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TRANSGENIC ANIMAL STUDIES OF HUMAN RETINAL DISEASE CAUSED BY MUTATIONS IN PERIPHERIN/RDS

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1. INTRODUCTION

The photoreceptor disk membrane protein peripherin/*rds* is essential for the outer segment morphogenesis and integrity. Peripherin/*rds* associates with itself and with its homologue Rom-1 to form homo- and hetero-complexes, which are necessary for its structural role (Goldberg et al., 1995; Molday, 1998). More than seventy different pathogenic mutations in the peripherin/*rds* gene have been identified. These mutations are divided primarily into two categories: those associated with classic retinitis pigmentosa (RP), and those associated with various forms of macular dystrophy (MD). In fact, mutations in peripherin/*rds* account for 5-10% of RP causes, and is a major cause for MD (Kohl et al., 1998; Molday, 1998; <http://www.sph.uth.tmc.edu/RetNet>; <http://www.retina-international.org/sci-news/rdsmut.htm>). Insights into the functional significance, structural role, and pathogenic effects of this protein have been accumulating considerably since its initial description; this is largely accomplished by the use of laboratory animal models. Use of transgenic or knockout animals holds great potential for the investigation of retinal disease pathogenesis and the exploration of therapeutic interventions. Table 21.1 summarizes the animal models used to investigate the disease-causing mutations in peripherin/*rds*. In addition to the pathogenesis study, transgenic mouse and *Xenopus laevis* expressing the wild type peripherin/*rds* or the C-terminus have also been used to explore the structural and functional significance of the protein (Loewen et al., 2003; Ritter et al., 2004).

2. TRANSGENIC ANIMAL MODELS OF RP CAUSED BY MUTATION IN PERIPHERIN/RDS

The majority of the RP-causing mutations in peripherin/*rds* exerts a dominant effect such as the P216L (Kajiwar et al., 1991) and C214S mutations (Saga et al., 1993); a few fall into the digenic group for example the double heterozygous for a L185P mutation in peripherin/*rds* and a second null mutation in Rom-1 (Dryja et al., 1997). In the first transgenic mouse model for peripherin/*rds* mutation-linked autosomal dominant RP, Kedzierski et al., (1997) described an expression-level-dependent photoreceptor degeneration and outer segment shortening in the P216L transgenic mice. Expression of the P216L transgene on the *rds*^{+/-} and *rds*^{-/-} background resulted in a faster rate of photoreceptor degeneration and outer segment dysplasia than that seen in the non-transgenic controls. Thus, the phenotype seen in P216L retina is caused by both direct dominant effect of the mutant protein and a consequence of haploinsufficiency. In the study by Stricker et al., (2003), the pathogenesis of the C214S mutation was examined in several transgenic lines with different expression levels of the transgene. Although, comparable amount of transgene message was formed in the transgenic retinas, only a very

small amount of the C214S protein was detected. Moreover, ectopic expression of the C214S mutant protein was observed in the inner retinal cells of transgenic mice (Stricker et al., 2003). The phenotype of photoreceptor degeneration seen in these transgenic mice resembles the symptom in patients with the same mutation. Thus, the haploinsufficiency resulted from the fatal mutation contributes to the retinopathy caused by the C214S mutation.

In a digenic RP mouse model (Kedzierski et al., 2001), in which both the L185P mutation and levels of peripherin/*rds* and Rom-1 closely matched those predicted for the corresponding human diseases, photoreceptor degeneration in these mice was shown to be faster than that in the monogenic controls. From this model, it was proposed that deficiency of peripherin/*rds* and Rom-1 might be the main cause of photoreceptor degeneration and that the threshold level for the combined abundance of peripherin/*rds* and Rom-1 is approximately 60% of the wild type. Below this level, the extent of outer segment disorganization may result in clinically significant photoreceptor degeneration.

Transgenic Xenopus, which express wild type peripherin/*rds* and the autosomal dominant RP-linked mutants as GFP-fusion proteins in rod photoreceptors, was recently established to identify the determinants required for peripherin/*rds* targeting to disk membranes and to elucidate the mutation pathogenesis (Loewen et al., 2003). The wild type and the P216L mutant were properly assembled as tetramers and targeted to disk membranes as visualized by confocal and electron microscopy. In contrast, the C214S and L185P mutants, which form homodimers but not tetramers, were retained in the inner segments. The finding of mislocalization of the C214S mutant was consistent with that observed in transgenic mice (Stricker et al., 2003). From these studies, it was proposed that tetramerization is required for peripherin/*rds* targeting and incorporation into disk membranes and that a checkpoint between the photoreceptor inner and outer segments allows only correctly assembled peripherin/*rds* tetramers to be incorporated into nascent disk membranes. Thus, the tetramerization-defective mutants (C214S and L185P) cause RP through a deficiency in wild type peripherin/*rds*, whereas tetramerization-proficient P216L peripherin/*rds* causes RP through a dominant negative effect. Further studies on this model indicated that the introduction of a new N-linked oligosaccharide chain might contribute to the defect of the P216L mutant protein (Loewen et al., 2003).

