Original Article

Melittin Prevents Metastasis of Epidermal Growth Factor-Induced MDA-MB-231 Cells through The Inhibition of The SDF-1α/CXCR4 Signaling Pathway

Fatemeh Salimian, M.Sc., Mohammad Nabiuni, Ph.D.*, Ensieh Salehghamari, Ph.D.

Department of Cell and Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran

Corresponding Address: P.O.Box: 31979-37551, Department of Cell and Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
Email: devbiokharazmi@gmail.com

Abstract —

Objective: Melittin is one of the natural components of bee venom (*Apis mellifera*), and its anticancer and antimetastatic properties have been well established, but the underlying mechanism remains elusive. The MDA-MB-231 is a triple-negative cell line that is highly aggressive and invasive. Besides, many critical proteins are involved in tumor invasion and metastasis. In this study, we investigated whether melittin inhibits the migration and metastasis of epidermal growth factor (EGF)-induced MDA-MB-231 cells via the suppression of SDF-1α/CXCR4 and Rac1-mediated signaling pathways.

Materials and Methods: In this experimental study, cells were treated with melittin (0.5-4 μg/ml), and the toxicity of melittin was assessed by the MTT assay. Afterward, the migration assay was conducted to measure the degree of the migration of EGF-induced cells. The western blot technique was performed to analyze the rate of Rac1, p-Rac1, SDF-1α, and CXCR4 expression in different groups.

Results: The results demonstrated that melittin markedly suppressed the migration of EGF-induced cells and decreased the expression of p-Rac1, CXCR4, and SDF-1α proteins.

Conclusion: The results of the present study suggested that the anti-tumor properties of melittin could be through the blocking of the SDF-1α/CXCR4 signaling pathway, which is beneficial for the reduction of tumor migration and invasion.

Keywords: CXCR4, Melittin, Rac1, SDF-1α

Introduction

Bee venom consists of a variety of biologically active peptides, including melittin, apamin, adolapin, and mast cell degranulating peptide (MCDP) (1). Melittin is a major constituent of bee venom, which is a 26-amino acid polypeptide containing 40-60% of the whole bee venom (2). It belongs to amphipathic, α-helical, and cell-penetrating peptides, possessing anti-inflammatory, anti-bacterial, anti-thrombosis, and anti-tumor properties (3).

Organs have restricted boundaries that are identified by the basement membrane that can surround the cells with a particular matrix (4). Metastasis is known as the dissemination of cancer cells from a specific organ or region to another location that is not generally connected to it (5). The process of metastasis occurs via the blood vessels or lymphatic system or both (6). It is now known that cell migration is a critical step in metastasis and tumor invasion, and the regulation of this process can control the pathogenesis of cancer. The understanding of the molecular mechanism underlying the process of cancer cell migration and metastasis is a prerequisite for designing new therapies for the elimination of cancer cells.

The stromal cell-derived factor1α (SDF1α) and its cognate receptor, CXC chemokine receptor type 4 (CXCR4), play a crucial role in tumor metastasis (7). Chemokines are a group of peptides with molecular weights between 8 and 12 kDa (8). They are divided into four groups based on the position of the cysteine motif at the NH2 terminus (9). CXCL-12 (SDF-1) is the most significant member of this family in numerous types of cancer, which exists in two forms, namely SDF-1α/CXCL-12a and SDF-1β/CXCL-12b (10). The binding of CXCL-12 to CXCR-4 can activate multiple molecular signaling pathways (11). Through one of these pathways, the association of SDF-1 with CXCR-4 could activate the Rho family GTPases, such as Rac1, which plays a critical role in tumor progression and modulation of other signaling pathways, such as cell-cell adhesion (12). In this way, the GTP-bound form of the Rac1 protein binds to the PAK family and induces the kinase activity of these types of proteins. PAKs phosphorylate and activate actin-binding LIM kinases present in two types, namely LIMK1 and LIMK2. These kinases can phosphorylate cofilin, which leads to the deactivation of this protein. The activity of cofilin, which participates in the actin cytoskeleton formation, is essential for tumor cell

metastasis and migration (13). Indeed, actin cytoskeleton reorganization is considered a principal mechanism for triggering cell motility, and necessary for numerous kinds of cell migration (14). Rac1 also causes dissociation of the WAVE-1 protein from its regulatory complex to drive Arp2/3 complex-mediated actin polymerization, and it induces the formation of the lamellipodium. Therefore, Rac1 regulates multiple elements involved in the invasion and metastasis of cancer cells (13).

