MÜLLER CELL-DERIVED NEUROTROPHIC FACTORS. A ROAD TO NEUROPROTECTIVE THERAPY IN THE RETINA

FACTORES NEUROTRÓFICOS DERIVADOS DE LAS CÉLULAS DE MÜLLER. EN EL CAMINO HACIA LA TERAPIA NEUROPROTECTORA EN LA RETINA

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In a large group of inherited retinal degenerations such as Retinitis Pigmentosa (RP) as well as in Age-Related Macular Degeneration (AMD), protecting photoreceptors from degeneration can be regarded as the key objective for future therapeutic strategies. Similarly, in another group of blinding diseases that lead to retinal ganglion cell death (glaucomatous optic neuropathies), protecting these neurons from degeneration is equally crucial. Despite the diversity of mutations conferring susceptibility or causing neurodegeneration, the final unifying event leading to blindness is apoptosis of retinal neurons. This fatal process has led to intensive studies on both the nature and molecular mechanisms of neuroprotective agents that may antagonize apoptosis.

Glial cell line-derived neurotrophic factor (GDNF) has been demonstrated as one of the most effective neurotrophic agents to rescue photoreceptors from degeneration in the rd1 mouse model of RP (1). However, we have recently demonstrated that GDNF does not directly act on the dying photoreceptors in the diseased retina. Specifically, GDNF activates retinal Müller glial cells (RMG) which in turn release molecules that support photoreceptor survival (2).

This finding adds yet another functional feature to the manifold role of Müller glial cells in the context of retinal health and disease. Müller cells are recognized to provide water and ion homeostasis to the retinal tissue, provide recycled neurotransmitter glutamate, protect neurons against oxidative stress and secrete vasoactive, neuroactive and neuroprotective substances (3). Additionally, their morphology enables them to act as «optical fibers» transmitting the light from the inner limiting membrane through the whole thickness of the retina to the light receptive outer segments of the photoreceptors (4). This plethora of molecular mechanisms essential for proper retinal function and maintenance underlines the importance of Müller cells in retinal pathologies. Indeed, each retinal pathology presents with a certain reactivity of Müller cells, which can be either complicating or compensating the molecular pathologic processes.

Since Müller cells are intrinsic sources for neuroprotective proteins, they support prolonged survival of retinal neurons. However, it has been observed that the secretion of such neuroprotective factors may be lost during certain pathologies which go along with reactive Müller cell gliosis. On the other hand, enhancing the neuroprotective support during neurodegenerative retinal pathologies could proof efficient to prolong survival of retinal neurons for extended times in the life of patients. A prerequesite for this strategy is the identification of the Müller cell-derived neuroprotective proteins. Genomic as well as proteomic approaches are currently employed to achieve this. For this purpose, primary Müller cells, isolated

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from porcine retina are cultured for short periods of time in vitro and secreted protein pools are collected from the culture medium. These pools of proteins do support photoreceptor survival on primary photoreceptors in vitro (5). They were also proven to promote sprouting of retinal ganglion cells (6). RMG secrete approximately 1000 distinct protein entities. Defining the active neuroprotective factors within that pool appears like looking for the «needle in the haystack». Thanks to well established proteomic techniques and the power of mass-spectrometry (MS), large pools of different proteins can be separated two-dimensionally using their isoelectric point in one dimension and their molecular weight in the second dimension (fig. 1). After separation and staining of those 2D gels, protein spots of interest can be excised and identified by MS. Since this method has evolved tremendously during the last 10 years, both with respect to accuracy as well as sensitivity, it is nowadays possible to identify the majority of all secreted proteins within such a complex protein mixtures as cell-conditioned media. The strategy for identification of candidate neurotrophic factors is to apply native protein fractionation methods such as ion exchange liquid chromatography for fractionation and combine that with sensitive functional screening assays and mass spectrometric protein identifications of the resulting subfractions. Thus we can systematically correlate neurotrophic activities with specific isolated proteins identified by MS. After cloning and recombinant expression of the respective proteins, we can functionally evaluate single candidates, as well as create rationally combined mixtures of bioactive compounds for their survival supporting activity (5).

Given the strong neuroprotective capacity of Müller cell-derived factors, these molecules are likely to bare enormous potential for therapeutic application: i) Their endogenous expression within the retina as the target tissue promise minor side effects when applied therapeutically. ii) Given their general neuroprotective properties, these molecules can be applied to treat hereditary forms of RD independent of its underlying genetic defect. Effective neuroprotection by novel neuroprotective factors also depends on development of effective delivery strategies. Since proteins are not as easily diffusible as small compounds due to their large molecular size, it is crucial to create intraocular depots, which deliver the protein in close proximity to the affected photoreceptors or ganglion cells. These depots should produce the therapeutic protein for extended periods of time avoiding repeated surgical invasion. Gene-therapy as well as cell-based delivery strategies are therefore needed to bring new molecules to the retina of patients waiting for treatment of their yet incurable disease.

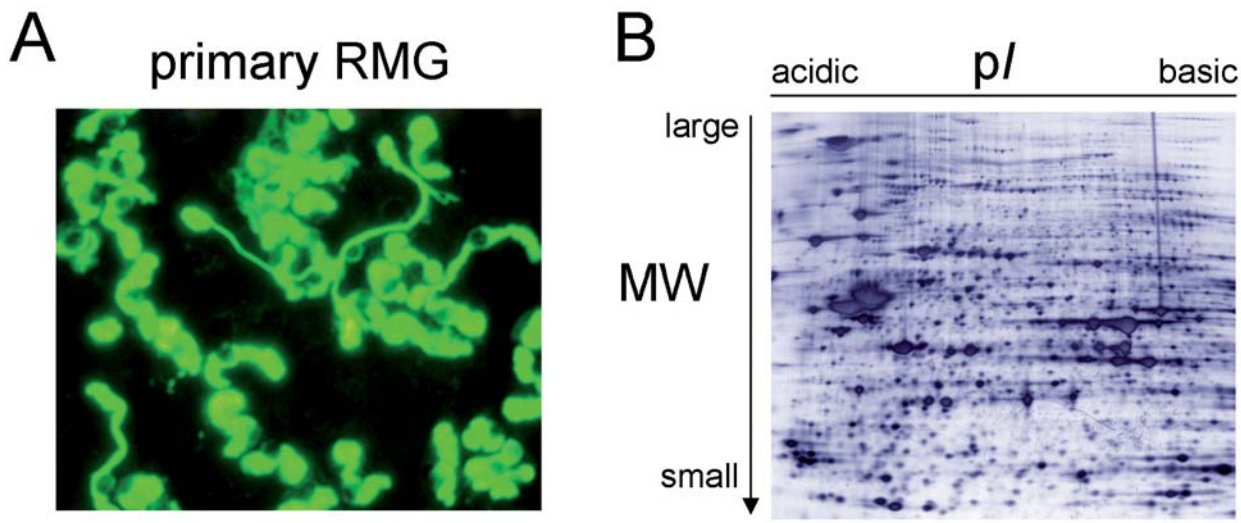

Fig. 1: A: primary retinal Müller cells labelled with anti-Glutaminsynthetase antibody. B: 2D gel of proteins derived from primary retinal Müller cells. Proteins are first separated by their isoelectric point (pI) and then transferred onto second dimension polyacrylamid gels, where they are separated upon molecular weight (MW). After the separations, proteins are visualised by staining with coomassie blue.

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