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Exosomal miRNAs from highly metastatic cells can induce metastasis in non-metastatic cells

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ABSTRACT

Aims: Breast cancer is a high prevalence cancer among women worldwide. 15–20% of breast cancer cases are triple-negative with a poor prognosis. miRNA aberrant expression is one of the reasons of cancer development and metastasis. Exosomes are vesicles that carry cargos such as miRNAs to other cells. Therefore, we hypothesized that miRNAs transported by exosomes to other cells can induce malignant transformation.

Materials and methods: We extracted exosomes from highly metastatic MDA-MB-231 cells and characterized them using Dynamic light scattering, scanning and transmitting electron microscopy as well as western blot. Then, we treated non-metastatic MCF-7 cells with the exosomes. Afterwards, we evaluated exosome uptake by MCF-7 cells using PHK67 staining. Finally, we used soft agar colony formation, migration, and invasion assays to explore any increase in/induction of metastatic behavior of exosome-treated MCF-7 cells.

Key findings: Our result indicated that the particles extracted from MDA-MB-231 cells' supernatant were actually exosomes. PKH67 staining and confocal microscopy showed that the exosomes were actively taken up by MCF-7 cells. Treatment of MCF-7 cells with the exosomes resulted in increased ability of MCF-7 cells to grow independent of anchorage. In addition, migration and invasion capacity of exosome-treated MCF-7 cells increased in a dose-dependent manner.

Significance: Along with our previous study, we here indicate that highly metastatic MDA-MB-231 cells' exosomes and exosomal miRNAs may induce malignant transformation in non-metastatic MCF-7 cells, thus introducing a novel route of cancer development and metastasis.

1. Introduction

Breast cancer is the major cause of women's mortality. Almost 1.2 million cases of the disease were newly diagnosed in 2012 [1]. In 2015, 40,290 breast-cancer-caused deaths were reported according to American Cancer Society. There are several molecular subtypes of breast cancer which are classified based on estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status. These subtypes are basal-like, HER2-enriched, luminal A, luminal B, and normal-like [2–4]. Triple negative breast cancer (TNBC) is classified as basal-like breast carcinoma [5] and accounts for 15–20% of cases of breast cancer in women under 40 years old [6,7]. Importantly, it is associated with poor outcomes [8] i.e. pathological complete response is observed in 35–40% of patients [9,10]. Moreover, TNBC cells frequently metastasize to lung and brain [11,12].

microRNAs (miRNAs) are small non-coding RNAs (19–25 nucleotides) and post-transcriptionally affect gene expression. They either repress translation or degrade mRNA [13] depending on the level of their complementary with the 3′-UTR of target mRNAs. Since one group of miRNAs' targets are genes involved in signaling pathways [14], they

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control cell fate, for example, differentiation, proliferation, survival, metastasis, and so forth [15]. They can target both tumor suppressor genes and oncogenes. Thus they are classified as tumor suppressor miRNAs and oncogenic miRNAs (oncomiRs) [16]. miRNAs mainly act within the cytoplasm, however, researches have demonstrated that they are also exported to extracellular space by exosomes [17].

Exosomes are 30–150 nm membranous vesicles, and mediate intercellular communication by carrying biomolecules such as miRNAs [18–20]. They transport miRNAs in physiological and pathological settings. Regarding physiological settings, Mittelbrunn and colleagues demonstrated T cells export miRNA-containing exosomes to antigen-presenting cells and modify the gene expression in recipient cells [21]. Li et al., showed monocyte secrete exosomes containing miR-150 towards epithelial cells and promote angiogenesis [22]. In case of pathological settings, Pang et al. found that exosomal miR-155 of pancreatic cancer cells shed miR-155-containing exosomes, which convert normal fibroblast to cancer-associated fibroblasts (CAF) [23]. In fact, exosomal miRNAs play a part in malignant transformation of normal cells by inducing migration [24], angiogenesis [25,26], and metastasis [27].

In the present study, we hypothesized that miRNA-containing exosomes of highly metastatic breast cancer cells (MDA-MB-231) can promote migration, invasion, and anchorage-independent growth of non-metastatic breast cancer cells (MCF-7).

In our previous study [28], we used a bioinformatic approach to find over-expressed miRNAs of metastatic cells' exosomes. GEO datasets, GeneCards, miRBase, DIANA tools, TargetScan, and miRWalk, and were used for several purposes: (i) finding over-expressed exosomal miRNAs, (ii) tumor-suppressor gene selection, (iii) miRNA confirmation, (iv) pathway analysis (v) miRNA:mRNA interaction prediction, (vi) validation of miRNA:mRNA interaction. Based on bioinformatic studies, miR-9 and miR-155, which target *PTEN* and *DUSP14*, respectively, were selected for further evaluation.