Previous studies have demonstrated that EGF-induced cell migration is associated with Rac1 activation and promotes tumor cell motility and invasion (15). Besides, it was reported that interaction between the chemokine receptor CXCR4 and its ligand SDF 1α plays a significant role in cell proliferation, angiogenesis, tumorigenicity, and metastasis in various types of cancer cell lines, such as breast cancer cells (16). Likewise, it has been shown that EGF can increase the expression of CXCR4, thereby the PI-3 kinase pathway (17). To date, there is no report on the stimulation of EGF in response to SDF 1α expression.

In the past few years, the anticancer properties of melittin have attracted much attention (18). Thus, due to the side effects of conventional therapies, such as chemotherapy, natural components possessing fewer side effects and enormous anti-cancer properties are currently used as complementary therapies (19). Data obtained during previous studies confirmed that melittin inhibits tumor cell metastasis via the suppression of the Rac1-dependent pathway in different types of cancer cell lines; however, the precise mechanisms of this event are still unclear (18). Since MDA-MB-231 is a triple-negative cell line (20), the effect of melittin on ER, PR, and HER2 receptors is rejected. According to the significant role of SDF-1α and CXCR4 in the migration and metastasis of cancer cells, this study aimed to examine the effect of melittin on the possible inhibition of the CXCR4/ SDF-1α pathway in MDA-MB-231 cells stimulated by EGF.

In this study, the effects of melittin on the motility and migration of a triple-negative breast cancer cell line were investigated. It was observed that melittin suppresses the level of CXCR4, SDF-1α, and Rac1 expression. Therefore, the CXCR4/SDF-1α signaling pathway could be one of the main ways through which melittin puts its effects. To confirm this, the expression level of Rac1, one of the key proteins in this pathway, was also examined. Evidence showed that the phosphorylation rate of this protein is decreased parallel with an increase in the concentration of melittin.

Materials and Methods

Cells and materials

In this experimental study, the breast cancer cell line MDA-MB-231 was purchased from the Pasteur Institute of Tehran, Iran. Melittin, at a concentration of 2 µg/ml, was purchased from Sigma-Aldrich (Sigma Aldrich, St. Louis, MO, USA). The monoclonal antibodies against Rac1 and its phosphorylated form were obtained from Abcam (Abcam, Cambridge, MA). Antibodies against CXCR4 and SDF-1α were obtained from Santa Cruz Biotechnology (Santa Cruz Biotechnology, Santa Cruz, CA). The anti-β-actin and anti-IgG HRP-conjugated (as a secondary antibody) antibodies were procured from Abcam (United Kingdom). The study was approved by the Kharazmi University of Tehran (616/9415).

Cell culture

MDA-MB-231 cancer cells were first cultured in the RPMI-1640 medium (Gibco, USA) that was supplemented with 100 U/ml penicillin-streptomycin and 10% fetal bovine serum (FBS). The cells were incubated at 37°C in a 5% CO_2-95% air atmosphere for 24 hours. The medium was replaced with the fresh cell culture medium every 48 hours when the color of the medium was changed.

Morphological observations

For the evaluation of the impact of melittin on the morphological alterations of MDA-MB-231 cells, approximately 5×10^4 cells were cultured in a 24-well plate. Upon reaching 65-70% confluence, cells were treated with various concentrations of melittin (0.5-4 µg/ml). After 24 hours, morphological changes were observed under an inverted microscope (Biomedia, EU) and compared to the control cells.

Cell viability assay

The toxicity of melittin against cancer cells was examined by the MTT assay. To this aim, cells were seeded onto a 24-well plate at the density of 30×10^3 cells per well in the RPMI-1640 medium and allowed to adhere for 24 hours and then treated with different concentrations of melittin for 24, 48, and 72 hours. Then, the medium was discarded, and the cells were incubated with 0.5 mg/ml of 3- [4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) (Sigma, USA). After 3-4 hours of the incubation period at 37°C and 5% CO_2 atmosphere, the number of formazan crystals was quantified at a wavelength of 570 nm.

