PERSPECTIVE

Onecut transcription factors in retinal development and maintenance

The retina and its development: The retina is an essential part of the visual system. A myriad of eye diseases are characterized by retinal degeneration, caused by either genetic or environmental factors. This underscores the importance of studying the genetic and molecular mechanisms that regulate the generation and maintenance of neurons in the mammalian retina. Our recent studies demonstrate that two related transcription factors Onecut1 (*Oc1*) and Onecut2 (*Oc2*) regulate multiple cell fates in the mouse retina, and their absence results in progressive retinal neurodegeneration (Wu et al., 2012, 2013; Sapkota et al., 2014). Here we summarize these studies and provide our perspective on these findings.

As part of the central nervous system, the vertebrate retina originates from the anterior neural plate during development. The mature retina possesses seven different cell types-rod and cone photoreceptors, horizontal cells (HCs), bipolar cells, amacrine cells, ganglion cells, and Müller glial cells, all arising from a common pool of retinal progenitor cells (RPCs) in two overlapping but distinct waves of neurogenesis (Livesey and Cepko, 2001). The first wave gives rise to retinal ganglion, horizontal, cone, and amacrine cells, which in the mouse form largely prenatally (early-born cell types), whereas the second wave generates rod, bipolar, and Müller cells, which form mainly postnatally (late-born cell types). With the exception of rods, of which there is only one type, all other retinal cell types exist as two or more subtypes, making the retina a mosaic of around seventy diverse cell (sub) types with a stratified structure. Since retinal neurogenesis, nevertheless, begins with a single pool of multipotent RPCs, there are three major tasks for the regulators of retinal cell fates: driving the birth of the seven major cell types, ensuring the proper temporal cell differentiation, and generating the subtype diversity for each major cell type. Our studies demonstrate that *Oc1* and *Oc2* take part in all three tasks.

The onecut transcription factors and retinal development: *Oc1* and *Oc2* are members of the onecut transcription factor family, which is characterized by the presence of a bipartite DNA-binding region that is composed of an atypical homeodomain and a "cut" domain. They share a high sequence homology in their DNA-binding domains and often exhibit overlapping expression patterns in different tissues during development. We first reported that *Oc1* and *Oc2* are present in early retinal progenitor cells, developing retinal ganglion cells (RGCs), and developing and mature HCs in the mouse retina (Wu et al., 2012). A subsequent loss-of-function study in our lab found that the *Oc1*-null retina has an 80% reduction in HC development but no defect in other retinal cell types (Wu et al., 2013). Similarly, the *Oc2*-null retina exhibits defects also only in HCs, but to a lesser degree, with around a 50% reduction in their development (Sapkota et al., 2014). Given the similar expression patterns of *Oc1* and *Oc2* in the developing retina, it is likely that the relatively minor defects in the single knockout retinas are due to their redundancy in function. This proved to be the case, as in the *Oc1/Oc2* double-null retina, HC and starburst amacrine cell development was completely abolished, and RGC and cone development was significantly reduced (Sapkota et al., 2014). These defects are consistent with the expression patterns of *Oc1* and *Oc2* during development. In addition, there is precocious formation of rod photoreceptors, a late cell type, in the double-null retina. Moreover, the double-null retina has perturbed proportions of various RGC subtypes and increased numbers of S-cone subtype at the expense of M-cone subtype. These observations suggest that the onecut transcription factors function to promote the early cell fates and repress at least one late cell fate (rods), likely by participating in the competence of RPCs for the early retinal cell fates.

