ORIGINAL ARTICLE

Exosomes from adriamycin-resistant breast cancer cells transmit drug resistance partly by delivering miR-222

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Abstract Breast cancer (BCa) is one of the major deadly cancers in women. However, treatment of BCa is still hindered by the acquired-drug resistance. It is increasingly reported that exosomes take part in the development, metastasis, and drug resistance of BCa. However, the specific role of exosomes in drug resistance of BCa is poorly understood. In this study, we investigate whether exosomes transmit drug resistance through delivering miR-222. We established an adriamycin-resistant variant of Michigan Cancer Foundation-7 (MCF-7) breast cancer cell line (MCF-7/Adr) from a drug-sensitive variant (MCF-7/S). Exosomes were isolated from cell supernatant by ultracentrifugation. Cell viability was assessed by MTT assay and apoptosis assay. Individual miR-222 molecules in BCa cells were detected by fluorescence in situ hybridization (FISH). Then, FISH was combined with locked nucleic acid probes and enzyme-labeled fluorescence (LNA-ELF-FISH). Individual miR-222 could be detected as bright photostable fluorescent spots and then the quantity of miR-222 per cell could be counted. Stained exosomes were taken in by the receipt cells. MCF-7/S acquired drug resistance after co-culture with exosomes from MCF-7/Adr (A/exo) but did not after co-culture with exosomes from MCF-7/S (S/exo). The quantity of miR-222 in A/exo-treated MCF-7/S was significantly greater than in S/exo-treated MCF-7/S. MCF-7/S transfected with miR-222 mimics acquired adriamycin resistance while MCF-7/S transfected with miR-222 inhibitors lost resistance. In conclusion, exosomes are effective in transmitting drug resistance and the delivery of miR-222 via exosomes may be a mechanism.

Keywords Breast cancer · Exosome · Drug resistance · LNA-ELF-FISH

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Introduction

Breast cancer (BCa) is the most common life-threatening tumor in women around the world. BCa treatment is largely realized by chemotherapy, but one major obstacle is drug resistance [1]. There are many mechanisms about how BCa cells evade chemotherapy. One vital mechanism is exosomes which act as a mediator of intercellular communication. Exosomes (40–100 nm in diameter) released by various cells are small lipid bilayer membrane vesicles that carry and transfer cargoes of proteins and nucleic acids [2]. Growing evidence shows that exosomes function in the development, metastasis, and drug resistance of BCa [3]. Exosomes from drug-resistant BCa cells transmit chemoresistance by transferring miRNAs, according to measurement of miRNA expression by reverse transcription polymerase chain reaction (RT-PCR) [4, 5]. However, RT-PCR requires the lysis of a cell population and cannot quantify miRNA abundance at single-cell level. RT-PCR results represent the average miRNA expression of a cell population, which may lose important information about

gene expression on phenotypic diversity. At this point, fluorescence in situ hybridization (FISH) can be used to verify that exosomes transmit chemoresistance through delivering miRNAs at single-cell level.

FISH allows the detection of miRNA expression in an effective and toilless way. As reported, locked nucleic acid (LNA) oligonucleotides which can be used as hybridization probes allow to image the spatial localization of miRNAs at the tissue, cellular, and subcellular levels. LNA probes have surprisingly high affinity and specificity against RNA targets and even can discriminate single-base mismatches. However, the combination LNA-FISH still lacks sensitivity for detection of single miRNAs and is unable to provide a qualitative assessment of miRNA abundance within single cells. Fortunately, the low sensitivity of LNA-FISH can be overcome by the combination with enzyme-labeled fluorescence (ELF). Moreover, LNA-ELF-FISH allows the visualization of individual miRNAs within single cells. The phosphatase substrate of ELF yields a brilliant yellow green fluorescent precipitate at the site of enzymatic activity [6, 7]. Besides the extremely higher photostability than commonly used fluorophores, the ELF precipitate also results in a labeling up to 40 times brighter than signals achieved by fluorophore-labeled probes or by hapten-labeled probes combined with fluorophore-labeled secondary detection reagents. In consequence, individual miRNAs are recognized as bright photostable fluorescent spots that can be counted on a fluorescence microscopy image.

MiRNAs are evolutionally conserved, small, and noncoding RNAs. They can regulate the expressions of multiple genes through post-transcriptional repression or mRNA degradation [8]. MiRNAs take part in many bioprocesses, including development, angiogenesis, cell differentiation, proliferation, apoptosis, metabolic pathways, and signal transduction. Several studies show that the dysregulation of miRNAs is closely associated with the acquired drug resistance of breast cancer [9].

Previous research shows that exosomes from drug-resistant BCa cells serve as mediators of intercellular communication by transferring miRNAs. Our team has identified miR-222 to investigate the role of exosomes in acquiring drug resistance of BCa. MiR-222 was overexpressed in both docetaxel- and adriamycin-resistant BCa cells and in exosomes derived from these cells (microarray results from our team). In this study, we utilize LNA-ELF-FISH to verify whether exosomes from drug-resistant BCa cells transmit chemoresistance by transferring miRNAs.

