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E-cadherin promotes retinal ganglion cell neurite outgrowth in a protein tyrosine phosphatase-mu-dependent manner

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During development of the visual system, retinal ganglion cells (RGCs) require cell–cell adhesion molecules and extracellular matrix proteins for axon growth. In this study, we demonstrate that the classical cadherin, E-cadherin, is expressed in RGCs from E6 to E12 and promotes neurite outgrowth from all regions of the chick retina at E6, E8 and E10. E-cadherin is also expressed in the optic tectum. E-cadherin adhesion blocking antibodies specifically inhibit neurite outgrowth on an E-cadherin substrate. The receptor-type protein tyrosine phosphatase, PTPμ, associates with E-cadherin. In this manuscript, we demonstrate that antisense-mediated down-regulation of PTPμ, overexpression of catalytically inactive PTPμ and perturbation of endogenous PTPμ using a specific PTPμ inhibitor peptide results in a substantial reduction in neurite outgrowth on E-cadherin. Taken together, these findings demonstrate that E-cadherin is an important adhesion molecule for chick RGC neurite outgrowth and suggest that PTPμ expression and catalytic activity are required for outgrowth on an E-cadherin substrate.

Keywords: Neurite outgrowth; Receptor protein tyrosine phosphatase; PTPμ; Cadherin; Retina; Tectum; Axon guidance

Introduction

The chick visual system serves as a well-established model to investigate the molecular mechanisms involved in axon growth and guidance. Retinal ganglion cells (RGCs) are the first cells to differentiate within the retina at embryonic (E) day 4 (reviewed in Mey and Thanos, 2000, 2001). Development within the retina proceeds in a central-to-peripheral gradient, with cells in the temporal region of the retina being the most differentiated. RGCs first extend an axon toward the optic fissure and then travel out of the eye along the optic nerve to the chiasm where they cross and continue on the retinofugal pathway to their target, the optic tectum. Retinal axons reach the anterior portion of the tectum by E6 and extend along the tectal surface to form the stratum opticum (SO). Temporal axons innervate the anterior surface while nasal axons extend to the posterior tectum at E10. RGCs extend axons toward the optic tectum in response to various molecular cues on the surface of other cells or in the extracellular environment (Mey and Thanos, 2000).

Cell adhesion molecules are important for the formation of the visual system (Hirano et al., 2003; Thiery, 2003; Kiryushko et al., 2004). Classical cadherins are cell surface integral membrane glycoproteins that mediate cell–cell adhesion, cell migration and cell sorting via calcium-dependent, homophilic interactions (Gumbiner, 2005). Cadherins are tethered to the actin cytoskeleton by their association with the catenins, α-catenin, β-catenin, plakoglobin and p120 (Lilien and Balsamo, 2005). N-cadherin is predominantly expressed in the developing nervous system and mediates axon guidance and synapse formation (Redies, 2000; Kiryushko et al., 2004; Takeichi and Abe, 2005). Previous studies have demonstrated that N-cadherin promotes neurite outgrowth *in vitro* and *in vivo* (Bixby and Zhang, 1990; Riehl et al., 1996). Within the chick retina, N-cadherin has been shown to be regulated by tyrosine phosphorylation (Lilien et al., 2002; Lilien and Balsamo, 2005).

Receptor protein tyrosine phosphatases (RPTPs) are expressed in the developing chick visual system and a subset of RPTPs have been suggested to play a role in retinotectal pathfinding (Brady-Kalnay, 2001; Ensslen-Craig and Brady-Kalnay, 2004; Johnson and Van Vactor, 2003). RPTPmu (PTPμ) is comprised of CAM-like extracellular domains that mediate cell–cell adhesion and associates with E-, N-, R- and VE-cadherin and the catenins, α-catenin, β-catenin and p120 (Brady-Kalnay et al., 1995, 1998; Hiscox and Jiang, 1998, 1999; Zondag et al., 2000; Sui et al., 2005).

Another classical cadherin, E-cadherin is expressed by mouse RGCs (Faulkner-Jones et al., 1999; Xu et al., 2002). However, a role for E-cadherin in neurite outgrowth has not been examined. In this

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study, we used a retinal explant model system to demonstrate that E-cadherin promotes neurite outgrowth of RGCs when used as a culture substrate *in vitro*. E-cadherin is expressed in the chick retina from E6 to E12 and promotes neurite outgrowth from all regions of the retina. Neurite outgrowth is specific to E-cadherin since outgrowth on an E-cadherin substrate is inhibited by addition of E-cadherin adhesion blocking antibodies. We have shown previously that PTPμ is present in a complex with E-cadherin in other systems (Brady-Kalnay et al., 1995, 1998). In order to determine the physiological significance of an association between PTPμ and E-cadherin in neurite outgrowth, the expression level of PTPμ was perturbed in retinal explants. The phosphatase activity of PTPμ was also perturbed in retinal explants. Down-regulation of PTPμ expression through antisense techniques and overexpression of catalytically inactive PTPμ resulted in a substantial reduction in neurite outgrowth on an E-cadherin substrate. In addition, perturbation of endogenous PTPμ in retinal explants using a specific PTPμ inhibitor peptide also resulted in a decrease in both N-cadherin- and E-cadherin-mediated neurite outgrowth. These findings indicate that PTPμ expression and catalytic activity are required for neurite outgrowth by RGCs on an E-cadherin substrate.

