COMMITTEE FOR AID AND EDUCATION IN NEUROCHEMISTRY (CAEN) CATEGORY 1B: Research supplies for use in the applicant's home laboratory, International Society for Neurochemistry.

August 2014 Round

Researcher: M. Alejandra Lopez Verrilli, PhD

This project aimed to study the effect of exosomes secreted by mesenchymal stem cells (MSCs) in neuronal growth and axonal regeneration.

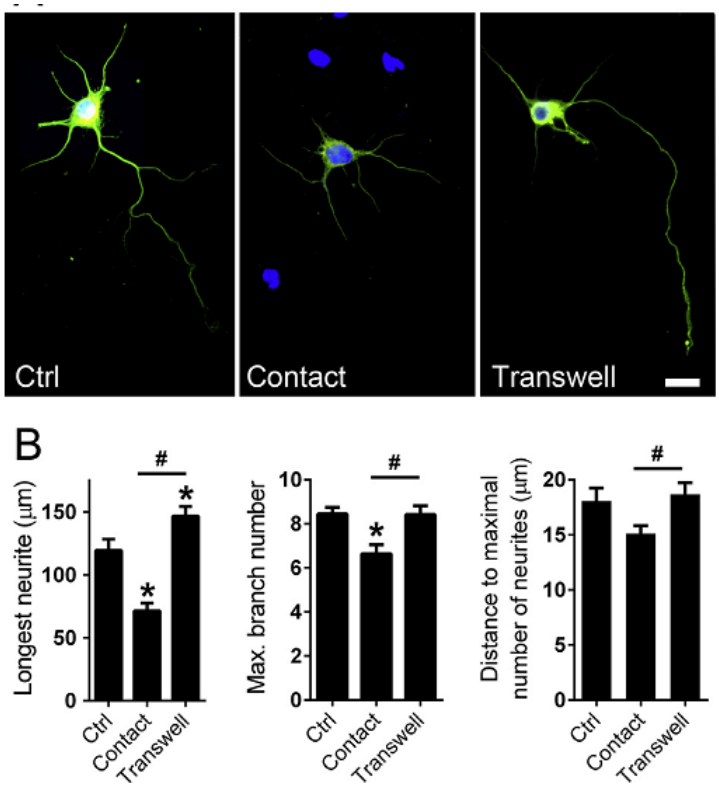
INTRODUCTION

Mesenchymal stem cells (MSCs) obtained from bone marrow (BM) have been shown to promote neuronal growth and survival. However, the comparative effects of MSCs of different sources, including menstrual MSCs (MenSCs), BM, umbilical cord and chorion stem cells on neurite outgrowth have not yet been explored. Moreover, the modulatory effects of MSCs may be mediated by paracrine mechanisms, i.e. by molecules contained in the MSC secretome that includes soluble factors and extracellular vesicles such as microvesicles and/or exosomes. The biogenesis of microvesicles, characterized by a vesicle diameter of 50 to 1000nm, involves membrane shedding while exosomes, of 30 to 100nm in diameter, originate in the multivesicular bodies within cells. Both vesicle types, which can be harvested from the conditioned media of cell cultures by differential centrifugation steps, regulate the function of target cells due to their molecular content of microRNA, mRNA, proteins and lipids.

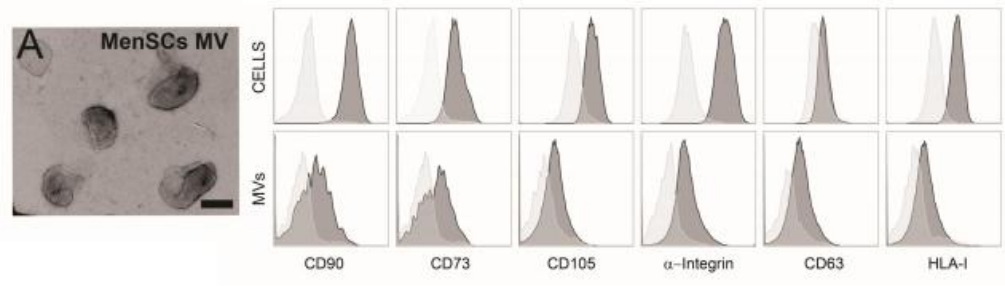
Here, we compared the effect of human menstrual MSCs (MenSCs) mediated by cell-cell contact, by their total secretome or by secretome-derived extracellular vesicles on neuritic outgrowth in primary neuronal cultures from the central and peripheral nervous system.