Materials and methods

Cell culture

Human breast cancer cell line Michigan Cancer Foundation-7 (MCF-7) (Cell Bank of Chinese Academy of Sciences, Shanghai) was used in this study. The adriamycin-resistant subline at 500-nM adriamycin (MCF-7/Adr) was successfully established by exposing the parental MCF-7 to gradually increasing concentration of Adr in vitro [10]. Parental MCF-7 cultured synchronously with the absence of adriamycin was used as a control (called MCF-7/S). MCF-7/Adr was cultured in a drug-free medium for 2 weeks before subsequent experiments to avoid the influence of adriamycin. The IC_{50} (inhibitory concentration to produce 50 % cell death) of Adr was 398.42 and 0.62 μM for MCF-7/Adr and MCF-7/S cells, respectively [11].

All cells were cultured in high-glucose (HyClone) Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. The cultures were conducted at 37 °C and 5 % CO_2 in a humidified atmosphere. For all experiments, exosome-depleted FBS was prepared by centrifuging FBS at 100,000g overnight to spin down any preexisting vesicular content.

Establishment of green fluorescent protein (GFP-S)

We established GFP-S-expressing MCF-7/S according to the manufacturer's directions (GenePharma, China) [12]. When MCF-7/S cells grew to 70 % confluence at the logarithmic growth phase, they were transfected with GFP-encoding lentivirus in petri dishes. Then, puromycin was used to filter out the positive cells because the successfully transfected cells acquired puromycin resistance. At last, the expression of green fluorescence was examined under a fluorescence microscope (Carl Zeiss, Germany).

Exosome isolation and identification

Exosomes isolation was described in previous studies [13]. The supernatants of MCF-7/Adr and MCF-7/S cultured in DMEM with 10 % exosome-depleted FBS were collected by differential centrifugation and ultracentrifugation. The cell supernatants were centrifuged at 300g for 10 min, 2000g for 15 min, and 12,000g for 30 min to remove the floating cells and debris. The remaining supernatants were further ultracentrifuged at 100,000g and 4 °C for 2 h on an Avanti J-301 device (Beckman Coulter Inc., USA). Then, the exosomal pellets were collected, washed with phosphate-buffered saline (PBS) and ultracentrifuged at 100,000g and 4 °C for another 2 h. The final exosomal pellets were used immediately or resuspended in 100 μL of PBS and stored at −80 °C. The exosomes extracted from the supernatants of MCF-7/S and MCF-7/Adr were named as S/exo and A/exo, respectively.

The morphology of the exosomes was observed under transmission electron microscopy as previously described [14, 15]. Briefly, 10 μL of an exosome sample was added onto

parafilm and covered with 300-mesh copper grids for 45 min. Then, the copper meshes were washed with PBS three times, fixed in 3 % glutaraldehyde for 10 min, washed with double distilled water, and contrasted in 2 % uranyl acetate. Images were acquired using a JEM-1010 electron microscope (JEOL Ltd., Japan) at an accelerating voltage of 80 kV.

Uptake assays

Exosomes were labeled with a PKH26 red fluorescent dye (Sigma-Aldrich, USA) according to manufacturer's instructions [13]. After that, the exosomes were co-incubated with GFP-S in complete media for 24 h. Then, they were observed using an LSM710 confocal laser scanning microscope (CLSM, Carl Zeiss, Germany). The excitation wavelengths of GFP and PKH26 were 488 and 543 nm laser line, respectively, and the emission light passed through a 530/30 and 573/26 nm band filter, respectively.

Transfection experiment

The miR-222 mimics and inhibitors were synthesized (Shanghai GenePharma Co., Ltd., China). Then, MCF-7/S and MCF-7/Adr cells were seeded into six-well plates and incubated under normal growth conditions overnight, until reaching 70 % confluency. MCF-7/S and MCF-7/Adr cells were transfected with miR-222 mimics and inhibitors, respectively, using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Meanwhile, MCF-7/S and MCF/Adr cells were transfected with miR-222 mimics and inhibitor negative controls provided by the same vendor, and blank controls were also performed. The cells were trypsinized after 24 h of transfection and used in the following experiments.

LNA-ELF-FISH

The samples were divided into three groups: MCF-7/S, MCF-7/S + S/exo, and MCF-7/S + A/exo. MCF-7/S cells were seeded in six-well plates for 24 h, and the supernatants of MCF-7/S were added separately with S/exo and A/exo. One sample was for blank control (without exosomes added). After full reaction (72 h), the three samples were ready to be sent into LNA-ELF-FISH. At the same time, negative control (no LNA probe was added) and scrambled control (scrambled LNA probe was added) were also carried out. Steps of LNA-ELF-FISH were described in previous studies [6, 7]. MCF-7 cells were seeded into multi-chambered cover glass slides (Lab-Tek, Chamber Slide™ system 177437, USA) and incubated under normal conditions overnight, until reaching 50–70 % confluency. The cells were washed in 500 μL 1× PBS three times, 5 min per wash. Then, the cells were fixed with 500 μL of 4 % formaldehyde for 30 min at room temperature, washed three times with 500 μL 1× diethyl

