Neuroprotective therapy for retinal neurodegenerative diseases by stem cell secretome

Ricardo Usategui-Martín, Ivan Fernandez-Bueno*

Retinal neurodegenerative diseases like age-related macular degeneration, glaucoma, diabetic retinopathy or retinitis pigmentosa are the most frequent causes of incurable low vision and blindness worldwide. It had been estimated that the prevalence of these diseases varies between 1/750 and 1/5000 depending on the region, the level of consanguinity or ethnicity (Na et al., 2017). The functional and structural complexity of the retina makes it susceptible to multiple types of pathogenic damage. The retinal neurodegeneration may be caused by genetic defects, increased intraocular pressure, high levels of blood glucose or other types of stress or aging. All of them cause progressive neuronal death which is accompanied by a response of glial cells. Although the etiology, pathogenesis and clinical characteristics of retinal neurodegenerative diseases are very different, they have common features because the cellular and molecular response to retinal neurodegeneration is closely similar (Cuenca et al., 2014). Thus, it had been proposed that several neuroprotective therapeutic approaches may be adequate for the retinal neurodegenerative process. Retinal neurodegeneration is characterized by an inflammatory response, oxidative stress and activation of cell death pathways (Cuenca et al., 2014). Besides, the neurodegenerative process of the retina is commonly divided into four different phases and changes that occur during the retina degeneration can be associated with the stage of neurodegeneration (Vugler, 2010; Cuenca et al., 2014; Gagliardi et al., 2019). During phase 1 the function and morphology of the retina appear normal but cell stress induces molecular changes and eventual cell death. In phase 2, cellular stress and the activation of apoptotic pathways leads to progressive cell loss and activation of glial cells. In phase 3, it could be observed a large-scale neuronal cell death which leads glial cells hypertrophy and microglial activation. Phase 4 is characterized by the global retinal alteration with the neuronal cell death, hypertrophy of glial cells, epiretinal membrane formation, invasion by blood vessels and by the migration of the retinal pigment epithelium cells (Cuenca et al., 2014).

Despite the impact on patients' daily life, social and economic consequences, there is no curative treatment for most of the retinal neurodegenerative diseases. Moreover, retinal neurodegeneration, once initiated, is irreversible. Currently, advanced therapies are being a therapeutic option that is being highly researched for retinal neurodegenerative diseases. According to The European Medicines Agency, advanced therapies could be classified into three groups depending on the origin of their products: genes (gene therapy), cells (cell therapy) or tissues (tissue engineering). The eye is an ideal organ for cell therapy due to the easy accessibility, is a highly compartmentalized organ, is immune privileged because the blood-retina barrier separates it from the rest of the body, and there are high-resolution and non-invasive imaging techniques which allow longitudinally studying the retina changes after treatment (Gagliardi et al., 2019). The principal therapeutic approach of cell therapy in retinal neurodegenerative diseases is the neuroprotection through the paracrine stem cells properties. Although, it has been reported that the retina might not provide an adequate environment for the replacement of damaged neurons due to stem cells cannot adequately migrate, differentiate, integrate and be functional (Hill et al., 2009; Johnson et al., 2009), the potential therapeutic effect depends on the quality of these cells. Cell therapy for retinal neurodegenerative diseases is currently using embryonic stem cells, induced pluripotent stem cells and mesenchymal stem cells (MSCs). MSCs have several advantages to be the most used stem cells in retinal pathologies. They are free of ethical dilemmas, are immunoprivileged or immune evasive and show immunomodulatory capacities (Nauta and Fibbe, 2007). As mentioned, the clinically different retinal diseases share common neurodegenerative pathways, such as an inflammatory response, oxidative stress, and activation of apoptotic pathways. Therefore, the main objective of the neuroprotective stem cell-based therapies is to provide an adequate cellular environment, through their secretome action, in which the retinal cells prolong their viability and functionality over time.