RESULTS

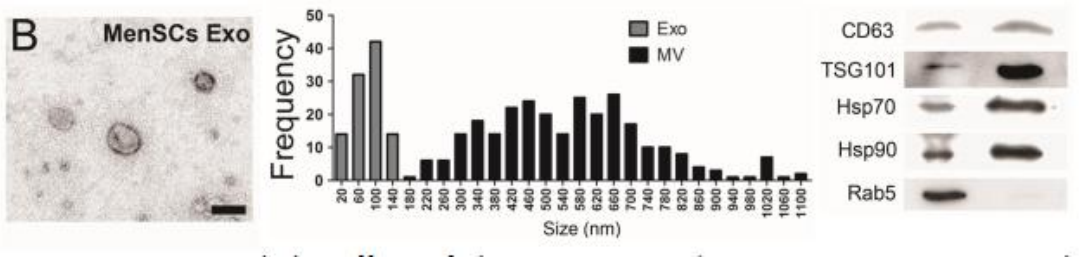
First, we investigated whether the MenSCs or their secretome modulate neuritic growth in primary cultures. Neurons were cultured alone or with MenSCs in direct contact condition, or separated by a 0.4 µm microporous membrane (transwell condition). At 3 days post-culture, neurons in contact with MenSCs showed a dramatic change in neuronal morphology. Conversely, the secretome of MenSCs enhanced neurites number and length.



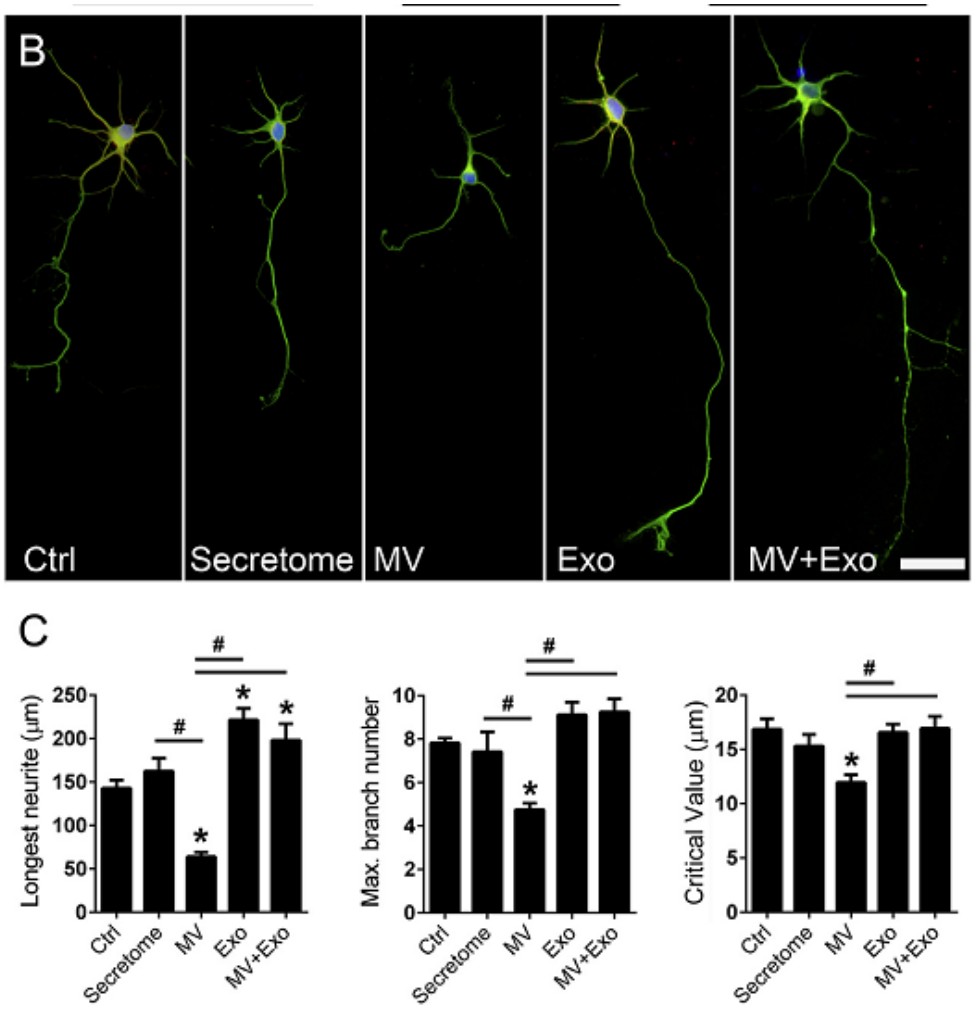
We thus focused our study on identifying what component of MenSCs secretome is involved in this beneficial effect. We analyzed microvesicles (the pellet after a centrifugation at 10,000 g) and exosomes (the pellet after centrifugation at 100,000 g). Men-SCs microvesicles showed a heterogeneous size with a mean diameter of 552+20nm by EM, and were positive for CD73, CD90, CD105, CD49a.