pyrocarbonate (DEPC)-treated PBS (5 min per wash), and permeabilized at 4 °C in 500 μL 70 % ethanol at least overnight. After ethanol was removed, the slides were washed in 500 μL 1× DEPC-treated PBS once for 5 min. Cells were prehybridized in 250 μL of a hybridization buffer (SuperHyb Solution, type B, Tiandz Inc.) and incubated in a humid chamber (Boekel InSlide Out™ Hybridization oven) at 55 °C for 2–4 h. After that, the cells were hybridized with a 10-nM LNA probe (5′-ACCCAGTAGCCAGAT GTAGCT-3′ for hsa-miR-222-p, with digoxigenin on the 3′ end) at 50 °C for 2 h. Then, the cells were stringently washed in 4× saline sodium citrate buffer (SSC) (briefly), 2× SSC (30 min), 1× SSC (30 min), and 0.1× SSC (20 min) at 37 °C. Then, the cells were subjected to an ELF 97 mRNA In-Situ Hybridization Kit (Molecular Probes, Inc., Eugene, OR, USA), according to the manufacturer's instructions. Briefly, the cells were washed in 500 μL 1× wash buffer (200 mL of 10× wash buffer was diluted tenfold by adding 1800 mL of sterile distilled water) three times (5 min each) at room temperature and incubated in 200 μL of a blocking buffer (component B of ELF kit) for 1 h. Then, the cells were added with 2 μg/ml of a goat anti-DIG-AP antibody (Jackson ImmunoResearch) in the blocking buffer (diluted 1–250) and incubated at room temperature for 1 h. After three washes in 1× wash buffer (5 min per wash), the cells were incubated in 200 μL of an ELF 97 phosphatase substrate working solution, which was prepared by diluting tenfold the developing buffer (component C of ELF kit). For long-term in situ signal preservation, the cell samples were quickly washed with 1×wash buffer two times and postfixed by incubating the slides in a post-fixation solution (2 % formaldehyde, 20 mg/ml BSA in 1× PBS) at room temperature for 30 min. The cells were then counterstained in a 1 μg/ml Hoechst 33342 solution and mounted in a mounting solution. After mounting, the slides can be checked quickly for 1 h of fluorescence imaging. However, to represent the best results for visualization, fluorescence should be retrieved overnight. Slides kept at 4 °C can be stored for months without signal loss. Control experiments were conducted using the same procedure except that only a single hybridization step was performed.

Image acquisition and analysis

After in situ hybridization, the cells were imaged using a Zeiss Axio Scope.A1 upright fluorescence microscope. A UPLN ×60 oil immersion objective (N.A. 0.9) was used for all imaging experiments. In brief, after random selection of cells in a field, a 3-day stack viewed image was taken at an increment of several micrometers in the z-direction and with a total of 20 sections. Then, the 3-day images were stacked to 2-day images. The images were then processed on ImageJ using the following commands: (1) Process—sharpen, (2) Image—type—8-bit, and (3) Process—binary—make binary. The total number of

isolated signals was counted in ImageJ using the particle analysis counter program (Analyze—analyze particles).

Apoptosis assay

Cell apoptosis was determined using an Annexin-V-FITC apoptosis detection kit (BD Biosciences, Franklin Lakes, NJ, USA). First, MCF-7/S cells were seeded in six-well plates for 24 h. Then, the supernatants of MCF-7/S were added separately with S/exo and A/exo. Another six-well plate was used for transfection with miR-222. Meanwhile, a negative control and a blank control were set. After 24 h of co-incubation, the cells were incubated with Adr for 24 h. Then, after two washings with PBS, the cells were incubated with Annexin-V-FITC and propidium iodide (PI) in the dark for 30 min. Cellular apoptosis was analyzed by a FACScan flow cytometer (BD Biosciences).

MTT assay

Four groups were prepared as below: MCF-7/S, MCF-7/S + S/exo, MCF-7/S + A/exo, and MCF-7/S transfected with miR-222 mimics. Meanwhile, a negative control and a blank control were set. The cells were seeded in 96-well plates and incubated for 24 h. Then, the cells were added with serial dilutions of Adr (quadruplicate of the wells per condition). After 2-day co-incubation, 20 μL of a 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT (Sigma, Germany) solution was added to each well. After 4 h of incubation, the culture medium was removed. Then, the cells in each well were mixed with 150 μL of dimethyl sulfoxide (DMSO, AMRESCO, USA). The absorbance at 550 nm was measured by CliniBio 128 (ASYS-Hitech GmbH, Austria). The half maximal inhibitory concentration (IC_{50}) was calculated on SPSS 16.0 (SPSS Inc., Chicago, USA).

Statistical methods

Statistical analyses were performed on SPSS 16.0. All experiments were carried out in triplicates, and the data representative of three independent experiments were presented. P was calculated using Student's t test with significance level at $p < 0.05$. Graphs were plotted on GraphPad and RStudio.

