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Experimental Eye Research 83 (2006) 484–492
EXPERIMENTAL EYE RESEARCH
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Aberrant retinal tight junction and adherens junction protein expression in an animal model of autosomal recessive Retinitis pigmentosa: The Rho(−/−) mouse

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Received 27 July 2005; accepted in revised form 23 January 2006
Available online 27 April 2006

Abstract

Retinitis pigmentosa (RP) comprises a heterogenous group of inherited diseases that are characterised by primary degeneration of rod photoreceptors and secondary degeneration of cone photoreceptors in the retina. Additional pathological changes include vascular changes and invasion of the inner retina by retinal pigment epithelial (RPE) cells. RP represents a major cause of progressive retinal disease worldwide. Using a mouse model of autosomal dominant Retinitis pigmentosa (adRP) with retinopathy induced by targeted disruption of the rhodopsin gene Rho(−/−), we have analysed the levels of expression of a range of tight and adherens junction associated proteins, in order to further elucidate the pathogenic mechanisms occurring at an early stage of this condition. Using western blot analysis and indirect immunostaining of retinal cryosections from 6-week-old mice from a C-129 background we have determined changes, if any, in the levels of expression and localisation of a series of tight and adherens junction associated proteins, including Zonula Occludens-1 (ZO-1), occludin, N-Cadherin, p120-Catenin, α-Catenin, γ-Catenin, β-Catenin, and E-Cadherin. We have found an up-regulation of the tight junction and adherens junction associated protein Zonula Occludens-1 (ZO-1) in the neural retina of 6-week-old Rho(−/−) knockout mice compared with 6-week-old Wild-Type (WT) mice. Following immunohistochemistry, however, it appears, that ZO-1, β-Catenin and p120-Catenin expression at the Outer Limiting Membrane (OLM) of the Rho(−/−) retina is compromised, in part, compared to WT animals of the same age. We hypothesise that these retinal changes following photoreceptor cell death may contribute to the pathogenesis of adRP. Our findings of changes in the levels of expression of ZO-1 and associated adherens junction proteins β-Catenin and p120-Catenin at the OLM in 6-week-old Rho(−/−) mice provide evidence for tight junction and adherens junction associated protein modifications in an animal model of autosomal dominant RP (adRP).

Keywords: Retinitis pigmentosa; tight junctions; adherens junctions; Zonula Occludens-1; β-Catenin; p120-Catenin; Outer Limiting Membrane; retina; photoreceptor; retinal vasculature

1. Introduction

Tissues of the central nervous system, including the brain and retina, depend on intact blood brain and blood-retinal barriers (BRB), respectively, to partition them from the systemic circulation (Takata et al., 1997). The BRB consists of the inner BRB (iBRB) and the outer BRB (oBRB) with the endothelial membranes of the retinal vessels forming the iBRB, whilst the RPE and Bruchs membrane are the main structures involved in forming the oBRB (Gonzalez-Mariscal et al., 2003).

The endothelial cells of the inner BRB (iBRB), and the blood brain barrier (BBB), contain tight junctions (TJs) that confer highly selective properties on these vessels. Tight junctions make a seal around the circumference of cells and function as barriers preventing free diffusion of solutes through the paracellular pathway (Balda et al., 1996). The tight junction is composed of a complex of several proteins. Occludin and the

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0014-4835/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2006.01.032

claudins are integral membrane proteins that span the plasma membrane 4 times (Tsukita and Furuse, 1999).

Junctional Adhesion Molecules (JAMs) have also been shown to be located close to TJ strands (Bazzoni, 2003). TJ associated proteins which have been proposed as mediating a scaffolding function include Zonula Occludens-1, -2, and -3 (ZO-1, ZO-2 and ZO-3). They are members of the Membrane Associated Guanylate Kinase (MAGUK) family of proteins, and in particular, ZO-1 has been shown to bind to claudins at its PDZ-1 domain, JAMs at its PDZ-3 domain and occludin at its GUK domain (Hamazaki et al., 2002; Itoh et al., 1999). Of the known integral membrane proteins of the tight junction, ZO-1 in particular may play an integral role in the scaffolding of transmembrane proteins, whilst also creating a link to the perijunctional actin cytoskeleton (Riesen et al., 2002). The fact that ZO-1 in particular has three PDZ domains could potentially mediate its binding to a wide variety of protein partners and allow for the control of tight junction and adherens junction assembly, whilst the overall molecular structure of ZO-1 facilitates a capacity for multiple protein–protein interactions (Zahraoui, 2004).

