Characterization of Exosomes

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Introduction

Exosomes are nano-vesicles secreted by cells. They are found in extracellular space and all body fluids. The frequency of release depends on the environment, and cancer cells are known to release more exosomes. They carry non-coding RNAs and it has been reported that the majority of miRNAs in the blood are within exosomes. Exosomes play a role in intercellular signaling by fusing with recipient cells and releasing their RNA. Exosomes can be distinguished from other vesicles by biomarkers and their small size.

This poster describes the methods for the isolation and characterization of the exosomes and my plans for future research.

Samples

Sera samples were collected from a 75-year-old female pancreatic cancer patient and a healthy 42-year-old female. Both samples were obtained from ARUP Laboratories Inc.

Exosome Isolation Method

Exosomes were isolated from 1mL serum using an ExoQuick kit (System Biosciences) following manufacturer's instructions.

- Centrifuge serum at 3,000xg for 15 minutes to remove cells and cell debris.
- Transfer supernatant to a sterile vessel and add 252 micro liters of ExoQuick.
- Refrigerate mixture for 30 minutes then centrifuge at 1500xg for 30 minutes at room temperature.
- Discard supernatant and save the exosome pellet.
- Resuspend pellet in 200 micro liters of 2mM ammonium acetate buffer.

Data Analysis

Size-frequency measurements for the samples obtained with different techniques were converted into probability density functions (pdf) of particle sizes expressed as a histogram.

Acknowledgements

Scanning Electron Microscopy (SEM)

- Dilute both samples to 1:100 in DI water.
- Clean glass slide with nitrogen gas and place on specimen stage.
- Place sample on the glass slide and allow to dry.
- Image samples at 0.98 Torr using a low vacuum secondary electron detector.
- Analyze images using MATLAB software to determine exosome size distribution.

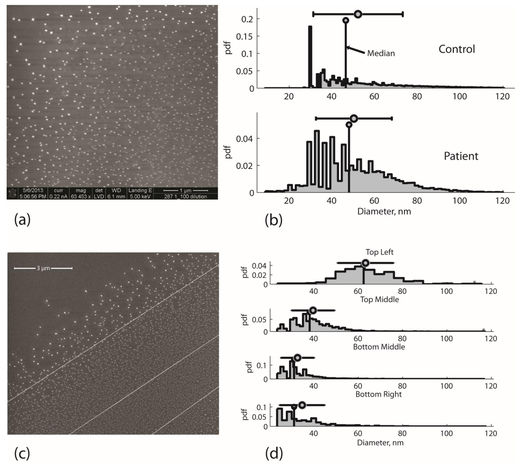

Figure 1: (a) Typical SEM image of desiccated exosomes. (b) The exosome size distribution is based on image analysis of 24,024 and 12,298 exosomes derived from control and patient samples, respectively. Panel (c) shows non-uniform surface deposition pattern. Size segregation was also observed. With reference to four areas in panel (c) delineated by diagonal lines, we see that the largest size is observed in the top left corner of the image and decreases towards its bottom right corner. This size segregation is quantified in panel (d).

Electrospray Differential Mobility Analysis (ES-DMA)

DMA was previously used to size biological particles including cold viruses.

- Dilute patient and control samples 1:100 in AA solution.
- Use the electrospray aerosol generator to confine individual exosomes inside charged droplets formed by atomizing the suspension in the Taylor cone formed at the end of an ID capillary.
- The bombardment by α-particles electrically neutralized most exosomes.
- The exosomes were carried by flowing gas into the differential mobility analyzer.
- Positively charged exosomes are deflected by a strong negative electrical potential towards a collection slit and are counted inside the condensation particle counter.
- The result is an average number of particles analyzed per volume of inlet gas.

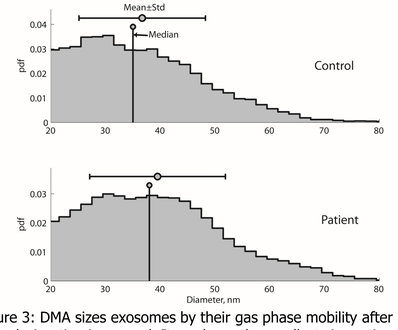

Figure 3: DMA sizes exosomes by their gas phase mobility after their desiccation in aerosol. It produces the smallest size estimate.