We also experimentally showed that (i) the miRNAs were over-expressed in exosomes of MDA-MB-231 cells (ii) the miRNAs target their selected target genes (iii) the target genes were down-regulated in MCF-7 cells after treatment with exosomes of MDA-MB-231 cells.

In the present study, we aimed to further analyze effects of MDA-MB-231 cells' exosomes on migration, invasion, and anchorage-independent growth of MCF-7 cells.

2. Materials and methods

2.1. Cell culture

MDA-MB-231 and MCF-10A cells were purchased from Pasteur Institute of Iran (Tehran, Iran). MCF-7 cell line was purchased from Iranian Biological Resources Center (Tehran, Iran). MDA-MB-231 and MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS. MCF-10A cells were cultured in DMEM containing 10% Horse Serum, EGF (20 ng/mL), Hydrocortisone (0.5 mg/mL), Cholera Toxin (100 ng/mL), and Insulin and (10 μg/mL final). All cell lines were incubated in a humidified atmosphere (95%) containing 5% CO_2.

2.2. Exosome extraction

Exospin Exosome Purification kit (Cell Guidance System, UK) was used to extract exosomes. The medium of the cells was replaced with serum-free media after the cells reached 60–80% confluency. After 48 h, the supernatant of the cells was harvested and subjected to two steps of centrifugation (i) 300 *g* (Eppendorf, 5810R, Germany) for 10 min to eliminate remaining cells, (ii) 16,000 g (Eppendorf, HERMLE z360k, Germany) for 30 min to eliminate cell debris and apoptotic bodies. Finally, exosomes were extracted and purified from these cell-free supernatants using ExoSpin exosome purification kit according to the manufacturer's instructions.

2.3. Exosome characterization

2.3.1. Scanning electron microscopy (SEM)

The quality and size of the exosomes were evaluated using SEM. Briefly, 5 μL of the purified exosome in PBS were spotted on a sterile glass slide and placed under a laminar flow hood until dried. The slides were then examined under KYKY EM3200 scanning electron microscope at 40 KV.

2.3.2. Transmitting electron microscopy (TEM)

20 μL of exosome extracted from MDA-MB-231 cells were gently placed in the middle of 50 μL of cooled low-melting agarose in a1.5 mL tube. The exosome-containing agarose gel transferred to a 1.5 mL microtube containing 3.7% formaldehyde and delivered to Biophysics Department (Shahid Beheshti University of Medical Sciences, Tehran, Iran) for further processing and imaging.

2.3.3. Dynamic light scattering (DLS)

Purified Exosomes were diluted 1:20 in phosphate buffer saline (PBS) and analyzed using Zetasizer Nano ZSP ZEN 6500 (Malvern, UK).

2.3.4. Exosome protein content

The protein content of exosomes was determined using BCA Protein Assay Kit (Ariatous, Mashad, Iran). 25 μL of BSA standards (eight 2-fold serially diluted standards starting from 1000 μg/mL) or samples were mixed with 75 μL of BCA working reagent in a 96-well ELISA plate. The plate was then incubated at 60 °C for 60 min. All tests were performed in duplicates. The OD was measured at 560 nm using ELISA reader system (BioTek, USA).

2.3.5. Western blot

The particles purified from the cells were subjected to western blot analysis to confirm they were actually exosomes. CD63 (25kd) and CD81 (22-26kd) are specific exosome markers. Twenty micrograms of the purified exosome was used for western blot. Mouse anti-human CD63 and mouse anti-human CD81 (Santa Cruz Biotechnology, USA) were used at the concentration of 1 μg/mL and 0.5 μg/mL, respectively. Goat anti-mouse IgG antibody conjugated with HRP (Santa Cruz Biotechnology, USA) was used as the secondary antibody at the concentration of 0.8 μg/mL. Enhanced chemiluminescence reagents (Cytomatingen, Tehran, Iran, cat. no. CMGECL) was used to visualize the bands on radiographic films.

2.4. MTT assay

MTT assay was performed to ensure the exosomes used for the treatment of MCF-7 cells have to adverse effect on their growth. 5000 cells/well were plated in a 96-well plate. Zero, 10, 50, 100 μg of MDA-MB-231 cells' exosomes were added to each well. After 24, 48, and 72 h, 100 μL of MTT (0.5 mg/mL) were added to each well. The plate was incubated for 3 h in dark at 37 °C. Then, 150 μl DMSO was added to each well, and the plate was incubated for 2 h in dark at 37 °C. The plates were examined using an ELISA reader (BioTek, USA) at 590 nm. Tests were performed in triplicate.

