INFLUENCE OF PC3-DERIVED EXOSOMES ON FIBROBLAST PROLIFERATION AND MIGRATION

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https://doi.org/10.37904/nanocon.2023.4802

Abstract

Nano-sized exosomes are essential for intercellular communication, especially in cancer biology. Human produces exosomes that may modulate the tumor microenvironment and cellular behaviour. This study examines how human prostate cancer cells (PC3) derived exosomes affect fibroblast proliferation and migration. Ultracentrifugation isolated exosomes from PC3 cell culture supernatants, which were characterized by confocal and transmission electron microscopy (TEM). Fibroblast cells were exposed to these PC3-derived exosomes. MTT [3–(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazoliumbromide] was used to measure fibroblast proliferation, while another assay measured migration. PC3 exosomes appear to boost fibroblast proliferation and migration. Investigations into the molecular mechanisms behind these observations are ongoing. The study shows that PC3-derived exosomes can regulate fibroblast proliferation and migration. This study illuminates the effects of cancer cell-derived exosomes on cellular interactions and therapeutic potential.

Keywords: Human prostate cancer cell (PC3), exosomes, fibroblast proliferation and migration

1. INTRODUCTION

Exosomes, which are classified as extracellular vesicles (EVs), are typically between 40 and 150 nm in size and originate from the endosomal system's multivesicular bodies (MVBs) within cells [1]. Beginning with primary endocytic vesicles, this system develops early endosomes (EE). These EEs develop into LEs (late endosomes). Some LEs transform into MVBs, which have the potential to fuse with the outer membrane of the cell, resulting in the release of exosomes [2] (**Figure 1**). Exosomes can be found in nearly all body fluids and organs and are secreted by almost every cell type [3]. They play an essential role in facilitating communication between cells, particularly during processes such as cellular movement [4]. Exosomes, a subset of EVs, transport a combination of proteins, nucleic acids, and lipids, thereby facilitating this intercellular communication [5]. In cancer research, exosomes have been identified as contributors to multiple stages of tumor development, including blood vessel formation, immune system evasion, and cancer dissemination [6]. Particularly, exosomes derived from cancer cells have a profound effect on the tumor environment, interacting with numerous cells, including fibroblasts [7]. It is known that the PC3 prostate cancer cell line produces exosomes that may affect the tumor environment, and prostate cancer remains one of the most common types of cancer [8]. The purpose of this study is to investigate how PC3-derived exosomes influence fibroblast activity, thereby shedding light on their role in the progression of tumors and potential treatment options.

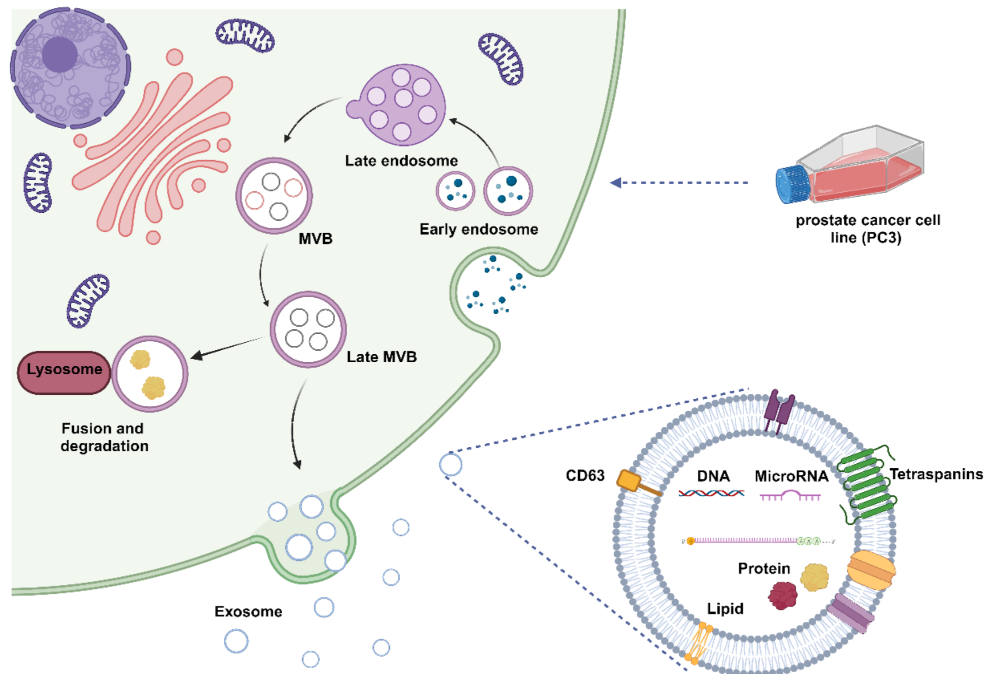

Figure 1 Diagram depicting the formation and release of exosomes from early endosomes (EE) to multivesicular bodies (MVBs)

