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BRAIN RESEARCH

Research Report

Expression of JAM-A, AF-6, PAR-3 and PAR-6 during the assembly and remodeling of RPE tight junctions

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ARTICLE INFO

Article history:
Accepted 19 June 2006
Available online 21 July 2006

Keywords:
Retinal pigment epithelium
Blood–retinal barrier
Tight junction
Adherens junction
Cell culture

Abbreviations:
RPE, retinal pigment epithelium
E3, embryonic day 3
CEF, chicken embryonic fibroblasts

ABSTRACT

The tight junctions of the endothelial and epithelial regions of the blood–brain barrier are regulated by interactions with the neighboring tissue. We examined how the neural retina regulates the assembly of tight junctions in the retinal pigment epithelium (RPE). The proteins JAM-A, AF-6, PAR-3 and PAR-6 have been implicated in the assembly of other epithelial tight junctions. Using chick embryos and primary cell culture, we examined gene expression of these proteins during embryonic development, and whether retinal secretions regulate their expression. Three highly conserved RNA splice sites of AF-6 were identified in chick ocular tissues, but only two were expressed in RPE. JAM-A and AF-6 were expressed at relatively high levels early in development when adherens junctions form, but before tight junctions form. Expression of JAM-A and the AF-6 isoforms actually decreased when tight junctions were forming and expanding. The expression of PAR-3 and PAR-6 was constant. Despite the expression of these proteins in vitro (along with claudins, occludin, ZO-1 and ZO-2), the tight junctional networks that form were discontinuous (Rahner, C., Fukuhara, M., Peng, S., Kojima, S., Rizzolo, L.J., 2004. The apical and basal environments of the retinal pigment epithelium regulate the maturation of tight junctions during development. J. Cell Sci. 117, 3307–3318). The expression of these assembly proteins was unaffected by a retinal conditioned medium that induced the completion of tight junction formation. These data indicate that the early expression of the assembly proteins corresponds to the initial establishment of the adherens and tight junctions, but secretory products of the neural retina must induce the expression of additional proteins to complete the maturation process.

1. Introduction

The retinal pigment epithelium (RPE) forms the outer blood–retinal barrier (Marmor and Wolfensberger, 1998). Barrier function depends upon the formation, maintenance and regulation of the apical junctional complex that joins the neighboring cells of the RPE monolayer (Rizzolo, 1997; Wilt and Rizzolo, 2001). The complex encircles each cell near the apical

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doi:10.1016/j.brainres.2006.06.059

end of the lateral membrane and includes two junctions whose functions are intertwined. The adherens junctions bind neighboring cells together, while the tight junctions form a partially occluding seal that semi-selectively retards diffusion through the paracellular spaces of the monolayer (Cereijido and Anderson, 2001; Gonzalez-Mariscal et al., 2003; Matter et al., 2005; Schneeberger and Lynch, 2004; Van Itallie and Anderson, 2006). Both portions of the complex participate in signal transduction pathways that regulate cell size and proliferation and help polarize the distribution of plasma membrane proteins. To enable these diverse functions, the apical junctional complex is an extraordinarily complex assembly of proteins. It is unclear how the apical junctional complex is assembled.

Many of the insights into the assembly of the complex come from studies using the MDCK, Caco-2 and T84 cell lines and mouse blastocysts. In MDCK (canine kidney), a primordial adherens junctions formed first, followed by the segregation of adherens and tight junctions over a 48 h period (Rajasekaran et al., 1996). Further studies suggested that nectin, E-cadherin and JAM-A triggered the formation of a primordial adherens junctions (Ando-Akatsuka et al., 1999; Asakura et al., 1999; Miyoshi and Takai, 2005). These are transmembrane proteins that homotypically bind their counterpart on the neighboring cell. Their cytoplasmic domains crystallized a complex of proteins by binding a scaffold, or adaptor, protein. The adaptor proteins included ZO-1, AF-6 and PAR-3. Their multiple protein binding domains enable them to assemble a complex. For example, PAR-3 binds PAR-6 and the atypical protein kinase C, which contribute to cell polarity (Mandell and Parkos, 2005; Matter et al., 2005). In MDCK, JAM-A localized ZO-1 to the apical side of the complex to initiate the formation of a tight junction by binding the transmembrane proteins occludin and members of the claudin family. A primary role for JAM-A was also observed in Caco-2 (human intestine) and during compaction of mouse blastocysts (Martinez-Estrada et al., 2001; Thomas et al., 2004). JAM-A rapidly assembled at sites of cell contact followed by its binding partners, AF-6 and ZO-1. In T84 cells (human colon), anti-JAM antibodies blocked the formation of tight junctions, as evidenced by the mislocalization of occludin and a low transepithelial electrical resistance. By contrast, the adherens junction did form, as evidenced by the localization of E-cadherin and ZO-1 (Liu et al., 2000). Taken together, these data suggest that JAM may play a role in assembling adherens junctions, but plays a more critical role in assembling tight junctions.

