

The extract methods and exosomes authentication

The method of macrophage-exosomes extraction and authentication were similar to the previous studies (1-3). Briefly, macrophages were cultured with BFM 24h, followed by DPBS washing 3 times. Then, macrophages were cultured with no-serum medium (99% L-DMEM, 1% penicillin and streptomycin) 48h. The supernatant of macrophages was harvest and centrifuged by 2,000g 30min at 4°C. The supernatant was harvested to a new centrifugal tube and centrifuged by 10,000g 45min at 4°C. Then, the supernatant was harvested and filtrated by a 0.45 µm sterile membrane. The filtrate was harvested and centrifuged by 100,000g 70min at 4°C. The precipitate was resuspended by 10 ml pre-cold DPBS and centrifuged by 100,000g 70min at 4°C again. The precipitate (macrophage-exosomes) was harvested and resuspended by 100 µl pre-cold DPBS.

Xiamen anti-hela biological company finished the transmission electron microscopy and nano-flowcytometry (NanoFCM) examination and imaging.

The operation process was as follows: briefly, Transmission electron microscopy was used to observe the exosomes. 10 µl exosomes solutions was added to the copper net to get the precipitation for 1 min and the surface liquid was absorbed by the filter paper. 10 µl uranyl acetate was added to the precipitation for 1 min and the surface liquid was absorbed by the filter paper, followed by dried at room temperature for several minutes. Then, electron microscopic was used and the voltage was set as 100 kV for examination and imaging (Fig.1).

NanoFCM was used to analyzed the particle size and concentration information of exosomes. The exosomes solution was 1:2 diluted by DPBS. The exosome sample was loaded after the instrument performance test qualified with the standard substance. The particle size and concentration information of exosomes were detected (Fig.2-3).

CD63, TSG101 and GAPDH were detected. The exosomes or macrophages were lysed with RIPA buffer which contained 1% phenylmethanolsulfonyl fluoride (PMSF) at 4°C 40min. BCA assay was used to measure the protein concentration of the lysates. SDS-PAGE Sample Loading Buffer (5X) was added to the lysates and the complex was heated at 100°C for 5 minutes to fully denature the protein. Then, the samples (30ug/well) were separated on a 15% SDS-polyacrylamide gels and transferred onto a PVDF membrane (0.2 µm). The membranes were blocked and incubated with rabbit anti- CD63 (1:1000), anti-TSG101 (1:2000) and anti-GAPDH (1:10000) overnight at 4°C. HRP-conjugated secondary antibody was used and the signals were detected by the ECL chemiluminescence kit (Fig.4-6).

