Large Scale Manufacturing and Functional Characterization of hTERT-Immortalized MSC Extracellular Vesicles

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Abstract

Extracellular Vesicles (EVs) isolated from primary mesenchymal stem cells (MSCs) are widely studied for potential therapeutic application across various tissue types [1]. We have previously demonstrated the anti-apoptotic and anti-inflammatory effects of MSC EVs in CNS cells [2,3]. However, there are challenges when working with primary stem cells and their by products. These include donor-to-donor variability and the finite cellular lifespan of the cells which makes large-scale manufacturing difficult to achieve. The development of hTERT immortalization protocols provided a scientific breakthrough in primary cell research. The application of this technology preserves telomere length and prevents cellular senescing, allowing for continuous replication of primary cells [4]. In this study, we used our large-scale EV manufacturing platform to produce multiple batches of EVs from hTERT-immortalized MSCs. We employed various methods to characterize and profile EV cargo to identify molecules which contribute to repair. Lastly, we evaluated the effects of EVs on irradiation-damaged retinal pigment epithelial (RPE) cells in vitro to better understand the underlying mechanisms which may drive their reparative properties. We have included side-by-side results with EVs from PC3 cancer cells to serve as a control.

Methods

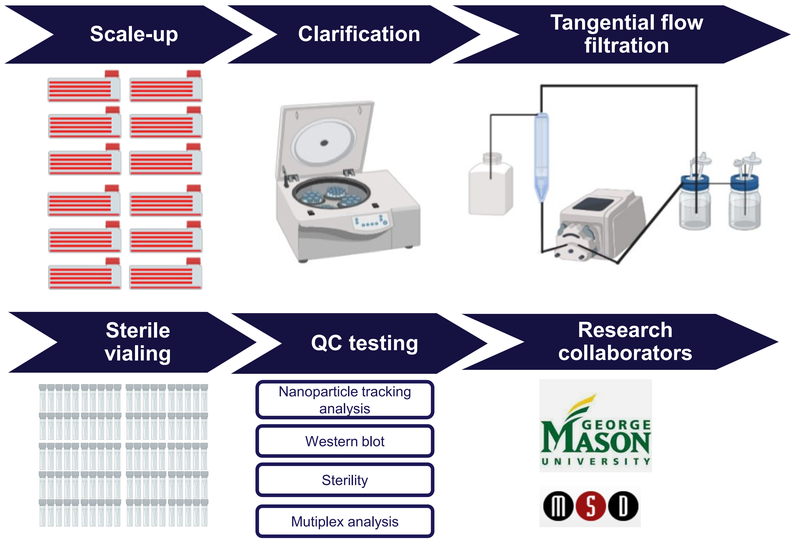


Figure 1. Large-scale EV manufacturing. Cells are scaled up, media is clarified by centrifugation, and the supernatant is concentrated and diafiltered by tangential flow filtration to enrich for EVs. EVs are vialed and downstream QC assays are performed for characterization. Research collaborations with George Mason University and MSD have allowed for extended EV characterization and functional assays to be performed in vitro.

Results

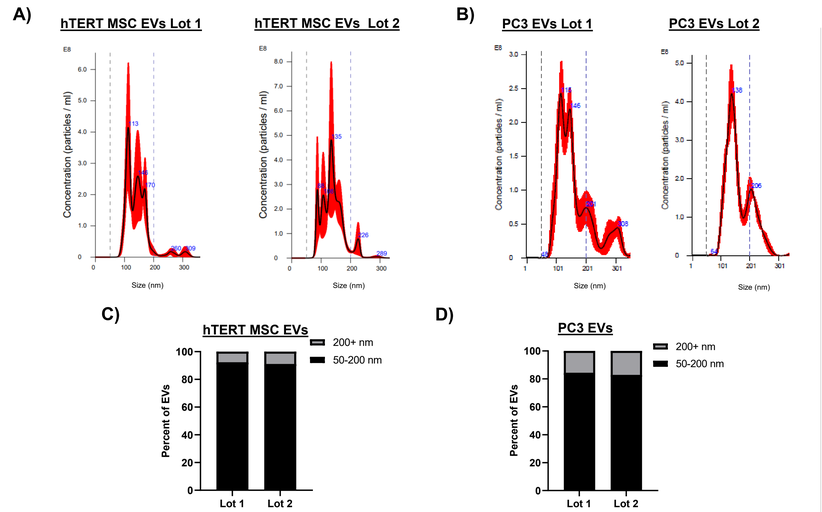


Figure 2. Nanoparticle Tracking Analysis. NTA was performed on two independent lots of **A**) hTERT MSC EVs and **B**) PC3 EVs to analyze relative concentration (particles/mL) and size distribution. Dashed lines indicate values of 50 nm and 200 nm. **C)** Percent of hTERT-immortalized MSC EVs within 50-200 nm. **D)** Percent of PC3 EVs within 50-200 nm.