The secretome of MSCs shows neuroprotective effects over retinal degeneration (**Figure 1**), but the mechanism of action is still unclear. In this sense, it had been reported that several proteins secreted by stem cells are associated with a slowdown of the retina neurodegenerative process. Some of these are glial cell-derived neurotrophic factor, brain-derived neurotrophic factor, pigment epithelium-derived factor, ciliary neurotrophic factor, nerve growth factor, basic fibroblast growth factor, platelet-derived growth factor, delta-like protein 4 and erythropoietin (Cuenca et al., 2014; Kolomeyer and Zarbin, 2014; Labrador-Velandia et al., 2019). These pro-survival neurotrophic factors promote cell proliferation, maturation and survival acting in an autocrine and paracrine mode, therefore they maintain general cell homeostasis preserving the viability of the retinal cells and, thus, functioning. Furthermore, it had been described that the combination of these factors may play a synergetic neuroprotective effect over retina degeneration (Cuenca et al., 2014). It had been observed, in animal models, that the combination of pro-survival neurotrophic factors is associated with an increase in the photoreceptor and retinal ganglion cells' survival, with better maintenance of the neuroretinal inner nuclear layer and improved electroretinogram response (Kolomeyer and Zarbin, 2014). However, despite the clinical potential of stem cell-based therapy, there are potential risks to be considered. Their effectiveness depends on the type of stem cells, the ability to differentiate, the proliferation status, the route of administration and/or the previous *in vitro* manipulation. Nevertheless, the main risks of cell therapy are associated with its safety, engraftment at another location, aggregate formation or inappropriate cell differentiation may occur. These incidents could trigger tumor formation, unwanted immune responses or the transmission of adventitious agents. In contrast, the majority of clinical trials conducted with MSCs have not reported important health concerns which suggest that MSCs therapies are relatively safe (Herberts et al., 2011). However, serious adverse events had also been reported (Herberts et al., 2011). Therefore, additional clinical studies are needed to improve knowledge about MSCs' biological mechanisms and long-term tolerance and efficacy.

In this scenario, the progress of investigations to determine the composition of the MSCs neuroprotective secretome is crucial and will be the basis for future production of "secretome cocktails" with effective antioxidants, anti-apoptotic and/or anti-inflammatory capacity over retinal diseases. Thus, the induction of an adequate

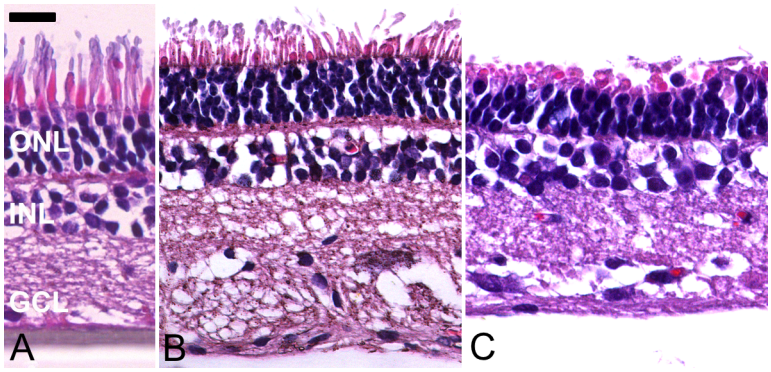

Figure 1 | Organ retinal explant cultures are considered useful tools for cellular and molecular research into retinal degeneration and neuroprotection.
Briefly, porcine neuroretina explants were cocultured with mesenchymal stem cells secretome (B) or cultured (C) in Transwell® plates, with the photoreceptor layer facing the supporting membrane. Semithin sections were evaluated after hematoxylin-eosin staining (A–C). Fresh porcine neuroretina (A) showed the typical layered morphologic organization and adequate cellular preservation before culturing. After 3 days of coculture (B), neuroretinas maintained the layered architecture and shortened photoreceptors. Nevertheless, neuroretinas cultured during 3 days (C) exhibited shorted, compacted, and edematous photoreceptors, which are indicators of neuroretina degeneration. Scale bar: 25 µm. GCL: Ganglion cell layer; INL: inner nuclear layer; ONL: outer nuclear layer. Figure 1 is our unpublished data.

