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Spotlight on vision

Therapeutic strategy for handling inherited retinal degenerations in a gene-independent manner using rod-derived cone viability factors

Une stratégie thérapeutique des dégénérescences rétiniennes héréditaires indépendante du gène causal : la voie des facteurs de survie des cônes

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ABSTRACT

The most common hereditary retinal degeneration, retinitis pigmentosa (RP), leads to blindness by degeneration of cone photoreceptors. Meanwhile, genetic studies have shown that a significant proportion of *RP* genes is expressed only by rods, which raises the question of the mechanism leading to the degeneration of cones. Following the concept of sustainability factor cones, rods secrete survival factors that are necessary to maintain the cones, named Rod-derived Cone Viability Factors (RdCVFs). In patients suffering from RP, loss of rods results in the loss of RdCVFs expression and followed by cone degeneration. We have identified the bifunctional genes nucleoredoxin-like 1 and 2 that encode for, by differential splicing, a thioredoxin enzyme and a cone survival factor, respectively RdCVF and RdCVF2. The administration of these survival factors would maintain cones and central vision in most patients suffering from RP.

RÉSUMÉ

La dégénérescence héréditaire rétinienne la plus fréquente, la rétinopathie pigmentaire (RP), conduit à la cécité par dégénérescence des photorécepteurs à cônes. Pourtant, les études génétiques ont montré qu'une proportion significative des gènes de *RP* n'est exprimée que par les bâtonnets, ce qui soulève la question du mécanisme entraînant la dégénérescence des cônes. Suivant le concept de facteur de viabilité des cônes, les bâtonnets sécrètent des facteurs de survie qui sont nécessaires au maintien des cônes. Chez les patients souffrant de RP, de la perte des bâtonnets résulte celle de ces facteurs et donc la dégénérescence des cônes. Nous avons identifié les gènes bi-fonctionnels

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nucleoredoxin-like 1 et *2*, qui codent chacun par épissage différentiel pour une enzyme de type thiorédoxine et un facteur de survie des cônes. L'administration de ces facteurs de survie permettrait de maintenir les cônes et la vision centrale chez la plupart des patients souffrant de RP.

1. Introduction

In patients suffering from retinitis pigmentosa (RP), the most common form of inherited retinal degeneration, the vision loss develops in two successive steps. Early in their adult life, these patients lose their ability to see in dim light conditions, which refers to a night vision lost, and corresponds to the loss of function and degeneration of rod photoreceptors. This is felt as a minor handicap, especially in individuals affected by congenital stationary night blindness, another type of inherited retinal disease characterized exclusively by lack of rod function; in our current well-illuminated environment, these people retain an almost normal way of life [1]. For patients with RP, the disease then progresses through another debilitating step resulting from loss of function and degeneration of the second class of photoreceptors, the cones, which dominate at the centre of the retina. Cones represent only 3–5% of all photoreceptors in most mammals, but their role for vision is essential. This secondary event leads to central vision loss and potentially complete blindness. Because the cones underlie all visual functions in lighted environment, cone rescue was deemed to be a clinically relevant target. Cone death being widespread cone death in the naturally occurring *rd1* mutant mouse, a model of RP, has been well described [2]. The degeneration does not arise in this model through a mutation within cone photoreceptor cells, but as a result of a recessive mutation in the rod photoreceptor-specific cGMP phosphodiesterase-β subunit, and is consequently non-cell autonomous [3]. This mutation also leads to rod-cone degeneration in humans [4]. Various hypotheses have been proposed to explain the secondary loss of cones: toxic by-products of rod cell death, structural alterations in the microenvironment, abnormal synaptogenesis, secondary changes at the level of the retinal pigmented epithelial or the glial cells, and loss of trophic interaction, reviewed in [5]. To discriminate among the possibilities, we demonstrated that grafting normal photoreceptors (97% rods) into the eye of the rodless *rd1* mouse before the cones degenerate exerts a positive effect on the host retina cones [6]. Subsequent work *in vitro* showed that this paracrine protective activity was carried by molecules, most likely proteins, secreted in the presence of rods [7,8]. Globally, this part of our work revealed the existence of proteins that we designated as Rod-derived Cone Viability Factors (RdCVFs) [9] and was recently further supported by the work of Punzo et al. [10]. If so, following rod death in the first phase of the disease, the degeneration of cones would be triggered by a mechanism reminiscent of the loss of trophic support. This cone degeneration mechanism is also likely to occur in human retinas presenting RP, when most rods have degenerated but before central vision is affected [11].

Since the cones represent only 3–5% of the photoreceptors in most mammals, we studied the survival of cones in cone-enriched cultures prepared from the retina of chicken embryos [12,13]. When plate at low density and chemically defined medium, the progenitor cells isolated from chicken embryos at embryonic day 6, stages 29 [14] differentiate into mostly photoreceptors in the absence of cell fade cues. Since the retinas of birds, contrarily to mammals, are dominated by cones, these primary post-mitotic cultures are enriched (60–80%) in cones. These cones develop *in vitro* a complex set of photoreceptor-specific properties, as polarized structural and opsin immunoreactivity. Interestingly, they are also capable of responding to light [15]. When maintained on a 12-h-light/12-h-dark cycle, approximately half of the cultured photoreceptors elongate in response to light, and contract in response to darkness [16]. These primary post-mitotic neurons degenerate over a period of a few days. We observed an increase in cell survival when cultured in the presence of fractions of secreted proteins isolated from the retina of wild-type mice [8]. The active molecule(s) are heat labile, with an apparent molecular weight of 25 kDa. Because of the simplicity of getting fertilized eggs, the chicken embryo retinal culture system is an easy, reproducible, and high-throughput cone viability assay. We made the hypothesis that RdCVF protein is encoded by a messenger RNA expressed in the normal retina and the encoded polypeptide would be secreted by any cell types. Based on this minimal two-term definition, we constructed an expression library from wild-type mouse retina and used expression cloning methods to screen genes for their potential to promote chicken cone survival. Briefly, pools of 100 clones from the expression library were used to transfect a cell line (COS-1). The conditioned media from the transfected COS-1 cells were added to primary chicken cone cells seeded into 96 well-plates. After seven days, viable cells from the cone-enriched cultures were counted using an in-house high content screening method and were compared to counts from the empty library vector. We screened 2100 pools, corresponding to 210,000 individual clones. Pool number 939 contained twice as many living cells as the negative controls. Using limiting dilution, we isolated clone 939.09.08 and found that it contained a 502 base pairs insert with an open reading frame encoding a putative 109 amino acids polypeptide, Rod-derived Cone Viability Factor [17]. This unbiased approach let us to identify a novel gene called now *nucleoredoxin-like 1* (*Nxnl1*). RdCVF is translation product made from an unspliced mRNA encoding the exon 1 with an in-frame stop codon of the nucleoredoxin-like gene (*Nxnl1*) and corresponds to a truncated thioredoxin-like protein with no thiol-oxidoreductase activity (Fig. 1a). The other product (RdCVFL),