Conversely, Men-SCs exosomes showed rounded shape and a diameter size of 90+2nm by EM, the exosomal markers CD63, TSG101, Hsp70 and Hsp90 and the absence of Rab5, an early endosome marker .



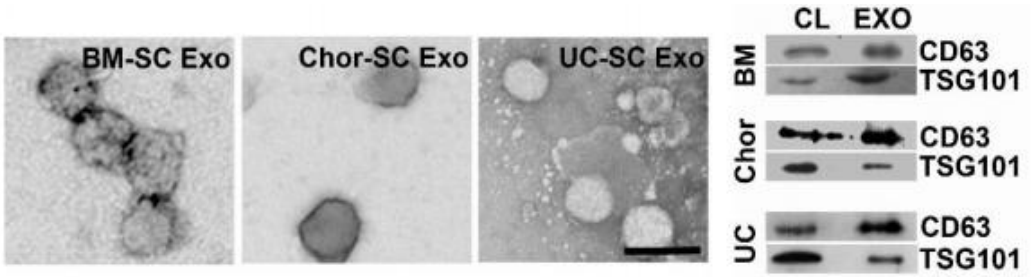
Next, we compared the effect of the MenSCs total secretome, exosomes and microvesicles on neuronal outgrowth. One day after culture, cortical neurons were supplemented with 3µg of protein from each fraction and incubated for three days.