2.5. Exosome uptake by MCF-7 cells

To show the uptake of exosomes by MCF-7 cells, PKH67 staining kit (Sigma-Aldrich) was used according to the manufacturer's instruction with slight modifications. Briefly, 50 μL of purified exosomes and 3 μL of PKH67 were separately resuspended in 500 μL of DC solution. 250 μL of each preparation was mixed and incubated for 5 min. Excess PKH67 dye was inactivated using 130 μL FBS. The labeled exosomes were re-purified using Exospin commercial kit. Afterwards, the labeled

exosomes were added to MCF-7 cells and incubated for 24 h. Finally, the cells were observed under confocal microscope (Leica, Germany).

2.6. Soft agar colony formation assay

To assess whether MDA-MB-231 cells' exosomes can induce anchorage-independent growth in MCF-7 cell, soft agar colony formation assay was used. MCF-7 cells were incubated with 0, 10, 50, and 100 μg of MDA-MB-231 cells' exosomes for 48 h. 1.5 mL of 1× DMEM containing 0.5% agar were added to each well of a 6-well plate. After casting of the bottom layer, 1.5 mL of an exosome-treated MCF-7 cell suspension in 1× DMEM containing 0.25% agar was added on top of the bottom layer. The plates were incubated for three weeks in cell culture incubator. 200 μL of culture medium was added to the wells every other day to prevent desiccation. After three weeks, the wells were stained using Nitroblue tetrazolium (1 mg/mL in PBS), and the number of colonies were counted. Results were analyzed using One-way ANOVA test.

2.7. Cell migration assay

MCF-7 cells were cultured in serum-free medium in a 24-well plate for 24 h. The cells were then detached using PBS-EDTA, and 80,000 cells/well in 250 μL medium were transferred to the upper chambers of transwells (SPL, south Korea). 700 μL medium containing 30% FBS was added to the lower chambers. Afterwards, 0, 10, 50, 100 μg exosomes were added to each well. After 48 h incubation, the cells in the upper chamber were removed, and 20 μL of ethidium bromide-acridine orange [29] was added to the lower surface of the transwell membrane. After 5 min, the migrated cells were counted using an invert fluorescent microscope (Leica, Germany).

2.8. Cell invasion assay

Cell invasion assay was performed like cell migration assay, but Matrigel (Sigma, Germany) was added before adding the cells. To do so, Matrigel and DMEM was mixed (1:1 *v*/v), and 50 μL of the diluted Matrigel was added to the upper chambers.

2.9. Statistical analyses

MTT, migration, and invasion assays were performed in triplicate. BCA and Soft agar colony formation assay was performed in duplicate. GraphPad Prism 7.0 was used for statistical analyses and plots. All tests were analyzed using One-way ANOVA statistical test.

3. Results

3.1. Exosome characterization

3.1.1. SEM and TEM analyses on purified exosomes

In order to characterize purified exosomes, several experiments should be performed. SEM analysis will indicate the shape and size of the purified particles. As described, exosomes are spherical particles with a size range between 30 and 150 nm. In our SEM micrographs spherical particles with a diameter of 30–130 nm was observed (Fig. 1A). This shape and size is agreement with the defined shape and size of exosomes.

TEM analysis is used to explore if the purified particles have a lipid bilayer membrane since exosomes are derived from multivesicular bodies originating from endoplasmic reticulum.

In our TEM micrographs vesicular structures with a diameter of 50–100 nm were observed. A distinct lipid bilayer was also present, which indicates the purified particles have the characteristics of exosomes (Fig. 1B).

3.1.2. DLS analysis of purified exosomes

Dynamic light scattering is used to determine the size of particles. Based on DLS analysis, purified particles had a size distribution between 30 and 105 nm with the mode of 80–100 nm (Fig. 1C).

3.1.3. Western blot

CD-63 and CD81 markers are abundantly found on endosomal membranes. However, they are enriched on exosomal membrane and are considered as exosomal specific markers. In order to characterize purified particles as exosomes, the presence of these marker on the purified particles should be assessed. Western blot analysis showed that the particles we purified carry these markers and thus can be considered as exosomes (Fig. 1D).

Taken together, SEM, TEM, DLS, and western blot analyses confirmed that the particles purified from MDA-MB-231 cells were actually exosomes.