2. MATERIALS AND METHOD

2.1 CELL CULTURE AND EXOSOME ISOLATION

The PC3 cells were grown in Dulbecco's modified Eagle medium (DMEM, Merck, Germany) and Ham's F12 with L-glutamine (F12, Serana, Germany) containing 10% fetal bovine serum (FBS, Biosera, France) and 2% penicillin-streptomycin (Merck, Germany). The NIH/3T3 mouse fibroblasts were cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine, and 2% penicillin-streptomycin. These cells were housed in a 37°C, humidity-controlled incubator (37°C, 5% CO_2). Once the cells reached a specific level of confluence, regular subculturing was performed using a 0.25% trypsin-EDTA solution.

2.2 EXOSOME ISOLATION

In 25T cell culture flasks, cells were cultivated until they reached 80% confluence. The medium was then replaced with medium devoid of exosomes to ensure that the isolated exosomes originated solely from the desired cells. After 48 hours, the medium was harvested and centrifuged for 10 minutes at 300g to remove cellular debris. This was followed by a 30-minute centrifugation at 10,000g to separate the microvesicles. The conditioned medium was ultracentrifuged at 100,000 g for 90 minutes at a temperature of 4 °C. The centrifuged supernatant was discarded and the exosome pellet was dispersed in PBS (Merck, Germany). Another ultracentrifugation wash at 100,000 g for 90 minutes at 4°C was performed. The final exosome preparation was resuspended in 500 µl of PBS and frozen at -80°C.

2.3 TRANSMISSION ELECTRON MICROSCOPY (TEM)

The purified exosomes were diluted to 1:1000 in PBS. We added 5 µL of diluted exosomes into Formvar-carbon-coated TEM grids. The grids were stained using 2% uranyl acetate, which was removed using filter paper. Finally, the grids were viewed using 80 kV-setting TEM (Titan Themis 60–300 Cubed, Thermo Fisher Scientific, USA).

2.4 EXOSOME UPTAKE ASSAY

For the analysis of exosome uptake, exosomes derived from PC3 cells were tagged with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) solutions (Merc, Germany) and allowed to incubate for 20 minutes at 37°C. The employed DiI concentration was 1 mM. Approximately 4,000 NIH/3T3 cells were grown in 35 mm glass bottom dishes with 20 mm well size. 24 hours of starvation were administered to the cells. After being rinsed twice with PBS, the cells were exposed to the tagged exosomes for 2 h at 37°C and 5% CO_2. After incubation, the cells were fixed in a 4% paraformaldehyde solution for 20 minutes at room temperature. The cells were then stained with a green fluorescent F-actin dye (ActinGreen, Invitrogen, USA) according to the provided instructions. DAPI was used to stain the nucleus. Confocal microscopy was used to observe the resulting fluorescence.

2.5 CELL PROLIFERATION CELL MIGRATION ASSAY

The NIH/3T3 cells were grown until 70-80% confluence was reached. After trypsinization, the cells were seeded at a density of 4,000 cells per well in 96-well plates. The cells were then exposed to PC3-Exo or PBS (as a control) and incubated for 1, 3, and 5 days at 37°C in a 5% CO_2 atmosphere. To reach a total volume of 100 µl, each well was supplemented with fresh DMEM and a 10 µl MTT solution (5 mg/mL stock in fresh medium). After three hours of incubation, purple crystals of formazan form. These crystals were dissolved in a solubilizing agent and the solution was transferred to a new 96-well plate. Using a UV-Vis spectrophotometer, the absorbance was measured at 570 nm after subtracting the 650 nm background. Using a standard curve, cell counts were determined. For migration experiments, 3 x 10^5 fibroblasts were seeded into 35 mm glass bottom dishes with 20 mm well size and incubated at 37°C with 5% CO_2 for at least 24 hours to permit cell adhesion and the formation of a confluent layer. After staining the cells with CellTracker™ Green CMFDA Dye (Invitrogen, USA), a 200 µl sterile pipette tip was utilized to scratch this monolayer. The culture medium was discarded immediately, and the cells were washed with PBS. PC3-Exo-containing fresh medium was added. Using a confocal microscope, images of the scratch were captured 24 and 48 hours after treatment with exosomes. Each scratch test was performed three times.

