Local administration of extracellular vesicles from bone marrow-derived mesenchymal stem cells restores homeostatic communication pathways and slows the progression of retinal degeneration

Yvette Wooff[1,2], X. Sango[3], S.M. Krishna[3], M. Shambrook[3], A. Cioanca[1,2], N. Reynolds[1,2], D. Haylock[3]*, R. Natoli[1,2]*.

[1]The John Curtin School of Medical Research, The Australian National University, Canberra, ACT, Australia
[2] ANU Medical School, The Australian National University, Canberra, ACT, Australia
[3] VivaZome Therapeutics, Melbourne, Australia

yvette.wooff@anu.edu.au, riccardo.natoli@anu.edu.au, david.haylock@vivazome.com

Purpose

Retinal degenerations, including Age-related macular degeneration (AMD), are a group of neurodegenerative diseases characterised by irreversible vision loss. In the degenerating retina, a loss of extracellular vesicle (EV) bioavailability is correlated with key pathological features of AMD including photoreceptor cell death and large-scale inflammatory cascades. We therefore hypothesise that replenishment of EV from cells shown previously to have therapeutic benefits to the eye, such as bone marrow-derived mesenchymal stem cell EV (BM-MSC-EV), and their molecular cargo, may restore these lost EV homeostatic communication pathways and slow the progression of degeneration.

Methods

Extracellular vesicles were isolated from human bone marrow-derived mesenchymal stem cells (RoosterBio; BM-MSC-EV) using tangential flow filtration (TFF) and size exclusion chromatography (SEC).

EV were characterised using Nanotracking analysis (NTA; ZetaView QUATT)

BM-MSC-EV or vehicle (PBS) were administered to the retina using intravitreal injection (2.0e9/μL) prior to degeneration.

C57Bl/6J mice were exposed to 100K lux light (photo-oxidative damage; PD) for 5 days to induce retinal degeneration

Electroretinography (ERG) was used to measure retinal function while optical coherence tomography (OCT) was used to visualise and measure retinal health.

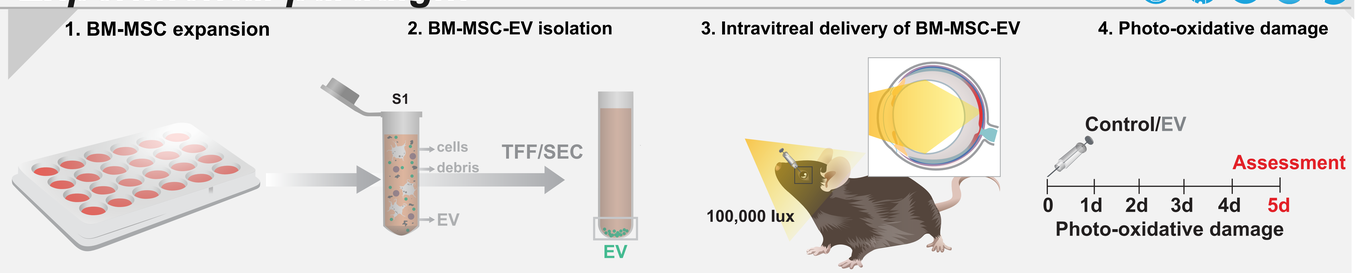
TUNEL assay, photoreceptor nuclei counts and retinal layer thickness measures were used to quantify photoreceptor cell death

Immunohistochemistry of IBA-1 was performed to detect migration of microglia/macrophage immune cells

Human Cytokine 7-plex assay (Crux Biolabs) was conducted on PBMC incubated with BM-MSC-EV to assess anti-inflammatory properties.