Migration assay

The process of cell migration was examined by the migration assay. Briefly, cells were seeded onto a 6-well plate and incubated at 37°C for 24 hours until a monolayer is formed. Monolayers were gently and slowly scratched with a pipette tip across the center of the well. Then, they were treated with various concentrations of melittin for 6 hours, followed by incubation with EGF (20 ng/ml) and 1% FBS medium for 24 and 48 hours. After the incubation period, the process of cell migration was tracked at the 24 and 48 hours of incubation. The open area was calculated

with the T scratch software, a novel and simple tool for the automated analysis of monolayer migration assay.

Western blot analysis

The western blot technique was performed to analyze the relative expression of the desired proteins after 6 hours of incubation of MDA-MB-231 cells with melittin. Briefly, cells were seeded onto a 6-well plate at a density of 5×10^5 cells per well and then treated with various doses of melittin for 6 hours, followed by the stimulation in the absence or presence of EGF (20 ng/ml) for 2 hours. The cell lysate was centrifuged at 13000 rpm at 4°C for 15 minutes. The supernatant was collected, and the concentration of total protein was determined by the Bradford assay. Approximately 25 µg of the extracted proteins were used for the sodium dodecyl sulphate-polyacrylamide electrophoresis gel (SDS-PAGE). The protein mixture was electrophoretically separated on 15% polyacrylamide gel and then transferred onto the PVDF membrane. The membrane was soaked in 5% non-fat dry milk and Tris-buffered saline and 0.1% Tween-20 (TBST) to prevent the binding of non-specific antibodies. The membrane was subsequently incubated with proper amounts of primary antibodies. After three times washing with TBST, the membrane was probed with horseradish peroxidase (HRP)-conjugated secondary antibody. The membrane was rinsed again for an additional three times in TBST, and finally, DAB (3,3'-diaminobenzidine) was used to visualize the protein bands. The densitometry analysis of protein bands was performed using the ImageJ software.

Statistical analysis

The obtained values were analyzed by Graphpad prism version 7.0 (San Diego, California USA). One-way analysis of variance (ANOVA), followed by Tukey's post hoc test was employed for the comparison of differences between experimental groups. Data are expressed as the mean ± standard error of the mean. The level of the statistical significance was set at $P<0.05$.

Results

Morphological alterations in MDA-MB-231 cells in response to melittin

The morphological changes induced by 0.5-4 µg/ml melittin after 48 hours was observed and compared with untreated cells. As shown in Figure 1, this type of cells exhibit a typical spindle-shaped morphology. Cells were treated with 0.5, and 1 µg/ml melittin did not show a significant morphological alteration when compared with untreated cells. Cells treated with melittin at a concentration of 2 µg/ml underwent some cellular damages such as cell shrinkage. The results demonstrated that 4 µg/ml melittin caused a significant morphological change as compared with untreated cells, showing a significant degree of cell death exposed to melittin.
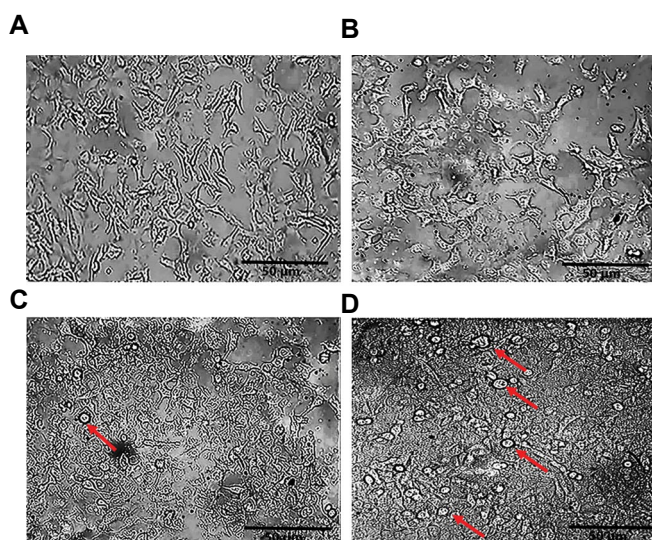

Fig.1: Morphological analysis of MDA-MB-231 cells in response to treatment with melittin after 48 hours. **A.** Untreated (control) cells, and **B.** treated with 1, **C.** 2, **D.** 4 µg/ml melittin (scale bar: 50 µm). Red arrows show dead cells (magnification ×10).

