Original Article

Potential Role of Royal Jelly and 10-Hydroxy-2-Decenoic Acid as Metastasis Inhibitors in Triple-Negative Breast Cancer Cells

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Background: Royal jelly (RJ) produced by honeybees has been reported to possess various pharmacological activities. For the role of antitumor, RJ has been demonstrated in several types of cancer cells which 10-hydroxy-2-decenoic acid (10-HDA) proposed as an active component. In the present study, the authors aimed to expand more knowledge of the antitumor effect of RJ and 10-HDA in the aspect of metastasis inhibition. Triple negative breast cancer cells (TNBC) that are often aggressive and associated with poor prognosis were selected as a model for the present study.

Materials and Methods: An MTT assay was used for determination of cytotoxicity, while migration, invasion and adhesion assays were used to evaluate metastatic potential. The effect of angiogenesis was assessed by the level of pro-angiogenic factor, VEGF.

Results: RJ decreased the cell viability of TNBC (MDA-MB-231) cells and other cancer cells (Colo205, HepG2, HeLa and KB). The blockage of TNBC migration was clearly seen when the cells were treated with RJ or 10-HDA. The invasion, adhesion and release of VEGF were significantly inhibited by 10-HDA.

Conclusion: RJ and 10-HDA have potential of anti-metastatic activity against aggressive breast cancer cells.

Keywords: royal jelly, 10-HDA, TNBC, Metastasis, Migration, Invasion, Adhesion, VEGF

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Royal jelly (RJ), is a white and viscous jelly like substance produced from the hypopharyngeal and mandibular glands of honey bees (*Apis mellifera*)[1]. RJ has been known as the superfood for honeybee larvae during the first three days after hatching and for the queen bee for its entire life[2]. RJ has been used as a traditional health food and has been reported to have various pharmacological activities, including anti-inflammatory, antioxidant, antibacterial, antiallergic, antitumor, antiaging, immunomodulatory, hepatoprotective, hypotensive, vasodilative and antihypercholesterolemic effects. Currently, RJ is widely used as a dietary supplement, commercial medical product, and cosmetic in many countries[3]. RJ is comprised of water (60% to 70%), proteins (9% to 18%), fructose (3% to 13%), glucose (4% to 8%), sucrose (0.5% to 2%) lipids (3% to 8%), mineral salts (1.5%), vitamins and 10-hydroxy-2-decenoic acid (10-HDA, >1.4%). A major unsaturated fatty acid component, 10-HDA, which is found exclusively in RJ[4], has been reported to be an active component of RJ[5]. Many biological activities of 10-HDA has been demonstrated; antitumor[6,7], melanogenesis inhibitor[8], antiaging[9], antibiotic, anti-inflammatory[10], antiangiogenic[11], promotion of collagen production[12], and skin protection[13] effects. However, the roles of RJ and 10-HDA as metastasis inhibitors have not been elucidated.

Breast cancer is the most commonly occurring cancer in women worldwide. The data from the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) reveals that 2 million new cases (12.19%) were diagnosed in 2018, and mortality claimed more than 600,000 cases (7.04% of all cancers). Triple-negative breast cancer (TNBC), which lacks the expression of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), is a subgroup of breast cancer, and it represents approximately 15% to 20% of all breast cancers[14]. TNBC is aggressive, with high proliferation and metastasis. Patients, mostly young women, have poor prognoses. In addition, TNBC has a high rate of early relapse, approximately 34% of which is diagnosed in the first three years of follow-up. The metastasis of TNBC to lungs and brain is high compared to other breast cancer subtypes[15,16]. TNBC is sensitive to cytotoxic chemotherapy agents, especially anthracyclines and taxanes[17]; however, these drugs have serious side effects[18]. In addition, resistance to the drugs can lead to more aggressive cancer that causes unsuccessful treatment and recurrence that

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ultimately contributes to mortality[19].

