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Scaffolding the retina: The interstitial extracellular matrix during rat retinal development

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ABSTRACT

Purpose: To examine the expression of interstitial extracellular matrix components and their role during retinal development.
Material and methods: Fibronectin (FN), collagen IV (Coll IV) and laminin 5 (Lam 5) expression in rat retinas from developmental stages E17 to adult were studied. In addition, PN5 full-thickness retinas were cultured for 7 days with dispase, which selectively cleaves FN and Coll IV, at either 0.5 U/ml or 5.0 U/ml for 3 or 24 h. Eyecups and retinal cultures were examined morphologically using hematoxylin and eosin staining and immunohistochemistry.
Results: Coll IV, Lam 5 and FN were all transiently expressed in the interstitial matrix of the retinal layers during development. The retinal layers in dispase treated explants was severely disturbed in a dose and time dependent manner.
Conclusions: FN, Lam 5 and Coll IV, are present in the interstitial extracellular matrix during rat retinal development. Enzymatic cleavage of FN and Coll IV early in the lamination process disrupts the retinal layers implicating their pivotal role in this process.

1. Introduction

The extracellular matrix (ECM) is essential for tissue development and homeostasis by providing mechanical and chemical cues for bidirectional signaling between cells within a tissue, by binding to a number of cell surface receptors including integrins. The ECM exists in two general compartments, basement membranes that delineate epithelia and blood vessels, and non-basement membranes i.e., interstitial matrix which is found between cells. During embryogenesis within the CNS, molecular interactions between the ECM and cell surface receptors orchestrate important events including neuroblast migration, cell fate determination, axon outgrowth and synapse formation (Libby et al., 2000a).

The retina is a highly organized structure with cell bodies and synapses defined in distinct layers. The basis of lamination formation relies on a defined relationship between the Müller glia and subpopulations of neurons involving cell-cell and cell-ECM contact, but the exact molecular mechanism behind this phenomenon has yet to be elucidated (Reichenbach et al., 1994; Drazba and Lemmon, 1990). ECM macromolecules in the retina, including laminins, collagens, and fibronectin (FN), have been described in the inner limiting membrane (ILM) of the developing and adult eye (Li et al., 2002; Kohno et al., 1987; Ponsioen et al., 2008). However, although several ECM-specific integrin receptors have been found within the retinal cell layers, very little is understood of the ECM macromolecules, with the exception of laminins, and their presence in the interstitial matrix (Clegg et al., 2000; Libby et al., 2000b).

Cellular fibronectin (FN), collagen IV (Coll IV) and laminin 5 (Lam 5) are relatively large ECM glycoproteins involved in various biological processes such as tissue development, growth, and maintenance of structural integrity. In the eye, these proteins have been linked to development of anterior segment structures including corneal, lens and ciliary body (Kohno et al., 1987; Kurkinen et al., 1979; Sramek et al., 1987; Peterson et al., 1995). Interestingly, FN has been found to interact intimately with radial glia during formation of cortical and tectal laminae in the brain, and inhibition of FN-specific integrin binding during development is associated with retinal structural disruption (Pearlman and Sheppard, 1996; Stettler and Galileo, 2004; Li and Sakaguchi, 2004).

For the present paper, we wanted to explore FN, Coll IV and Lam 5 expression in the developing retina, its relationship with Müller cells, and the fate of the retinal architecture as well as development of neuronal subtypes when ECM mediated cellular interaction is disturbed. We have thus explored ECM immunohistochemistry in

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Table 1
Table of primary and secondary antibodies used for immunohistochemical analysis.

Antigen	Antibody name	Target cell	Species	Dilution	Source
Vimentin	Anti-vimentin	Müller cells	Mouse monoclonal	1:500	Chemicon International, Temecula, CA, USA
Fibronectin	Rabbit anti-Fibronectin	ECM/Inner limiting membrane	Rabbit polyclonal	1:100	Sigma, St. Louis, MO, USA
Collagen IV	Rabbit polyclonal to CollagenIV	ECM/Inner limiting membrane	Rabbit polyclonal	1:100	abCam Cambridge, UK
Laminin 5	Rabbit polyclonal to Laminin 5	ECM/Inner limiting membrane	Rabbit polyclonal	1:100	abCam Cambridge, UK
Recoverin	Anti-recoverin	Rod and cone photoreceptors	Rabbit polyclonal	1:10000	Chemicon International, Temecula, CA, USA
Parvalbumin	Mouse anti-parvalbumin	AII amacrine cells	Mouse monoclonal	1:10000	Sigma, St. Louis, MO, USA
GFAP	anti-glial fibrillary acidic protein	Müller cells and astrocytes	Mouse monoclonal	1:500	Chemicon International, Temecula, CA, USA
NeuN	Anti-neuronal nuclei	Ganglion cells, displaced amacrine cells	Mouse monoclonal	1:100	Millipore, Billerica, MA, USA

Secondary antibody	Antibody name	Target	Species	Dilution	Source
FITC (fluorescein isothiocyanate)	Anti-mouse IgG FITC conjugate	Anti-mouse	Goat	1:200	Sigma–Aldrich, St. Louis, MO, USA
Texas red	Texas red dye-conjugated AffiniPure	Anti-rabbit	Donkey	1:200	Jackson ImmunoResearch, PA, USA

the developing rat retina, as well as in an explant model in which the protease dispase was used to disrupt the FN and Coll IV during early retinal lamination formation (Ghosh et al., 2009).

