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ECM enrichment restores the morphology and functionality of Müller Glial Cells in the *rd1* Model of Retinitis Pigmentosa

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Abstract

Purpose : In Retinitis Pigmentosa the progressive loss of photoreceptors (PRs) leads to vision impairment. Our previous work showed that Müller glial cells (MGCs), specialized retinal cells that support PRs survival, are affected in the retinal degeneration mouse model (*rd1*). Even before PRs degeneration, *rd1* MGCs evidence a reduced regenerative potential, incapacity to form lamellipodia and impairment of their neuroprotective role on PRs. Whether the extracellular matrix (ECM) is affected in *rd1* retinas, altering MGCs adhesion and functionality and contributing to PRs death remains unclear. The aim of this study was to investigate ECM protein expression in *rd1* retinas prior to the peak of PRs degeneration and assess whether ECM pretreatment can restore the morphology and functionality of *rd1* MGCs *in vitro*.

Methods : Mixed neuron-glial cultures were prepared from postnatal day 2 *rd1* and wt mouse retinas. We analyzed SPARC and Fibronectin expression (ECM proteins), Paxillin (focal adhesions, FAs), and actin by immunocytochemistry, and measured the neuron-glial cluster area with Image J software. We obtained ECM (SPARC)-enriched conditioned media (CM) from retinoblastoma RN22 cell line cultures. We then grew *rd1* cultures on dishes pre-treated or not with ECM-CM to

evaluate its effects on neuron-glial cluster area, FAs, actin cytoskeleton, cell proliferation, measured through BrdU nucleotide incorporation, and cell death, assessed by DAPI staining.

Results : Neuron-glial clusters were smaller, whereas expression of SPARC was significantly lower (p=0.0059) and that of fibrillary fibronectin higher in *rd1* than in wt neuron-glial cultures (p=0.0002). *Rd1* MGCs had shorter FA (p= 0.0059), which showed mainly a cortical distribution (p=0.001), contrary to wt MGCs. Actin displayed a cortical distribution, and higher fluorescence intensity (p= 0.004) in *rd1* compared to wt cultures. Noteworthy, dish pre-treatment with ECM-CM increased the size of neuron-glial clusters (p= 0.0006), restored the distribution of glial FAs, and reduced phalloidin fluorescence intensity (p= 0.0005). Moreover, ECM-CM pre-treatment also stimulated MGCs proliferation (p= 0.021) and increased PRs survival (p= 0.0003) in *rd1* cultures.

Conclusions : Our results suggest that expression of ECM proteins and FAs assembly are altered in *rd1* MGCs and enrichment with ECM restores MGCs functionality, contributing to PRs survival.

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