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A common microRNA signature in mouse models of retinal degeneration

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ABSTRACT

Perturbed microRNA (miR) expression is a feature of, and may play a fundamental role in, certain disease states such as different forms of cancer. Retinitis pigmentosa (RP) a group of inherited retinal degenerations is characterised by a progressive loss of photoreceptor cells and consequent visual handicap. We have previously reported an altered pan-retinal expression of miR-96, -183, -1 and -133 in a P347S-Rhodopsin transgenic mouse model of RP. As many different mutations in Rhodopsin and other genes such as RDS/Peripherin can lead to RP, it was of interest to explore whether the characterized retinal miR expression signature was observed in three other mouse models of RP linked to rhodopsin and rds/peripherin. Therefore, pan-retinal expression of miR-96, -182, -183, -1, -133 and -142 was analysed using quantitative real-time RT-PCR. A common signature of altered miR expression was found; expression of miR-96, -182 and -183 decreased by 14.1-53.2%, while expression of miR-1, -133 and -142 was up-regulated by 186.1-538.5%. Significantly, the detected pan-retinal miR signature was mirrored by similar miR expression profiles in FACS-isolated rod photoreceptors from these mice. In an attempt to understand the function of these miRs, corresponding target genes were predicted using computational means. Many 'enriched' targets (with binding sites for at least two of the above miRs) were found to be regulatory molecules and members of intracellular signalling circuits. However, further studies are required to highlight which of the large number of *in silico* predicted targets are actually controlled by these miRs.

1. Introduction

Mature microRNA (miR) transcripts are small (~22 nucleotides) non-coding RNAs that control eukaryotic gene expression at the post-transcriptional level by interacting with target mRNAs, which either results in mRNA cleavage or translational repression (Bartel, 2004). Tissue-specific expression of miRs is well documented; however, a clear understanding of the functions of the majority of miRs remains elusive. The critical roles played by miRs in developmental and, in particular disease processes, has attracted immense attention (Kloosterman and Plasterk, 2006; Chang et al., 2008). While a number of miRs that are highly expressed in the mouse retina have been identified (Ryan et al., 2006; Karali et al., 2007), the fundamental roles of miR regulation in this tissue are not yet understood (Arora et al., 2007; Loscher et al., 2007; Xu et al., 2007). A group of inherited retinal degenerations with different genetic origins but similar clinical manifestations involving progressive photoreceptor loss and visual impairment is known by the umbrella term retinitis pigmentosa (RP; RetNet, http://www.sph.uth.tmc.edu/Retnet/). Our recent data (Loscher et al., 2007) from studies using P347S-Rhodopsin transgenic mice (R347; Li et al., 1996), a model of RP, provided evidence of altered pan-retinal expression of miR-96, -183, -1 and -133 suggesting a potential involvement of miRs in retinal disease. As RP is characterized by both intergenic and intragenic mutational heterogeneity, the aim of this study was to determine whether the identified retinal miR expression signature was present in other rhodopsin (rho) and rds/peripherin (rds)-linked mouse models of RP. For instance, a rho knockout (rho−/−) mouse line exhibits a severe retinal degeneration (Humphries et al., 1997). Additionally, mutations in the RDS/Peripherin gene, which encodes the photoreceptor specific protein rds/peripherin, are also causative of RP (RetNet, http://www.sph.uth.tmc.edu/Retnet/). Notably, transgenic mice carrying a deletion in codon 307 of the rds gene (Δ307; McNally et al., 2002), modelling a similar human mutation responsible for a dominant form of RP (RetNet, http://www.sph.uth.tmc.edu/Retnet/), and mice with a naturally occurring null mutation in this gene (rds−/−; Sanyal et al., 1980) display progressive retinopathy. A further objective was to clarify whether

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the observed miR expression signature was a result of genuine alterations in miR expression in retinal cells. Particularly, a common signature of miR dysregulation in the analyzed diseased retinas, reflected by similar miR expression profiles in rod photoreceptors of Δ307 and R347 mice, has emerged from the study.

2. Materials and methods

P347S-Rhodopsin (R347; Li et al., 1996), Δ307-rds (Δ307; McNally et al., 2002), rho knockout (rho−/−; Humphries et al., 1997), rds null mutant (rds−/−; Sanyal et al., 1980), Rhodopsin-GFP (RGFP; Chan et al., 2004), wild type balbC and 129 mice were bred under specific pathogen free (SPF) housing conditions. Mice at one month of age were sacrificed and three retinal samples/mouse line were prepared by pooling six retinas/group. For fluorescence-activated cell sorting (FACS) analysis, RGFP+/−, RGFP+/− Δ307+/− and RGFP+/− R347+/− mice at one month of age were sacrificed and three samples/mouse line were prepared by pooling two retinas/group. Retinas were dissociated using trypsin and cells analyzed and separated by FACS as previously described (Palfi et al., 2006). Total RNA from pan-retinal samples and FACS-isolated retinal cells was extracted and quantitative real-time RT-PCR (qRT-PCR) performed as described (Palfi et al., 2006; Loscher et al., 2007). Equal amounts of RNA (10 ng/reaction) were used in each RT reaction. SnoRNA-202 and -234, with strong correlation in their expressions across all samples ($r^2 = 0.8958$ and $p < 0.0001$), were used as internal controls. For histology, eyes from two animals/strain were fixed in 4% paraformaldehyde, cryoprotected, sectioned (12 μm), and nuclei counterstained with DAPI. Outer nuclear layer (ONL) thickness was measured using microscopic images from sections in three planes 250 μm apart in the medial part of the eye using Photoshop (Adobe Systems Europe Ltd., Glasgow, UK). Analysis of statistical significance was performed using ANOVA, Student's *t*-test and Pearson's correlation. *In silico* target predictions using miRanda (John et al., 2004) and PicTar-Dog (Krek et al., 2005) were retrieved from miRGator (Nam et al., 2008). The intersections of the predicted miR targets, retinal transcriptome and human eye disease genes were obtained as described (Loscher et al., 2007); an additional retinal gene list was included in the analysis (Arora et al., 2007).