2. Material and methods

All proceedings and animal treatment were in accordance with the guidelines and requirements of the Government Committee on Animal Experimentation at Lund University and with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.1. In vivo

Adult, pre- and postnatal retinal tissue was obtained from normal Sprague–Dawley rats (Scanbur, Sweden; and Harlan Netherlands B.V., The Netherlands). Prenatal eyes were derived from embryos collected after caesarean section of time pregnant

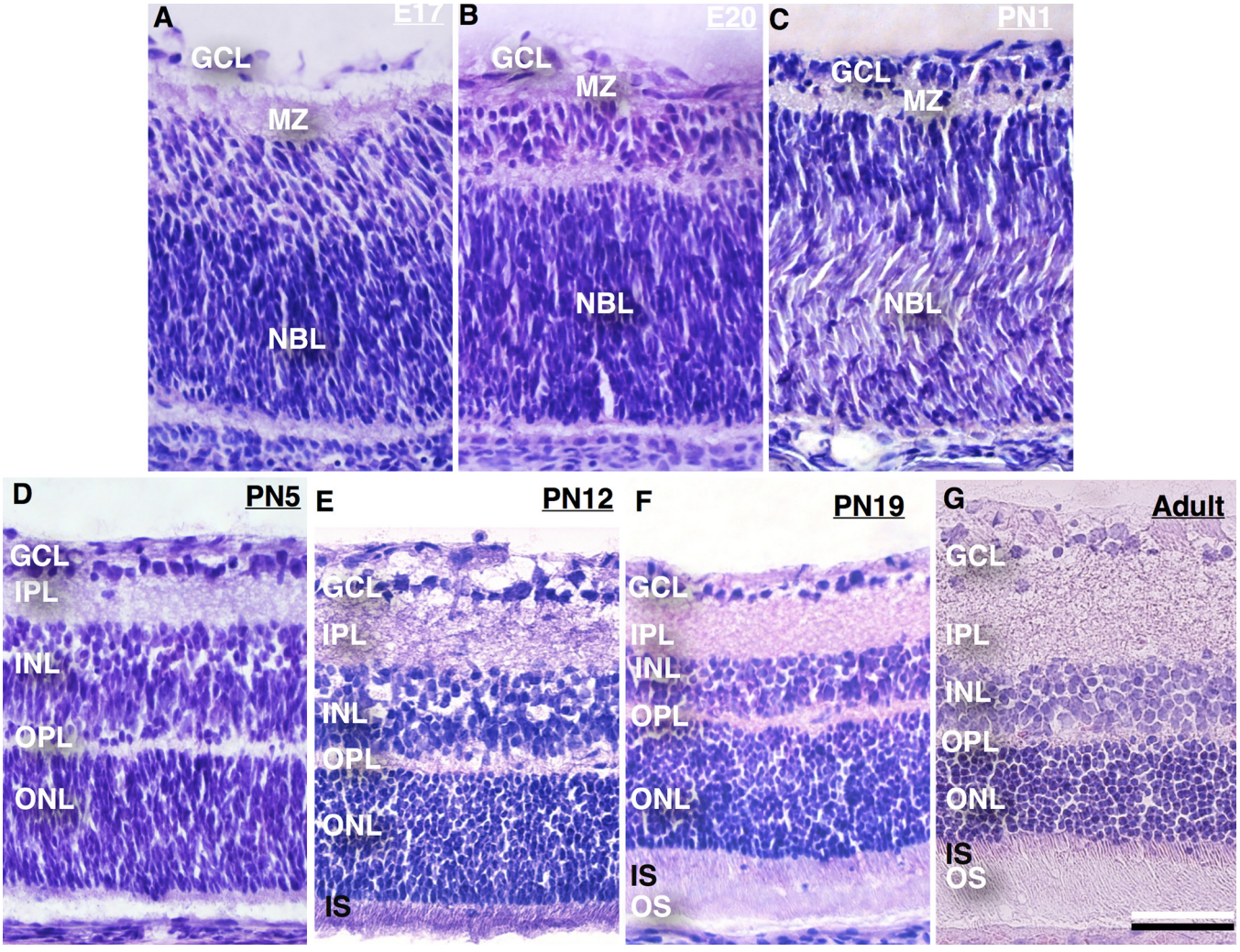

Fig. 1. Normal retinal development in vivo. Hematoxylin and eosin staining of E17-adult rat retina. MZ = marginal zone, NBL = neuroblastic layer, GCL = ganglion cell layer, IPL = inner plexiform layer, INL = inner nuclear layer, OPL = outer plexiform layer, ONL = outer nuclear layer, IS = inner segments, OS = outer segments. Scale bar = 50 μm.