extracellular environment that maintains retinal homeostasis, will preserve the viability and functionality of the retinal neurons. Besides, manufacturing, handling, and storage of these "secretome cocktails", based on MSCs secreted factors, present important advantages over the handling and application of living cells. Nevertheless, the final success of this new therapeutic approach understood as the maintenance or improvement of visual function also depends on other factors such as the age of the patient, the etiology of the disease and/or the stage of the retinal neurodegeneration. In this sense, neuroprotection via antioxidative, antiapoptotic and/or anti-inflammatory factors may be crucial in phases 1 and 2; furthermore, neuroprotection should always be maintained because retinal neurodegeneration is irreversible (Cuenca et al., 2014). On the other hand, it is important to report the presumable intraocular short-life of the neurotrophic factors described above, which would require frequent intravitreal injections. To solve this problem, new approaches/devices for the long-term delivery of the paracrine factors into the vitreous should be developed.

In summary, it had been reported that stem cells secretome exhibits neuroprotective effects over retinal degeneration, therefore the development of "secretome cocktails" could be a new approach for the treatment of retinal degeneration diseases. These cocktails could supply an adequate environment for preserving the retinal homeostasis, slow the neurodegeneration and maintenance or improve the visual function. To achieve this goal, determining which neurotrophic factors are crucial for providing neuroprotective effects, and also their optimal concentration seems essential. Finally, we want to highlight that the key to the effective treatment of neurodegenerative diseases of the retina would be the use of combinations of different neurotrophic factors, where the intravitreal injection of the stem cell secretome would play a crucial role.

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References

Cuenca N, Fernández-Sánchez L, Campello L, Maneu V, De la Villa P, Lax P, Pinilla I (2014) Cellular responses following retinal injuries and therapeutic approaches for neurodegenerative diseases. Prog Retin Eye Res 43:17-75.
Gagliardi G, Ben M'Barek K, Goureau O (2019) Photoreceptor cell replacement in macular degeneration and retinitis pigmentosa: A pluripotent stem cell-based approach. Prog Retin Eye Res 71:1-25.
Herberts CA, Kwa MS, Hermsen HP (2011) Risk factors in the development of stem cell therapy. J Transl Med 9:29.
Hill AJ, Zwart I, Tam HH, Chan J, Navarrete C, Jen LS, Navarrete R (2009) Human umbilical cord blood-derived mesenchymal stem cells do not differentiate into neural cell types or integrate into the retina after intravitreal grafting in neonatal rats. Stem Cells Dev 18:399-409.
Johnson TV, Bull ND, Martin KR (2009) Transplantation prospects for the inner retina. Eye (Lond) 23:1980-1984.
Kolomeyer AM, Zarbin MA (2014) Trophic factors in the pathogenesis and therapy for retinal degenerative diseases. Surv Ophthalmol 59:134-165.
Labrador-Velandia S, Alonso-Alonso ML, Di Lauro S, García-Gutierrez MT, Srivastava GK, Pastor JC, Fernandez-Bueno I (2019) Mesenchymal stem cells provide paracrine neuroprotective resources that delay degeneration of co-cultured organotypic neuroretinal cultures. Exp Eye Res 185:107671.
Na KH, Kim HJ, Kim KH, Han S, Kim P, Hann HJ, Ahn HS (2017) Prevalence, age at diagnosis, mortality, and cause of death in retinitis pigmentosa in Korea-A nationwide population-based study. Am J Ophthalmol 176:157-165.
Nauta AJ, Fibbe WE (2007) Immunomodulatory properties of mesenchymal stromal cells. Blood 110:3499-3506.
Vugler AA (2010) Progress toward the maintenance and repair of degenerating retinal circuitry. Retina 30:983-1001.

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