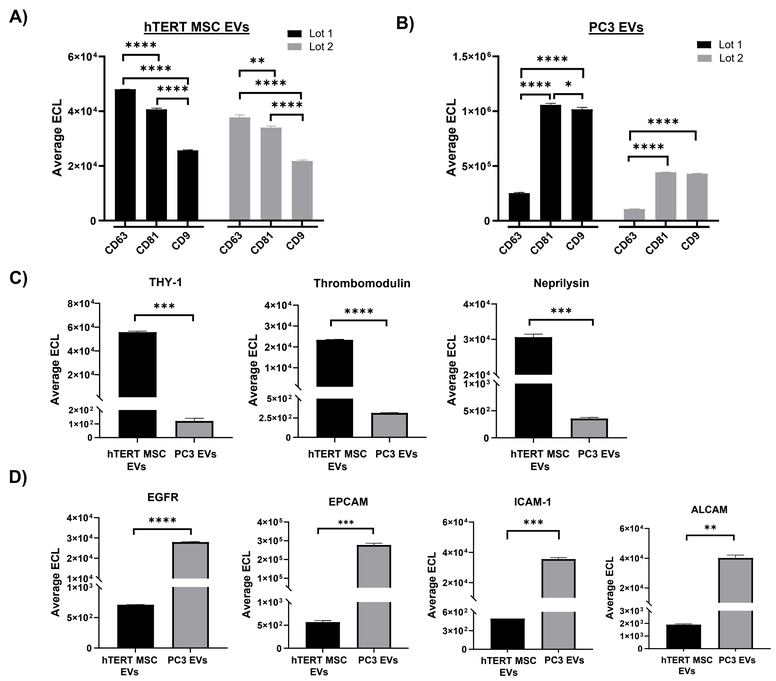
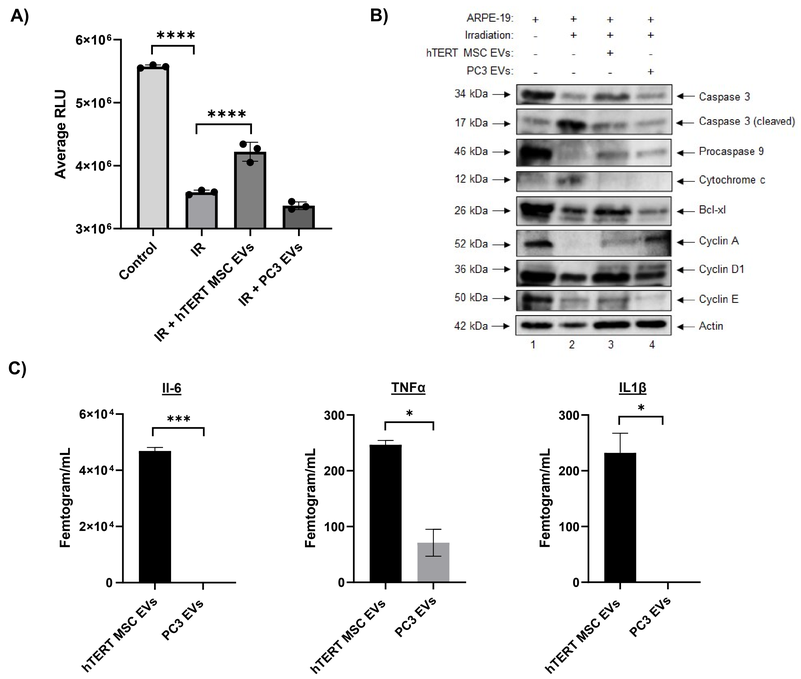


Figure 3. EV surface marker profiling. Multiplex analysis was performed to analyze various EV-associated surface markers. **A)** Average ECL of tetraspanins from two independent lots of hTERT MSC EVs. **B**) Average ECL of tetraspanins from two independent lots of PC3 EVs. **C)** Average ECL values of surface markers enriched in hTERT MSC EVs. **D)** Average ECL values of surface markers enriched in PC3 EVs. All assays run in duplicate. **** p <0.0001; *** p <0.001; ** p <0.01, * p < 0.05.



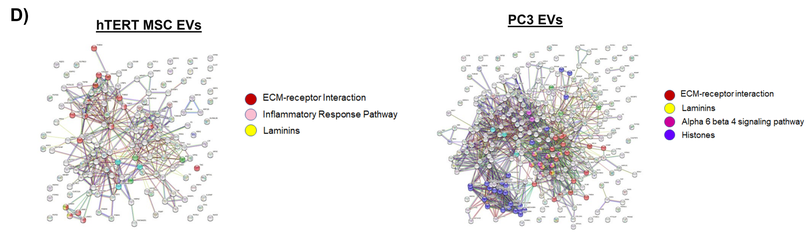


Figure 4. EV functionality and cargo analysis. A) RPE cells were exposed to IR and treated with EVs (day 0). On day 5, a CellTiter- Glo® assay was performed to quantify the effects of EVs on cellular viability. n =3. **** p <0.0001. **B)** Western blot was performed to evaluate the effects of EVs on different proteins involved in apoptosis and cell cycle regulation. **C)** EVs were assayed for cytokines using the S-PLEX® Kit. Assays were run in duplicate. Analyte concentrations were calculated using a 4-PL fit of a standard curve of the calibrators. Data shown represent samples assayed without lysis. *** p <0.001; * p <0.05. **D)** The STRING databased was used to calculate the PPIs of peptides extracted from each EV preparation. Proteins are represented by nodes that are connected by lines representative of their confidence level. Colors represent specific functions that are associated with proteins.

Summary

- EVs can reproducibly be isolated at-scale from hTERT-immortalized MSCs.
- hTERT MSCs display unique phenotypic and biochemical properties compared to cancer-derived EVs.
- hTERT MSCs are functional in vitro and exert anti-apoptotic effects and regulatory effects on damaged eye cells.
- hTERT MSC EVs may be enriched in cytokines and other proteins that contribute to their reparative properties.

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