3.1.4. Exosome protein content

Exosomal protein concentration was used as a clue to estimate the amount of exosome required for the other assay such as migration, invasion, and so forth. Using a BCA protein assay kit, it was determined that the exosome preparation had a concentration of nearly 1000 μg/mL.

3.2. MTT assay

MTT assay is used to determine (i) the proliferation rate of cells or (ii) the toxic effects of treatments on cell proliferation. In order to determine the toxic effects of purified exosomes on the proliferation of MCF-7 cells, MTT assay was used. In fact, we aimed to explore if the exosomes had any proliferation-limiting effects on the recipient cells. MCF-7 cells were treated with 0, 10, 50, 100 μg exosomes and evaluated after 24, 48, and 72 h. After 24 and 48 h, there was not any statistically significant difference between the mean OD of the treatment groups ($p > 0.05$). After 72 h, the cells that received 100 μg exosome showed greater proliferation than the other groups compared to non-treated cells ($p < 0.0001$). Note that exosome treatment had no adverse effect on cell growth and proliferation (Fig. 2).

3.3. PKH67 staining and exosome uptake by MCF-7 cells

In our previous study, using luciferase assay, we showed that miR-9 and miR-155 targeted *PTEN* and *DUSP14*, respectively. After exosomal treatment of MCF-7 cells with MDA-MB-231 exosomes, RT-qPCR results showed that *PTEN* and *DUSP14* level decreased in MCF-7 cells (compared to untreated MCF-7 cells) [28].

In the present study, we aimed to show miR-9 and miR-155 contained in the exosomes of MDA-MB-231 cells were responsible for the promotion of cancerous phenotype in MCF-7 cells. We thus labeled the exosomes with PKH67 dye (green) to evaluate exosome uptake by MCF-7 cells. Fig. 3 shows that exosomes were taken up by MCF-7 cells. The green color indicates the labeled exosomes. The 3D confocal micrographs indicate that the exosomes are present inside MCF-7 cells, in the cytoplasm, and around nucleus.

3.4. Soft agar colony formation assay

Cancer cells are capable of unlimited proliferation. Metastatic cells are also capable of unlimited proliferation. Importantly, metastatic cells can grow and proliferate independent of anchorage. Soft agar colony formation assay is used to evaluate anchorage-independent growth of cells. In this assay, since there is a solidified agar-containing layer on culture surface, the cells cannot attach to surface. Therefore, if the cells are metastatic, they can grow on the agar-containing media, i.e. anchorage-independently. Colony count after three weeks showed that the difference of the mean number of colonies in treatment groups was