In the present study, we aimed to investigate the role of RJ and 10-HDA as antitumor agents by focusing on the role of metastasis inhibition in breast cancer cells. The aggressive TNBC was selected as a model due to its resistance to clinical treatment. Our work demonstrated cytotoxic effect of RJ and 10-HDA together with their inhibition activity on migration, invasion, adhesion and possibility angiogenesis in TNBC. These findings revealed novel biological effects that support the value of RJ and 10-HDA as antitumor agents.

Materials and Methods

Chemicals and reagents

DMEM (Dulbecco's Modified Eagle Medium), Medium 199 (M199), RPMI 1640, Foetal Bovine Serum (FBS), 0.25% Trypsin-EDTA, and Penicillin-streptomycin were obtained from Thermo Fisher Scientific (Waltham, MA USA). 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazo lium bromide (MTT) was obtained from USB Corporation (Ohio, USA). The 10-HDA was purchased from Cayman Chemical (Michigan, USA).

Cell lines and cell culture conditions

The triple-negative breast cancer cell line (MDA-MB-231, ATCC HTB-26), cervical cancer cell line (HeLa, ATCC CRM-CCL-2), colon cancer cell line (Colo205, ATCC CCL-222), liver cancer cell line (HepG2, ATCC HB-8065), and the normal kidney monkey cell line (Vero, ATCC CCL-81) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The oral cancer cell line (KB) was kindly provided by Dr. Duangporn Srisuparbh, Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand. The MDA-MB-231, HeLa, and KB cells were maintained in Dulbecco's Modified Eagle's medium (DMEM). HepG2 cells were maintained in RPMI 1640. Vero cells were maintained in M199. Colo205 cells were maintained in RPMI1640 and supplemented with glucose, sodium pyruvate, and HEPES. All culture media were supplemented with 10% FBS and 100 U/mL of penicillin-streptomycin. The cells were cultured at 37°C in an atmosphere of humidified air with 5% CO_2.

RJ and 10-HDA preparation

RJ from *Apis mellifera* was obtained from Supa Farm, Chiang Mai, Thailand, and stored at -20°C until used. RJ preparation was described previously[20]. Briefly, the same lot number of RJ was used throughout the study to avoid the variability of the biological activity. RJ was extracted with PBS at a stock concentration of 1,000 mg/mL for 1 h on a rotating shaker and centrifuged at 15,000 RPM, 4°C for 10 min. The supernatant was collected by syringe filter and kept at -20°C until use. 10-HDA was dissolved in DMSO, which was recommended by the manufacturer, and stored at -20°C until use.

Cytotoxicity measurement

The cytotoxic effect of RJ was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium bromide) assay as described previously[21]. Briefly, all cells were seeded at a density of 1 x 10^4 cells per well of 96-well plate. Cells were treated for 24 h with final concentrations of RJ ranging from 0 to 100 mg/mL; PBS was used as the negative control. 10-HDA was used only on MDA-MB-231 and Vero cells with the final concentrations ranging from 0 to 20 mM, and 1% DMSO was used as the vehicle control. The MTT solution was added at 0.5 mg/mL to each well and incubated for 2 h at 37°C. The formazan crystal products were dissolved with DMSO, and the concentrations were determined by optical density (OD) measurements using an ELISA microplate reader at 595 nm.

Wound healing assay

The effect of RJ and 10-HDA on the migration of MDA-MB-231 cells was determined by a monolayer wound healing assay[22]. MDA-MB-231 cells were grown in serum-free medium in 6-well tissue culture plates at a density of 8 x 10^5 cells per well. After the cells formed 95% confluent monolayers, the cells were scratched with 10 µL sterile pipette tips. The medium was immediately replaced with or without RJ or 10-HDA at 25% and 50% of IC_{50} for 24 h. The wound closure was monitored and imaged with an inverted microscope at 10x magnification at 0 and 24 h.