Results

Confirming successful isolation of exosomes

After total ultracentrifugation, the residual vesicles were observed under transmission electron microscopy (TEM, Fig. 1). On the TEM images, the vesicles are homogeneous in morphology and their diameter is less than 100 nm, indicating that the vesicles are obviously exosomes.
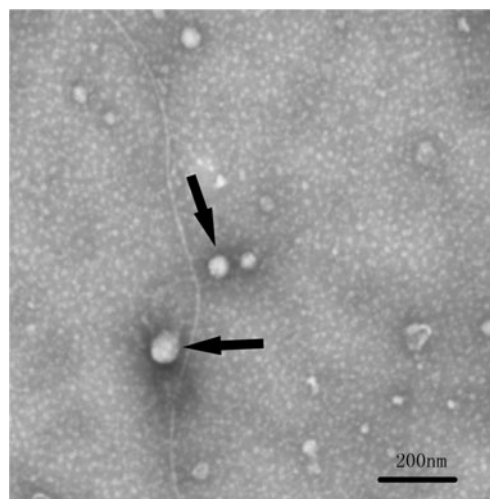

Fig. 1 Representative TEM graph of A/exo. The *spheroids with arrow* are exosomes (scale bar, 200 nm)

Uptake of exosomes

It is argued whether exosomes could be taken in by recipient cells. To solve this problem, we employed GFP-S and dyed A/exo with PKH26. Then, A/exo was added into GFP-S, and after 24 h, GFP-S was observed under fluorescence microscopy (Fig. 2), where red fluorescence and green fluorescence represent exosomes and GFP-S, respectively. In this image, some exosomes were taken in by the cells, while some were located on their surfaces. These results indicate that A/exo was not only bound to MCF-7/S but also engulfed and internalized.

Exosomes transmitted drug resistance

To investigate whether A/exo could spread chemoresistance, we compared their effects with S/exo. The chemoresistance of exosome-treated MCF-7/S cells was assessed by IC_{50} and apoptotic rate. As showed in Fig. 3, MCF-7/S + A/exo has a lower apoptotic rate than MCF-7/S + S/exo ($P = 0.011$). The apoptotic rates between MCF-7/S and MCF-7/S + S/exo are not significantly different ($P = 0.62$). The IC_{50} of Adr value of MCF-7/S + A/exo was greater than that of MCF-7/S + S/exo ($P = 0.001$). These two sets of data suggest that exosomes from drug-resistant cells could transmit drug resistance to drug-sensitive cells.

Exosomes transferred miR-222 (detecting individual miR-222 transcripts using LNA-ELF-FISH)

We carried out LNA-ELF-FISH of three groups and each group had a negative control and scrambled control. The three groups are MCF-7/S, MCF-7/S + S/exo, and MCF-7/S + A/exo. Individual miR-222 could be detected in situ by hybridizing with LNA probes, which were labeled with anti-dig-

Fig. 2 Confocal microscopy images of GFP-S exposed to PKH26-labeled A/exo. **a** *Green* signal from GFP-S under high magnification. **b** *Red* signal from PKH26-labeled A/exo. **c** *Overlap* of images **a** and **b**. **d** *Green* signal from GFP-S cultured with unlabeled A/exo. **e** Unlabeled exosomes *without red signal*. **f** *Overlap* of images **d** and **e**
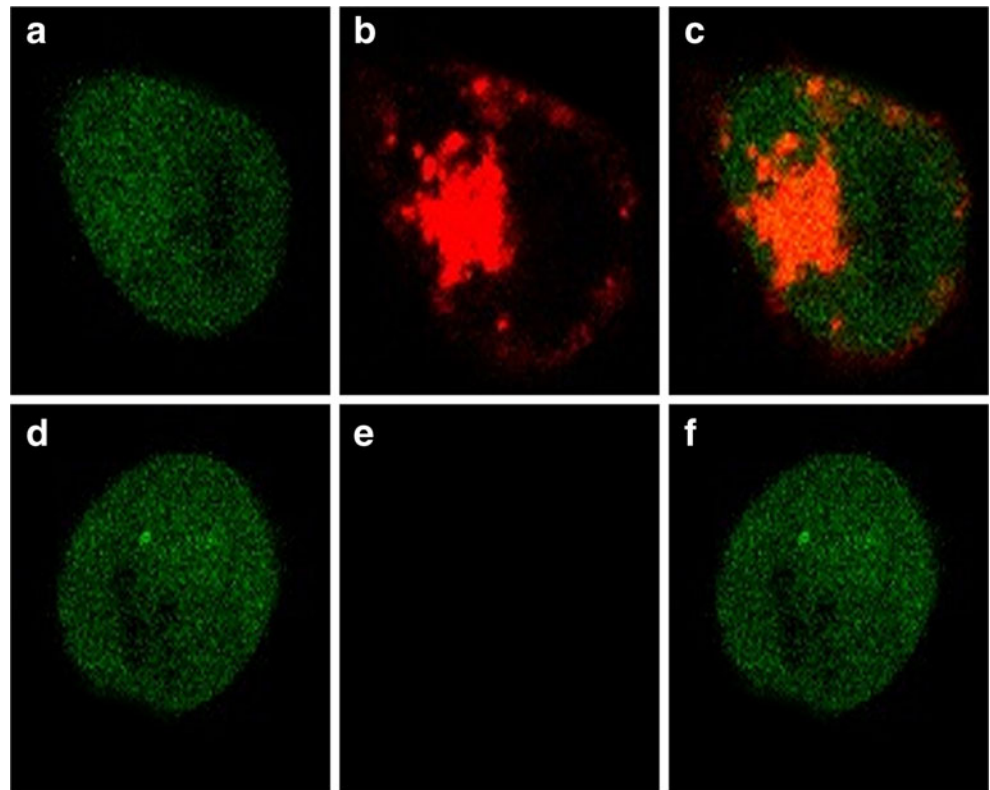