Small RNAseq (CATS RNA-seq Kit v2) was performed on BM-MSC-EV

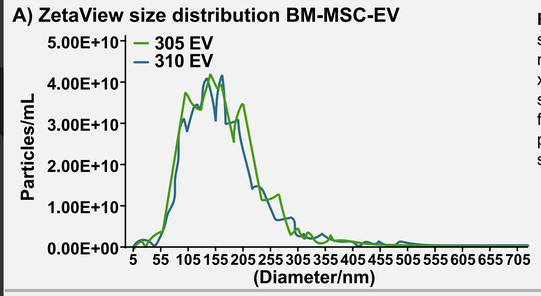
Experimental paradigm



Experimental paradigm: (A) BM-MSC were grown until confluence. **(B)** Cell culture supernatant from BM-MSC was collected and spun at 2000xg for 10 minutes to remove cells and debris. Following, EV were isolated using tangential flow filtration (50R; Sartorious) and size exclusion chromatography (qEV 10mL; Izon). The collected EV pellet was then spun at 4000xg (Amicon 10kDa cut off). **(C)** To test therapeutic efficacy, BM-MSC-EV or PBS were then delivered using intravitreal injection. **(D)** Photo-oxidative damage (PD 100k lux, 5 days) was used as a model of retinal degenerations as it recapitulates key disease facets of AMD. Following PD, functional and morphological assessments were performed.