Transwell migration and invasion assays

The 24-well plate transwell inserts were used with 8.0 µm pore polycarbonate membranes (BD Biosciences, New Jersey, USA) and precoated with (for invasion assays) or without (for migration assays) Matrigel[23,24]. MDA-MB-231 cells were added at a density of 5 x 10^4 cells in serum-free medium to the upper insert, and culture medium containing 10% FBS was added to the lower chamber. The 10-HDA was added at 25% (3 mM) and 50% (6 mM) of the IC_{50} to the upper insert. After incubation for 24 h, the cells that passed through to the lower chamber were fixed with 100% ice-cold methanol for 20 min and stained with 500 µL of 0.5% crystal violet at room temperature for 15 min. Cell invasion or migration was then photographed. Quantification of the stained cells was done by dissolving the stain in 100% methanol and measuring the colour at OD_{540}.

Adhesion assay

CHEMICON ECM Cell Adhesion Array Kits, colorimetric, were used to measure adhesion following the manufacturer's protocol. In brief, the plate strips containing adhesion molecules were rehydrated with PBS for 10 min at room temperature. MDA-MB-231 cells were suspended in medium at the density of 2x10^5 cells/mL, and 100 µL of the suspension was added to the wells of a 96-well plate. 10-HDA was applied immediately, and incubated for 2 h at 37°C in 5% CO_2. Samples were then gently washed twice with assay buffer and stained for 5 min. After staining, the plate strips were washed and air dried. Then, the extraction buffer was applied to each well and OD_{560} was measured with a microplate reader.

VEGF-A enzyme-linked immunosorbent assay (ELISA)

MDA-MB-231 cells were seeded in a 6-well plate at a density of 1 x 10^6 cells per well in complete medium overnight. The medium in each well was replaced with 2 mL serum-free medium containing 25% (3 mM) or 50% IC_{50} (6 mM) of 10-HDA for 24 h. Then, the medium was collected and concentrated with Amicon Ultra-4 10 K filters (Millipore, Darmstadt, Germany). The protein concentrations were measured by the Bradford method. The quantification of VEGF-A was performed according to the manufacturer's instructions (VEGFA Human ELISA Kit, Abcam).

Statistical analysis

The results were expressed as the mean ± standard error (SD, for each group n = 3). All data were processed with GraphPad Prism 5 software. Data analysis was performed by using nonlinear regression to evaluate the inhibitory concentration at 50% (IC_{50}). The statistical significance of the adhesion, migration and invasion assays was assessed by one-way Analysis of Variance (ANOVA). Two-way ANOVA was used for wound healing and evaluated for the significance differences between the groups; $p<0.05$ was considered significant. Specific *p*-values are indicated in figure legends.

Results

The cytotoxic effects of RJ and 10-HDA on cancer cells

The authors first investigated the antitumor effects of RJ on various types of cancer cells. As shown in Figure 1, the cytotoxic effects of RJ were clearly observed in all cancer cells (MDA-MB-231, KB, HepG2, Colo205 and HeLa) compared to the continuously proliferating epithelial Vero cells. The IC_{50} values for RJ were 17.50, 22.66, 24.92, 25.43, 46.12 and 63.26 mg/mL, respectively, whilst the vehicle control had no effect. Because 10-HDA has been known to be an active component of RJ, we asked whether 10-HDA had the same cancer cell cytotoxicity as RJ. The cytotoxicity of 10-HDA was evaluated against the MDA-MB-231 breast cancer cell line, which was derived from an aggressive and untreatable cancer. Vero cells were used as the control. As depicted in Figure 2, 10-HDA reduced the viability of MDA-MB-231 cells more than the viability of the non-cancerous Vero cells. The IC_{50} values for MDA-MB-231 and Vero cells were 12 mM and 19 mM, respectively. The difference in cytotoxicity was not significant; however, the effect of 10-HDA at 5 mM was clearly more pronounced in MDA-MB-231 than in Vero cells. When the concentration of 10-HDA was increased to 20 mM, proliferation of both cell lines was inhibited, but the difference in the response remained.