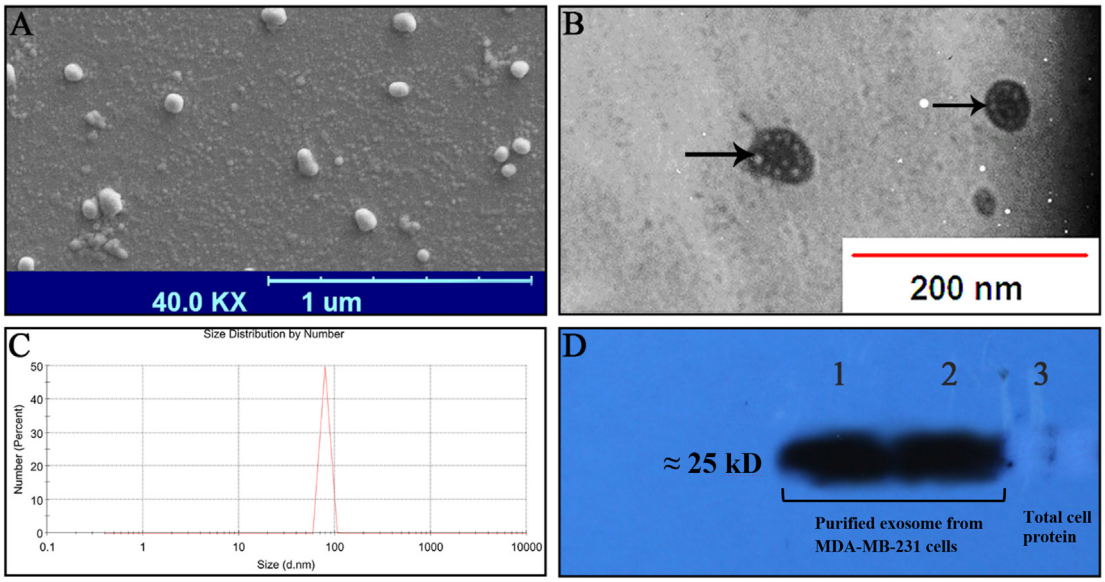

Fig. 1. Exosome characterization assays. A) SEM. SEM micrograph shows that the extracted particles have a size range between 30 and 130 nm which is comparable to that of exosomes B) TEM. TEM micrograph shows particles within the range of exosome size. In addition, the lipid bilayer (Black arrows) is perfectly visible C) DLS. DLS analysis shows that the size of purified particles is between 30 and 105 nm. This size range is within the acceptable size of exosomes D) Western blot. Using anit-CD63 and CD81 antibodies, we found that the extracted particles are $CD63^+$ (lane 1, ≈25 kd) and $CD81^+$ (lane 2, ≈25 kd), which is the characteristic of exosomes. Cell extract was $CD63^-$ (lane 3) since CD63 is enriched on the surface of exosomes. Taken together, these results indicate that the purified particles were actually exosomes.

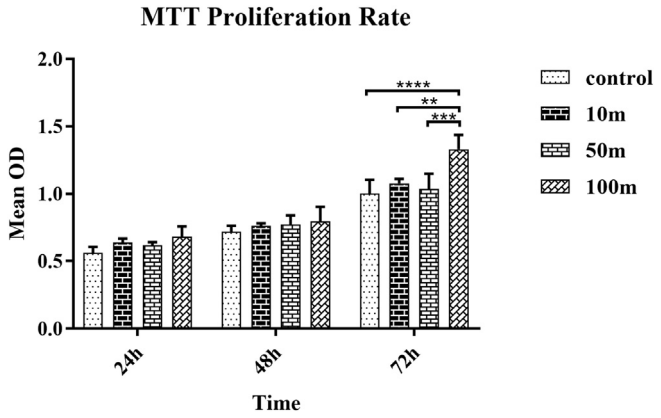

Fig. 2. MTT assay. After 24 and 48 h, the mean difference between the treatment groups was not statistically different ($p > 0.05$). However, after 72 h, 100 μg exosome significantly increased cell growth and proliferation compared to other groups. Importantly, MTT assay indicates that MDA-MB-231 cells' exosomes do not have any adverse effect on the growth of MCF-7 cells. *p*-values: **: 0.0014, ***: 0.0003, ****: < 0.0001.

statistically significant ($p < 0.0001$). These result may indicate that exosome treatment confers MCF-7 cells the ability of anchorage-independent growth, which is a characteristic of metastatic cells (Fig. 4). The mean ± SD of the migrating cells in group 0, 10, 50, and 100 was 53.5 ± 4.95, 92 ± 4.2, 163 ± 9.8, 689 ± 11.3, respectively.

3.5. Cell migration and invasion assays

Migration assays indicates if cells can detach from their original place and move. This is a characteristic of metastatic cells. Invasion assay shows the ability of cells to degrade basement membrane of the tissue they reside in. Thus, these two assays could indicate if cells are capable of degrading basement membrane and move to other locations. We performed migration and invasion assays, to determine of highly metastatic cells' exosome can transfer metastatic phenotype to non-metastatic MCF-7 cells.

The migration and invasion assays results indicated that exosome treatment of MCF-7 cells promoted the cells ability to migrate (Fig. 5A) and invade the basement membrane (Matrigel) (Fig. 5B). Note that exosomes increased migration and invasion ability of MCF-7 cells in a dose-dependent manner. In fact, the greatest migration/invasion was observed when the cells were treated with 100 μg exosome. Fig. 6 shows the migration and invasion assays micrographs.

The mean ± SD of the migrating cells in group 0, 10, 50, and 100 was 6.6 ± 2.5, 11 ± 2.2, 19.6 ± 3.2, 57.8 ± 4.3, respectively. The mean ± SD of the invading cells in group 0, 10, 50, and 100 was 6.6 ± 2.5, 7 ± 1.5, 15 ± 1.8, 47.8 ± 2.55, respectively.

4. Discussion

Genetic and Epigenetic factors contribute to cancer progression. Exosomes are a new route by which cancer cells and other cells in tumor microenvironment communicate [30–32]. However, this phenomenon is in its infancy and further studies are required to prove the concept and decipher the underlying mechanisms. We previously reported that miR-9 and miR-21were enriched in MDA-MB-231 cells' exosomes. In addition, we showed that mir-9- and miR-155-containing exosomes of highly metastatic MDA-MB-231 cells can decrease *PTEN* and *DUSP14* expression in non-metastatic MCF-7 cell. miR-9 and miR-155 respectively target *PTEN* and *DUSP14,* the tumor suppressors involved in PI3K and MAPK pathways, respectively [28]. Based on our previous study, we hypothesized that highly metastatic MDA-MB-231 cells' exosomes, which contain miR-9 and miR-155, can increase cancerous phenotype in non-metastatic MCF-7 cells. Since we were interested in exploring the transfer of metastatic behavior, MDA-MB-231 cells' exosomes were used. In addition, we used cancerous but non-metastatic MCF-7 cells to be able to assess if the metastatic traits have been transferred to these non-metastatic breast cancer cells. However, to further examine the hypothesis, cells irrelevant to the exosome source should also be tested. This will be included in our future projects.