3. Results

The retinal miR expression of the previously reported miRs; miR-96, -183, -1 and -133 (Loscher et al., 2007) and two additional miRs; miR-182 and -142, were explored in the current study. MiR-182 belongs to a sensory organ specific miR cluster along with miR-96 and -183 (Xu et al., 2007), while miR-142 exhibited marked changes in previous array experiments (Loscher et al., 2007). We aimed to test whether the reported miR expression signature detected in R347 animals involving a Rhodopsin mutation (Loscher et al., 2007), characterize other photoreceptor-linked mouse models of RP such as rho−/−, Δ307 and rds−/−. Therefore, the retinal expressions of these six miRs were analyzed by qRT-PCR in the above mice.

An early time-point of one month of age was chosen for the study, to minimize the possibility that miR expression changes resulted from altered cell compositions in degenerating mouse retinas. At this time-point, there is at maximum a 31.8% reduction in the ONL thickness of the retina in the various mutant mouse lines used for the study as determined by retinal histology (Fig. 1). As the rds−/− mice are on a balbC background and the remaining mouse lines evaluated are on a 129 background, wild type balbC and 129 mice were used as controls. ONL thickness of wild type balbC and 129 mice did not differ significantly from each other (Fig. 1).

Particularly, a common trend in pan-retinal miR expression profiles was observed among the analyzed mouse models (Fig. 2). The expression of miR-96, -182 and -183 decreased by 14.1–53.2%

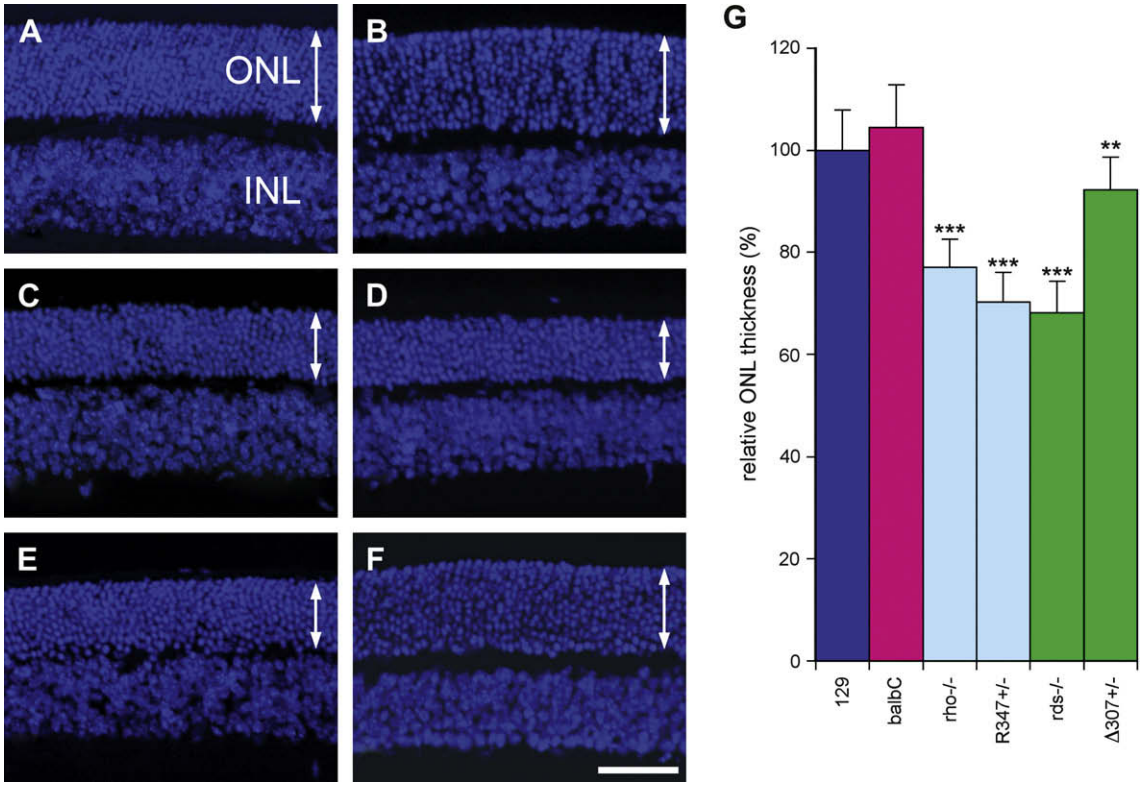

Fig. 1. Comparative histology of retinas from rho- and rds-linked RP mouse models. Representative microscopic images illustrate retinas of (A) 129, (B) balbC, (C) rho−/−, (D) R347+/−, (E) rds−/− and (F) Δ307+/− mice. Cell nuclei were counterstained by DAPI and outer nuclear layer (ONL) thickness was measured in microscopic images and corresponding average values plotted (G). Arrows indicate the thickness of the ONLs. INL, inner nuclear layer; scale bar represents 25 μm; $^{**}p < 0.01$; $^{***}p < 0.001$.