RJ and 10-HDA inhibited TNBC cell migration

To evaluate the effect of RJ and 10-HDA on cancer cell metastasis, a wound healing assay was performed. The MDA-MB-231 cell line, derived from an aggressive, metastatic breast cancer, was selected as the model for studying metastasis inhibition. Monolayers of MDA-MB-231 cells were scratched and treated with 25% or 50% IC_{50}

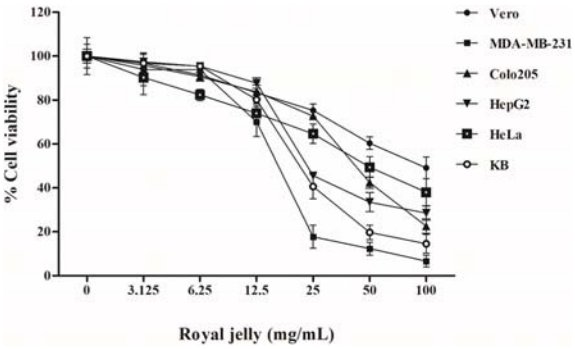

Figure 1. The effect of RJ on cell viability was determined by MTT assays. MDA-MB-231, Colo205, HeLa, HepG2, KB, and Vero cells were treated with various concentrations of RJ (0 to 100 mg/mL) or with the vehicle control (1% PBS) for 24 h. The data are expressed as a percentage of cell viability, and each bar represents the mean ± SD of three independent experiments.

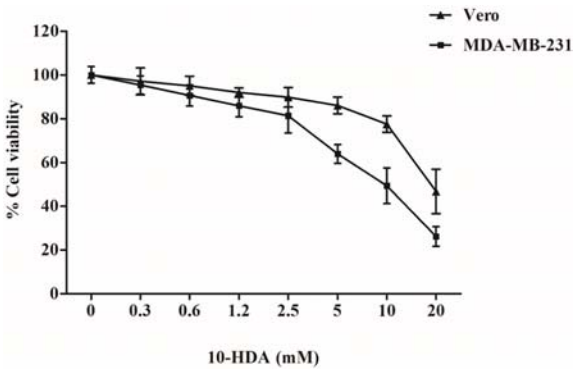

Figure 2. The effect of 10-HDA on cell viability was determined by MTT assays. Breast cancer cells (MDA-MB-231) and African green monkey kidney cells (Vero) were treated with various concentrations of 10-HDA (0 to 20 mM) or with the vehicle control (1% DMSO) for 24 h. Each bar represents the mean ± SD of three independent experiments. The data are expressed as a percentage of cell viability.

of RJ or 10-HDA, and the effects were measured at 0 and 24 h. As shown in Figure 3 to 4, the inhibition of cell migration was clearly observed in both the RJ and 10-HDA treated samples. The wound width as a function of RJ or 10-HDA treatment indicated that RJ and 10-HDA significantly inhibited cell migration in a dose-dependent manner ($p<0.001$). These results confirmed that both RJ and 10-HDA have an anti-migration effect on MDA-MB-231 cells.

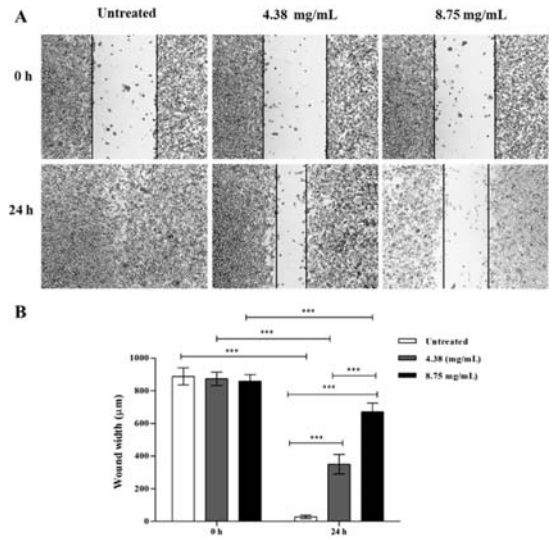

Figure 3. The effect of RJ on breast cancer cell migration was evaluated by a wound healing assay. The confluent MDA-MB-231 cells were scratched and treated with RJ at 0, 4.38 and 8.75 mg/mL for 24 h. The morphology (A) and the wound width (B) of untreated and treated samples were measured in at least three independent locations in each wound, and the final data are shown as the mean ± SD of three independent experiments. Two-way ANOVA was used for evaluating the significance of differences between the groups and within each group. *** Significantly different from the control (p<0.001).