To examine the hypothesis, we extracted exosomes from MDA-MB-

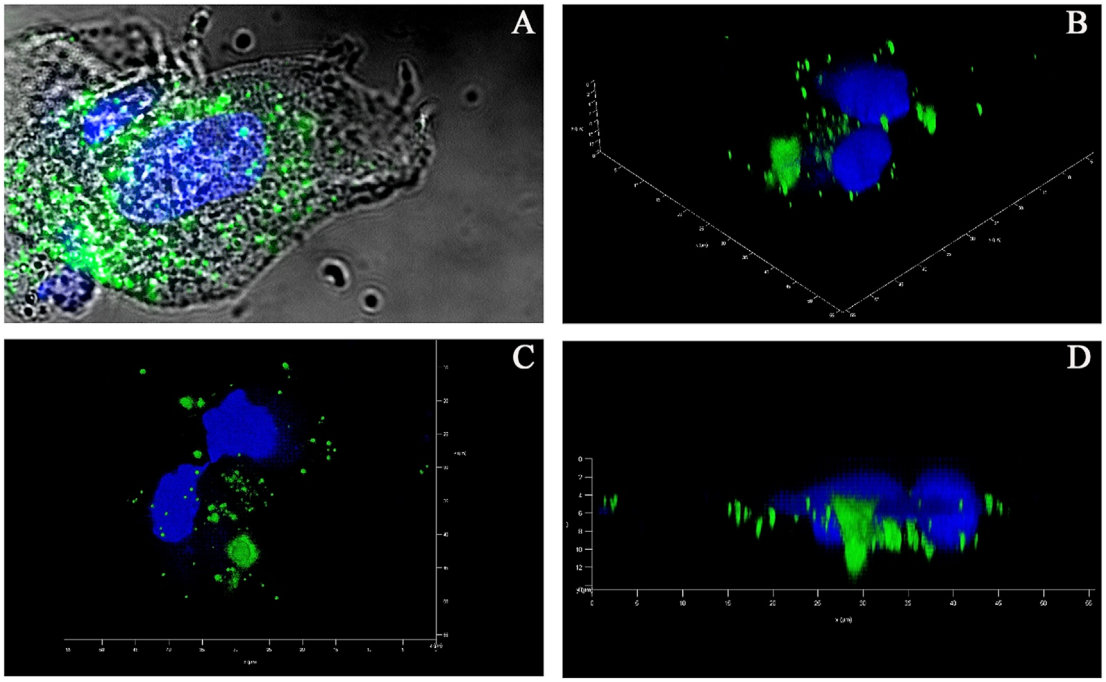

Fig. 3. PKH67 staining and exosome uptake. Confocal microscopy shows that PKH67-labeled exosomes have been taken up by MCF-7 cells. A) 2D view of exosomes (Green) in the cytoplasm and around cell nucleus (Blue). B, C, D) 3D view shows that exosomes have been taken up by the cells and exist in the cytoplasm and around cell nucleus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

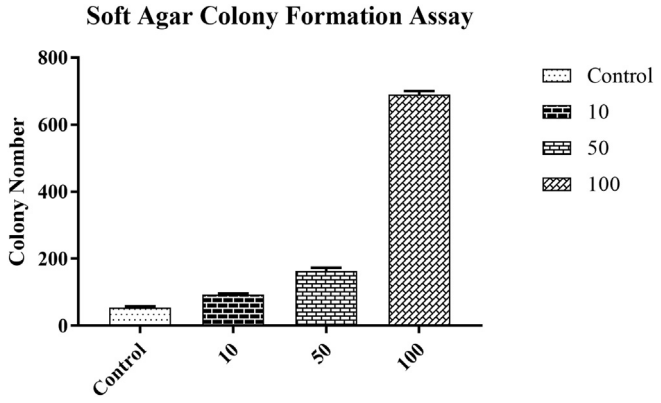

Fig. 4. Soft agar colony formation assay. Results indicate that exosome treatment promotes anchorage-independent growth of the cells. The mean difference between the groups was statistically significant ($p < 0.0001$). Multiple comparison showed that there was a statistically significant difference between the control group and the groups receiving 10 μg ($p = 0.02$), 50 μg ($p = 0.004$), and 100 μg ($p = 0.001$) exosome.

