Author Response: Possibility of Cytoplasmic Transportation Between Donor–Host Cell Following Photoreceptor Transplantation

We thank Liang and colleagues[1] for their interest and thoughtful analysis of our recent article describing the integration of stem cell–derived photoreceptor transplants into mouse models of cone–rod dystrophy.[2] The authors raise a crucial issue in the field of photoreceptor transplantation: Do donor photoreceptors following transplantation into the subretinal space structurally integrate into the host tissue, fuse with the endogenous cells (cell–cell fusion), or do the latter cells take up cytoplasmic biomaterials from donor cells?

We have already considered these potential pitfalls, and a series of experiments has been conducted to clarify this concern.[3] These include (1) single-cell analysis following transplantation of enhanced green fluorescent protein (eGFP)–labeled donor photoreceptors into Discosoma sp. red fluorescent protein (DsRed)–labeled hosts by imaging flow cytometry; (2) Cre/lox technology in which photoreceptors isolated from a conditional mouse reporter line containing a loxP-flanked stop cassette upstream of the tdTomato reporter gene were transplanted into photoreceptor-specific Cre-recombinase–expressing hosts; and (3) independent labeling of cytoplasm (using transgenic mice expressing eGFP under a neural retina zipper [Nrl] promoter) and nucleus (either by 5-ethynyl-2′-deoxyuridine [EdU] or Y-chromosome staining) of male donor cells following transplantation into female wild-type hosts. Collectively, our new data demonstrate that cytoplasmic content is transferred from donor to host photoreceptors without translocation of the nucleus. Thus, the majority of potentially newly integrated photoreceptors with fluorescently stained cytoplasm in the outer nuclear layer represent host photoreceptors rather than donor photoreceptors, thus contradicting the common view that transplanted photoreceptors structurally integrate into the retinal tissue. The mechanism by which donor and host photoreceptors exchange cytoplasmic biomaterials is currently unknown and does not represent "classical" fusion events that lead to the formation of binucleated heterokaryons, as previously described, for example, following transplantation of bone marrow–derived donor cells.[4] Instead and excitingly, the observed cell material transfer might involve tunneling nanotubes and/or extracellular membrane vesicles such as exosomes. However, all new experiments mentioned above have been performed using primary donor photoreceptors isolated from, and transplanted into, wild-type mice. It remains to be clarified whether pluripotent stem cell–derived donor photoreceptors and host photoreceptors in disease models show a similar propensity for intercellular material transfer.

In view from a different angle, the intercellular interaction observed between donor and host photoreceptors might represent an unexpected mechanism for the treatment of blinding diseases that need to be further explored. Given the improvements in visual performance following transplantation of rod[5,6] or cone-like photoreceptors,[7] transplanted cells might be useful vectors, and hence therapeutic biological tools, for supporting and repairing affected photoreceptors without substituting them. Another potential approach to regenerate murine photoreceptors, the reprogramming of Müller cells by spontaneous cell fusion with transplanted hematopoietic stem and progenitor cells was recently reported.[8]

Altogether, our recent observations further support our strategy that for the development of a photoreceptor replacement therapy, the selection of appropriate animal models is of utmost importance. Severely degenerated models with significant loss of cone and rod photoreceptors, like the Cpfl1/rho$^{-/-}$ double transgenic mouse line described in our study,[2] might represent benchmark models for photoreceptor replacement approaches, as they recapitulate the potential disease stage of patients most likely recruited for initial clinical trials and do not show the described transfer of cytoplasmic materials.[2,9,10]

Again, we thank the authors for their critical analysis and suggestions, as we believe that such debates are essential in this growing field for paving the way to successful clinical translation of cell-based therapies to treat retinal degeneration.

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