Otx2-Genetically Modified Retinal Pigment Epithelial Cells Rescue Photoreceptors after Transplantation

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In the retina of vertebrates, the capture of photons of incident light, the initial step of visual perception by photoreceptor cells, results from the trans-isomerization of a derivative of vitamin A covalently linked to a seven-transmembrane, G protein–coupled receptor, the opsin molecule. Opsins are highly concentrated in a subcellular structure, the outer segment, made of multiple disks or of invaginations of lipid bilayers, in rod and cone photoreceptors, respectively. This cyto-architecture permits maximal light perception. The trans-isomerization of the vitamin A-derivative triggers a conformation change in the seven surrounding helixes of opsin and initiates the phototransduction cascade. This is facilitated by the optimal fluidity of the lipids of the outer segments that are rich in poly-unsaturated fatty acids (PUFA). The sophistication of this photon-capture system is the cause of its vulnerability. PUFA are prone to oxidation, either by reactive oxygen species produced by metabolic leakage of the mitochondrial respiratory chain or directly by photo-oxidation. The outer segment must constantly be renewed to keep the system operational.

Photoreceptor outer segments point in the direction of the retinal pigment epithelium (RPE), corresponding to the opposite direction to the incident light. RPE cells phagocytize daily 10% of the outer segments that are then renewed by synthesis in the photoreceptor inner segment. Glucose supply from blood circulation through the RPE is metabolized by photoreceptors through aerobic glycolysis to produce triglycerides entering in the composition of phospholipids of the outer segments. For this gigantic energetic task, rods assist metabolically cones. This *ménage à trois* (RPE-rods-cones) is reflected in the pathophysiology of retinal diseases. Retinitis pigmentosa (RP), the most prevalent Mendelian retinal degeneration, occurs when any of the 63 genes identified so far is mutated. Rods degenerate through apoptosis leading to night vision, but also leaving cones without metabolic assistance. The predominance of rods over cones, a contingence linked to the evolutionary history of the mammalian retina, results in the dysfunction of cones and ultimately to their death, the consequence is an irreversible blindness for RP patients. In primates, cones are concentrated at a specific point, called the fovea within the central region of the retina, the macula. This region that sustains most of our visual acuity is also affected in another and more frequent blinding disease, aged-related macular degeneration (AMD). RP genes have nothing in common with the 34 loci where AMD risk alleles are located indicating that the function of cones is harmed in these retinal diseases by distinct pathophysiological mechanisms that converge.

The genetic heterogeneity of RP and the genetic complexity of AMD were the key arguments for the development of therapeutic approaches that target common pathways that would alleviate the need for gene- and mutation-restricted cures to treat these threatening diseases. The restoration of metabolic and redox signaling between rods and cones by the products of the *NXNL1* is one of such approaches for RP. Neovascularization is a clinical sign that is amenable to anti-VEGF therapy of AMD, but concerns only a minority of patients. More generally, oxidative damages accumulation with age and the chronic non-resolving inflammation are environmental risk factors. The production of cytotoxic byproducts of vitamin A close to RPE cells might participate in disease progression that is initiated in genetically predisposed people by local and abnormal activation of the complement cascade. Because the dysfunction and progressive destruction of the RPE are considered as instrumental, replacing the affected RPE by transplantation of unaffected one has been envisioned for years as a potential treatment of AMD.

In medical practice, cell transplantation requires the production of healthy RPE cells. Consequently, the expected therapeutic benefit on AMD will depend on the ability to produce RPE cells that must be amplified *in vitro* before transplantation. Our study reveals that the expansion of primary pig RPE cells is accompanied by the reduction of the expression of messenger RNAs of 27 out of 37 genes

involved in RPE function, designated later as RPE markers. Among these genes, two transcription factors of the homeobox family, OTX2 and CRX were selected as candidate upstream regulators of a gene network preventing the down-regulation of the RPE markers. OTX2 was further studied since it was shown to regulate CRX expression and because its inactivation in mature RPE of the mouse leads to retinal dysfunction. We demonstrated the ability of OTX2 to restore the expression of 10 of these RPE markers after the infection of pig primary RPE cells with a recombinant adeno-associated vector (AAV) serotype 1. A direct effect of OTX2 on the activity of promoter of these RPE markers was demonstrated in a subset of four genes. To ensure that the observed phenomenon was not restricted to the studied species, we performed a similar analysis with human induced pluripotent (iPS) cells differentiated into RPE and confirm that OTX2 enhances the expression of *CRX*, but also of the potassium voltage-gated channel *KCNJ13* and the lactate transporter *SLC16A8*.

Because the retina of rodents do not have a macula, we pursued our study *in vivo* with the Royal College of Surgeon (RCS) rat, a spontaneous model of a recessive form of RP with a mutation in the gene *Mertk,* expressed specifically by RPE cells and involved in photoreceptor outer segments phagocytosis. Pig primary RPE cells were cultured for one week while infected with AAV2/1-OTX2 or AAV2/1-GFP as negative control. Those cells (50,000 cells/eye) were then injected in the subretinal space of the RCS rat aged 18 days. In this model of RP, both rods and cones degenerate, starting from day 19. The function of photoreceptors was measured using electroretinograhy (ERG). The rod response was maintained in the grafted animals as compared to the unoperated ones, in accordance to previous observations. More importantly, the over-expression of OTX2 in the transplanted cells improve significantly rod function, but also that of cones. The transplantation of RPE-GFP cells increases cone response by 186% and that of RPE-OTX2 cells by 305%. OTX2 increases the effect of RPE transplantation by 164% compared to GFP. Given the average loss of cone vision of about 4% per year for RP patients, 164% would refer to 41 years of maintenance of central vision, a medically significant situation.

The preservation of rod function is correlated with a reduction of the death of rod photoreceptors as scored by optic coherence tomography (OCT). The protection of rods is likely the source of the maintenance of cone function through the *NXNL1* metabolic signaling. The mechanism of action of OTX2 in those experimental conditions is not completely elucidated. The fact that rod preservation was observed at distance from the transplanted cells indicates that the visual benefit does not entirely rely on the restoration of photoreceptor outer segments phagocytosis that is defective in the RCS rat. Our analysis supports the presence of unidentified protective factors secreted by RPE cells transduced with OTX2. We also highlight that the up-regulation of proteins regulating metabolites transport and transmembrane ion gradients could also produce the non cell-autonomous protection of rods.

The maintenance of a proper differentiation status of the grafted cells by OTX2 is relevant for the treatment of AMD by iPS cell transplantation. In that perspective, the lactate transporter *SLC16A8* whose expression in the RPE is directly or indirectly regulated by OTX2 carries a risk allele for AMD.