231 cells. The exosomes were first characterized using SEM, TEM, DLS, and western blot methods. Then, we assessed if the exosomes are taken up by MCF-7 by using PKH67 staining and confocal microscopy. We then treated MCF-7 cells with the exosomes and then evaluated cancerous and metastatic phenotypes of the treated cells.

Our result showed that migration and invasion of non-metastatic MCF-7 cell increased after treatment with exosomes extracted from highly metastatic breast cancer cell line, MDA-MB-231. The exosomes increased MCF-7 cells migration in a dose-dependent manner. In fact, 100 μg exosome significantly increased migration and invasion in MCF-7 cell. We previously showed that miR-9 and miR-155 were enriched in MDA-MB-231 cells' exosomes. We also confirmed that miR-9 and miR-155 target *PTEN* and *DUSP14*, respectively. These genes are tumor suppressors in PI3K/Aky and MAPK pathways, and inhibition of these genes will result in increased cell proliferation and survival as well as decreased expression of cell adhesion molecules. Therefore, it could be inferred that this increase in migration/invasion is due to *PTEN* and *DUSP14* suppression by exosomal miR-9 and miR-155. Various studies have shown similar results. Wang et al., in 2016 showed that exosomes purified from human non-small lung cancer cells (NCI-H2228) and classic small cell lung cancer (NCI-H1688) increased the migration of HMEC-1 cells. In addition, they showed that NCI-H2228 cells had a greater effect in HMEC-1 migration than NCI-H1688 cells [33]. In 2015, Menck and colleagues showed that MCF-7 cells migration capacity increased upon treatment with MDA-MB-231 cells' microvesicles (not exosomes). They mentioned that the exosomes were less effective in migration induction than microvesicles. However, it means that exosomes also induce migration in recipient cells, which is in line with our experiments [34]. In a study led by Dutta et al., the scientists showed that treatment of HMEC-1 cells with breast cancer cells' exosomes induces the release of cancer-cell-promoting growth factors from HMEN-1 cells [35]. Scores of studies have shown that cancers or cells lines' exosomes increased migration, angiogenesis, and invasion in normal or non-metastatic cell lines [22,25].

MDA-MB-231 cells' exosomes also increase anchorage-independent growth of non-metastatic MCF-7 cells. Based on soft agar colony formation assay results, we found that MDA-MB-231 cells-derived exosomes increased colony formation on MCF-7 cells in a dose dependent manner. In fact, 100 μg exosome significantly increased anchorage-independent growth of MCF-7 cell. In agreement with our results, Li and colleagues in 2018 found that exosomes derived from ovarian cancer cells increased the proliferation capacity of ovary epithelial cells in a dose dependent manner [36]. In another study led by Cheng et al. in 2018, the researchers showed that inducing miR-9 in MCF-7 cells or inhibiting miR-9 in MDA-MB-231 cells led to increased and decreased anchorage-independent growth of the cells, respectively.

In conclusion, our results indicate that of MDA-MB-231 cells' exosomes are taken up by recipient cells such as MCF-7 cells. The exosomes also have metastasis-inducing effects and increase migration, invasion, and anchorage-independent cell growth. Our previous and present study together shows that these exosomes induce metastatic phenotype in recipient cells probably through the interaction of miR-9 and miR-