Fig. 3 Effects of exosomes, miR-222 mimics, and inhibitors on sensitivity of MCF-7 cells to Adr. **a** MCF-7/S cells and **b** MCF-7/Adr cells were added with a final concentration of 0.5 μM Adr; IC_{50} of Adr was determined **c** after MCF-7/S cells were treated with exosomes, miR-222 mimics, or the negative control, and **d** after MCF-7/Adr cells were transfected with miR-222 inhibitors or the negative control. *$P < 0.05$ versus negative control
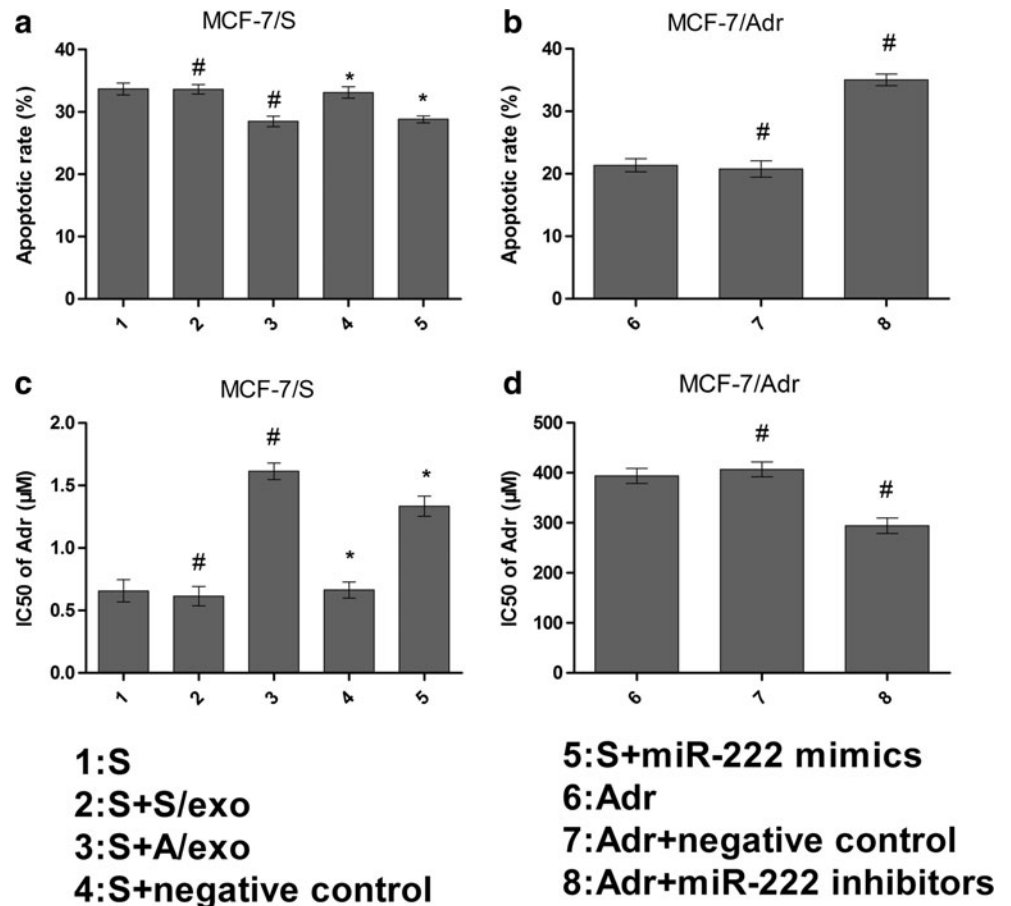