In contrast to these models, the assembly of the apical junctional complex of chicken RPE was a much slower process. There was a substantial delay between assembly of the adherens junction and assembly of the tight junction. Although they would be remodeled, adherens junctions were already present in the neuroepithelium that formed the RPE on embryonic day 3 (E3), days before rudimentary tight junctions began to form on E7 (Grunwald, 1996; Liu et al., 1997; Rahner et al., 2004; Sandig and Kalnins, 1990; Williams and Rizzolo, 1997). Despite the presence of the tight junctional proteins, ZO-1 and occludin, on E3, tight junctional strands were absent until claudins are expressed on E7 (Fujisawa et al., 1976; Rahner et al., 2004). Claudin family members form the strands of the tight junction and determine its selectivity and permeability (Van Itallie and Anderson, 2006). Slowly, between E7 and E14, tight junctional strands grew in number and length to gradually coalesce into a complete network that encircled the cell (Rahner et al., 2004). The tight junction first becomes functional between E10 and E12 (Williams and Rizzolo, 1997). After the active period of growth, structural modifications continue between E14 and E18 (Kniesel and Wolburg, 1993). This slow process allowed us to explore the assembly of the adherens junction and tight junction semi-independently.

The assembly of tight junctions has been modeled using primary cultures of E7 and E14 RPE (Ban and Rizzolo, 2000; Peng et al., 2003; Rahner et al., 2004). Tight junctional strands are formed, but like the intermediate stage of tight junction assembly in vivo, the network is discontinuous despite the over-expression of the claudins normally found in chick RPE. These discontinuities can be sealed, if the cultures are incubated in a medium that was conditioned by E14 neural retinas (the retina with the RPE layer removed). Perhaps the junctions are discontinuous because of inadequate expression of the proteins responsible for the assembly of junctions, and retinal conditioned medium increases their expression. Previous studies indicated that ZO-1 and occludin are expressed in normal amounts and are unaffected by retinal conditioned medium (Ban and Rizzolo, 1997).

We took advantage of the slow assembly process in chick RPE to examine the hypothesis that the expression of another group of putative assembly proteins is regulated by retinal conditioned medium. We found that JAM-A and its ligands, AF-6 and the PAR-3/PAR-6 complex, were expressed before RPE tight junctions formed in vivo. Surprisingly, the expression of AF-6 and JAM-A decreased during the period of tight junction formation, and the expression of PAR-3 and PAR-6 was constant. Retinal condition medium had minimal effects on the expression of these proteins, despite its ability to regulate claudin expression and alter the fine-structure of the tight junctions.

2. Results

2.1. *Expression during development in vivo*

A blast search of the chicken genome using human and rodent sequences revealed JAM-A and JAM-B like sequences. Primer pairs were designed for each isoform and used to amplify total RNA that was isolated from embryonic RPE of varying age. Only JAM-A was detected, as confirmed by sequencing the amplification product. Quantitative RT-PCR was used to follow the expression of the mRNA of JAM-A (Fig. 1A). Expression was high on E7 but decreased 2.8× by E18. By immunoblotting, a similar decrease was observed for the expression of JAM-A itself (Fig. 1B).

A predicted transcript of AF-6 is reported in Ensembl, an annotated database of the chicken genome (Hubbard et al., 2005). Three alternative splice sites for AF-6 were predicted in earlier versions of Ensembl that are described in Fig. 2. The inserts for regions 1 and 2 are included in the current prediction, but the insert for region (3) is found in intron 34–35. The insert^{+} and insert^{-} versions of these regions