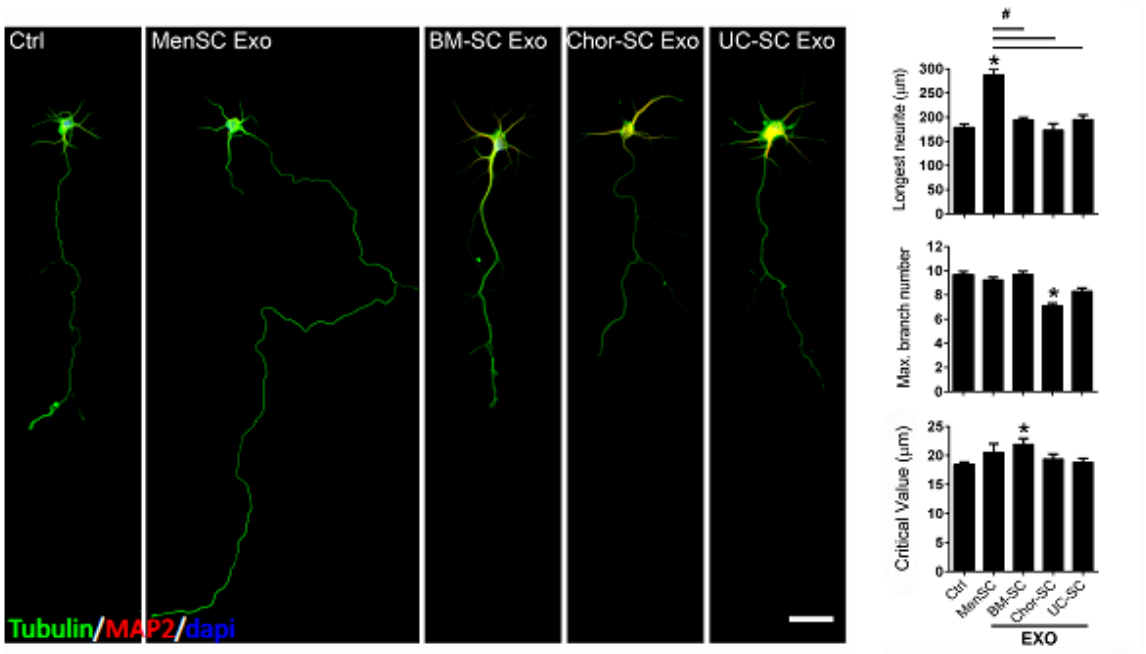
MenSC microvesicles inhibited neuronal outgrowth while the combination of microvesicles and exosomes restored neurons morphology. Exosomes alone showed the same effects as their combination with microvesicles, being exosomes per se beneficial for neuronal outgrowth.

Screening of MSC exosomes on neurite outgrowth

Considering the beneficial effect of MenSCs-derived exosomes on neurite outgrowth, we aim was to establish a comparative study classifying the potential effect of exosomes derived from the most clinically relevant MSC sources. EM analysis of exosomes from umbilical cord (UC), chorion (Chor) and bone marrow (BM) showed the expected round shape and mean diameters of 87+3nm for BM-, 101+5 for Chor- and 62+2 nm for UC-MSCs derived exosomes, respectively, together with the presence of the exosome markers CD63 and TSG101.

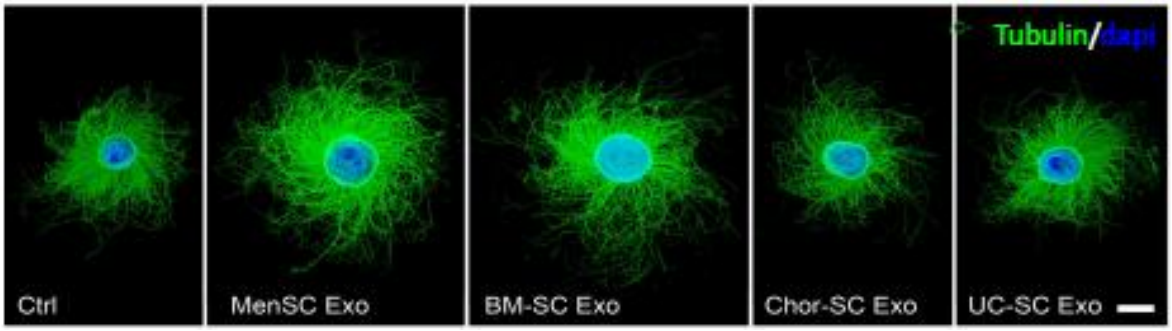


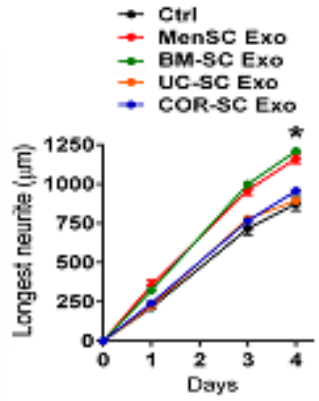
Exosomes from these MSCs sources were supplemented to the cortical neurons culture at a concentration of 3 µg exosomes/5,000 neurons. MenSCs exosomes showed superior effects on neurite outgrowth. Chor-SCs exosomes decreased the total branch number respect to control condition, while BM-SC-derived exosomes increased the distance of ramifications from the cell soma compared to neurons alone.