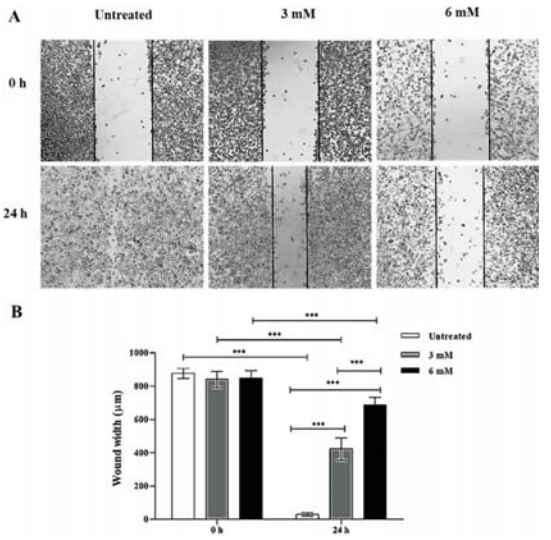

Figure 4. The effect of 10-HDA on breast cancer cell migration was evaluated by a wound healing assay. Confluent MDA-MB-231 cells were scratched and treated with 0, 3 or 6 mM 10-HDA for 24 h. The morphology (A) and the wound width (B) of untreated and treated samples were measured at least three locations in each wound, and the final data are shown as the mean±SD of three independent experiments. Two-way ANOVA was used for evaluating the significance of differences between the groups and within each group. ***Significantly different from the control (p<0.001).

10-HDA suppressed 3D cell migration and invasion

A transwell migration and invasion assay was performed to analyse the ability of 10-HDA to inhibit metastasis. Both assays used 10% FBS as the chemoattractant in the lower chamber to induce the cancer cells in the upper chamber to pass through the pores of the transwell. As shown in Figure 5, the number of MDA-MB-231 cells that moved through to the lower chamber was significantly reduced by 10-HDA, and the effect was dose-dependent (p<0.001 and p<0.01). For cell invasion assays, the transwell upper chamber was pre-coated with Matrigel as a source of extracellular matrix (ECM) proteins before it was seeded with cancer cells and treated with 10-HDA. As demonstrated in Figure 6, 10-HDA significantly reduced cancer cell invasion in a dose-dependent manner compared to the untreated control cells (p<0.001 and p<0.01). These results indicate that 10-HDA clearly affected both the cell migration and invasion of these breast cancer cells.

Anti-adhesion effect of 10-HDA

Cell adhesion is one important step in cancer cell metastasis. To determine the effect of 10-HDA on cancer cell adhesion, the in vitro ECM cell adhesion array assay was performed. Different ECM proteins, including collagen I, collagen II, collagen IV, fibronectin, laminin, tenascin, and vitronectin were used, and 10-HDA was tested at 0.75, 1.5, 3 and 6 mM for 2 h on MDA-MB-231 cells. The effect of 10-HDA as an anti-adhesion agent was demonstrated as the number of cell adhesions declined at the concentrations of 3 mM (p<0.01) and 6 mM (p<0.001) compared to untreated cells (Figure 7). Interestingly, the anti-adhesion effect of 10-HDA was clearly seen from 0.75 mM for the binding of ECM proteins and with different levels for each ECM protein. These data suggest that 10-HDA has a strong effect on the adhesion property of MDA-MB-231 cells. In addition, the data show that specific ECM component proteins, especially collagen IV, fibronectin, and vitronectin, are preferred for MDA-MB-231 cell adhesion[25,26].