Melittin at low concentrations does not affect cell survival

Before the examination of migration and metastasis of cancer cells, the cell viability of the cultured cells, as well as the impact of melittin on cell survival was examined by the MTT assay. Melittin significantly caused cell death in MDA-MB-231 cells when used at a concentration of 4 µg/ml, and the cytotoxicity of the drug was mediated in a dose-dependent fashion. However, melittin, at concentrations lower than 2 µg/ml, cannot significantly change the cell survival of MDA-MB-231 cells compared with untreated cells (Fig.2). So, melittin was used at a lower concentration of 2 µg /ml for subsequent experiments.
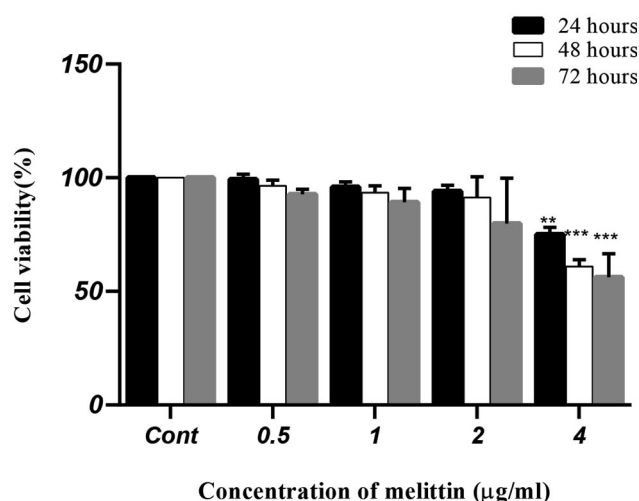

Fig.2: The percentage of the cell viability of MDA-MB-231 cells determined by the MTT assay. After 24, 48, and 72 hours, the impact of melittin on 0.5, 1, 2, and 4 µg/ml on the survival rate of breast cancer cells (MDA-MB-231) was measured. The graph shows that, in parallel with an increase in the concentration of melittin, the cell viability of MDA-MB-231 cells is markedly decreased. The data are expressed as the means and standard errors (mean ± SE) of three independent experiments. ***; P<0.001, **; P<0.01 compared to the untreated control (the first bar).

Inhibitory effect of melittin on the migration of the MDA-MB-231 cancer cell line

The cell migration assay was carried out to assess the preventive role of melittin in migration and cell motility of the MDA-MB-231 cancer cell line induced by EGF at 24 and 48 hours. As illustrated in Figure 3, along with an increase in the levels of melittin the rate of cell migration is decreased.

Melittin halts the invasion of the MDA-MB-231 cancer cell line induced by epidermal growth factor by reducing the expression of Rac1, CXCR4, and SDF-1α

The inhibitory effect of melittin on the expression of Rac1, p-Rac1, CXCR4, and SDF-1α was examined by the western blot analysis of whole-cell lysates of MDA-MB-231 cells treated with melittin for 6 hours, followed by stimulating with EGF (20 ng/ml) for 2 hours. As depicted in Figure 4., the results showed that the rate of the expression of the proteins mentioned above was substantially diminished in a dose-dependent manner in response to the treatment of EGF-induced MDA-MB-231 cancer cells with melittin.
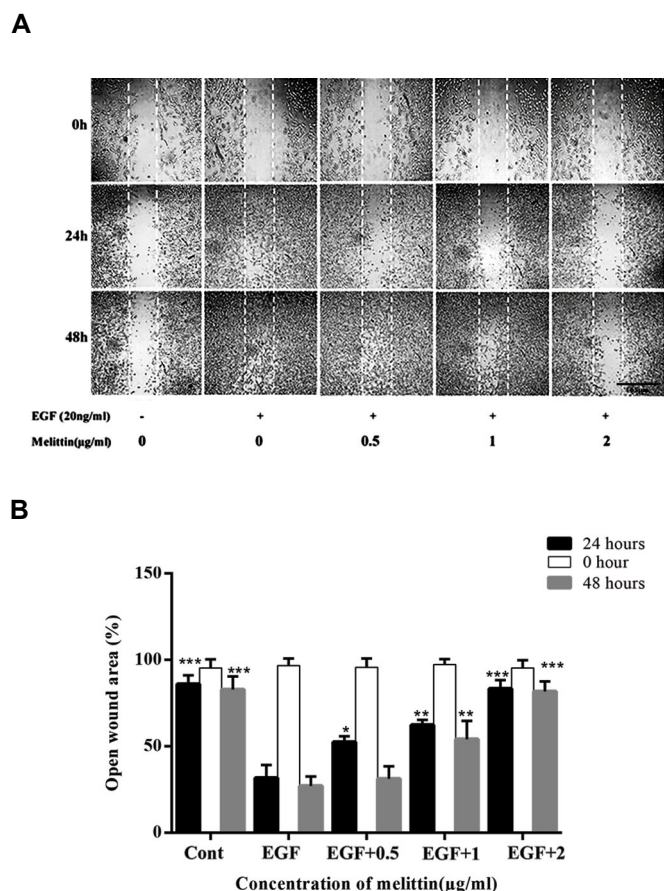
A