1:S
2:S+S/exo
3:S+A/exo
4:S+negative control

5:S+miR-222 mimics
6:Adr
7:Adr+negative control
8:Adr+miR-222 inhibitors

Fig. 4 Detection of individual miRNA molecules using LNA-ELF-FISH. Each miR-222 was labeled with a single dig-labeled LNA probe which was labeled with anti-dig-alkaline phosphatase conjugates subsequently. Then, ELF signals were amplified. The bright photostable fluorescent spots represent miR-222 molecules. The nucleus was stained *blue*. Images **a**–**e** were from the negative control (no LNA probe was added), MCF-7/S, MCF-7/S + S/exo, MCF-7/S + A/exo, and scrambled control (scrambled LNA probe was added) groups, respectively
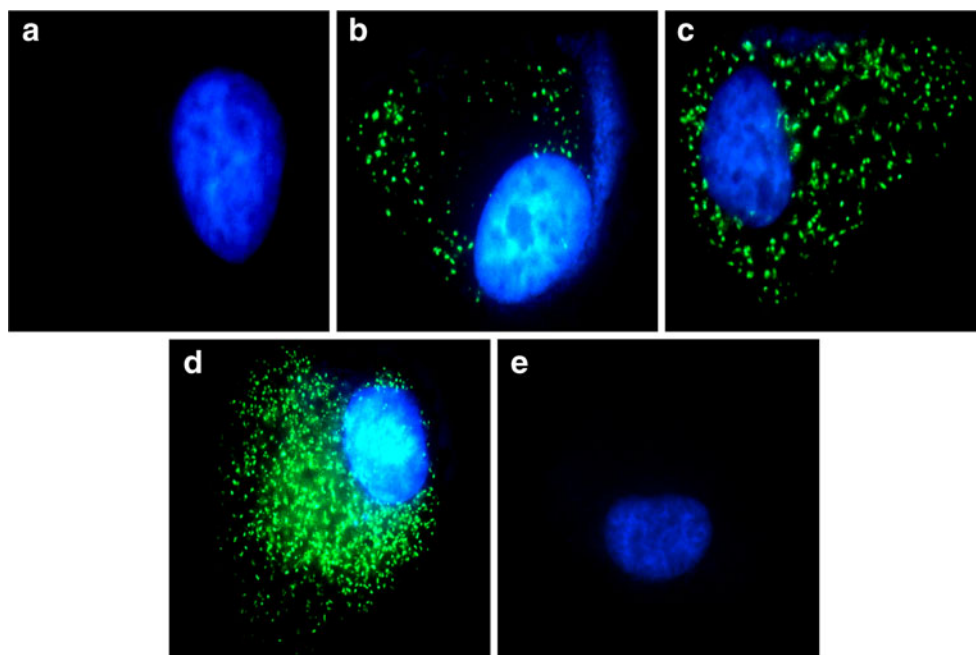
alkaline phosphatase conjugates. Then, ELF signals were amplified. Each bright fluorescent spot represented one miR-222

and could be visualized by fluorescence microscopy. We randomly selected a number of cells from each group.

Fig. 5 Fluorescent images of individual miR-222 in MCF-7 cells. The total numbers of miR-222 were shown in the *lower right corner* of each image
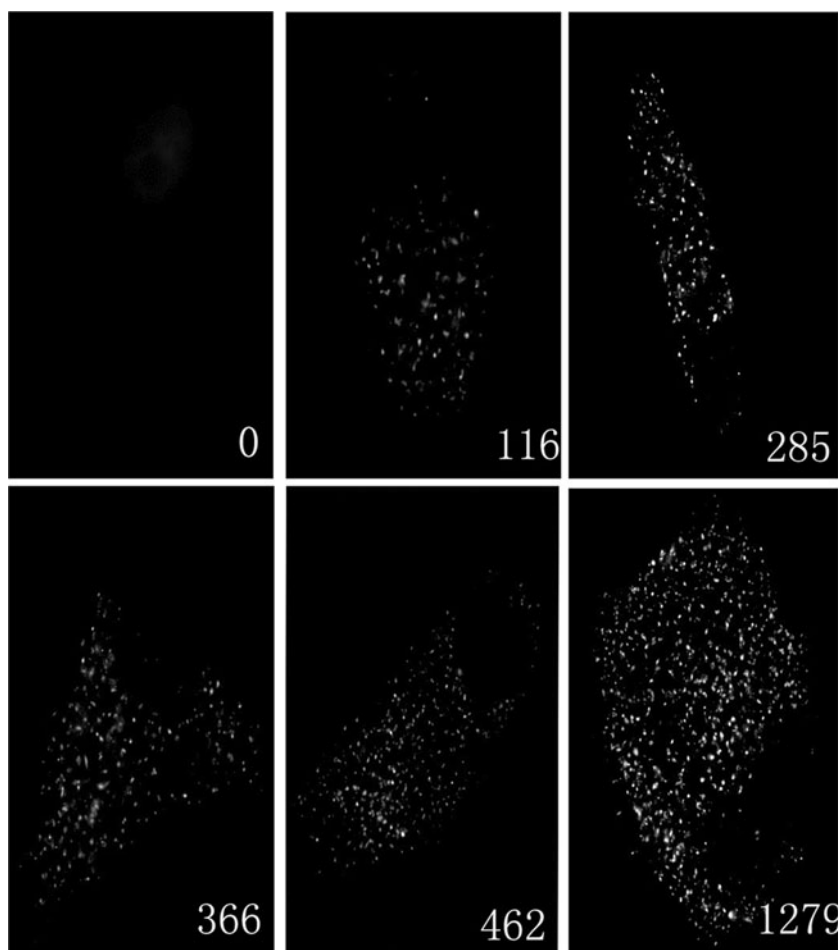
Fluorescence images selected from each group and one image from the corresponding negative control were shown in Fig. 4.

