

## **Neuroprotection of photoreceptor cells in rod-cone dystrophies: from cell therapy to cell signaling**

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Neuroprotection of photoreceptor cells in rod-cone degenerations is primarily targeted at preventing the loss of function. Strategies for protecting rod cells should therefore aim not only at structural preservation but also must be assessed using functional parameters (e.g. ERG). Given the number of mutations leading to an impaired visual response of rods, the preservation of cones is a realistic approach since 1) numerous mutations do not affect proteins expressed by cones; 2) the secondary degeneration of cones is the main event leading to profound visual impairment; 3) even a small proportion of functional cones is sufficient for major visual functions.

Our group has 1) established and confirmed the existence of non cell autonomous mechanisms promoting cone cell viability; 2) shown that rod cell protection or replacement provides a mean to extend the survival of cones; 3) demonstrated that rod-cone trophic interactions are mediated by diffusible proteins; 4) identified by expression cloning a protein mediating such interactions: RdCVF (Rod-derived Cone Viability Factor).

**1)** The rd1 mouse is a model of rod-cone degeneration. A recessive mutation carried by the gene encoding the beta subunit of the rod-phosphodiesterase is leading to the rapid degeneration of rod photoreceptors through apoptosis and a secondary degeneration of cones, while these neurons do not express the mutated gene and are not directly suffering from the enzymatic deficit. This model does mimic the sequence of events in patients affected with Rod-Cone dystrophies i.e. primary loss of dark-adapted vision followed with loss of central and light-adapted vision. By transplanting normal photoreceptors in the subretinal space of the rd1 mouse immediately after rod death, we have demonstrated that the cones from the grafted animal are surviving significantly longer than the shamed animals (Mohand-Saïd et al., 2000). This confirms the existence of non cell autonomous mechanisms promoting cone viability (Mohand-Saïd et al., 1998).

**2)** The most straightforward interpretation of our data is that cone degenerate when rods are missing. We have further validated this model by showing that the trophic factor GDNF, and the pharmacological treatment of the rd1 mouse with the calcium channel diltiazem are generating rod protection that is translated by the persistence of cone function (Frasson et al., 1999<sup>a</sup>; 1999<sup>b</sup>). It should be noticed that future therapeutic approaches aimed at preserving cone function would be optimal.

**3)** In order to study the effect of rods on cones, we have used a co-culture system. Rod-less retinal explants from the rd1 mouse co-cultured with rod-enriched retina show a 40% rescue of cones. Not only this experiment demonstrated that rods are exerting a trophic activity toward cones in vitro, but it also established that the trophic activity is diffusible (Mohand-Saïd et al., 1998). The activity is present in the conditioned media prepared from rod-enriched retina. Using a partial purification scheme, we have shown that the activity is carried by molecules with the physical properties of proteins (Fintz et al., 2003).

**4)** We have adopted a systematic high content screening approach based on a cone-enriched cultures system and an expression cloning protocol to identify from an expression library

clones that encode for cone viability factor. By screening 210,000 clones, we have identified a factor Rod-derived Cone Viability Factor (Léveillard et al., 2004). RdCVF is expressed in a rod dependant manner and the activity of rod-enriched conditioned medium is largely decreased by RdCVF antibodies. The current model is that cones degenerate in the rd1 mouse by trophic support withdrawal after rod degeneration and RdCVF loss of expression. RdCVF is a truncated thioredoxin produced by a novel gene Txnl6. Our laboratory is currently working on RdCVF delivery and signaling.

These studies provide a rationale for protecting non functional rods and clues for broad neuroprotective therapies of rod-cone dystrophies. Therefore, planning for clinical trials implies the identification of genotyped patients with symmetrical progressive disease and the implementation of outcome measurements that allow early and reproducible detection of both rod and cone function and structure. Such facilities and programs (from fine matrix mapping, autofluorescence confocal imaging, optical coherence tomography and adaptative optics are currently implemented at the Clinical Investigation Center of the Centre Hospitalier National d'Ophthalmologie des Quinze-Vingts.

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