B

Fig.3: The inhibitory effect of different concentrations of melittin on the migration of epidermal growth factor (EGF)-induced MDA-MB-231 cells. **A.** The migration assay was carried out after 24 and 48 hours; then the migrated cells were imaged. **B.** Semi-quantification of protein bands in the migration assay (scale bar: 165 μm) (n=3, mean ± SE, ***; P<0.001, **; P<0.01, *; P<0.05, compared to the 0 hour (the white bars).
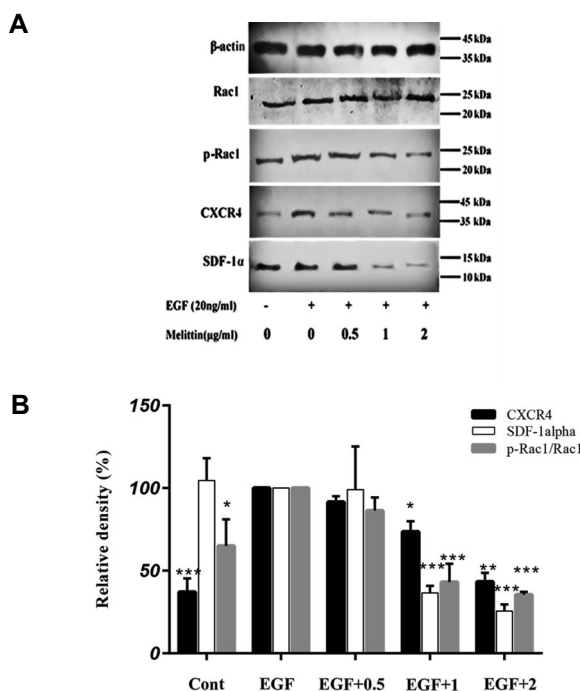
A

B

Fig.4: The inhibitory effect of melittin on epidermal growth factor (EGF)-induced Rac1, p-Rac1, CXCR4, and SDF-1α, in EGF-induced MDA-MB-231 cells. **A.** The western blot analysis indicated that melittin treatment reduces the expression of all metastasis-related proteins. β-actin was used as a loading control. **B.** Semi-quantification of the density of protein bands showed a significant decrease in the expression of all proteins in a dose-dependent manner (n=2, mean ± SE, ***; P<0.001, **; P<0.01 *; P<0.05, compared to the untreated control which is getting EGF and no melittin (the second group bar).

Discussion

Breast cancer is the most common type of cancer among women. In recent decades, the number of patients diagnosed with breast cancer, as well as the mortality of patients, has been significantly increased, implying that there is an urgent need to seek more efficient therapeutic strategies to cure patients who have breast cancer.

One of the main problems in breast cancer, especially in MDA-MB-231 cells, is the occurrence of metastasis. Several lines of evidence demonstrated that EGF is capable of promoting the migration of the MDA-MB-231 cancer cell line (21). EGF can stimulate F-actin polymerization, which leads to the formation of lamellipodia (22). Our results also confirmed that EGF stimulates the motility of MDA-MB-231 cells. The migration assay also revealed that this EGF-induced motility could be inhibited by melittin.

It has been indicated that a large number of genes contribute to the signaling pathways related to the metastasis process. Among those genes, Rac1, SDF-1α, and CXCR-4 have an undeniable impact on the function of cofilin, which contributes to stimulus-induced actin filament assembly during the formation of the lamellipodium.

The Rac1 protein belongs to the Rho family proteins, and it is one of the most common proteins, regulating the adhesion and migration of various types of cells. Rac1

is crucial for tumor growth, invasion, metastasis, and angiogenesis. This protein binds to either GDP or GTP, Rac1-GDP (inactive), and Rac1-GTP (activated) (23). The activation of the Rac1 leads to actin polymerization and lamellipodium formation during the migration of cells (24). Rac1 is overexpressed in numerous types of cancers, including testicular cancer, gastric, and breast cancers (25). Therefore, Rac1 may be a useful target for therapeutic purposes to halt the process of metastasis in cancer.

