Brain Research 1646 (2016) 522–534

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research report

Human neural progenitor cells decrease photoreceptor degeneration, normalize opsin distribution and support synapse structure in cultured porcine retina

Tanzina Mollick [a,1], Camilla Mohlin [b,1], Kjell Johansson [a,*]

[a] School of Health and Medicine, Örebro University, Sweden
[b] Department of Chemistry and Biomedicine, Linnaeus University, Kalmar, Sweden

ARTICLE INFO

Article history:
Received 7 April 2016
Received in revised form
27 May 2016
Accepted 27 June 2016
Available online 28 June 2016

Keywords:
Photoreceptor degeneration
Synapse
Opsin
Gliosis
Neuroprotection

ABSTRACT

Retinal neurodegenerative disorders like retinitis pigmentosa, age-related macular degeneration, diabetic retinopathy and retinal detachment decrease retinal functionality leading to visual impairment. The pathological events are characterized by photoreceptor degeneration, synaptic disassembly, remodeling of postsynaptic neurons and activation of glial cells. Despite intense research, no effective treatment has been found for these disorders. The current study explores the potential of human neural progenitor cell (hNPC) derived factors to slow the degenerative processes in adult porcine retinal explants. Retinas were cultured for 3 days with or without hNPCs as a feeder layer and investigated by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), immunohistochemical, western blot and quantitative real time-polymerase chain reaction (qRT-PCR) techniques. TUNEL showed that hNPCs had the capacity to limit photoreceptor cell death. Among cone photoreceptors, hNPC coculture resulted in better maintenance of cone outer segments and reduced opsin mislocalization. Additionally, maintained synaptic structural integrity and preservation of second order calbindin positive horizontal cells was also observed. However, Müller cell gliosis only seemed to be alleviated in terms of reduced Müller cell density. Our observations indicate that at 3 days of coculture, hNPC derived factors had the capacity to protect photoreceptors, maintain synaptic integrity and support horizontal cell survival. Human neural progenitor cell applied treatment modalities may be an effective strategy to help maintain retinal functionality in neurodegenerative pathologies. Whether hNPCs can independently hinder Müller cell gliosis by utilizing higher concentrations or by combination with other pharmacological agents still needs to be determined.

1. Introduction

The retina is a highly sophisticated neurosensory organ that transduces light energy to electrical impulses that are transmitted to the visual cortex of the brain to be reconstructed to form visual perception. This phototransduction cascade is possible due to the organized arrangement and collective functioning of retinal cells, and any abnormality or damage to the neuronal circuitry leads to visual impairment. Retinal neurodegenerative disorders like retinitis pigmentosa, age-related macular degeneration (AMD), diabetic retinopathy and retinal detachment decrease retinal functionality, which leads to severe and usually incurable visual impairment (reviewed by Hanus et al., 2015; Klassen, 2016; Murakami et al., 2013). Although the underlying disease mechanisms related to these disorders may differ, the neurodegenerative events are generally characterized by the degeneration and loss of the rod and cone photoreceptors, remodeling of postsynaptic second order neurons and the activation of glial cells, all of which result in altered retinal morphology and considerable loss of functional capacity. The apoptotic loss of photoreceptors and the remodeling of inner retinal neuron dendrites lead to synaptic anomalies in the outer plexiform layer (OPL) by the loss of pre- and post-synaptic connections and the formation of ectopic synapses (Cuenca et al., 2014; Jones and Marc, 2005; Khodair et al., 2003; Soto and Kerschensteiner, 2015).

Müller cells, the primary glia of the retina, respond to such

Abbreviations: EGF, epidermal growth factor; FGF-2, fibroblast growth factor 2; GFAP, glial fibrillary acidic protein; hNPC, human neural progenitor cell; IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; IL-8, interleukin-8; INL, inner nuclear layer; MCP-1, monocyte chemotactic protein 1; ONL, outer nuclear layer; OPL, outer plexiform layer; OS, outer segment; PNA, peanut agglutinin; PSD-95, postsynaptic density protein 95; RPE, retinal pigment epithelium; TGF-α, transforming growth factor alpha; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; VEGF, vascular endothelial growth factor

* Corresponding author.
E-mail address: kjell.johansson@oru.se (K. Johansson).
[1] Equal contributors.

http://dx.doi.org/10.1016/j.brainres.2016.06.039
0006-8993/

injuries by undergoing reactive gliosis where the cells tend to have altered morphological and biochemical features that compromise the otherwise beneficial neuron-glia interactions (Hippert et al., 2015; Vecino et al., 2016). The characteristic gliotic response may prove to initially be neuroprotective, while continued gliosis may tend to be quite the contrary (Xue et al., 2011). Activation of Müller cells may trigger an endogenous neuroprotection cascade that includes the production of neurotrophic factors by the Müller cells (Harada et al., 2002).

Interestingly, *in vitro* culture paradigms of adult retinal tissue have been reported to portray morphological changes similar to the characteristic degenerative events of human retinal dystrophies (Englund-Johansson et al., 2010b; Fernandez-Bueno et al., 2012; Kaempf et al., 2008; Liljekvist-Soltic et al., 2008; Mohlin et al., 2011; Taylor et al., 2013; Winkler et al., 2002). In conjunction to the progression of rapid degeneration, which otherwise takes weeks to months in an *in vivo* system, the *in vitro* model may serve as an essential experimental approach for direct manipulation and investigation of pharmacological and bioengineering treatment modalities (Johnson and Martin, 2008; Kobuch et al., 2008). Up to date most studies have been focused on rodent models, but the porcine retinal model may prove to be advantageous since it shares similar traits to those of humans (Di Lauro et al., 2016; Guduric-Fuchs et al., 2009). In particular, the porcine retina has a cone-enriched visual streak (Hendrickson and Hicks, 2002), making it possible to study degenerative events and/or rescue paradigms in these cells.