Mechanisms of the action of the onecut transcription factors in retinal development: Our studies also shed light on the mechanisms by which *Oc1* and *Oc2* regulate the different retinal cell fates. Through in utero electroporation experiments, we demonstrated that the onecut transcription factors collaborate with Ptf1a, a bHLH transcription factor, to determine the HC fate. Our analysis shows that both Ptf1a and *Oc1* function downstream of the fork head factor FoxN4, but are parallel to each other. Cells expressing just Ptf1a become amacrine cells, whereas cells expressing both *Oc1* and Ptf1a assume the HC fate. These results indicate that onecut transcription factors and Ptf1a together dictate the HC fate. In order to understand how *Oc1* and *Oc2* function at the molecular level, we analyzed the transcriptomes from retinas null for *Oc1* and/or *Oc2* by RNA-seq (Sapkota et al., 2014), which identified alterations in gene expression in the mutant retinas. The results are consistent with the idea that *Oc1* and *Oc2* function redundantly, as they regulate largely overlapping sets of downstream genes. The functions of these downstream genes are consistent with the phenotypes observed in the single and double mutant retinas and confirmed that the Onecut factor promote the early retinal cell fates and repress the late rod fate. Consitent with the reduced number of HCs, downstream genes specifically expressed in HCs, such as *Prox1* and *Lim1*, are significantly down-regulated. In the context of photoreceptors, *Oc1* and *Oc2* activate *Rxrγ* (Retinoid X receptor γ) and *Thrb* (Thyroid hormone receptor β) to facilitate cone genesis and M-cone differentiation, respectively, and repress *Nrl* (Neural retina-specific leucine zipper) to prevent precocious rod generation. Finally, in the context of RGCs, they activate some RGC subtype-specific genes but repress others to fine tune the proportions of different ganglion cell subtypes being generated. Thus, the onecut transcription factors control the specification of early retinal cell lineages by either collaborating with other transcription factors, or directly regulating cell fate determining genes.

Horizontal cells and retinal maintenance: An unexpected finding from our studies is that *Oc1* and *Oc2* are involved in the maintenance of photoreceptors in the mature retina through HCs. HCs synapse with photoreceptors in the outer plexiform layer. Functionally HCs provide inhibitory feedback to photoreceptors to help integrate and regulate the input from multiple photoreceptor cells. However, it has not been known whether HCs play any roles in the survival of photoreceptors. In the absence of *Oc1*, although the only developmental defect is HC deficiency and other retinal cell types appear normal in young mice, photoreceptors begin to degenerate at around 5 months of age. However, photoreceptors in the *Oc2*-null retina do not degenerate despite the fact that the major defect in the *Oc2*-null retina is also HC deficiency. Since *Oc1* and *Oc2* are not expressed in the photoreceptors of mature retinas, this effect might be indirect, resulted from the loss of HCs. The differences of photoreceptor degeneration between *Oc1*- and *Oc2*-null retinas may

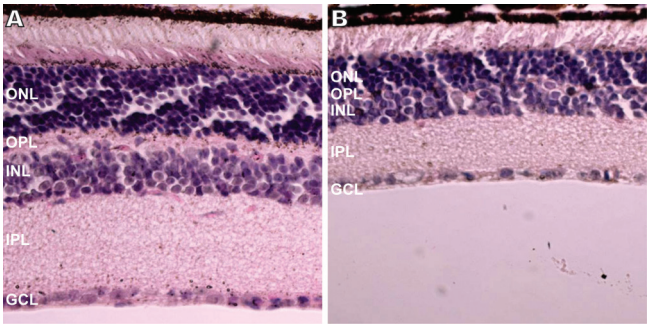

Figure 1 The Oc1/Oc2 double-knockout (DKO) retina undergoes neuronal degeneration.
Hematoxylin and eosin stained wild-type (A) and DKO (B) retinal sections from 8-month-old mice are shown. Note the loss of neurons, especially in the ONL Wu et al., 2013 region, and the absence of the OPL in the DKO retina. The loss of photoreceptors is more severe than that in the Oc1 single knockout (Wu et al., 2013). ONL: Outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer.

be due to the degree of HC loss; only 20% are left in *Oc1*-null retinas, but 50% remain in *Oc2*-null retinas. This indicates that HCs are essential for photoreceptor maintenance and a minimum (threshold) number of HCs are required to keep the photoreceptor cells healthy. Consistent with this idea, in the *Oc1/Oc2* double null retina, in which HCs do not form, photoreceptors degenerate more severely than in *Oc1*-null retina (**Figure 1**). Degeneration of photoreceptors is the most common cause of vision loss in many retinal diseases, but in many cases the underlying mechanisms are unknown. Our findings highlight a novel role for HCs in maintaining retinal integrity (Wu et al., 2013; Sapkota et al., 2014). Currently we do not know the exact mechanisms by which HCs regulate the survival of photoreceptors. It is possible that they carry out this role by secreting some trophic factors essential for photoreceptor survival, or by maintaining the normal structure of photoreceptors. Since the causes of many retinal degeneration diseases are currently unknown, it is possible that some of them are due to defects in HCs. Thus, our studies provide a new mechanism involved in retinal degeneration. Further investigation may lead to better understanding, and even treatment, for some of these diseases.