Chemokines are small proteins that interact with a large superfamily of the G protein-coupled receptors (8). Previous studies have shown that binding of SDF 1α and its cognate receptor, CXCR4, is essential for tumor progression, angiogenesis, metastasis, and survival (26). Thus, the inhibition of this chemokine and its cognate receptor can result in the prevention of tumor metastasis (27). Any interference with the expression of master genes involved in actin cytoskeleton formation can contribute to a decrease in the motility of cancer cells (28). Due to the severe side effects of chemotherapy and surgery, in recent years, the use of natural compounds with anticancer properties has been proposed since they possess much fewer adverse effects on the human body. Melittin is one of these natural components that the biological potential of this compound has been extensively studied (19). Melittin has only mild allergic side effects (29). Besides, melittin can cause massive hemolysis. This poses significant limitations in clinical studies. These days, the discovery of new methods of melittin delivery has solved this problem (30).

On the other hand, EGF treatment increased the expression of CXCR-4 and the active form of Rac1 (p-Rac1) proteins, but it was not able to alter the expression of SDF1α and total Rac1 (Rac1) significantly. These data are in conformity with the previous evidence that EGF induces CXCR4 activation in other cancer cells (16) and Rac1 expression in MDA-MB-231 cells (15) particularly Rac1 and Rac1b in TGF-β-induced epithelial-mesenchymal transition (EMT. But there is not any evidence on the role of EGF in the modulation of SDF1α.

A large body of evidence has shown that melittin could induce cell death in ovarian cancer cells via the stimulation of the expression of death receptors, as well as the inhibition of the STAT-3 pathway (29). This peptide inhibits metastasis through the hindrance of MMP-9 expression (30). In a study performed by Huh et al., they have demonstrated that melittin inhibits the VEGFR-2 and COX-2-mediated MAPK signaling pathways, which have anti-angiogenesis and anti-tumor activities (31). In a study conducted on AGS gastric cancer cell line and HeLa cervical cancer cell line, it was shown that melittin inhibited the proliferation of both types of cell lines (32, 33). In another study performed on the expression of Rac1 in gastric carcinoma, Wu et al. reported that Rac1 expression is associated with increased metastasis in gastric cancer (34). Studies have also shown that the suppression of Rac1 activity by melittin halts the process

of metastasis in liver cancer cells in nude mice (35). Several lines of evidence indicated that the expression levels of the chemokine receptors are highly associated with the development of some cancers and have specific roles in cancer metastasis (36). It has been shown that melittin is capable of decreasing the expression of SDF1α and CXCR4 in the UMR-106 osteosarcoma xenograft mouse model (37). Another report showed that melittin could attenuate tumor invasion through the inhibition of the PI3K/AKT/mTOR signaling pathway in breast cancer cells (38). Our findings were in line with previous results showing that melittin is able to halt migration and invasion of the EGF-induced MDA-MB-231 cancer cells through mitigating the expression of *Rac1* and *CXCR4* genes. Although the comparison between the control group (receiving no treatment) and the EGF group (treated with EGF) shows that EGF was not capable of stimulating the expression of SDF1α, whereas melittin suppressed the expression of SDF1α in a dose-dependent fashion.

In summary, the present study indicated the anti-proliferative effect of melittin on EGF-induced MDA-MB-231cancer cells as a metastatic cell line. Our findings implicated that this natural compound is highly toxic and could impair the viability of MDA-MB-231 cells and reduce the migration of cancer cells in a dose-dependent manner.

Conclusion

In this study, it was shown for the first time that melittin can affect tumor cell migration through CXCR4/SDF-1α signaling pathway. All in all, we revealed that melittin, by blocking CXCR4 in the cell membrane and inhibiting the expression of SDF-1α, can exert an anti-motility potential. Altogether, further studies such as murine models of breast cancer will be required to unravel the inhibitory effects of melittin on the propagation of tumor cells.

Acknowledgments

Authors' Contributions

M.N.; Designed experiments, contributed to material preparation, and wrote the manuscript. F.S.; Performed experiments, analyzed the data, and wrote the manuscript. E.S.; Supervised the research, developed the theory, contributed to the final version of the manuscript, and read and approved the final manuscript. All authors read and approved the final manuscript.