10-HDA has an inhibitory effect on VEGF-A expression

The production of VEGF is a crucial step in physiological and pathological angiogenesis. VEGF induces the formation of new blood vessels and maintains blood vessel

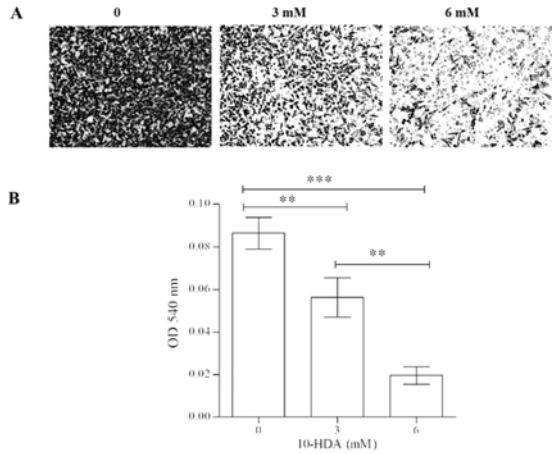

Figure 5. 10-HDA affected breast cancer cell migration. Transwell assays were performed by seeding MDA-MB-231 cells on the upper inserts and treating with 10-HDA at 0, 3, and 6 mM for 24 h. The lower chambers were loaded with 10% FBS as a chemoattractant. The cancer cells that migrated from the upper inserts through to the lower chambers were fixed with methanol, stained with crystal violet and photographed (A). The migrated cells were quantified by using methanol extraction of the stain and measuring the OD_{540} (B). The data are expressed as the mean ± SD, n = 3. ***p<0.001 and **p<0.01 compared with all pairs of columns.

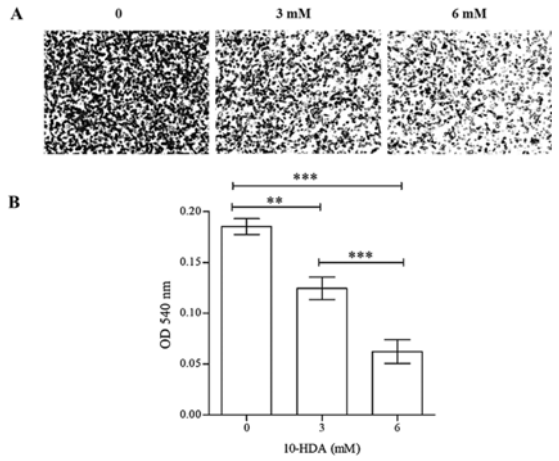

Figure 6. 10-HDA affected invasion by breast cancer cells. Transwell assays were performed by seeding MDA-MB-231 cells on the upper inserts and treating them with 10-HDA at 0, 3, and 6 mM for 24 h. The upper inserts were pre-coated with Matrigel. The lower chambers were loaded with 10% FBS as chemoattractant. The cancer cells that invaded from the upper inserts through the Matrigel to the lower chambers were fixed with methanol, stained with crystal violet and photographed (A). The cells were quantified by extracting the stain with methanol and measuring the OD at 540 nm (B). The data are expressed as the mean ± SD, n = 3. ***p< 0.001 and **p<0.01 compared to all pairs of columns.

structure; it also helps in wound healing. Amongst the VEGF proteins, VEGF-A plays the major role in tumour angiogenesis and helps in cancer progression and survival[27,28]. To observe the effect of 10-HDA on the angiogenic process, the release of VEGF-A from MDA-MB-231 cancer cells was used as a marker. The culture medium of 10-HDA treated samples was collected, concentrated and evaluated by VEGF-A Human ELISA assays. As shown in Figure 8, the release of VEGF-A significantly decreased in 10-HDA treated samples in a dose-dependent manner (p<0.001 and p<0.01) compared to vehicle controls. This result suggests that 10-HDA has an anti-angiogenic effect on highly metastatic breast cancer cells.

