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Progress in Gene Therapy for Rhodopsin Autosomal Dominant Retinitis Pigmentosa

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Abstract

This brief review summarizes the major proof-of-concept gene therapy studies for autosomal dominant retinitis pigmentosa (RP) caused by mutations in the rhodopsin gene (*RHO*-adRP) that have been conducted over the past 20 years in various animal models. We have listed in tabular form the various approaches, gene silencing reagents, gene delivery strategies, and salient results from these studies.

Keywords

Autosomal dominant retinitis pigmentosa; Rhodpsin; Gene therapy; Knockdown and replacement

19.1 Introduction

The P23H mutation in rhodopsin (*RHO*) was the first genetic mutation identified to be causally associated with retinitis pigmentosa (RP) (Dryja et al. 1990). Currently, over 150 unique mutations in *RHO* are known to cause ~40% of all autosomal dominant forms of RP (adRP), and *RHO* P23H accounts for ~10% of *RHO*-adRP among the US Caucasian population (https://sph.uth.edu/Retnet). Mutant RHO proteins cause disease via either a dominant negative or a toxic gain-of-function effect. While gene augmentation with a wild-type copy of *RHO* may be sufficient to dilute out the effects of a dominant negative mutant protein, a gene knockdown strategy is more likely to be beneficial for the toxic gain-of-function mutations. In the past 20 years, significant progress has been made in the field of gene therapy with very promising results in preclinical animal models. A number of transgenic rodent and pig models of *RHO*-adRP (listed in Table 19.1) have been used to evaluate gene knockdown, gene augmentation, gene editing, or combined gene knockdown and replacement strategies. The latter approach delivered within a single AAV vector was recently shown to successfully prevent the onset of photoreceptor degeneration in the *RHO*-T4R dog, the only currently available naturally occurring animal model of this disease (Cideciyan et al. 2018). Gene editing using CRISPR-Cas9 may be an elegant approach to specifically correct common *RHO* mutants such as P23H; however, due to the wide mutational heterogeneity in *RHO*, a mutation-independent strategy that combines knockdown with gene replacement could be an economically attractive therapy to target all

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forms of *RHO*-adRP. This review presents a tabular summary of all preclinical studies in this field, spanning 20 years, from 1998 to present.

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Table 19.1

Summary of all major gene therapy studies for treatment of *RHO*-adRP, grouped by therapeutic strategy

Allele specificity (target)	Silencing and/or replacement reagents	Delivery vector	Animal model	Salient results	References
Treatment strategy: knockdown					
Mutation dependent (mouse P23H)	Ribozyme *Hp11*	AAV2-BOPS-*Hp11*	P23H-3 rat	15% KD of mutant RNA compared to control eye. 12% ONL loss at P60–90 vs. 40% in control eyes. Scotopic ERG b-wave 30% >control eye	(Lewin et al. 1998)
	Ribozyme *Hh13*	AAV2-BOPS-*Hh13*		11% KD of mutant RNA compared to control eye. 20% ONL loss at P60–90 vs. 40% in control eyes. Scotopic ERG b-wave 45% >control eye	
Mutation dependent (mouse P23H)	Ribozyme *Hp11* Ribozyme *Hh13*	AAV2-BOPS-*Hp11*; AAV2-BOPS-*Hh13*	P23H-3 rat	Long-term (8 months) ONL and ERG rescue PI at P15 (before RD onset) 0.3-month ONL and ERG rescue PI at P60–P90 (40% PR loss)	(LaVail et al. 2000)
Mutation dependent (mouse P23H)	*siRNA0, shRNA0* (*shP23H*)	AAV2/5-U1-*shP23H*	P23H-3 rat	68% KD (at 3–4 months) 61% KD (at 4–7 months) of mouse P23H RNA; ERG decline and no ONL rescue	(Tessitore et al. 2006)
Mutation dependent (mouse P23H; mouse RHO)	Antisense oligonucleotide: *ASO2, ASO3*	Intravitreal ASO injection	WT $RHO^{+/+}$mouse	50% (ASO3) −70% (ASO2) KD of mouse RHO	(Murray et al. 2015)
			P23H-1 rat	30% KD of mouse P23H RHO (ASO3); limited ERG rescue; ONL and OS rescue	
Mutation independent (mouse, dog RHO)	Ribozyme *Rz397*	AAV2-mOP-*Rz397*	$RHO^{+/+}$ mouse	50% KD of RHO protein (compared to control eye); reduced ERG b-wave amplitude but no ONL or OS loss	(Gorbatyuk et al. 2005)
			$RHO^{+/-}$ mouse	80% KD of RHO protein (compared to control eye); reduced ERG b-wave amplitude and 30% ONL loss	
Mutation independent (mouse, dog, human RHO)	Ribozyme: *Rz525*	AAV2/5-mOP-*Rz525*	P23H-3 rat	46% KD of mouse P23H RNA; no change in protein levels; ONL rescue; ERG rescue but decline over time	(Gorbatyuk et al. 2007a)
Mutation independent (mouse, dog, human RHO)	shRNA: *shRNA301*	AAV2/5-H1-*shRNA301*	$RHO^{+/+}$ mouse	49% KD of mouse RHO RNA	(Gorbatyuk et al. 2007b)
			$RHO^{+/-}$ mouse	30% KD of mouse RHO RNA; 60% KD of RHO protein; reduced ERG amplitudes and ONL loss	
Mutation independent (human RHO)	shRNA: *shBB*	AAV2/5-H1-*shBB*	$NHR^{+/-}$ $RHO^{-/-}$ mouse	90% KD of human RHO RNA in FACS sorted PRs	(O'Reilly et al. 2007)
Mutation independent (human RHO)	shRNA: *shQ1*	AAV2/5-H1-*shQ1*	$NHR^{+/-}$ $Rho^{-/-}$ mouse	95% KD of human RHO RNA in FACs sorted PRs; reduced ERG and loss of rod OS and RHO immunostaining	(Chadderton et al. 2009)
			$hP347S^{+/-}$ $RHO^{+/-}$	Improved ONL thickness and ERG up to 10 weeks PI but not stable: loss of ONL thickness between 5 and 10 weeks PI	

