and treating metastasis of ER+ breast cancer VEGF-A overexpression.

VEGF-A has been shown to be able to induce both physiological and pathological angiogenesis (21, 22). Besides its involvement in angiogenesis, VEGF-A is deemed as a strong inducer of cancer metastasis (23). VEGF-A needs to bind with VEGFRs to exert its function, these are tyrosine kinase receptors and mainly include VEGFR1, VEGFR2, and VEGFR3, with VEGFR2 being the dominant receptor triggering angiogenesis (24). Originally, VEGFRs were thought to be expressed only in endothelial cells, however, recent studies clearly indicate that VEGFs are present in a variety of cancer cells, such as those of the brain, and bladder. For breast cancer, VEGFR2 has been shown to correlate with worse outcome (9). Of note, in cancer cells, neuropilins binds with VEGFRs to increase the affinity of VEGF-A to VEGFRs (25). Moreover, the autocrine phenomenon of VEGF-A noted in cancer cells

further strengthens the invasiveness and stemness of cancer cells (23). Our results clearly show that VEGF-A increased MCF-7 cell migration and invasion and did induce an autocrine loop in MCF-7 cells. Expression of VEGFR2 and neuropilin1 was up-regulated by VEGF-A (Figure 2C). $1\alpha,25(OH)_2D_3$ and MART-10 effectively blocked VEGF-A effects on MCF-7 cells regarding migration, invasion, autocrine loop, as well as expression of VEGFR2 and neuropilin1, with MART-10 possessing more potent effect than $1\alpha,25(OH)_2D_3$.

The synthesis and dynamic reorganization of actin is crucial for cell motility, thereby influencing cancer metastasis (26). LIMK-1, phosphorylated by Rho-associated coiled-coil-containing protein kinase (ROCK) to be active, plays a vital role regarding regulation of cell actin (26). It has been shown that LIMK-1 activity is positively associated with cancer invasiveness (27, 28). As shown in Figure 2A and 2B, the results of both western blot and immunofluorescent stain show that VEGF-A increased F-actin synthesis in MCF-7 cells. VEGF-A further induced LIMK-1 phosphorylation without influencing LIMK-1 expression. Both phenomena led to increased MCF-7 cell migration when cells were treated with VEGF-A as noted in Figure 1. MART-10 and $1\alpha,25(OH)_2D_3$ effectively blocked the effect of VEGF-A on F-actin and LIMK-1 phosphorylation in MCF-7 cells (Figure 2).

After stimulation, β-catenin, existing in the cytoplasm mainly in the latent form, can enter the nucleus and act as a co-activator of LEF/TCF transcription factors, which stimulate expression of genes to trigger cell proliferation and migration (11, 13). The increased expression or nuclear translocation of β-catenin is also deemed as a marker for epithelial–mesenchymal transition, a process rendering cancer cells more invasive (29). Figure 4 shows that VEGF-A treatment increased both β-catenin expression and its nuclear translocation in MCF-7 cells, which was blocked by $1\alpha,25(OH)_2D_3$ and MART-10.

The NF-κB transcription factor family has been reported to be involved in regulation of the expression of a variety of genes regarding inflammation, immune, cell growth and cancer metastasis. Thus, the aberrant activation of NF-κB signaling has been linked to a number of cancer development and progress (30-32). The activation of NF-κB takes place mainly when cells receive a number of signals, such as cytokines, and stress (31). In the latent state, NF-κB is sequestered in the cytosol. Once activated, NF-κB is phosphorylated and translocated into the nucleus (33). It has been shown that the VEGF–NPR1 axis promotes breast cancer cell metastasis through activation of NF-κB (34). Figure 5A clearly shows that VEGF-A increased NF-κB phosphorylation, leading to nuclear translocation of NF-κB. Both $1\alpha,25(OH)_2D_3$ and MART-10 significantly attenuated this effect (Figure 5).

Conclusion

In addition to angiogenesis, the VEGF-A–VEGFR2–neuropilin-1 axis has been implicated in cancer metastasis, which is the main cause of breast cancer-related death. We showed that VEGF-A stimulation increased migration, invasion, *VEGF-A* mRNA expression and secretion, as well as VEGFR2 and neuropilin 1 expression in MCF-7 cells. Synthesis of F-actin and phosphorylation of LIMK-1 were also strengthened. In addition, the expression and nuclear translocation of β-catenin and NF-κB were all up-regulated by VEGF-A in MCF-7 cells. $1\alpha,25(OH)_2D_3$ and MART-10 blocked all these effects of VEGF-A on MCF-7 cells, with MART-10 being much more potent than $1\alpha,25(OH)_2D_3$. Our findings strongly suggest that MART-10 could be a potential drug to prevent and treat metastasis of ER+ breast cancer with VEGF-A overexpression.

Conflicts of Interest

The Authors declared that they have no competing interests.

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