Results

Expression of E-cadherin in the visual system

Molecules that regulate axon outgrowth can be expressed in a gradient within the chick visual system. Since RGCs from nasal versus temporal regions of the retina extend axons to distinct locations in the tectum, we examined nasal versus temporal E-cadherin expression at several developmental time points corresponding to peak RGC axon growth in the retina and tectum (Mey and Thanos, 2000). Lysates were made, separated by SDS–PAGE and immunoblotted for E-cadherin (Fig. 1). E-cadherin is expressed during development from E6 to E12, the earliest and latest time points examined (Fig. 1), and is expressed in the nasal and temporal regions of the retina. N-cadherin is expressed in the retina from E8 to E10 as tested by immunoblot analysis

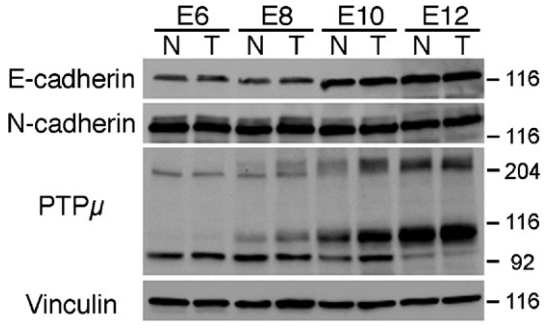

Fig. 1. Immunoblot of E-cadherin, N-cadherin and PTPμ in the developing chick retina. Lysates from nasal or temporal retina were prepared from E6, E8, E10 and E12 chicks, separated by SDS–PAGE, transferred to nitrocellulose membrane and probed with an antibody to E-cadherin, N-cadherin or PTPμ (SK18). E-cadherin protein migrates at ~120 kDa, while N-cadherin migrates at ~130 kDa. Full-length PTPμ is ~200 kDa whereas the proteolytically processed form of PTPμ containing the cytoplasmic domain migrates at ~100 kDa (Brady-Kalnay and Tonks, 1994). A 95-kDa immunoreactive band is also present. Each immunoblot was stripped and reprobed with antibodies against vinculin to verify equal protein load.

(Matsunaga et al., 1988; Lagunowich and Grunwald, 1989; Burden-Gulley and Brady-Kalnay, 1999). PTPμ is also expressed in the retina (Burden-Gulley and Brady-Kalnay, 1999; Burden-Gulley et al., 2002). Full-length PTPμ migrates at ~200 kDa whereas the proteolytically processed form of PTPμ that contains the cytoplasmic domain migrates at 100 kDa (Brady-Kalnay and Tonks, 1994). In retinal lysates, an additional 95 kDa immunoreactive band is also present (Burden-Gulley and Brady-Kalnay, 1999; Burden-Gulley et al., 2002). Full-length PTPμ increases in size, possibly due to glycosylation or alternative splicing. To ensure equal protein loading, immunoblots were stripped and reprobed with antibodies to vinculin (Fig. 1).

To further characterize the expression of E-cadherin in the developing retina, E8 retinas (stage 32) were sectioned and immunohistochemically labeled with an anti-E-cadherin antibody (Fig. 2A). E8 retinas were used since this time point in development coincides with peak RGC axon extension (Mey and Thanos, 2001). Coronal sections of the retina were taken in order to view both the dorsal and ventral region of the retina. E-cadherin is expressed in the retinal ganglion cells and optic fiber layer (Figs. 2A, B). Serial sections of retina were stained with hematoxylin to indicate the nuclear location of the RGC cell bodies (Figs. 2C, D) or incubated in the absence of primary antibody (Figs. 2E, F) as a control.

We then examined the expression of E-cadherin in the optic tectum. By E8, RGC axons have migrated out of the retina, across the optic chiasm and are innervating the anterior region of the tectum (Mey and Thanos, 2001). Retinal axons extend along the tectal surface to form the stratum opticum (SO). Temporal axons innervate the anterior surface while nasal axons extend to the posterior tectum at E10. E-cadherin is expressed in E8 optic tectum in the stratum opticum (SO), the outermost layer of the tectum, and the stratum griseum et fibrosum superficiale (SGFS), where RGC axons innervate (Fig. 2G). E-cadherin was also expressed in the neuroepithelium of the tectum (Fig. 2G). At E8, undifferentiated neuroepithelium is most prominent in the anterior portion of the tectum and gives rise to differentiating cells which migrate to the pial surface (LaVail and Cowan, 1971).

E-cadherin promotes neurite outgrowth

Early in embryogenesis, one or two leading RGC axons migrate along the optic stalk toward the optic tectum (Mey and Thanos, 2001). As development continues, successive waves of axons project along the neuronal and glial cells within the optic nerve (Mey and Thanos, 2001). Thus, cadherins expressed on the surface of these cells can serve as a "substrate" for axonal migration. To determine whether E-cadherin promotes neurite outgrowth, we used a well-established *in vitro* model lab to investigate neurite outgrowth (Lagenaur and Lemmon, 1987; Burden-Gulley and Brady-Kalnay, 1999). Purified recombinant E-cadherin-Fc chimera was coated on tissue culture dishes and used as a substrate to culture chick retinal explants. Neurite outgrowth on an E-cadherin substrate was observed from retinal explants taken at E6, E8 and E10, after 20 h in culture (Figs. 3A–C). Neurite length and density were similar between all time points examined, suggesting that E-cadherin is equally effective at promoting neurite outgrowth at these ages. Neurite outgrowth on E-cadherin was similar in length and density to that observed on N-cadherin (Figs. 6D, G).

Growth cones located at the distal tip of the axon allow neurons to interact with the extracellular environment. Each growth cone