Cryo-Transmission Electron Microscopy (Cryo-TEM)

- Dilute samples 1:100 in DI water.
- Place sample on a holey carbon-coated copper grid.
- Plunge freeze the aqueous sample into liquid ethane maintained at the temperature of liquid nitrogen to vitrify the sample and store in liquid nitrogen.
- Transfer stored samples to a cryoholder to maintain their temperature at -180 °C during imaging.
- Obtain the cryo- TEM images using FEI Tecnai F20 transmission electron microscope camera.
- The images were analyzed using MATLAB software to determine exosome size distribution.

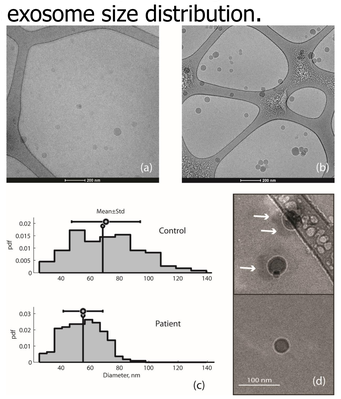

Figure 2: Cryo-TEM images of control (a) and pancreatic patient (b) exosome. The exosome size distribution in (c) is based on computer analysis of 106 control and 212 patient exosomes. Patient exosomes have a tendency to cluster, as seen in the lower right quadrant of panel (b). Exosomes at the highest obtained resolution are shown in (d). Arrows shows faint, extra density surrounding some particles, which suggests the presence of macromolecules conjugated to their surface.

Nanoparticle Tracking Analysis (NTA)

- Nanosight instrument illuminated the sample with a 40 mW violet laser, capturing the light scatted by exosomes with a high-sensitivity sCMOS camera.
- The results were analyzed using the software provided by the manufacture.
- Dilute the samples 1:1,000 in DI water (filtered using Nanopure Filtration System) and allow it to equilibrate to room temperature.
- Analyze samples within 5 minutes of the initial dilution.
- Inject each sample with a 1mL sterile syringe into the test cell.
- Record a 60 second video for each sample and analyze using NTA software, which reported the exosome size distribution, its mode, mean and the standard deviation.

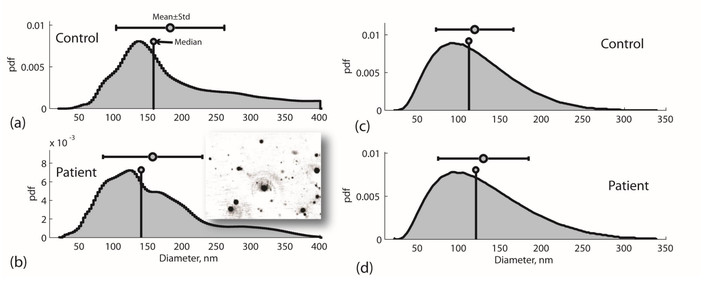

Figure 4: Hydrodynamic sizes measured by BTA (a,b) and DLS (c,d).

Dynamic Light Scattering (DLS)

- Dilute samples 1:100,000 in DI water and filter through syringe filters.
- Place 1mL of the sample into a low volume disposable sizing cuvette for analysis.
- Perform DLS measurements on a Malvern Zetasizer Nano ZS at 173° angle.
- Measurements were interpreted with a refractive index of the solution equal to 1.33 and a refractive index for exosomes set to 1.35.
- Samples were analyzed 3 repeated times, each time with 12 scattering measurements.
- The data was processed using a General Purpose Model in the Zetasizer software to obtain size distribution, its mean, and standard deviation.

Discussion

The sizes of exosomes overlap with many other biological particles. They overlap with lipoproteins and protein agglomerates in the lower range of size distribution and extracellular macrovesicles and cell debris at the higher range of their sizes. This means the exosome isolation method influences the population of the isolated particles, which affects the sizing results. Exosome isolation is not a standardized procedure, but differential ultracentrifugation is the most widely used exosome isolation approach followed by gradient ultracentrifugation and precipitation techniques like ExoQuick.

These sizing techniques will be used to determine many different characteristics including the biophysical difference of the exosomes.

Future Research

We will use several techniques, including those outlined in this poster, to characterize biophysical, biochemical and molecular properties of the exosomes. We will start with the characterization of the cell culture exosomes and eventually progress toward the characterization of the exosomes isolated from the pleural effusions and sera of cancer patients.

References

Chernyshev,V. et al., "Size and Shape Characterization of Hydrated and Desiccated Exosomes," Anal. Bioanal. Chem., 407:3285–3301, 2015.