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Allele specificity (target)	Silencing and/or replacement reagents	Delivery vector	Animal model	Salient results	References
Mutation independent (human RHO CRE)	Zinc finger artificial transcription factors: *ZF-R2*; *ZF-R6*	AAV2/8-RKp-*ZF-R6*	hP347S$^{+/-}$ Rho$^{+/+}$ mouse	26% KD of hP347S RHO RNA in Tx area; partial ERG and ONL rescue	(Mussolino et al. 2011)
Mutation independent (human and pig RHO CRE)	Zinc finger DNA-binding domain: *ZF6-DB*	AAV2/8-CMV-*ZF6*	RHO$^{+/+}$ pig	45% KD of WT pig RHO at 15 days PI, collapse of OS	(Botta et al. 2016)
			hP347S$^{+/-}$ Rho$^{+/+}$ mouse	ERG rescue at P30 (injection at P14)	
Mutation independent (dog, human RHO)	shRNA: *shRNA$_{820}$*	scAAV2/5-H1-*shRNA$_{820}$*	RHO$^{+/+}$ dog	8 weeks PI: RHO RNA 0–3%, RHO protein 15% of control at highest safe viral dose. Shortening of OS, loss of immunolabeling	(Cideciyan et al. 2018)
			Light sensitive RHO$^{T4R/+}$ dog	6–8 weeks PI: RHO RNA and protein levels, structural changes, similar to seen in treated RHO$^{+/+}$. ONL preservation in treated area after 8–10 weeks PI, 2 weeks after light exposure	
Treatment strategy: replacement					
Mutation independent	RHO-M (resistant human RHO)	Tg RHO-M mouse	RHO-M$^{+/-}$ RHO$^{-/-}$ mouse	Rescue of rod ONL and ERG loss	(O'Reilly et al. 2007)
				Single copy of resistant human RHO transgene rescues ONL, OS, and ERG loss; leads to RHO RNA expression (~ 75% of RHO$^{+/+}$) and expression of RHO in OS	(O'Reilly et al. 2008)
Mutation independent	Various *RHO-BB* (resistant human RHO)	AAV-mOP *RHO-BB24*	Rho$^{-/-}$ mouse	ONL rescue + OS formation; rescue of rod ERG but decline from 6 to 12 weeks of age	(Palfi et al. 2010)
Treatment strategy: augmentation					
Mutation independent	*RHO301* (mouse RHO resistant to shRNA301)	AAV2/5-mOP-*RHO301*	hP23H$^{+/-}$ RHO$^{+/+}$ mouse	Twofold increase in total RHO RNA and 58% increase in RHO monomer protein; ERG and ONL rescue up to 6-month PI (at Pl5)	(Mao et al. 2011)
Mutation independent	*RHO-BB* (human RHO resistant to shBB)	AV2/8–1.7 RHOp-*RHO-BB*; AAV2/rh10–1.7 RHOp-*RHO-BB*	RHO$^{-/-}$ mouse	75% of RHO RNA levels as in NHR$^{+/-}$ Rho$^{-/-}$; ONL rescue; rod expression in OS, formation of OS, ERG rescue, visual acuity rescue	(Palfi et al. 2015)
Treatment strategy: knockdown and replacement					
Mutation independent (mouse RHO)	shRNA: *shMR3* siRNA: *siMR3* Resistant RHO: *MR7*	*shMR3* and resistant RHO *MR7* (as plasmids)	WT mouse (liver)	shMR3 + mouse RHO, 90% KD (in liver); shMR3 + MR7, 0% KD	(Kiang et al. 2005)
Mutation independent (human RHO)	shRNA: *shBB* Resistant RHO: *rBB*	AAV2/5-H1-*shBB*-mOP-*rBB*	hP23H$^{+/-}$ Rho$^{+/-}$ mouse	ONL: 33% thicker than control eye at P10	(O'Reilly et al. 2007)
	shRNA: *shQ1* Resistant RHO: *rQ1*	AAV2/5-H1-*shQ1*-mOP-*rQ1*	WT mouse (liver)	ONL: 33% thicker than control eye at P10	
Mutation independent (mouse, dog, human RHO)	shRNA: *shRNA301* Resistant mouse RHO: *RHO301*	AAV2/5-H1-*shRNA301*-mOP-*RHO301*	hP23H$^{+/-}$ RHO$^{+/-}$ mouse	74% KD of endogenous (human P23H and mouse RHO) RNA; 2X increase in total RHO RNA (compared to control eye); 2X increase in RHO protein (compared to control eye); long-term (9 months) ERG, and ONL and OS rescue	(Mao et al. 2012)