Human neural progenitor cell (hNPC) and stem cell therapy may be a potential strategy to promote survival in neurodegenerative disorders of the retina. Such a provision is attributed due to the many beneficial characteristics of hNPCs which include their capacity to highly expand in culture, ability to migrate and integrate with damaged host tissue and provide neuroprotection (reviews by Cuenca et al., 2014; Jayakody et al., 2015). One important neuroprotective mechanism of these cells is through the supply of neurotrophic factors that offer a supporting niche for surviving photoreceptors. Accumulating data suggest that hNPCs aid in the preservation rather than restoration of retinal neurons in retinal dystrophies by producing a multitude of neurotrophic factors which may include the concomitant and synergistic effects of a combination of growth factors and cytokines. (Bull et al., 2008; Englund-Johansson et al., 2010b; Gamm et al., 2007; Liljekvist-Soltic et al., 2008; McGill et al., 2012; Mohlin et al., 2011; Ortin-Martinez et al., 2014; Wang et al., 2008). Such a treatment mechanism may be beneficial in genetically complex and multifactorial retinal disorders.

The major aim of this study was to characterize and evaluate the protective effects of hNPCs against the neurodegenerative events in cultured porcine retinal explants in association to photoreceptor degeneration, synaptic disassembly, horizontal cell remodeling and Müller cell gliosis. Human neural progenitor cells had the potential to prevent the apoptotic loss of photoreceptors in general and maintain cone photoreceptor structure in particular. Cone rescue was evaluated by better regulation of opsin trafficking and structural preservation of synaptic terminals. Such events also translated to better horizontal cell morphology and survival. Although Müller cell gliosis was somewhat alleviated, some retinas continued to show elevated expression of glial fibrillary acidic protein (GFAP).

2. Results

2.1. Retinal morphology

The morphology of adult porcine retinas has been previously described in detail (Chandler et al., 1999; Hendrickson and Hicks, 2002; Taylor et al., 2013; Winkler et al., 2002). Cryosections of cultured and cocultured retinas counterstained with DAPI were used to visually assess the cytoarchitecture of the retinal explants. The retinal lamination pattern was retained in all explants with separated nuclear and plexiform layers, but in the 3 div cultures the thickness and compactness of the inner nuclear layer (INL) and outer nuclear layer (ONL) were decreased and accompanied by the formation of tissue cavities and folds indicative of increased neuronal cell death (Fig. 1A and inset). Although the neurodegenerative features described in the cultured explants were not entirely eliminated in the cocultured explants, the nuclear layers maintained better integrity and were more compact with much fewer vacuole formations (Fig. 1B).

2.2. Neurotrophic factors

Earlier observations on rodent retina culture paradigms have shown that hNPC-conditioned medium provide significant neurotrophic support to photoreceptors in cocultured rat and rd1 mouse retinas (Englund-Johansson et al., 2010b). Here, conditioned medium from commercially available hNPC was analyzed and significant amounts of EGF, eotaxin, FGF-2, IL-2, IL-4, IL-6, IL-8, MCP-1, TGF-α and VEGF were detected.

2.3. TUNEL

In this study, we analyzed whether the plethora of neurotrophic factors released from hNPCs could support sufficient preservation and survival of photoreceptor cells in cultured porcine retinal explants. Initially, we analyzed retinal explant cultures at different time points, namely 1 div, 2 div and 3 div and found gradual increase in $TUNEL^+$ photoreceptor cells with increasing time *in vitro* (Fig. 1C). When compared with normal uncultured retinas a significant increase in photoreceptor cell death was observed at 3 div (3402 ± 1002 cells/mm^2, $n=8$, $p<0.01$).

Next, the 3 div time point was selected to set the optimal concentration of hNPCs required to show significant photoreceptor survival. After coculturing retinal explants with hNPC concentrations of 1×10^4, 1×10^5 and 2×10^5 cells/well for 3 div, it was established that 2×10^5 cells resulted in significant photoreceptor survival relative to only cultured explants (cultured 3402 ± 1002 cells/mm^2, $n=8$; cocultured 863 ± 364 cells/mm^2, $n=8$, $p<0.05$) (Fig. 1D). A final culture time period of 3 days and hNPC concentration of 2×10^5 cells were selected for further investigations in this study.

In cultured retinas most $TUNEL^+$ photoreceptor cells seemed to localize near the inner aspect of the ONL where rod photoreceptor cell bodies reside (Fig. 1A and B). Other regions had a more scattered distribution that included the outer aspect of the ONL, indicating cone photoreceptor death (see Pow and Sullivan, 2007). This could suggest that rod photoreceptors are more susceptible to injury than the cones; accelerated rod photoreceptor death prior to cone death has also been reported in other models of retinal degeneration (Genové et al., 2014; Jones et al., 2003; Nir et al., 1989; Rohrer et al., 2005). However, some regions of the cultured retina seemed to be well preserved and lacked $TUNEL^+$ photoreceptors, indicating that cell death may occur in microenvironments. In case of the cocultures, the presence of hNPCs and protective factors reduced cell death and most $TUNEL^+$ cells were observed near the inner aspect of the ONL indicating the vulnerability of rod photoreceptors (see Fig. 1A and B).

2.4. Cone L/M opsin mislocalization

Opsin is the primary visual pigment in the cone photoreceptor