References

1. Wehbe R, Frangieh J, Rima M, El Obeid D, Sabatier JM, Fajloun Z. Bee venom: overview of main compounds and bioactivities for therapeutic interests. Molecules. 2019; 24(16): 2997.
2. Chen J, Guan SM, Sun W, Fu H. Melittin, the major pain-producing

substance of bee venom. Neurosci Bull. 2016; 32(3): 265-272.

3. Wu Y, Han MF, Liu C, Liu TY, Feng YF, Zou Y, et al. Design, synthesis, and antiproliferative activities of stapled melittin peptides. RSC Adv. 2017; 7(28): 17514-17518.

4. Sekiguchi R, Yamada KM. Basement membranes in development and disease. Curr Top Dev Biol. 2018; 130: 143-191.

5. Fares J, Fares MY, Khachfe HH, Salhab HA, Fares Y. Molecular principles of metastasis: a hallmark of cancer revisited. Signal Transduct Target Ther. 2020; 5(1): 28.

6. Rahman M, Mohammed S. Breast cancer metastasis and the lymphatic system. Oncol Lett. 2015; 10(3): 1233-1239.

7. Kircher M, Herhaus P, Schottelius M, Buck AK, Werner RA, Wester HJ, et al. CXCR4-directed theranostics in oncology and inflammation. Ann Nucl Med. 2018; 32(8): 503-511.

8. Miller MC, Mayo KH. Chemokines from a structural perspective. Int J Mol Sci. 2017; 18(10): 2088.

9. Choi J, Selmi C, Leung PS, Kenny TP, Roskams T, Gershwin ME. Chemokine and chemokine receptors in autoimmunity: the case of primary biliary cholangitis. Expert Rev Clin Immunol. 2016; 12(6): 661-672.

10. Adelita T, Stilhano RS, Han SW, Justo GZ, Porcionatto M. Proteolytic processed form of CXCL12 abolishes migration and induces apoptosis in neural stem cells in vitro. Stem Cell Res. 2017; 22: 61-69.

11. Busillo JM, Benovic JL. Regulation of CXCR4 signaling. Biochim Biophys Acta. 2007; 1768(4): 952-963.

12. Lopez-Haber C, Barrio-Real L, Casado-Medrano V, Kazanietz MG. Heregulin/ErbB3 Signaling enhances CXCR4-driven Rac1 activation and breast cancer cell motility via hypoxia-inducible factor 1α. Mol Cell Biol. 2016; 36(15): 2011-2026.

13. Clayton NS, Ridley AJ. Targeting Rho GTPase signaling networks in cancer. Front Cell Dev Biol. 2020; 8: 222.

14. Zeng Y, Cao Y, Liu L, Zhao J, Zhang T, Xiao L, et al. SEPT9_i1 regulates human breast cancer cell motility through cytoskeletal and RhoA/FAK signaling pathway regulation. Cell Death Dis. 2019; 10(10): 720.

15. Ungefroren H, Witte D, Lehnert H. The role of small GTPases of the Rho/Rac family in TGF-β-induced EMT and cell motility in cancer. Dev Dyn. 2018; 247(3): 451-461.

16. Xu C, Zhao H, Chen H, Yao Q. CXCR4 in breast cancer: oncogenic role and therapeutic targeting. Drug Des Devel Ther. 2015; 9: 4953-4964.

17. Wang L, Li X, Zhao Y, Fang C, Lian Y, Gou W, et al. Insights into the mechanism of CXCL12-mediated signaling in trophoblast functions and placental angiogenesis. Acta Biochim Biophys Sin (Shanghai). 2015; 47(9): 663-672.

18. Rady I, Siddiqui IA, Rady M, Mukhtar H. Melittin, a major peptide component of bee venom, and its conjugates in cancer therapy. Cancer Lett. 2017; 402: 16-31.

19. Schirrmacher V. From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment (Review). Int J Oncol. 2019; 54(2): 407-419.

20. Huang Z, Yu P, Tang J. Characterization of triple-negative breast cancer MDA-MB-231 cell spheroid model. Onco Targets Ther. 2020; 13: 5395-5405.

21. Park JH, Cho YY, Yoon SW, Park B. Suppression of MMP-9 and FAK expression by pomolic acid via blocking of NF-κB/ERK/mTOR signaling pathways in growth factor-stimulated human breast cancer cells. Int J Oncol. 2016; 49(3): 1230-1240.