Unanswered questions: Our findings have revealed new mechanisms underlying the formation of the diverse retinal cell types. However, many key aspects of retinogenic functions of the onecut transcription factors remain to be understood. For example, how do the onecut factor function in multiple lineages? Do they regulate common target genes in all the cell lineages involved or specific set of genes in specific lineages? What regulates them in photoreceptor and RGC precursors? These issues may be addressed by genetic and biochemical experiments to identify the regulators of *Oc1* and *Oc2*, ChIP-Seq to identify direct onecut targets, and fate-mapping to catalog retinal cell (sub) types of onecut ancestry. It is possible that both shared and cell-specific mechanisms are at play to convey the function of the onecut transcription factors in retinal development. For instance, the homeodomain factor Otx2 regulates horizontal and cone lineages and collaborates with *Oc1* to do so (Emerson et al., 2013). Similarly, the Foxn4-Ptf1a cascade regulates horizontal and amacrine lineages (Li et al., 2004; Fujitani et al., 2006), and, as mentioned above, it does interact with the onecut transcription factors. On the other hand, a factor that regulates two or more lineages including RGCs and interacts with *Oc1* and *Oc2* is not reported yet. Therefore, we think that the future holds an integrated picture of the gene regulatory networks regulating the early retinal lineages, wherein *Oc1* and *Oc2* occupy both intersections and diversions.

Concluding remarks: Through a series of studies, we establish *Oc1* and *Oc2* as key regulators for early retinal cell lineages, likely through modulating the competence of RPCs. The two factors function redundantly by regulating the same set of target genes and contribute quantitatively to the expression of cell fate-determining genes in the developing retina (Sapkota et al., 2014). Since *Oc1* and *Oc2* are expressed in several tissues during development, including the brain and spinal cord, it is likely that they regulate cell fates in those tissues in a similar fashion. Our findings from the retina may facilitate our understanding of the roles of these factors in other regions of the central nervous system. Our discovery that HCs are essential for photoreceptor survival is both novel and clinically relevant. Clearly further studies on the function of onecut transcription factors in normal retinal development and retinal degeneration are required to address many of the unanswered questions.

Research in the Mu lab is supported by grants from the National Eye Institute (EY020545), the SUNY/RF Research Collaboration Fund, and an unrestricted grant from Research to Prevent Blindness.

Darshan Sapkota, Xiuqian Mu*

Department of Ophthalmology/Ross Eye Institute; Department of Biochemistry; New York State Center of Excellence in Bioinformatics and Life Sciences; SUNY Eye Institute, University at Buffalo, Buffalo, NY, USA

***Correspondence to:** Xiuqian Mu, Ph.D., xmu@buffalo.edu.*
***Accepted:** 2015-04-14*
***doi:**10.4103/1673-5374.158350* ***http**://www.nrronline.org/*
Sapkota D, Mu X (2015) Onecut transcription factors in retinal development and maintenance. Neural Regen Res 10(6):899-900.

References

Emerson MM, Surzenko N, Goetz JJ, Trimarchi J, Cepko CL (2013) Otx2 and Onecut1 promote the fates of cone photoreceptors and horizontal cells and repress rod photoreceptors. Dev Cell 26:59-72.

Fujitani Y, Fujitani S, Luo H, Qiu F, Burlison J, Long Q, Kawaguchi Y, Edlund H, MacDonald RJ, Furukawa T, Fujikado T, Magnuson MA, Xiang M, Wright CV (2006) Ptf1a determines horizontal and amacrine cell fates during mouse retinal development. Development 133:4439-4450.

Li S, Mo Z, Yang X, Price SM, Shen MM, Xiang M (2004) Foxn4 controls the genesis of amacrine and horizontal cells by retinal progenitors. Neuron 43:795-807.

Livesey FJ, Cepko CL (2001) Vertebrate neural cell-fate determination: lessons from the retina. Nat Rev Neurosci 2:109-118.

Sapkota D, Chintala H, Wu F, Fliesler SJ, Hu Z, Mu X (2014) Onecut1 and Onecut2 redundantly regulate early retinal cell fates during development. Proc Natl Acad Sci U S A 111:E4086-4095.

Wu F, Sapkota D, Li R, Mu X (2012) Onecut 1 and Onecut 2 are potential regulators of mouse retinal development. J Comp Neurol 520:952-969.

Wu F, Li R, Umino Y, Kaczynski TJ, Sapkota D, Li S, Xiang M, Fliesler SJ, Sherry DM, Gannon M, Solessio E, Mu X (2013) Onecut1 is essential for horizontal cell genesis and retinal integrity. J Neurosci 33:13053-13065.