Images for quantification of individual bright fluorescent spots in single cells were provided in Fig. 5. Individual miRNAs were unidentifiable in some cells with high miRNA copy numbers. So, we estimated miRNA copy number in cells with high miRNA expression by drawing a linear correlation between total fluorescence intensity and miRNA copy number (Fig. 6d). Histograms showing the per cell distribution of miR-222 in MCF-7 cells are shown in Fig. 6. Mean numbers of miR-222 per cell of MCF-7/S (Fig. 6a), MCF-7/S + S/exo (Fig. 6b), and MCF-7/S + A/exo (Fig. 6c) detected by LNA-ELF-FISH were 1409 ± 15 (SE), 1472 ± 19 (SE), and 2669 ± 17 (SE), respectively. The approximate 80 % increase of mean miR-222 copy number of MCF-7/S + A/exo compared with MCF-7/S + S/exo suggests that A/exo could transfer miR-222 to MCF-7/S cells. There is little difference between mean miR-222 copy numbers of MCF-7/S and MCF-7/S + S/exo.

MiR-222 changed drug resistance of MCF-7 cells

The focus of this paper is whether miR-222 is involved in drug resistance of MCF-7 cells. MCF-7/S transfected with miR-222 mimics has an apoptotic rate significantly lower than MCF-7/S ($P=0.015$). MCF-7/Adr has an apoptotic rate significantly lower than MCF-7/Adr transfected with miR-222 inhibitors ($P=0.001$). The IC_{50} of Adr value of MCF-7/S + miR-222 mimics was greater than that of MCF-7/S ($P=0.013$). The IC_{50} of Adr value of MCF-7/Adr + miR-222 inhibitors was less than that of MCF-7/Adr ($P=0.016$). These results demonstrate that miR-222 could change the drug resistance of MCF-7 cells.

Discussion

Adriamycin is one major chemotherapeutic agent used in treatment of breast cancer (BCa). Adriamycin can induce cancer cell apoptosis by intercalating among the base pairs of the DNA double-helical structure and interfering with gene transcription. However, BCa patients treated with anti-cancer drugs often develop drug resistance, which means they fail to respond to the drugs. In this study, we are committed to investigating one MCF-7/Adr resistance mechanism: the role of exosomes in development of drug resistance.

Exosomes are recently discovered as a new form of cell-to-cell communication. Exosomes can be detected by TEM according to their sizes. Exosomes can be taken in by recipient

Fig. 6 Quantitative analysis of miR-222 in MCF-7 cells. The total numbers of miR-222 in **a** MCF-7/S ($n=512$), **b** MCF-7/S + S/exo ($n=522$), and **c** MCF-7/S + A/exo ($n=559$) were quantified. Individual miRNAs were unidentifiable in some cells with high miRNA copy numbers. Therefore, they were estimated by linear correlation between total fluorescence intensity and miRNA copy number **(d)**
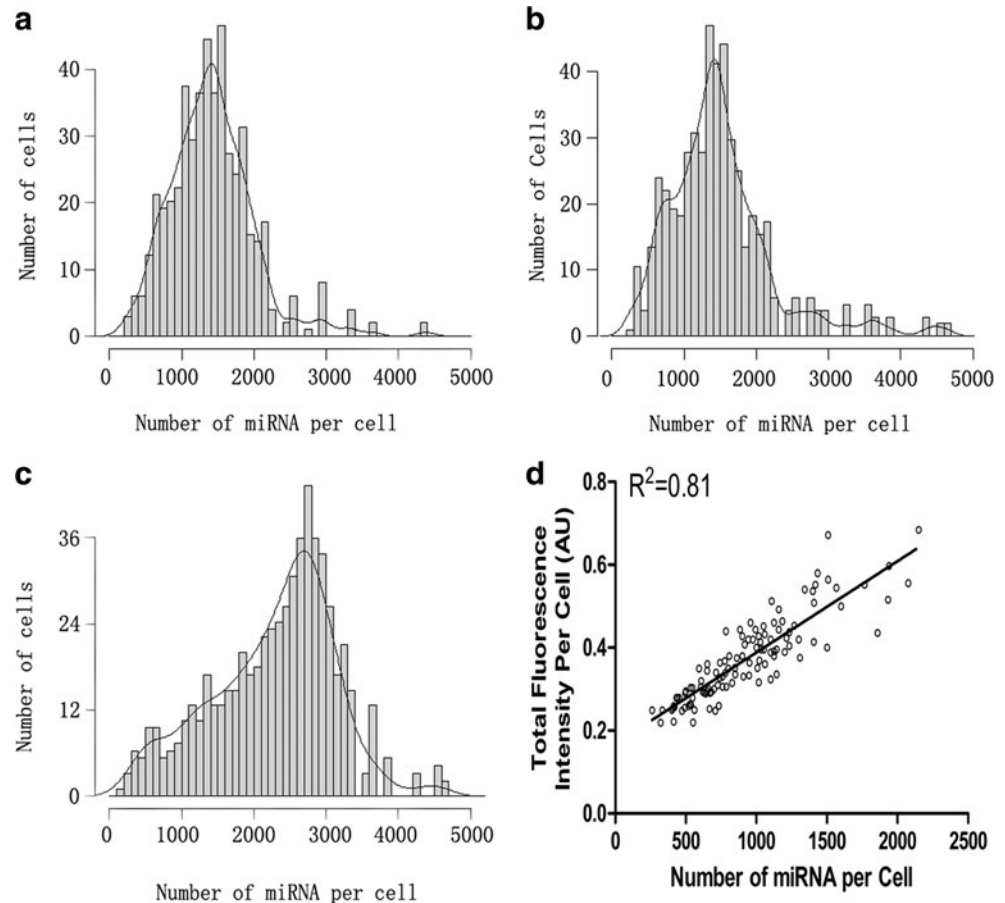
cells and transmit drug resistance from MCF-7/Adr to MCF-7/S. MiR-222 expression was high in A/exo but low in S/exo (microarray results from our team). The miR-222 abundance in MCF-7 cells was increased after treatment with A/exo. MCF-7/S acquired drug resistance after transfection miR-222. In conclusion, exosomes from adriamycin-resistant BCa cells transmit drug resistance partly by delivering miR-222.