McNally et al., (2002) introduced a targeted single-base deletion at codon 307 of the peripherin/*rds* gene in mice, similar to a human mutation reported by Apfelstedt-Sylla et al., (1995) in which patients suffered from a slowly progressive form of autosomal dominant RP. The mutation in the human gene causes a frameshift which results in a stop codon after a further 16 triplets, and the expected protein is 26 amino acids shorter than the wild-type peripherin/*rds*. The frameshift in the mouse gene is predicted to result in alteration of the last 40 amino acids of the C-terminus of the protein and the addition of an extra 11 amino acids. In heterozygous and homozygous peripherin/*rds*-307del mice, the induced retinopathy, as evaluated by histopathologic and electroretinographic analysis, appeared to be more rapid when compared with *rds*^{+/-} or *rds*^{-/-} mice. Thus, the pathogenesis of this mutation in patients may involve both the dominant-negative effect and the haploinsufficiency.

3. TRANSGENIC ANIMAL MODEL OF MD CAUSED BY MUTATIONS IN PERIPHERIN/RDS

The second category of the disease-causing mutations contains those mutations affecting the central macular regions of the retina. To date, more than thirty different mutations in peripherin/*rds* have been identified in patients diagnosed with MD or different forms of cone-rod dystrophy. Wells et al., (1993) first described a substitution of arginine with tryptophan in codon 172 (R172W mutation) in patients with MD. Later, the same mutation was reported in patients of English, Japanese, Swiss, and Spanish origins, with similar patterns of retinopathy

(Wroblewski et al., 1994; Nakazawa et al., 1995; Jacobson et al., 1996; Milla et al., 1998; Payne et al., 1998). In addition, other mutations in this position, including R172G and R172Q, were also found to associate with MD (Nakazawa et al., 1995; Payne et al., 1998). Although symptoms in a majority of the patients suggest a cone-dominant defect, one report has shown a more diffuse and progressive retinal degeneration in patients with this mutation (Ekstrom et al., 1998).

The pathogenesis of MD related to the R172W mutation was explored in the transgenic mouse model (Li et al., 1999; Ding et al., 2004). The phenotype in these mice resembles the clinical symptoms of patients carrying this mutation. Functional, structural and biochemical analyses showed a direct correlation between transgene expression levels and the onset/severity of the phenotype. Transgenic mice from the low expresser line (40% of wild type) showed a mild, late-onset cone dystrophy in which cone functional deficits were associated with reduction in cone density as early as nine months of age (Li et al., 1999). However, the high expresser line (75% of the wild type) revealed an early onset, autosomal dominant cone-rod dystrophy (Ding et al., 2004). The cone-dominant degeneration induced by the R172W mutation was well documented in the transgenic mice with mutant protein expressed on the different *rds* background. When expressed on the wild type background, both cone and rod structure and function were significantly diminished with more severe cone defect. The phenotype seen in the transgenic retina on the wild type background is not an effect of peripherin/*rds* over-expression. This has been demonstrated in a study by Nour et al., (2004) in which over-expression of wild type peripherin/*rds* did not alter retinal function and structure. When the R172W transgene expressed on the *rds*^{+/-} background, cone ERG responses were diminished to 41% of the wild type level while rod function and outer segment structure were improved. Expression of the R172W mutant in *rds*^{-/-} mice rescued the rod function to 30% of the wild type level but no rescue of cone function was observed. The functional and structural characteristics of the transgenic retinas from the high expresser line on different *rds* genetic background are summarized in Table 21.2. Biochemical studies of the mutant protein isolated from transgenic mice on the *rds*^{-/-} background showed no abnormalities in complex formation and association with Rom-1. However, the R172W protein was more sensitive to limited tryptic digestion, suggesting a change in the protein conformation that possibly contributes to the cone-specific phenotype (Ding et al., 2004). As the first animal model for peripherin/*rds*-associated cone-rod dystrophy, the R172W mice provides a valuable tool for studying the pathophysiology of human macular dystrophies and for development of the therapeutic interventions.