3. RESULTS

3.1. Exosome Identification

Exosomes were isolated from PC3 cell culture supernatants using ultracentrifugation and characterized using confocal and transmission electron microscopy. The exosomes' morphology and size range of 50-150 nm confirmed their identity as exosomes (see **Figure 2**). To analyse the uptake of PC3-EVs by NIH/3T3 cells, PC3-Exo were fluorescently labeled with DiI and incubated with NIH/3T3 cells for 2 hours. **Figure 3** depicts the dose-dependent increase in PC3-Exo uptake by NIH/3T3 cells as visualized by fluorescence microscopy. Our findings indicate that PC3-EVs are effectively internalized by NIH/3T3 cells.

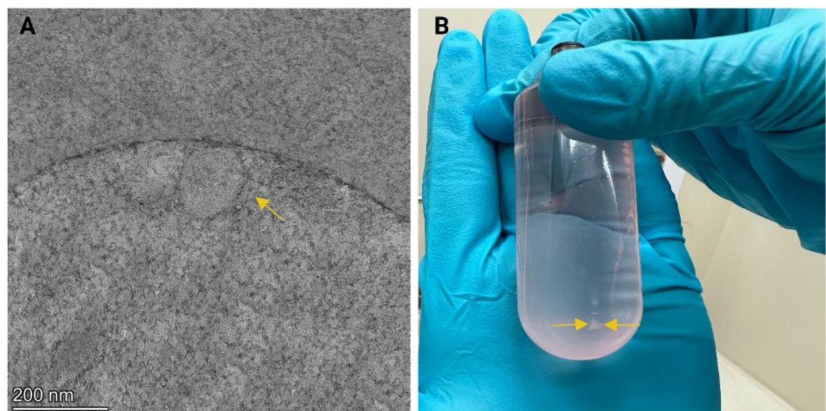

Figure 2 Transmission Electron Microscopy (TEM). Exosome characterization. (A) Transmission Electron Microscopy (TEM). TEM images of exosomes derived from prostate cancer cell lines (PC3). Exosomes were negatively stained with 2% uracyl acetate after removing the extra moisture. Cup-shaped structures, with 30–200 nm size were identified as being exosomes. Exosomal pellets. (B) Pellet obtained using the ultracentrifugation (UC) method. It was in the form of a very thin, barely visible withe film (in between the arrows).

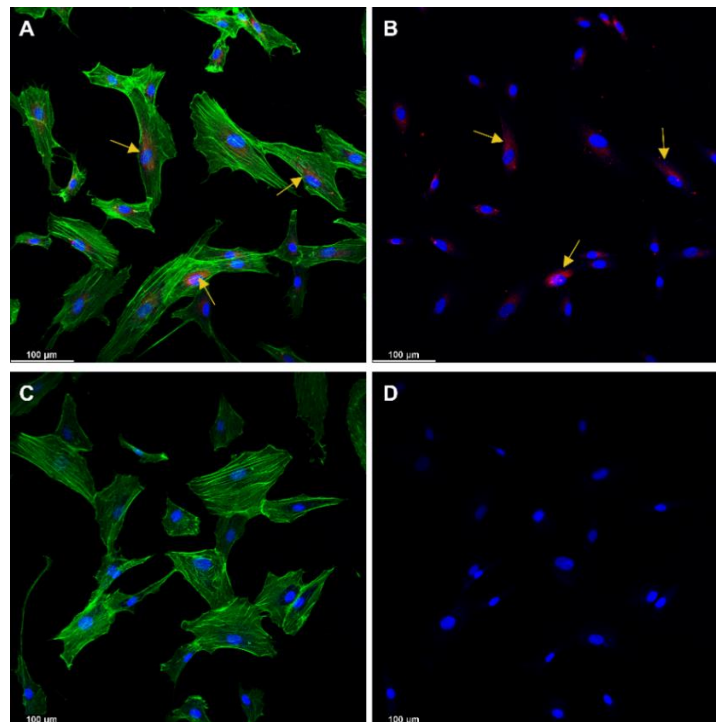

Figure 3 Confocal microscopy visualization of exosome fused (A,B) and untreated (C,D) NIH/3T3 cell line. Yellow arrows show PC3- exosomes in NIH/3T3 cell line.