Numerous publications document that labeled exosomes can be absorbed by cells [16–18]. The present study shows that A/exo was not only bound to MCF-7/S but also engulfed and internalized by a GFP-S model [19]. This result was based on CLSM. There are diverse mechanisms behind the absorption of exosomes by recipient cells, such as ligand-receptor binding, direct fusion with plasma membrane, endocytosis, and phagocytosis [20].

Growing evidence indicates that exosomes contribute to drug resistance of BCa cells through various pathways. Exosomes transferred from stromal to BCa cells contribute to chemotherapy and radiation resistance [21]. Exosomes from docetaxel-resistant BCa cells transmit chemoresistance through the delivery of p-gp and miRNAs [4, 22]. This result is similar to our observation that exosomes from adriamycin-resistant BCa cells transmit chemoresistance to sensitive cells. According to the results of apoptotic rate and IC_{50}, the A/exo-treated MCF-7/S cells acquired drug resistance compared with S/exo-treated cells. There is no remarkable difference in drug resistance between MCF-7/S and S/exo-treated MCF-7/S.

It is generally accepted that exosomes transfer RNAs and proteins to mediate the communication among cancer cells. It was proposed that the exosome-mediated miRNA transfer may be a new method of gene flow among cells [23]. In the present study, gene microarray was used to investigate whether the A/exo-induced change of MCF-7/S chemoresistance was partly due to the exosome-carried miRNAs. We found 374 miRNAs with differential expression in A/exo compared with S/exo. miR-222 was selected for this experiment because it was overexpressed in both MCF-7/Adr and A/exo. PCR is a commonly used but indirect method to measure miRNA expression. Thus, we used LNA-ELF-FISH to quantify miR-222 abundance in situ. LNA-ELF-FISH is a highly sensitive and specific way of detecting single miRNAs in individual cells. It is an excellent idea to combine LNA hybridization probes with ELF signal amplification, which allows the visualization of single miRNAs. Compared with PCR, LNA-ELF-FISH avoids cell lysis, miRNA purification, and enrichment to retain the spatial information. LNA-ELF-FISH has many advantages, such as the need of only one hybridization probe, long stoke shift, and high photostability of fluorescent precipitate. These advantages mean cost saving, low autofluorescence, and imaging repeatability. The average miRNA copy number per cell acquired by LNA-ELF-FISH was within 17.5 % of measurements from quantitative RT-PCR [7]. However, only one ELF substrate can be used, which limits LNA-ELF-FISH to image only a single RNA in cells. As showed in Fig. 6, the average amount of miR-222 in A/exo-treated MCF-7/S was significantly higher than exo-treated MCF-7/S. This result is direct evidence that A/exo could transfer miR-222 to MCF-7/S cells.

It is hard to say whether distinctively expressed miRNAs between A/exo and S/exo contribute to drug resistance. MiR-222 was chosen to study whether it contributed to drug resistance of BCa. According to the results of apoptotic rate and IC_{50}, MCF-7/S cells transfected with miR-222 mimics acquired drug resistance compared with those transfected with the negative control. At the same time, MCF-7/Adr cells transfected with miR-222 inhibitors lost drug resistance.

In conclusion, our experiments verify that exosomes from drug-resistant BCa cells can be absorbed by recipient cells and transmit chemoresistance partially by transferring miR-222. This study indicates that the delivery of miRNAs by exosomes may be a significant mechanism of exosomes-mediated drug resistance transfer.

Compliance with ethical standards

Conflicts of interest None

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