ZO-1 has also been shown to be present in the Outer Limiting Membrane (OLM) (see Diagram 1) of the mammalian retina, which provides a barrier function at the photoreceptor (Meuleman et al., 2004). The OLM, which lies at the apical site of the Outer Nuclear Layer (ONL) of the photoreceptors, contains specialized adherens junctions (AJs), which are present between the photoreceptor cells and the Müller glial cells. The multi-protein complexes, which comprise these adherens junctions, allow for the maintenance of the cell skeleton, shape and its overall health and integrity (Tepass, 2002).

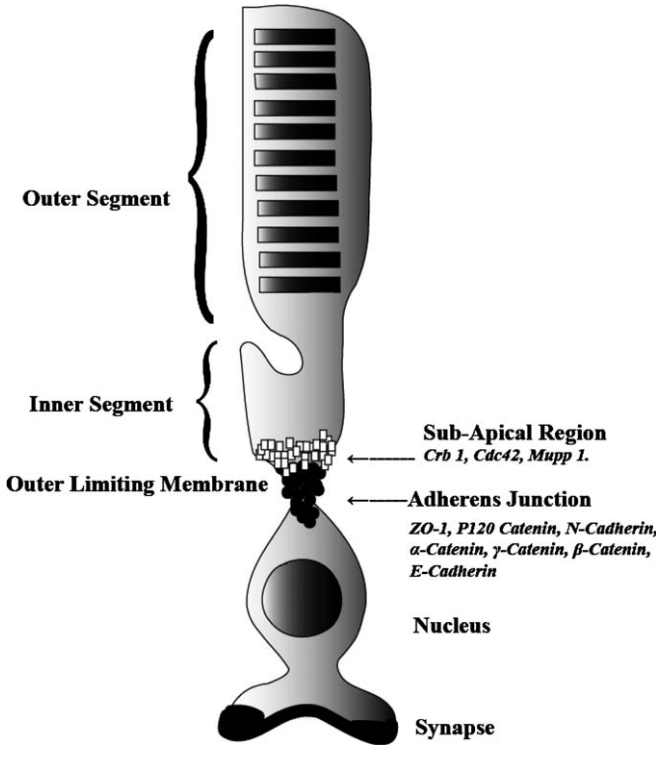

Diagram 1. A schematic diagram of the mammalian photoreceptor gives an overview of the localisation of the OLM, showing ZO-1 and a series of other adherens junction associated proteins mediating the formation of a distinct adherens junction.

In this study, we were interested in TJ and AJ protein expression in an animal model of autosomal dominant Retinitis pigmentosa (adRP), namely the Rho(−/−) knockout mouse, in which mice carry a targeted disruption of the rhodopsin gene, and present with rapid photoreceptor degeneration.

RP represents a major cause of progressive retinal disease worldwide and comprises a heterogenous group of inherited diseases that are characterised by primary degeneration of rod photoreceptors and secondary degeneration of cone photoreceptors in the retina (Farrar et al., 2002).

Additional pathological changes include vascular compromisation and invasion of the inner retina by Retinal Pigment Epithelial (RPE) cells (Li et al., 1995).

We hypothesised that both BRB and photoreceptor adherens junction integrity may be affected following photoreceptor cell death and that retinal changes following this photoreceptor cell death may contribute significantly to the pathogenesis of RP.

Retinal expression and localisation of the tight junction protein occludin and the tight junction and adherens junction associated protein ZO-1, as well as a series of adherens junction proteins including β-Catenin, E-Cadherin, p120-Catenin, γ-Catenin, α-Catenin and N-Cadherin were determined in retinas from 6-week-old C-129 WT and Rho(−/−) mice. Retinal histology of these animals at 6 weeks reveals that the outer segments become visibly shorter in older mice (Fig. 1) and no rod Electroretinogram (ERG) response is detected in 8-week-old animals (Humphries et al., 1997).

We found consistent up-regulation of the tight/adherens junction associated protein ZO-1 in retinal lysates of Rho(−/−) mice compared with WT animals of the same age and background, while increased transcripts of ZO-1 were also shown to be present in retinas from the Rho(−/−) knockout mice compared with C-129 WT mice. A decrease in the levels of expression of ZO-1, β-Catenin and p120-Catenin was also observed at the OLM of 6-week-old Rho(−/−) mice compared to WT mice of the same age, suggesting altered integrity of the adherens junction at the OLM of the Rho(−/−) knockout animals.

2. Materials and methods

2.1. Animal models and experimental groups

The studies adhered to the ARVO statement for the use of Animals in Ophthalmic and Vision Research. Mice developed by Humphries et al. (1997), carrying a targeted disruption of the rhodopsin gene, the Rho(−/−) mouse provides an animal model of autosomal dominant Retinitis pigmentosa (adRP) that enables comparison of protein expression profiles in mouse retinas with and without functional photoreceptors (Kennan et al., 2002). Six-week-old mice from a C-129