RD Genes Associated with High Photoreceptor cGMP-Levels (Mini-Review)

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Abstract

Many RD-causing mutations lead to a dysregulation of cyclic guanosine monophosphate (cGMP), making cGMP signalling a prime target for the development of new treatment approaches. We showed previously that an analogue of cGMP, which inhibited cGMP signalling targets, increased photoreceptor viability in three rodent RD models carrying different genetic defects, in different RD genes. This raises the question of the possible generality of this approach as a treatment for RD. Here, we review RD genes that can be associated with high cGMP and discuss which RD genes might be amenable to a treatment aimed at inhibiting excessive cGMP signalling.

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40.1 Introduction

The degeneration and loss of photoreceptors in retinal degeneration (RD)-type diseases are a major unmet medical problem. Development of treatments for RD is hampered by the vast genetic heterogeneity of this group of diseases, with disease-causing mutations known in over 260 genes (https://sph.uth.edu/retnet; information retrieved October 2018). Mutations causing RD often affect genes relating to the photoreceptor phototransduction cascade. Within this cascade, the signalling molecule cyclic guanosine monophosphate (cGMP) occupies a key position. cGMP is synthesized by retinal guanylyl cyclase (retGC) in a highly regulated way, allowing cGMP to activate and open its prototypic photoreceptor target, the cyclic nucleotide gated (CNG) ion channel. This raises intracellular Na^+ and Ca^{2+} levels and promotes the conversion of light to an electrochemical signal (Kulkarni et al. 2016). Light-dependent sequential activation of the photopigment rhodopsin and the G-protein transducin activate the enzyme phosphodiesterase 6 (PDE6), which hydrolyses cGMP, leading to the closure of CNG channels. Seminal research

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performed already in the 1970s established that high levels of cGMP were associated to and likely causal for photoreceptor degeneration (Farber and Lolley 1974; Lolley et al. 1977). Because of this pathologic aspect of cGMP in photoreceptors, we hypothesized several years ago that interventions in cGMP signalling might constitute a viable therapeutic avenue applicable to many different RD-causing mutations.

40.2 Inhibition of cGMP Signalling Protects Photoreceptors

In an initial study, we highlighted the possibility to use inhibitory analogues of cGMP to reduce photoreceptor cell death in vitro, in organotypic retinal explant cultures derived from the *Pde6b*-mutant *rd1* mouse and the *Prph2*-mutant *rd2* mouse (Paquet-Durand et al. 2009). In a comparative study, using ten different RD animal models, we subsequently found also that mutations in RD genes different from PDE6 could cause an excessive accumulation of cGMP in photoreceptors (Arango-Gonzalez et al. 2014), underlining the possibility that cGMP could represent a relative mutation independent target in RD. We then set out to develop a combination of inhibitory cGMP analogues with a drug delivery system (Maussang et al. 2016) that would enable these compounds not only to reach the photoreceptors in vivo, across the blood retinal barrier, but also to achieve favourable pharmacokinetics and improved bioavailability. Notably, the lead compound formulation protected rod photoreceptors in vivo in the *rd1*, *rd2* and *rd10* animal models for the RD-type disease retinitis pigmentosa (RP) (Vighi et al. 2018). Furthermore, the rod photoreceptor protection observed with this treatment resulted in marked preservation of cone photoreceptor functionality.

We thus found that animals suffering from mutations in very different genes (*rd1: Pde6b* – functioning in phototransduction; *rd2: Prph2* – important for outer segment structure) showed high levels of photoreceptor cGMP and benefitted from this treatment. Together with the fact that high cGMP indeed has been seen in yet other models (Arango-Gonzalez et al. 2014), this raises the question which else of the vast array of RD genes can be, in one way or the other, linked to cGMP dysregulation and which thus may be identified as additional targets for such treatment (Fig. 40.1).

To give us a better perspective of this, we here reviewed RD genes that can be surmised to associate with high levels of cGMP in photoreceptors. This in turn could help to produce future estimates as to how many RD patients might be amenable to a treatment targeting cGMP signalling.