Discussion

It is well known that metastasis is a major driver of mortality in cancer patients[29]. This process is defined as a complex and multi-step biological process. Metastasis consists of five steps, including invasion, migration, intravasation to the circulatory or lymphatic system, extravasation into the bloodstream by adhering to the endothelium, and growth in distant organs with angiogenesis induction[30]. All these steps must be well coordinated; a defect in any step may interfere with subsequent steps of the cascade. Therefore, the development of antimetastatic therapy for any step may help to improve patient survival. RJ is a nutritious product from honey bees used as a food for the bee larvae and for the whole life of the queen bee[31]. In humans, RJ has been used as a traditional medicine and cosmetic since ancient times[32]. Currently, RJ is consumed as a dietary supplement because of its various biological effects, including antioxidant, neuroprotective, prevention of insulin resistance, hepatoprotective, antiinflammatory, antibacterial, immunomodulatory, antiallergic, antiaging and antitumor effects[31]. For antitumor activity, RJ has been shown to prevent the development of leukaemia or ascitic tumours in a mouse model. The activity has also demonstrated in breast cancer cells[33]. These cytotoxic effects on cancer cells suggest that RJ is an interesting compound to use as an antitumor agent. In the present study, the authors confirmed the cancer cytotoxicity efficacy of RJ and provided more information on the effect. The cytotoxicity was demonstrated in breast cancer (MDA-MB-231), oral cancer (KB), hepatoma (HepG2), colon cancer (Colo205) and cervical cancer (HeLa) cells within the range of 20 to 45 mg/mL (Figure 1). Interestingly, these results demonstrated the cytotoxic effect

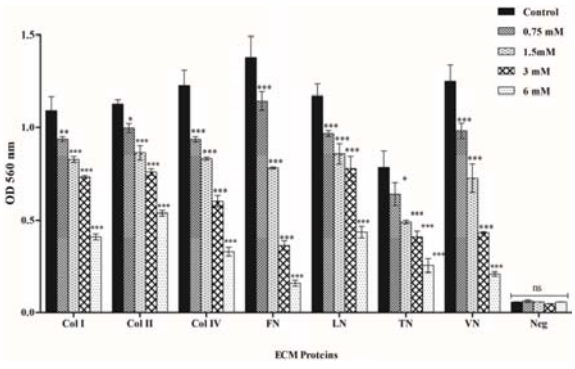

Figure 7. Inhibition of breast cancer cell adhesion by 10-HDA. ECM array plates coated with collagen I (Col I), collagen II (Col II), collagen IV (Col IV), fibronectin (FN), laminin (LN), tenascin (TN), or vitronectin (VN) were seeded with MDA-MB-231 cells and treated with 10-HDA at 0, 0.75, 1.5, 3 and 6 mM for 2 h. Adherent cells were stained and quantified by extracting the stain and measuring the OD at 560 nm. The data are represented as the mean ± SD, n = 3. ***p<0.001, **p<0.01, *p<0.05 compared to the vehicle control.

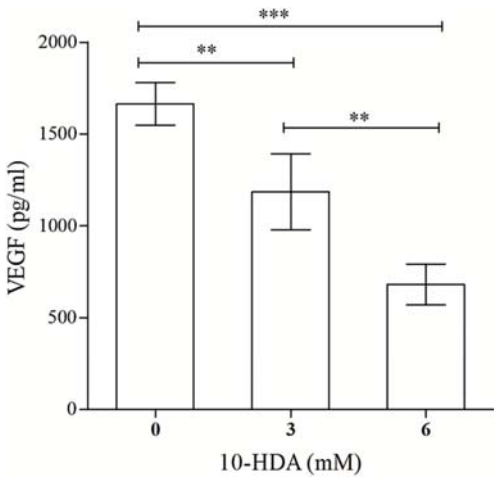

Figure 8. The release of VEGF from breast cancer cells is inhibited by 10-HDA. MDA-MB-231 cells were treated with 0, 3 or 6 mM of 10-HDA for 24 h. Culture supernatants were collected and concentrated, and the levels of VEGF-A was measured. The data are expressed as the mean ± SD, n = 3. ***p<0.001 and **p<0.01 compared to all pairs of columns.

in MDA-MB-231, a triple-negative breast cancer (TNBC) cell line, which is well known for aggressive metastasis and resistance to chemotherapy. The five year survival of TNBC patients tends to be lower than that of patients with other forms of breast cancer[34]. This result led us to determine whether RJ could reduce the metastasis of TNBC. We observed the effect of RJ against cancer cell migration using wound healing assays. As depicted in Figure 3, RJ significantly reduced the migration of TNBC cells. This work showed for the first time that RJ could act as anti-metastatic agent, especially in the aggressive breast cancer cell line MDA-MB-231.

