Large Scale Manufacturing and Evaluation of hTERT-immortalized MSC-derived Extracellular Vesicles for Reversal of Irradiation-induced Cellular Damage

Jessica Hindle[1], Zachary Cuba[1],Dongsung Kim[1], Sujata Choudhury[1], Yuriy Kim[2], Pooja Khatkar[2], Collin Nelson[3], David Routenberg[3], Fatah Kashanchi [2], Heather Branscome[1,2*]

[1]American Type Culture Collection, Manassas, Virginia, USA, [2]Laboratory of Molecular Virology, School of Systems Biology, George Mason University, Manassas, VA, USA, [3]Meso Scale Diagnostics, L.L.C., Rockville, MD, USA

ID # MHSRS-24-12266

INTRODUCTION

Comat-associated ocular trauma is a major cause of visual impairment and morbidity among military service members. In addition to imposing a significant cost burden to the Military Health System, these injuries also pose a significant threat to the retention and readiness of U.S. Service Members. Exposure to ionizing radiation (IR) during service can cause direct ocular damage and can also initiate an inflammatory response that can lead to further visual damage if not properly managed. Traditional ocular therapeutics have experienced limited efficacy and poor bioavailability due to the anatomic and physiologic barriers that protect the eye. Therefore, there is an urgent need to explore novel therapeutics for eye-related trauma. EVs derived from Mesenchymal Stem Cells (MSCs) have rapidly emerged as a promising therapeutic tool for regenerative medicine. These nano-sized particles can quickly penetrate biological barriers to deliver biological cargo to exert reparative functions without eliciting an immune response. Here, we report a large-scale platform for manufacturing of EVs from hTERT-immortalized MSCs and evaluate their biochemical and reparative properties on retinal cells before and after exposure to IR.

METHODS

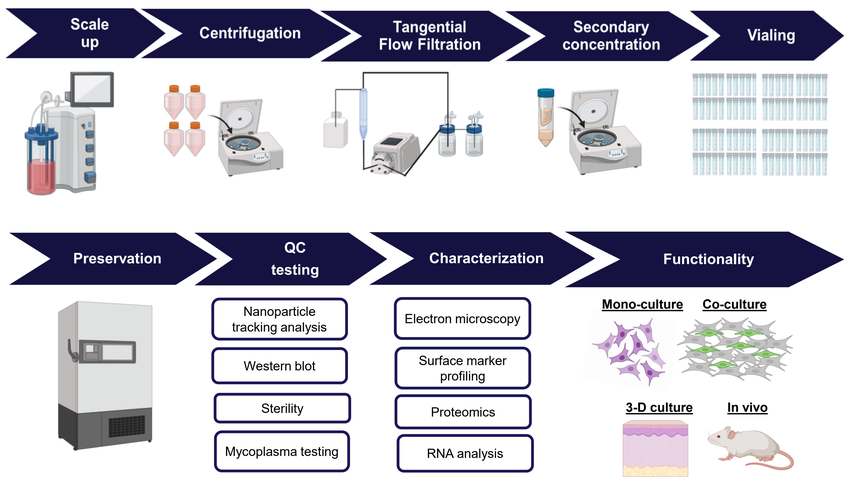

Figure 1. Large scale EV manufacturing and characterization. hTERT MSCs (ATCC® SCRC-4000™) are expanded at-scale (e.g. ≥ 5L) prior to EV isolation. EVs are isolated using a stepwise process of centrifugation, tangential flow filtration, and secondary concentration to obtain the final product. After preservation, EVs undergo stringent quality control testing to assess size, concentration, and sterility. Extended characterization of EVs has also been performed to better assess morphology and biochemical properties. Functionality of EVs has also been demonstrated both *in vitro* using multiple different cell types and *in vivo.*