Allele specificity (target)	Silencing and/or replacement reagents	Delivery vector	Animal model	Salient results	References
Mutation independent	*ZF6* and *hRHO*	AAV2/8-RHOΔ-*ZF6*-GNAT1-*hRHO*-WPRE	$RHO^{+/+}$ pig	38% KD of pig RHO; replacement with hRHO protein; OS structure better preserved than with ZF6 alone	(Botta et al. 2016)
Mutation independent (dog, human RHO)	shRNA: $shRNA_{820}$ Resistant human RHO: human RHO_{820}	scAAV2/5-hOP-RHO_{820}-H1-$shRNA_{820}$	Light-sensitive $RHO^{T4R/+}$ dog; complete ONL degeneration in 2 weeks post light exposure.	9 weeks PI: Dog RHO RNA 15% of untreated control eye; human RHO RNA 5–9% of canine RHO in untreated control eyes. Total RHO protein: 18% compared to untreated area 13 weeks PI: Dog RHO RNA 1–2% of untreated control eye; human RHO RNA 118–132% of canine RHO in untreated control eyes. 32% compared to untreated area Preservation of ONL, OS, and ERG in the treated area even after repeated light exposure (light exposure at 11, 15, 25, and 37 weeks PI; retinal assessment at 13, 17, 27, and 37 weeks)	(Cideciyan et al. 2018)
Treatment strategy: CRISPR-Cas9 gene editing					
Mutation dependent (mouse RHO, S334 locus)	*spCas9/sgRNA*	*sgRNA-spCAs9* plasmid	S334ter-3 rat	Cleavage efficiency: 33–36%; ONL rescue (8 rows vs 1 in Ctrl); OS formation, improved optokinetic response, no ERG rescue	(Bakondi et al. 2016)
Mutation independent (human RHO)	*hSpCas9/sgRNA1*, *sgRNA3*, or 2 *sgRNAs*	CRISPR-Cas9-2sgRNA plasmid	$hP23H^{+/-}$ $RHO^{-/-}$ (very fast RD) mouse	Editing efficiency, 4–33% in transfected rods; KD of RHO protein, 56–77% in transfected rods; no structural or functional rescue shown	(Latella et al. 2016)
Mutation dependent (human P23H)	*saCas9/sgH23*	AAV2/5-*sgH23*–2-*saCas9*	hP23H Tg pig	NHEJ editing in 2 out of 5 pigs but low efficiency (3.4–4.4% alleles showed NHEJ)	(Burnight et al. 2017)

KD knockdown, *PR* photoreceptors, *Tg* transgenic, *ONL* outer nuclear layer, *OS* outer segment, *PI* postinjection, *CRE* cis-regulatory element, *WPRE* woodchuck hepatitis virus posttranscriptional regulatory element. *Promoters listed: BOPS* bovine opsin promoter, *mOP* mouse proximal opsin promoter, *U1* human U1 small nuclear RNA promoter, *H1* human H1 RNA polymerase III promoter, *GNAT1* human guanine nucleotide-binding protein 1 promoter, *CMV* cyto-megalovirus promoter, *RKp* human rhodopsin kinase promoter, *1.7 RHOp* 1.7 kb mouse rhodopsin promoter