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Response to: Janssen et al., “Human ciliary epithelia do express genes with retinal progenitor cell characteristics *in vivo*”

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Dear Editor

We are responding to the letter from Janssen and colleagues, commenting on our recently published review article in this journal (Fischer et al., Exp. Eye Res.) regarding the involvement of the ciliary marginal zone (CMZ) in the development and regeneration of the vertebrate eye.

In our review, we argue that *in vivo* the cells of the pigmented epithelium of the ciliary body (PECB) and non-pigmented epithelium of the ciliary body (NPECB) simply do not function as retinal stem cells. We agree that there is good evidence for some overlap of gene expression between the retinal stem, PECB and NPECB cells, which is not at all surprising given their shared embryonic origin. Indeed, the PECB and NPECB cells in primates appear to express Chx10, Pax6, Six3, Sox2 and Sox9 (Martinez-Navarrete et al. 2008 J Comp Neurol and unpublished observations), homeodomain transcription factors with well-established, important roles during retinal histogenesis and key in maintaining “stemness” phenotype. However, retinal stem cells and CB cells clearly do not share functional overlap *in vivo*. We maintain that although gene expression patterns can imply the function and identity of a cell, the *in vivo* cell behavior, functions, anatomical location and morphology, taken together with gene expression, are comprehensive criteria by which to define the identity of a cell. By this standard, the PECB and NPECB cells are not retinal stem cells.

If one defines a retinal stem cell as a cell based on function, rather than shared expression of a few genes, then a retinal stem cell functions to proliferate, self-renew, and produce daughter cells that populate, and if properly stimulated regenerate, the different types of retinal neurons and Müller glia. One should cautiously apply gene expression profiles to define the identity of cell type. For example, compelling work from the Cepko lab has indicated that Müller glia and retinal progenitors share a significant (more than 60%) overlap in transcriptomes (Blackshaw et al. 2004 PLoS Biol; Roesch et al. 2008 J Comp Neurol). However, under normal conditions Müller glia do not act as retinal stem cells;

Müller glia normally act as glia, supporting metabolic, structural and synaptic needs (among many other function) of retinal neurons. Although Muller glia can be stimulated to become progenitors that regenerate neurons in the fish retina (Bernardos et al. 2007 J Neurosci; Fausett and Goldman 2006 J Neurosci), the capacity of these glia to become progenitors that regenerate neurons is very limited in birds and mammals (reviewed by Gallina et al. 2013 Exp Eye Res). Even in the fish retina, the Müller glia function normally as glia, not stem cells, and must be stimulated to acquire stem cell phenotype. Further, the potential of a cell should not define identity, particularly given that any cell could, in principle, be manipulated to express several key transcription factors to become an induced pluripotent stem cell (iPSC).

Although there is some evidence that NPECB cells in the intact chick eye can be stimulated to produce cells that appear neuronal (Fischer and Reh 2003 Dev Biol), it seems entirely unlikely that these newly generated neuronal cells contribute to visual function given their anterior location and apparent lack of input from photoreceptors or output to higher visual centers. It must be noted that significant stimulation, in the form of repeated intraocular injections of different growth factors was required to induce widespread proliferation of NPECB cells and subsequent generation of a few neuron-like cells (Fischer and Reh 2003 Dev Biol). Significant stimulation can also come in the form of sustained cell culture conditions. For example, clearly the plasticity of PECB cells can be enhanced by long-term culture, treatment with growth factors and proliferation (Ahmad et al. 2000 Biochem Biophys Res Commun; Tropepe et al. 2000 Science). However, the *in vitro* potential of PECB cells as retinal stem cells has been cast in doubt with findings indicating a lack of genuine neurogenesis and maintenance of pigmented phenotype (Cicero et al. 2009 Proc Natl Acad Sci U S A; Gualdoni et al. 2010 Stem Cells).

In conclusion, we maintain that the PECB and NPECB cells do not function as retinal stem cells in normal, healthy eyes, and these cells cannot be stimulated *in vivo* to produce retinal neurons that contribute to visual function in a meaningful manner. Shared expression of “stemness” factors does define the identity of a cell type. We maintain that defining the identity of a cell type should involve a combination of criteria including gene expression, function, morphology and anatomical location. These conclusions do not obviate the evidence that the PECB and NPECB cells can be stimulated *in vitro* to acquire some properties of retinal stem cells, nor do these conclusions obviate the possibility that PECB and NPECB cells could be manipulated and transplanted into the retina for purposes of regeneration.