3.2. PC3-Derived Exosomes Stimulate Proliferation and Migration of NIH/3T3 cells

To explore whether PC3-Exo could induce the growth NIH/3T3 cells, the fibroblasts were incubated with PC3-Exo for 1,3 and 7 days. Normal adult fibroblast growth, as measured by the MTT assay, was found to be significantly increased and in a dose-dependent manner as compared to vehicle and depleted medium at day 3 and 7 compared to control (**Figure 4A**).

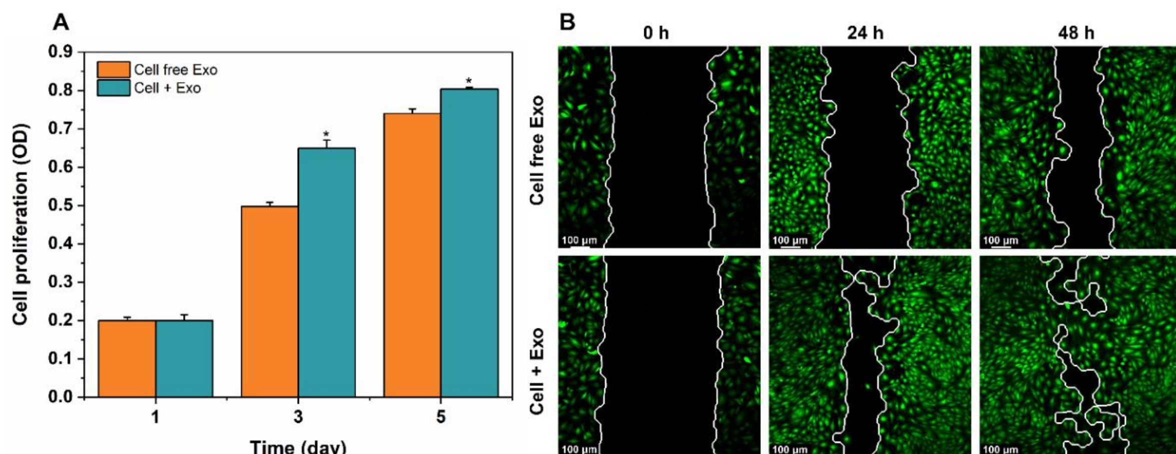

Figure 4 Proliferative and migration effect of exosomes derived from prostate cancer cell line. (A) NIH/3T3 cells were treated with PC3-Exo for 1,3 and 5 days and the proliferation was assessed with MTT assay. Exosomes increased the cellular proliferation of the NIH/3T3 cells compared to the untreated control cells. Bars represent mean ± standard deviation (SD); n = 3; * $p < 0.005$. (B) PC3-Exo enhance cell migration. Fibroblast monolayers were scratched (cleared areas outlined in white dashed lines) to simulate a wound. After 18 h, fibroblast proliferation began to close the wound in (top) untreated and (bottom) treated cultures. Treatment with PC3-Exo accelerated wound closure compared to control.

The migration rate of cells was significantly increased in the PC3-Exo group after 24 and 48 hours compared to the untreated sample (see **Figure 4B**). The mean values of scratch width for untreated NIH/3T3 cells (Cell free Exo) are 579.0 μm (immediately after scratching), 469.2 μm (after 24 hours) and 274.3 μm (after 48 hours). Meanwhile, the mean values of scratch width for PC3-Exo-treated cells (Cell+Exo) are represented as 674.0 μm (immediately after scratching), 207.7 μm (after 24 hours) and 156.0 μm (after 48 hours).

4. CONCLUSION

Exosomes, as crucial intercellular communication mediators, have been highlighted for their crucial role in cancer biology, particularly in the modulation of the tumor microenvironment. Our research highlights the impact of exosomes derived from the PC3 prostate cancer cell line on the proliferation and migration of fibroblasts. The observed increase in fibroblast activity upon exposure to exosomes derived from PC3 provides valuable insight into the complex cellular interactions within the tumor microenvironment. These findings not only enhance our understanding of the dynamics of tumor progression, but they also suggest potential therapeutic avenues based on exosomal interactions. Utilizing the therapeutic potential of exosomes in the treatment of cancer requires further investigation into the molecular mechanisms underlying these effects.

ACKNOWLEDGEMENTS

We acknowledge CzechNanoLab Research Infrastructure supported by MEYS CR (LM2018110).

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