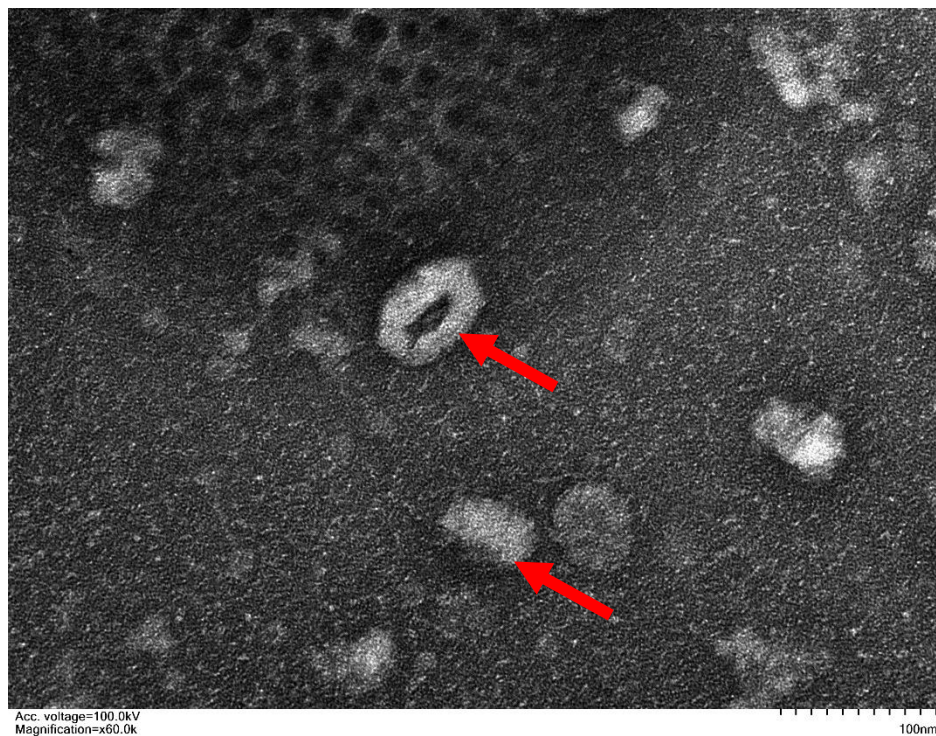


Fig.1 the transmission electron microscopy image. Macrophage-exosomes could be seen in the image (red arrows). The magnification times: 100nm.

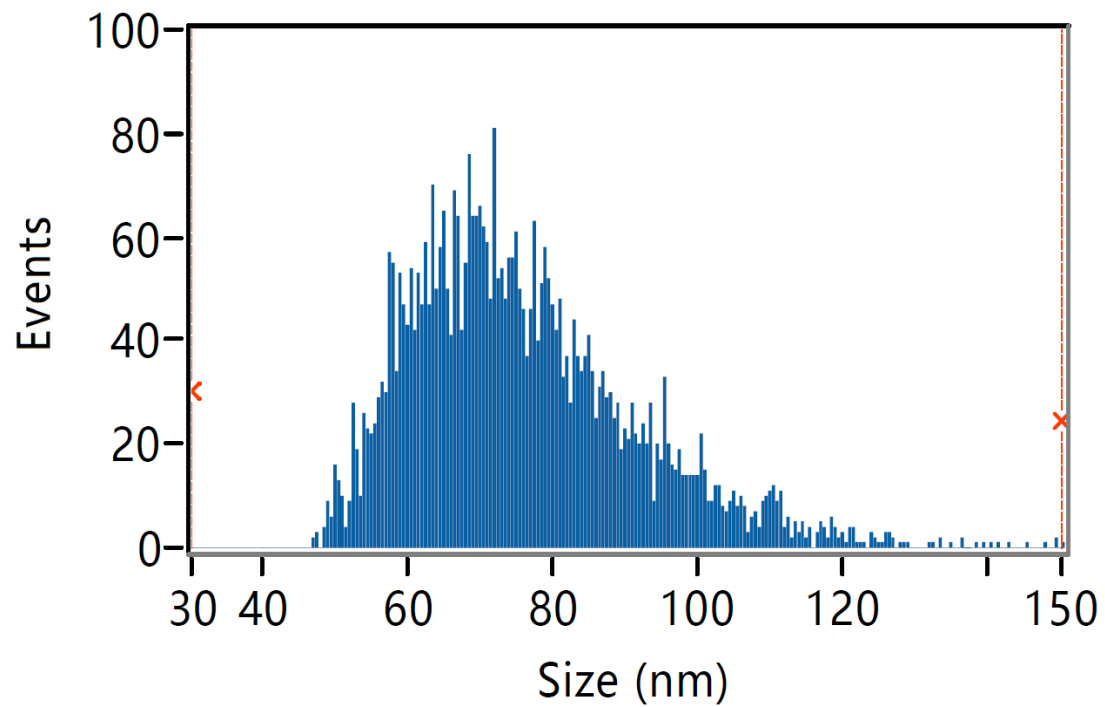


Fig.2 the particle size results. NanoFCM was used to detect the particle size and 99.64% events were in 30-150nm. The size was 75.60 ± 15.60 nm.