4. TRANSGENIC ANIMAL MODELS USED TO STUDY THE STRUCTURE AND FUNCTION RELATIONSHIP OF PERIPHERIN/RDS

Transgenic animal models have been applied to the study of the structural and functional role of peripherin/*rds*. Using transgenic mouse line expressing the wild type peripherin/*rds*, Nour et al., (2004) has demonstrated *in vivo* the critical level of peripherin/*rds* needed to maintain photoreceptor structure and ERG function. Total peripherin/*rds* levels in the retina were modulated by crossing the wild type transgenic mice into different *rds* genetic backgrounds and the consequences of peripherin/*rds* over-expression in both rods and cones were assessed morphologically and functionally. A positive correlation was observed between peripherin/*rds* expression levels and the structural and functional integrity of photoreceptor outer segments. Over-expression of peripherin/*rds* caused no detectable adverse effects on the structure and function of rods or cones (Nour et al., 2004). In the study by Kedzierski et al., (1999), the functional role of the intradiscal D2 loop of peripherin/*rds* was examined in transgenic mice expressing a chimeric protein containing the D2 loop of peripherin/*rds* in the context of Rom-1. The chimeric protein was able to form covalent homodimers and to interact non-covalently with itself, wild type peripherin/*rds*, and Rom-1, and displayed a more stable

interaction with peripherin/*rds* when compared to the authentic Rom-1. This study proposed that peripherin/*rds* is about 2.5-fold more abundant than Rom-1 and the complexes formed may extend the entire circumference of the disc (Kedzierski et al., 1999).

Through use of transgenic *Xenopus* expressing the GFP-C-terminus of peripherin/*rds*, the participation of the C-terminus in rod outer segment targeting and alignment of disk incisures was studied recently (Ritter et al., 2004; Tam et al., 2004). The C-terminus peripherin/*rds* fusion protein localized uniformly to disk membranes while the Rom-1 C-terminus did not promote rod outer segment localization. The GFP-fusion proteins did not immunoprecipitate with peripherin/*rds* or Rom-1, suggesting that this region does not form intermolecular interactions and is not involved in subunit assembly. Interestingly, presence of GFP-peripherin/*rds* fusions correlated with disrupted outer segments structure which may reflect competition of the fusion proteins for other proteins that interact with peripherin/*rds*.

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Table 21.1Transgenic animal models of retinal diseases caused by mutations in *peripherin/rds*.

Mutations and Diseases	Animal Models	Phenotype in the Animals	Pathogenesis of Retinal Degeneration	References
L185P/Rom-1 null (RP)	Transgenic mice	Rod degeneration	Haploinsufficiency	Kedzierski et al., 2001
	Transgenic enopus	Aggregated in the inner segment		Loewen et al., 2003
C214S (RP)	Transgenic mice	Rod degeneration	Haploinsufficiency	Stricker et al., 2003
	Transgenic Xenopus	Aggregated in the inner segment		Loewen et al., 2003
P216L (RP)	Transgenic mice	Rod degeneration	Dominant negative effect	Kedzierski et al., 1997
	Transgenic Xenopus	Rod degeneration	Haploinsufficiency	Loewen et al., 2003
307del (RP)	Target-deleting mice	Rod degeneration	Dominant negative effect	McNally et al., 2002
R172W (MD)	Transgenic mice	Cone-dominant degeneration	Haploinsufficiency Dominant negative effect	Li et al., 1999; Ding et al., 2004

Table 21.2

Functional and structural characteristics of the R172W transgenic mice. Retinal characteristics of R172W mice were compared with the age-matched, non-transgenics on the same *rds* genetic background. WT, wild type; OS, outer segment; IS, inner segment. (Adapted from Ding et al., Human Molecular Genetics, 2004, V.13, Issue 18, 2075–2087, by permission of Oxford University Press).

Transgenic Mice	Rod ERG Response	Cone ERG Response	Light-microscopic Appearance	Electron-microscopic Appearance
R172W ^{+/+} / <i>rds</i> ^{+/+}	40% reduction of the WT	75% reduction of the WT	Disorganization and shortening in OS length	Disruption of OS structure
R172W ^{+/-} / <i>rds</i> ^{+/-}	81% increase of the <i>rds</i> ^{+/+}	60% reduction of the <i>rds</i> ^{+/+}	Improvement on OS structure	Improvement on OS structure
R172W ^{+/+} / <i>rds</i> ^{-/-}	30% rescue of the WT	No rescue	Partial rescue and OS formation	Restoration in OS and IS structure