The antitumor activity of RJ has been reported to be due to 10-HDA, a major unsaturated fatty acid constituent. We hypothesized that 10-HDA may be involved with the cytotoxicity and probably the anti-metastatic effect of RJ. The hypothesis was tested by using MTT and wound healing assays on MDA-MB-231 cells and on Vero cells, the representative of normal cells. The results demonstrated that 10-HDA reduced the proliferation (Figure 2) and migration (Figure 4) of MDA-MB-231 cells. For cytotoxicity, the difference between MDA-MB-231 and Vero cells was clearly observed starting from 5 mM, which reduced the number of viable cancer cells to 60%. This millimolar range was quite high compared to that of other natural bioactive agents; however, it is in the same range as in other reports of 10-HDA. For example, 10-HDA inhibited LPS-induced NO production in RAW264 cells at 5 mM[35]. In rheumatoid arthritis synovial fibroblasts (RASFs), 10-HDA inhibited TNF-induced ERK, p38 and JNK at 1-2 mM[10] and 10-HDA inhibited human colon cancer cell proliferation at 5 mM[36].

The preliminary result of the effect of 10-HDA on wound healing was also clearly seen and was consistent with the effect of RJ. To evaluate the anti-metastatic effect of 10-HDA in more detail, the transwell migration and invasion assays were used. As shown in Figure 5 to 6, 10-HDA reduced both the migration and invasion properties of MDA-MB-231 cells, and the effect was dose-dependent. Migration, invasion and adhesion are important characteristics of cancer metastasis. Many molecules have been reported to be involved with the adhesion of cancer cells, including fibronectin, vitronectin, collagen, tenascin and laminin[36,37]. We asked whether 10-HDA could affect the interaction between MDA-MB-231 cells and these adhesive molecules. As depicted in Figure 7, 10-HDA inhibited the adhesion of MDA-MB-231 cells to the EMC proteins tested, and the effects were dose-dependent. The effects were clearly observed at 0.75 mM of 10-HDA on collagen IV, fibronectin, laminin and vitronectin. These results suggest that these molecules would probably contribute to the adhesive property of MDA-MB-231 cells. This finding is supported by the results of other studies, which demonstrated the influence of these adhesive proteins on MDA-MB231 cells[25,26,38].

To determine the effect of 10-HDA on the process of angiogenesis, a key regulator, VEGF, was selected to be assayed. VEGF is a family of proteins comprised of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF)[39]. VEGF-A has been shown to be a pre-metastatic factor in target organs[40]. It is a key regulator of cancer, and it

promotes angiogenesis, cell proliferation, and migration. Here, we found that 10-HDA significantly decreased the level of VEGF-A in MDA-MB-231 cells (Figure 8) implying that the angiogenic ability of breast cancer cells was inhibited. A similar effect has been reported in other work, in which 500 mM of 10-HDA suppressed cell migration, proliferation and tube formation in HUVECs[11].

Conclusion

The present study demonstrated that RJ and 10-HDA have the potential to inhibit the metastatic activity of triple-negative breast cancer cells. 10-HDA inhibited breast cancer cell migration, invasion, adhesion and possibly angiogenesis. This extends the effects of RJ and 10-HDA beyond those previously reported and suggests that RJ and 10-HDA may be useful for cancer therapy, especially for highly aggressive breast cancers.

What is already known about this topic?

It is already known that RJ has antitumor property in several types of cancer cells but have never tested in TNBC. While 10-HDA has shown to inhibit the migration of human umbilical vein endothelial cells (HUVEC) but have never investigated for antitumor or anti-metastasis activities.

What this study adds?

The present study adds more information of RJ that could act as antitumor and inhibit the migration of TNBC. For the activity of 10-HDA, it is firstly demonstrated in this work that 10-HDA could act as an antitumor in TNBC. Importantly, 10-HDA potentially acts as anti-metastasis by inhibit the process of migration, invasion, adhesion and the releasing of pro-angiogenic factor.

Acknowledgements

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Potential conflicts of interest

The authors declare no conflict of interest.

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