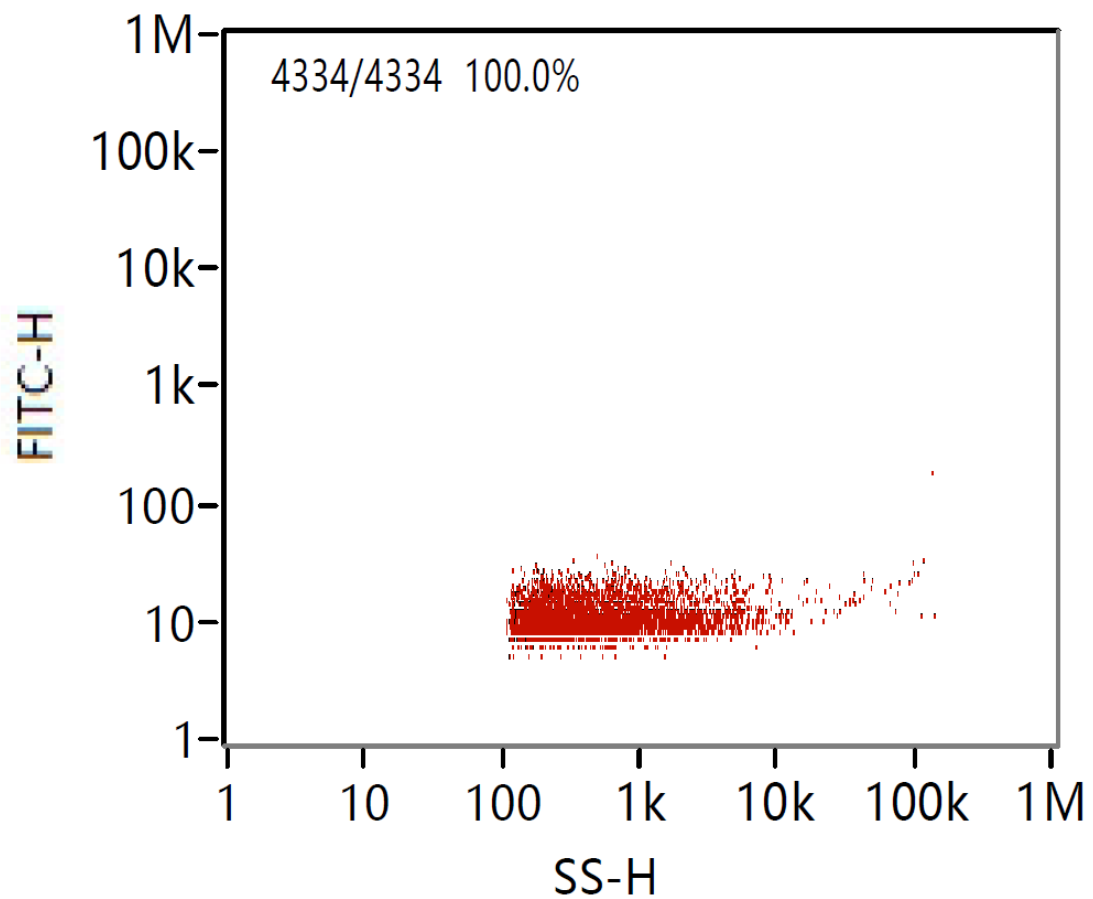


Fig.3 the concentration results of exosomes. NanoFCM was used and the concentration was 1.25×10^{11} particles/ml.

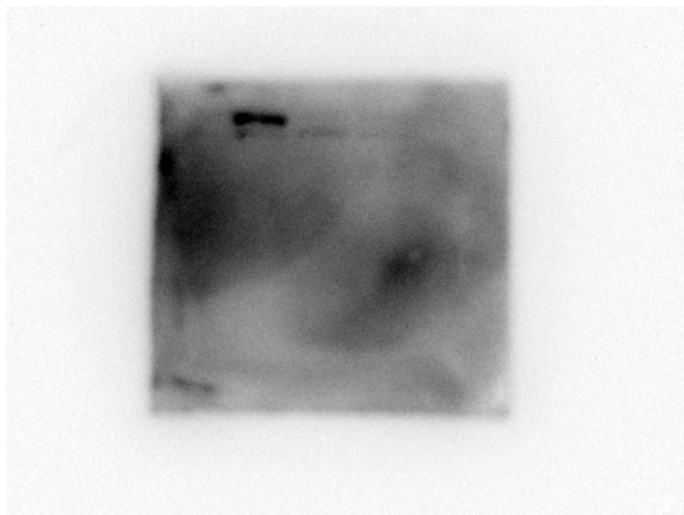


Fig.4 the CD63 gel image. Bio-Rad gel imaging system and Image lab were used to export the image. Left side was exosomes lysates and right was macrophages lysates.