40.3 RD Genes Connected to Excessive Photoreceptor cGMP Production

In photoreceptors, cGMP is produced by retGC, a protein encoded by the *GUCY2D* gene in human rods, and in which gain-of-function mutations cause rapid cGMP-dependent photoreceptor loss (Sato et al. 2018). The activity of retGC is regulated by a guanylyl cyclase activating protein (GCAP), encoded by the *GUCA1A* and *GUCA1B* genes in rods and cones. When Ca^{2+} levels are low, GCAP activates retGC to produce cGMP, while in high Ca^{2+} concentrations, GCAP inhibits retGC (Tucker et al. 1999).

This dependence of cGMP synthesis on Ca^{2+} levels provides for a regulatory feedback loop in which cGMP-dependent activation of CNG channels leads to Ca^{2+} influx, which in turn inhibits further cGMP synthesis and limits photoreceptor cGMP to physiological levels of 1–5 µM (Olshevskaya et al. 2002). This also means that when CNG channels are dysfunctional, the negative feedback control of retGC is missing, allowing cGMP levels to overshoot to extremely high concentrations. Such dysfunction may come from the known RD connected mutations in the CNG subunit genes *CNGA1*, *CNGA3*, *CNGB1* and *CNGB3* (Reuter et al. 2008; Biel and Michalakis 2009; Paquet-Durand et al. 2011). retGC is additionally inhibited by the RD3

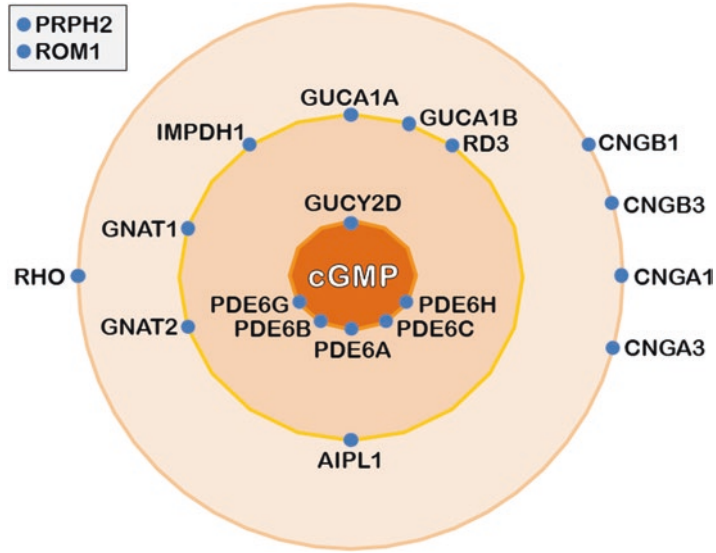

Fig. 40.1 RD genes associated with high levels of photoreceptor cGMP. The different circles show RD genes related to cGMP signalling in various ways: the innermost circle displays genes directly involved in cGMP synthesis and hydrolysis. The second circle relates to genes that regulate the synthesis/hydrolysis, while the third level indicates genes known to affect intracellular cGMP via genes shown in the second circle. The grey box shows genes found or reasoned to be associated with high cGMP but for which no clear causal relationship has been established so far

protein, so that loss-of-function mutations in the *RD3* gene will also cause high cGMP and photoreceptor death (Peshenko et al. 2016)

Furthermore, retGC activity critically depends on the availability of its substrate GTP. The rate-limiting step in the synthesis of the required guanine nucleotides is mediated by inosine monophosphate dehydrogenase 1 (IMPDH1). Remarkably, RD-causing mutations in the *IMPDH1* gene preserve its enzymatic activity; however, the mutant enzyme is no longer regulated by nucleotide binding (Xu et al. 2008), possibly causing an overproduction of retGC substrate.