RESULTS

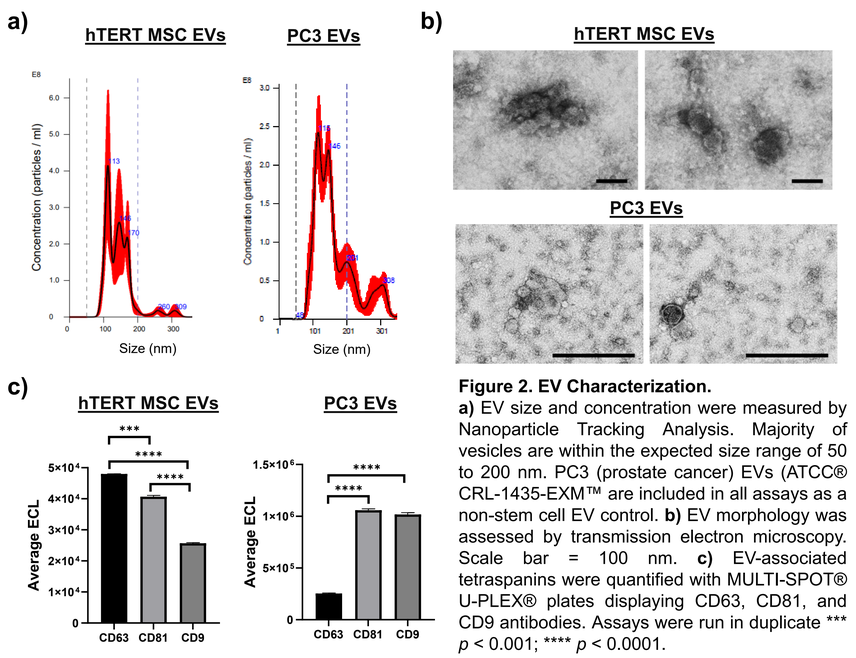

Figure 2. EV Characterization.
a) EV size and concentration were measured by Nanoparticle Tracking Analysis. Majority of vesicles are within the expected size range of 50 to 200 nm. PC3 (prostate cancer) EVs (ATCC® CRL-1435-EXM™ are included in all assays as a non-stem cell EV control. **b)** EV morphology was assessed by transmission electron microscopy. Scale bar = 100 nm. **c)** EV-associated tetraspanins were quantified with MULTI-SPOT® U-PLEX® plates displaying CD63, CD81, and CD9 antibodies. Assays were run in duplicate *** $p < 0.001$; **** $p < 0.0001$.

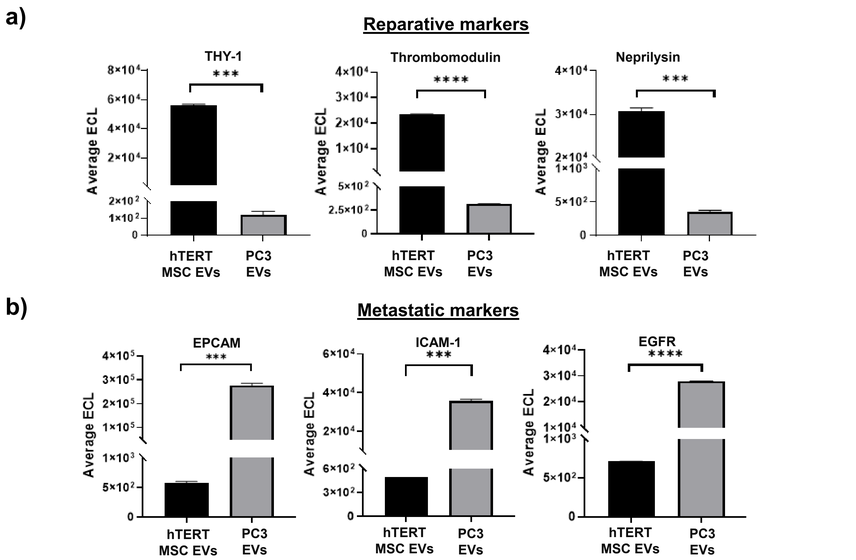

Figure 3. EV Surface Marker Profiling. Multiplex analysis was performed to analyze EV-associated surface marker proteins. Assays were run in duplicate. The average ECL values were analyzed and compared between hTERT-immortalized MSC and PC3 cancer EVs. **a)** THY-1, Thrombomodulin, and Neprilysin were significantly enriched in hTERT-immortalized MSC EVs. **b)** MCAM, EPCAM, ICAM-1, EGFR, and ALCAM were significantly enriched in PC3 EVs. **** p < 0.0001; *** p < 0.001; ** p < 0.01.