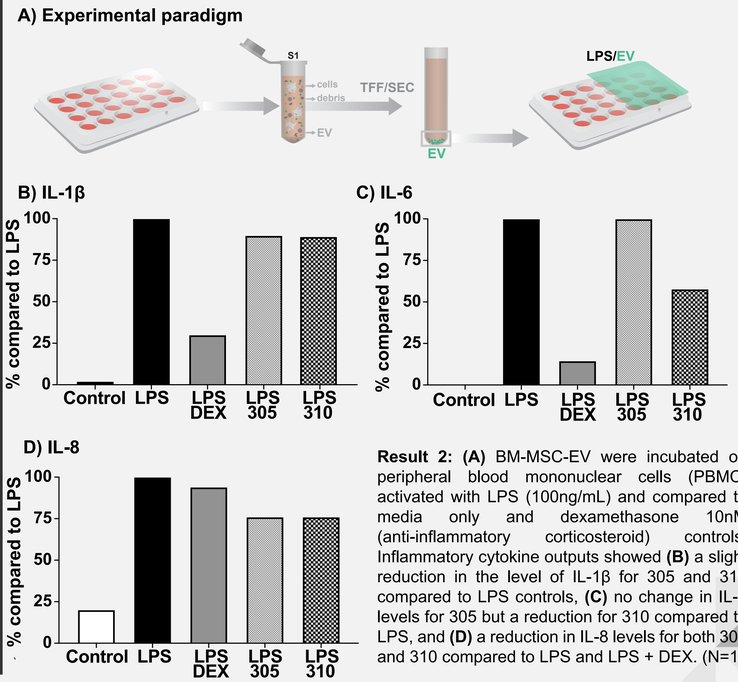
Results

Characterisation of BM-MSC-EV



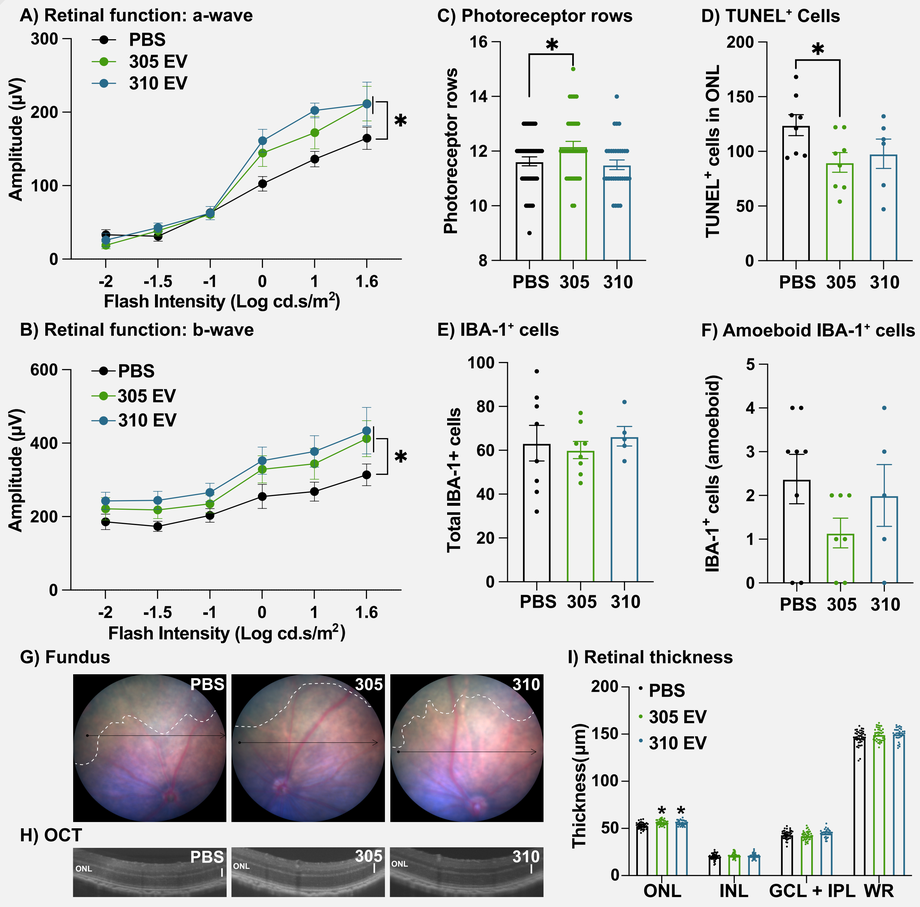
Result 1: BM-MSC-EV size distribution was measured using ZetaView x30 QUATT and showed a similar distribution profile for both 305 and 310 populations with a mean size of ~150nm.

BM-MSC-EV have anti-inflammatory properties



Result 2: (A) BM-MSC-EV were incubated on peripheral blood mononuclear cells (PBMC) activated with LPS (100ng/mL) and compared to media only and dexamethasone 10nM (anti-inflammatory corticosteroid) controls. Inflammatory cytokine outputs showed **(B)** a slight reduction in the level of IL-1β for 305 and 310 compared to LPS controls, **(C)** no change in IL-6 levels for 305 but a reduction for 310 compared to LPS, and **(D)** a reduction in IL-8 levels for both 305 and 310 compared to LPS and LPS + DEX. (N=1).

BM-MSC-EV confer protection against retinal degeneration



Result 3: Mice injected with BM-MSC-EV prior to 5 days photo-oxidative damage (both 305 and 310) were shown to have significantly improved retinal function for both **(A)** a-wave (photoreceptor function) and **(B)** b-wave (second order neuron function) measures, compared to PBS-injected controls ($p<0.05$, N=5).

Mice injected with 305, but not 310 were further shown to have a significant protection against photoreceptor cell death, as shown by **(C)** a significantly higher number of photoreceptor rows and **(D)** signficantly fewer numbers of TUNEL⁺ photoreceptor cells in the outer nuclear layer (ONL) compared to PBS-injected controls ($p<0.05$, N=5).

(E) No change was observed in the total number of IBA-1⁺ cells (marker of microglia/macrophages) in any group, **(F)** however a slight but non-significant reduction in the number of activated/amoeboid IBA-1⁺ cells was observed in 305-injected mice compared to PBS-injected controls ($p>0.05$, N=5).

(G) Representative fundus imaging shows large areas of degeneration (dotted area) in the superior retina in PBS-injected mice, with smaller areas of degeneration observed in mice injected with 305 and 310 BM-MSC-EV. **(H)** Representative OCT imaging and **(I)** retinal layer thickness measurements show significant ONL thickness in 305 and 310-injected mice compared to PBS-injected controls supporting photoreceptor survival following BM-MSC-EV administration, ($P<0.05$, N=5).

Conclusions

BM-MSC-EV are a potential therapeutic EV source to slow the progression of retinal degeneration, and can potentially be used to deliver current and future therapeutics.