22. Ho E, Dagnino L. Epidermal growth factor induction of front-rear polarity and migration in keratinocytes is mediated by integrin-linked kinase and ELMO2. Mol Biol Cell. 2012; 23(3): 492-502.

23. Haga RB, Ridley AJ. Rho GTPases: Regulation and roles in cancer cell biology. Small GTPases. 2016; 7(4): 207-221.

24. Kunschmann T, Puder S, Fischer T, Steffen A, Rottner K, Mierke CT. The small GTPase Rac1 increases cell surface stiffness and enhances 3D migration into extracellular matrices. Sci Rep. 2019; 9(1): 7675.

25. Porter AP, Papaioannou A, Malliri A. Deregulation of Rho GTPases in cancer. Small GTPases. 2016; 7(3): 123-138.

26. García-Cuesta EM, Santiago CA, Vallejo-Díaz J, Juarranz Y, Rodríguez-Frade JM, Mellado M. The role of the CXCL12/CXCR4/ACKR3 axis in autoimmune diseases. Front Endocrinol (Lausanne). 2019; 10: 585.

27. Gupta N, Duda DG. Role of stromal cell-derived factor 1α pathway in bone metastatic prostate cancer. J Biomed Res. 2016; 30(3): 181-185.

28. Gandalovičová A, Rosel D, Fernandes M, Veselý P, Heneberg P, Čermák V, et al. Migrastatics-anti-metastatic and anti-invasion drugs: promises and challenges. Trends Cancer. 2017; 3(6): 391-406.

29. Jo M, Park MH, Kollipara PS, An BJ, Song HS, Han SB, et al. Anti-cancer effect of bee venom toxin and melittin in ovarian cancer cells through induction of death receptors and inhibition of JAK2/STAT3 pathway. Toxicol Appl Pharmacol. 2012; 258(1): 72-81.

30. Park JH, Jeong YJ, Park KK, Cho HJ, Chung IK, Min KS, et al. Melittin suppresses PMA-induced tumor cell invasion by inhibiting NF-kappaB and AP-1-dependent MMP-9 expression. Mol Cells. 2010; 29(2): 209-215.

31. Huh JE, Kang JW, Nam D, Baek YH, Choi DY, Park DS, et al. Melittin suppresses VEGF-A-induced tumor growth by blocking VEGFR-2 and the COX-2-mediated MAPK signaling pathway. J Nat Prod. 2012; 75(11): 1922-1929.

32. Mahmoodzadeh A, Morady A, Zarrinnahad H, Pooshang Bagheri K, Ghasemi-Dehkordi P, Mahdavi M, et al. Isolation of melittin from bee venom and evaluation of its effect on proliferation of gastric cancer cells. Tehran Univ Med J. 2013; 70(12): 760-767.

33. Zarinnahad H, Mahmoodzadeh A, Pooshang Bagheri K, Mahdavi M, Shahbazzadeh D, Moradi A. Isolation of melittin from iranian honey bee venom and investigation of its effect on proliferation of cervical cancer- hela cell line. JSSU. 2013; 21(2): 226-238.

34. Wu YJ, Tang Y, Li ZF, Li Z, Zhao Y, Wu ZJ, et al. Expression and significance of Rac1, Pak1 and Rock1 in gastric carcinoma. Asia Pac J Clin Oncol. 2014; 10(2): e33-39.

35. Liu S, Yu M, He Y, Xiao L, Wang F, Song C, et al. Melittin prevents liver cancer cell metastasis through inhibition of the Rac1-dependent pathway. Hepatology. 2008; 47(6): 1964-1973.

36. Mollica Poeta V, Massara M, Capucetti A, Bonecchi R. Chemokines and chemokine receptors: new targets for cancer immunotherapy. Front Immunol. 2019; 10: 379.

37. Qin G, Chen Y, Li H, Xu S, Li Y, Sun J, et al. Melittin inhibits tumor angiogenesis modulated by endothelial progenitor cells associated with the SDF-1α/CXCR4 signaling pathway in a UMR-106 osteo-sarcoma xenograft mouse model. Mol Med Rep. 2016; 14(1): 57-68.

38. Jeong YJ, Choi Y, Shin JM, Cho HJ, Kang JH, Park KK, et al. Melittin suppresses EGF-induced cell motility and invasion by inhibiting PI3K/Akt/mTOR signaling pathway in breast cancer cells. Food Chem Toxicol. 2014; 68: 218.