RESULTS

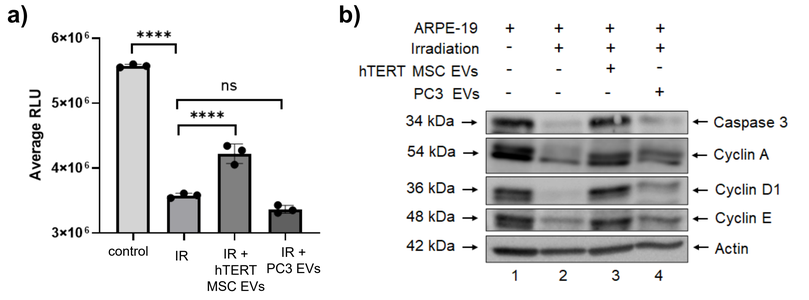

Figure 4. The Effect of hTERT MSC EVs on IR-damaged Retinal Epithelial (RPE) cells. RPE cells (ATCC® CRL-2302™) were exposed to IR and treated with EVs. **a)** CellTiter-Glo® assay was performed to assess viability. n = 3. **** p < 0.0001. **b)** Western blot was performed to evaluate the expression of proteins involved in apoptosis and cell cycle regulation.

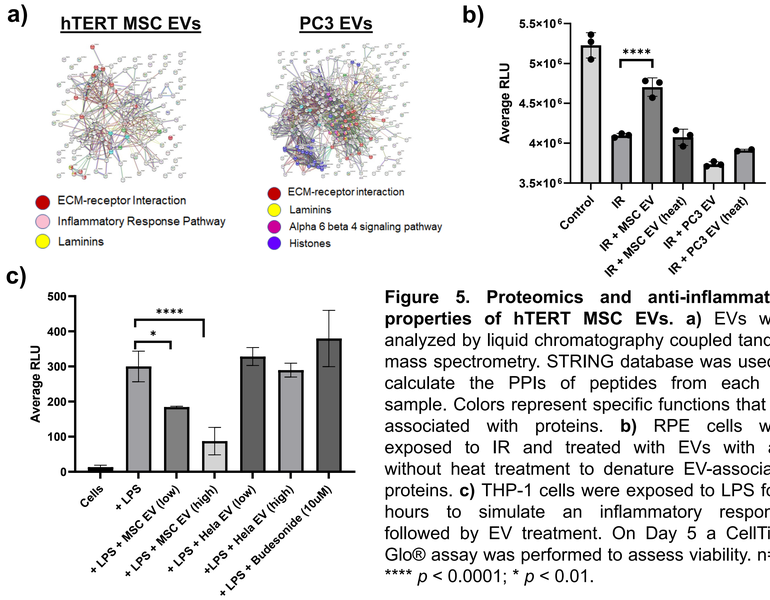

Figure 5. Proteomics and anti-inflammatory properties of hTERT MSC EVs. a) EVs were analyzed by liquid chromatography coupled tandem mass spectrometry. STRING database was used to calculate the PPIs of peptides from each EV sample. Colors represent specific functions that are associated with proteins. **b)** RPE cells were exposed to IR and treated with EVs with and without heat treatment to denature EV-associated proteins. **c)** THP-1 cells were exposed to LPS for 3 hours to simulate an inflammatory response, followed by EV treatment. On Day 5 a CellTiter-Glo® assay was performed to assess viability. n= 3. **** $p < 0.0001$; * $p < 0.01$.

CONCLUSION

hTERT MSC EVs meet well-established QC specifications and exert multi-functional effects (anti-apoptotic, cell cycle regulation, anti-inflammatory) in retinal cells that have been damaged by IR. Biochemical assays have identified surface marker proteins and proteins involved in the inflammatory response that may be contributing to their reparative properties. This work highlights the therapeutic potential of hTERT MSC EVs for reversing ocular damage.

Acknowledgements: This work was partially funded by NIH Grants AI078859, AI074410, AI127351-01, AI043894, NS099029, DA050176, and R01MH13438901. Ongoing work is being funded by the ATCC Internal Reserach and Development Program. We also thank Sofia Garcia-Hernandez and Anastas Popratiloff from the George Washington University for their contributions to this work.

AFS 10801 University Boulevard, Manassas, VA 20110-2209 **Phone:** 800.359.7370 **Email:** federalsolutions@atcc.org **Web:** https://www.atcc.org/federal-solutions