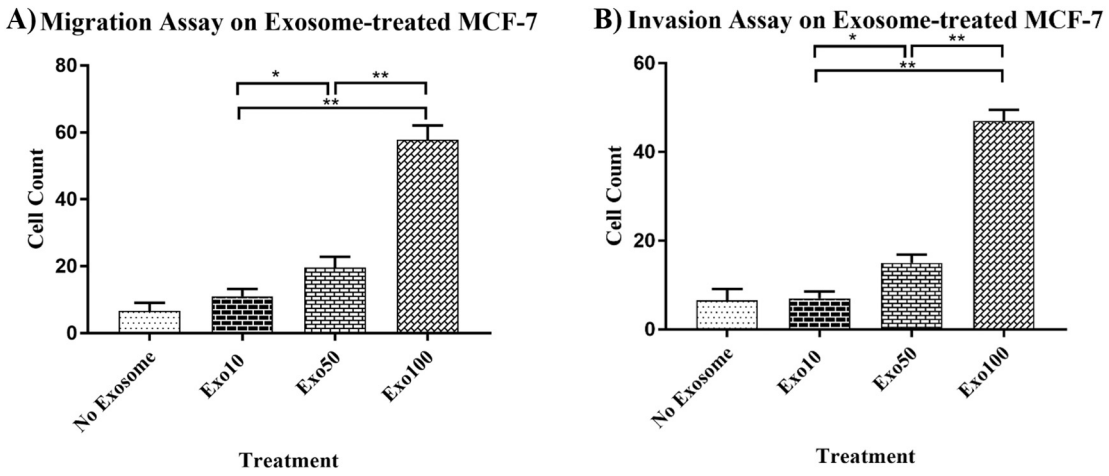

Fig. 5. A) Migration assay. The results indicate that there is a total statistically significant difference ($p < 0.0001$) between groups i.e. exosome treatment increases cell migration ability. Multiple comparison shows that there is no statistically significant difference between the control group and the group receiving 10 μg exosome ($p = 0.1$). However, there is a statistically significant difference between the control group and other groups as well as between the three groups receiving exosome ($p < 0.002$). **: 0.002, ****: < 0.0001. B) Invasion assay. The results indicate that there is a total statistically significant difference ($p < 0.0001$) between groups i.e. exosome treatment increases cell migration ability. Multiple comparison shows that although there is no statistically significant difference between the control group and the group receiving 10 μg exosome ($p = 0.9$), there is a statistically significant difference between the control group and other groups as well as between the three groups receiving exosome ($p < 0.001$). ***: 0.0001, ****: < 0.0001.

155 and their respective tumor suppressor genes. Therefore, preventing exosome secretion from metastatic and cancerous cells might be a novel approach to prevent metastasis. However, further experiments are required to elucidate the exact mechanism. Note that since exosomes contain lots of biomolecules (proteins, RNAs, lipids…), there is a challenging path towards deciphering the communication map of exosomes and cells in tumor microenvironment.

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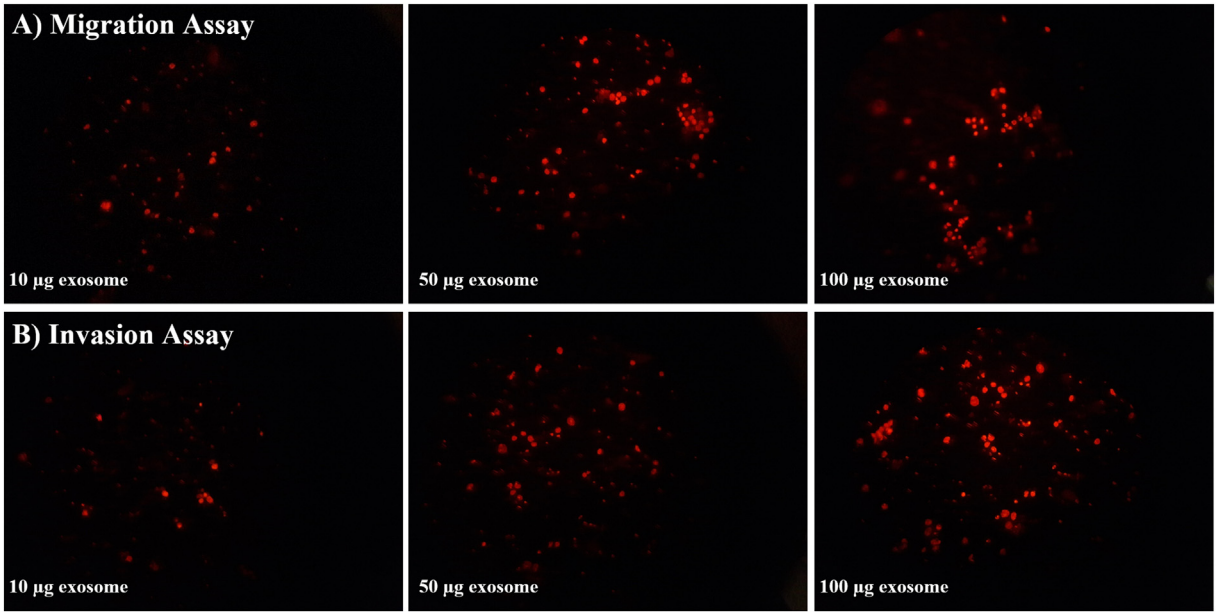

Fig. 6. A) Migration assay. The micrographs of the migration (A) and invasion (B) assay. MCF-7 cells were treated with different concentrations of MDA-MB-231 exosomes (zero, 10, 50, and 100 μg) and used in migration and invasion assays.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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