The effect of exosomes from different MSC sources was also assessed on sensory neurons from dorsal root ganglia (DRG). DRGs were supplemented with vehicle (PBS) or 3 µg of

exosomes on a daily basis for 4 days. We observed that MenSCs and BM-SCs exosomes increased the rate of neuritic growth compared to control. Chor-SC and UC-SC exosomes treated neurons did not present any difference in comparison with untreated control condition.





CONCLUSION

Men-SCs release microvesicles and exosomes, which exert opposite effects on neuronal growth. Exosomes from MenSCs and BM-SCs stimulated neuronal growth, while exosomes from other MSC sources were not effective. Conversely, the co-culture of MenSCs with cortical neurons inhibited neuronal growth. Thus, the growth-stimulating effects of exosomes derived from MenSCs as well as the opposing effects of both extracellular vesicle fractions provide important information regarding the potential use of MenSCs as therapeutic conveyors in neurodegenerative pathologies.

PURCHASES

The fund from this grant was spent on acquiring antibodies, cell culture reagents, use of electronic microscope and laboratory consumables and the cost of housing mice.

SCIENTIFIC PUBLICATIONS DERIVED FROM THE GRANT

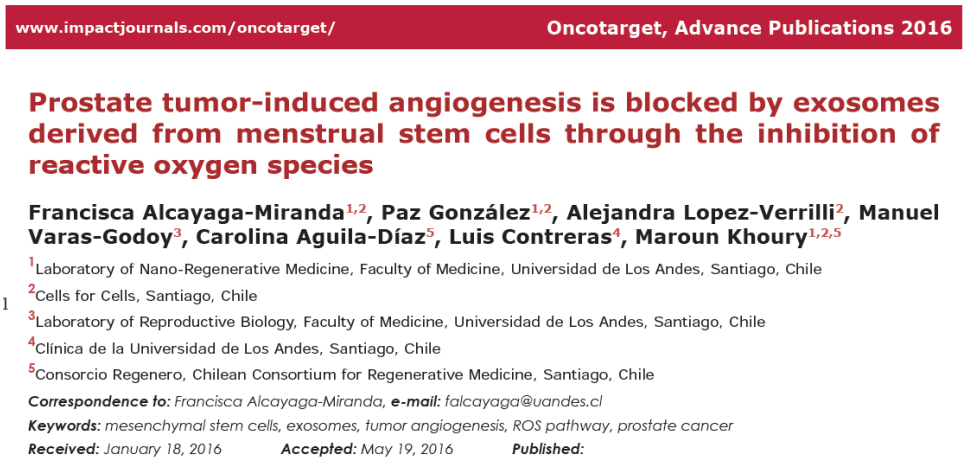
Our data was published and properly acknowledged in the following publications:

1) Mesenchymal stem cell-derived exosomes from different sources selectively promote neuritic outgrowth. **Lopez-Verrilli MA**, Caviedes A, Cabrera A, Sandoval S, Wyneken U, Khoury M. Neuroscience. 2016 Apr 21; 320:129-39.

doi: 10.1016/j.neuroscience.2016.01.061. Epub 2016 Feb 3.

2) Prostate tumor-induced angiogenesis is blocked by exosomes derived from menstrual stem cells through the inhibition of reactive oxygen species. Alcayaga-Miranda F, González PL, **Lopez-Verrilli M**, Varas-Godoy M, Aguila-Díaz C, Contreras L, Khoury M. Oncotarget, accepted May 19, 2016.

http://www.impactjournals.com/oncotarget/index.php?journal=oncotarget



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Prostate tumor-induced angiogenesis is blocked by exosomes derived from menstrual stem cells through the inhibition of reactive oxygen species

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3) **Poster Presentation: EXOSOMES OF MESENCHYMAL STEM CELLS FROM DIFFERENT SOURCES DIFFERENTIALLY MODULATE NEURITIC OUTGROWTH.** LOPEZ-VERRILLI MA, CAVIEDES A, CABRERA A, SANDOVAL S, WYNEKEN U, KHOURY M