40.4 RD Genes Involved in cGMP Hydrolysis

The enzyme hydrolysing cGMP in photoreceptors is PDE6, a heterodimer consisting of one alpha (*PDE6A*) and one beta (*PDE6B*) subunits in rods (McLaughlin et al. 1993; Dryja et al. 1995), which in turn form a complex with the small inhibitory gamma (*PDE6G*) subunit (Tsang et al. 1996). Cone PDE6 is a homodimer encoded for by the *PDE6C* gene (Thiadens et al. 2009), with the specific gamma subunit encoded by the *PDE6H* gene (Brennenstuhl et al. 2015). In both rods and cones, the functional assembly of the subunits is mediated by the AIPL1 protein. Hence, loss-of-function mutations in the *AIPL1* gene lead to PDE6 dysfunction, cGMP accumulation and rapid photoreceptor loss (Ramamurthy et al. 2004).

PDE6 is activated by transducin, a protein encoded by the *GNAT1* gene in rods and *GNAT2* in cones. Transducin in turn is activated by rhodopsin (*RHO*) and cone opsins, respectively, once their photopigment retinal has been activated by a photon of light. Thus, PDE6 activity depends on the function of both rhodopsin and transducin, explaining why certain mutations in these genes lead to insufficient cGMP hydrolysis (Kohl et al. 2002; Arango-Gonzalez et al. 2014; Mejecase et al. 2016).

40.5 Other RD Genes that May Be Associated with High cGMP in Photoreceptors

The gene *PRPH2* encodes for the peripherin-2 protein, which together with the rod outer segment membrane protein 1 (*ROM1*) is essential for the formation of photoreceptor outer segments (Goldberg et al. 2016). Mutations in *PRPH2* lead to an absence of outer segments, which likely leads to an ectopic and dysregulated expression of outer segment enzymes. Interestingly, *Prph2* mutations in the mouse cause high levels of photoreceptor cGMP (Paquet-Durand et al. 2009), suggesting that *Rom1* mutations would have similar effect.

We may expect that also photoreceptor-specific transcription factors such as *CRX*, *NRL* and *NR2E3*, which regulate the expression of genes linked to phototransduction (Pittler et al. 2004; Xu et al. 2013), when mutated may eventually lead to lack of phototransduction activity and consequently increased cGMP. Similarly, mutations in genes involved in the correct trafficking of phototransduction proteins could result in aberrant cGMP production. For instance, the REEP6 protein appears to be important for the trafficking of retGC to the photoreceptor outer segment (Agrawal et al. 2017). Whether *REEP6* gene mutations cause RD via aberrant cGMP ectopic production in the photoreceptor cytoplasm is currently not known.

40.6 Conclusion

The data available to date indicates that mutations in at least 20 different RD genes may lead to excessive accumulation of cGMP in photoreceptors. Further studies on RD models, with mutations in genes mediating other functions in photoreceptors, are needed to comprehensively assess the effects of mutations on photoreceptor cGMP levels. Given the antecedents, one may assume that such studies will in the future connect even more RD genes to high photoreceptor cGMP. Furthermore, we need to consider that in each RD gene, gain-of-function and loss-of-function mutations can have opposite effects on cGMP (Power et al. 2019). For instance, most RD-causing mutations in retGC appear to be gain-of-function mutations (Sato et al. 2018), while a retGC gene knockout in the mouse, with expected no photoreceptor cGMP production, also causes photoreceptor loss (Yang et al. 1999). Irrespective of this, it is likely that therapeutic approaches aimed at lowering cGMP signalling will be applicable to several RD genes and gene mutations causing high photoreceptor cGMP.

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Conflict of Interest Statement François Paquet-Durand, Valeria Marigo, and Per Ekström have filed for three patents on the synthesis and use of cGMP analogues (PCTWO2016/146669A1, PCT/EP2017/066113, PCT/EP2017/071859) and have obtained a European Medicine Agency orphan drug designation for the use of the cGMP analogue DF003 for the treatment of retinitis pigmentosa (EU/3/15/1462). François Paquet-Durand, Valeria Marigo, and Per Ekström are shareholders of, or have other financial interest in, the company Mireca Medicines GmbH (www.mireca.eu), which intends to forward clinical testing of DF003.

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