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Identification of extracellular vesicles released by Müller glial cells *in vitro*.

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Footnotes

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Abstract

Purpose : Müller glia play a major role in the neuroprotection of retinal neurons during disease and injury. Extracellular vesicles are thought to mediate neuroprotection through the transference of neurotrophic factors and anti-apoptotic nucleic acids from neuroprotective cells into target neurons. It was, therefore, the aim of this study to investigate whether Müller glia secrete extracellular vesicles into the extracellular microenvironment that could potentially exert the protective ability of these cells.

Methods : The Müller glia cell line MIO-M1 was cultured in the presence of DMEM containing foetal bovine serum (FBS) to obtain confluent monolayers. At 70% confluency media containing FBS was removed, and cells were washed 3 times with PBS before 24-hour incubation in DMEM serum-free medium. After collection, conditioned medium was differentially centrifuged at increasing force in order to isolate various vesicle fractions present, with a final spin at 100 000 x g for 90 minutes. Particles were characterised by dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA), and individual vesicles were imaged by electron microscopy.

Results : DLS and NTA analyses of purified extracellular vesicle fractions from Müller-conditioned media indicated the presence of two vesicle populations, one peak corresponding to particles with a diameter ~95nm, and the second ~450nm. These corresponded to the sizes predicted for endosome-derived exosomes (50-100 nm diameter), and membrane-shed microvesicles (200 - 1000nm diameter). Transmission electron-microscopy imaging identified single particles within those size ranges, in addition to confirming the size and classic double-membrane structure of these vesicles

Conclusions : This study is the first to demonstrate that Müller glia cells release discrete populations of membrane-bound extracellular vesicles *in vitro*. Proteomic and functional analyses of vesicle cargo will ascertain whether these populations contain biomolecules capable of direct or indirect promotion of neurotrophism and protection. We hope that these studies will contribute to the design of future therapies for diseases such as glaucoma, retinitis pigmentosa and macular degeneration, and that these may represent an exciting alternative to either direct delivery of exogenous proteins or stem cell transplant, by-passing issues such as short half-life, graft-host rejection, and risk of tumorigenesis.

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