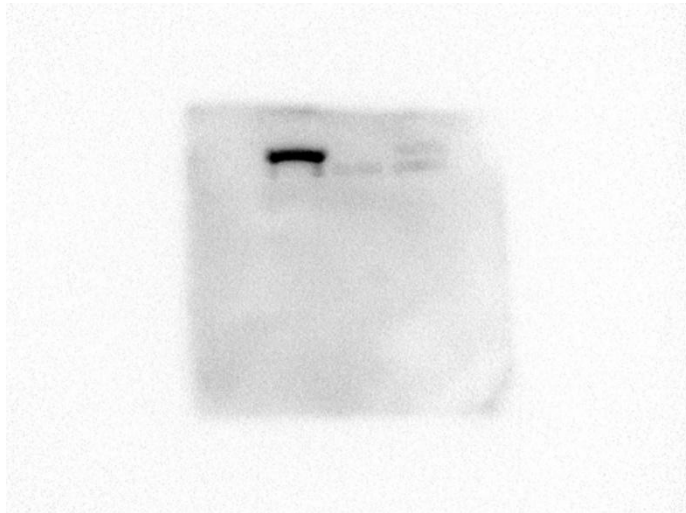


Fig.5 the TSG101 gel image. Bio-Rad gel imaging system and Image lab were used to export the image. Left side was exosomes lysates and right was macrophages lysates.

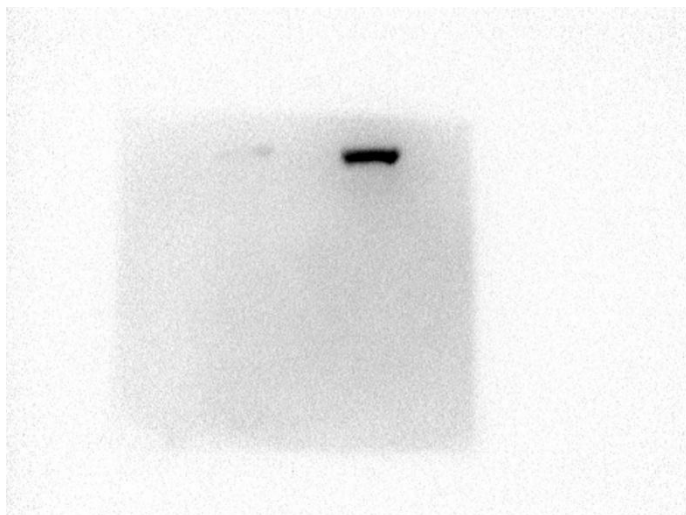


Fig.6 the GAPDH gel image. Bio-Rad gel imaging system and Image lab were used to export the image. Left side was exosomes lysates and right was macrophages lysates.

Reference

- 1.Zheng P, Luo Q, Wang W, et al. Tumor-associated macrophages-derived exosomes promote the migration of gastric cancer cells by transfer of functional Apolipoprotein E. *Cell Death Dis.* 2018;9(4):434.
- 2.Wang P, Wang H, Huang Q, et al. Exosomes from M1-Polarized Macrophages Enhance Paclitaxel Antitumor Activity by Activating Macrophages-Mediated Inflammation. *Theranostics.* 2019;9(6):1714-1727.

3.Zhu J, Liu B, Wang Z, et al. Exosomes from nicotine-stimulated macrophages accelerate atherosclerosis through miR-21-3p/PTEN-mediated VSMC migration and proliferation. *Theranostics*. 2019;9(23):6901-6919.