The protein OTX2 in the retina is necessary for the maintenance of normal physiologic processes, and may be a promising therapeutic agent for some ophthalmic diseases. In this Perspective, we first shortly introduce the organization of the retina and the importance of OTX2 expression. We then present an example of reduced OTX2 activity in the developed retina associated with structural and functional consequences. We next show that homeoproteins can have neuroprotective functions. We finally review several clear examples of OTX2 non-cell autonomous activity in the retina and the effects of diminishing or providing extracellular OTX2.

The retina is part of the brain that forms a light-transducing neural sheet at the back of the eye apposed to the retinal pigmented epithelium (RPE). Photons enter at the front of the eye and penetrate through the retina to the outermost layer of photoreceptors. The photoreceptor outer segments convert the photon into a neurochemical signal. This process submit the outer segments to a significant oxidative stress and leads to a turnover about every 5 days with the RPE phagocytosis the depleted or damaged outer segments. From the photoreceptors, the visual signal is transmitted to the bipolar cells and then to the retinal ganglion cells (RGCs) in the ganglion cell layer. The visual information is then conveyed by RGC axons from the retina to higher visual brain centers such as the lateral geniculate nucleus and superior colliculus. In addition to the vertical processing and transfer of visual information in the retina, there is also two levels of lateral processing by horizontal cells at the photoreceptor/bipolar synapse and by amacrine cells at the bipolar/RGC synapse. Notably, OTX2 protein is found in all the layers of the mature retina. OTX2 is a homeoprotein (HP) of the bicoid family of transcription factors whose namesake member, bicoid in the fly acts as a diffusible morphogen to pattern the anterior-posterior axis (see Di Nardo et al., 2018).

OTX2 plays an important role in vertebrate head development and patterning of the brain. During development of the mouse retina, OTX2 is first expressed in the optic vesicle around embryonic day 9 and then soon after in differentiated RPE cells and is essential for the final differentiation of photoreceptors and bipolar cells (see Bernard et al., 2014). Later, OTX2 continues to be expressed in RPE, photoreceptors and bipolar cells. In the RPE and photoreceptors, OTX2 regulates a number of genes for the maintenance of these cell types as well as genes involved in the phototransduction pathway (Omorri et al., 2011). In mice, ablation of the Otx2 gene results in an embryonically lethal phenotype while, in humans, mutations lead to several different eye and ocular malformations. Interestingly, OTX2 is found in RGCs and amacrine of the GCL even though the locus is silent.

We experimentally explored the effects of OTX2 hypomorphism by engineering mice to express different levels of OTX2 activity. We observed a strong relation between the graded loss of OTX2 activity and abnormal retinal structural, physiological dysfunction and visual function defects. Mice with a single intact allele of Otx2 developed normally and externally appeared normal whereas those with less OTX2 activity had increasingly severe external defects ranging from microphthalmia, anophthalmia to absence of the anterior part of the head. The severity of the defects was not 100% penetrant. When mice that appeared externally normal were tested for visual acuity, there was a worsening loss of acuity with decreasing OTX2 activity. The loss of visual acuity was paralleled by diminished electroretinogram function in photoreceptors and bipolar cells. Mice with the lowest level of OTX2 activity showed early and important deficits in the a- and b-wave function. Associated with the physiological changes were structural defects in the retina that was a significant thinning of the outer nuclear layer and the inner nuclear layer. Since photoreceptors and bipolar cells in these layers express OTX2, these important deficits point strongly to loss of cell autonomous OTX2 activity. However, the use of a mutant that reduces OTX2 affinity for cell surface binding sites raises the possibility of non-cell autonomous effects as well. In particular, the thinning of the GCL could be due to a reduced transfer and/or accumulation of mutated OTX2 in amacrine cells and RGCs. Alternatively, their loss may be secondary degeneration due to loss and dysfunction of bipolar cells (Bernard et al., 2014).

Several HPs including OTX2 have been shown to promote neuron survival. Homeoproteins ENGRAILED and OTX2 have been shown to protect CNS neurons from various stresses in models of Parkinson, glaucoma and amyotrophic lateral sclerosis (Di Nardo et al., 2018; Thomasson et al., 2018; Vargas Abonce et al., 2020). A recent study found that this neuroprotective activity may be a general property of HPs that is evolutionarily conserved and may have emerged hundreds of millions of years ago (Vargas Abonce et al., 2019). Also, it was shown that OTX2 protected CNS neurons in vitro against oxidative stress at concentrations in the mM range (Vargas Abonce et al., 2019).

As mentioned above, RPE vili interdigitate with the photoreceptor outer segments, and are central to the turnover of outer segments by phagocytosis, playing an essential role in photoreceptor health and function. OTX2 expression in RPE is necessary for photoreceptor survival. When OTX2 is specifically knocked out in mature retina including the RPE, photoreceptors degenerate and mice have a phenotype reminiscent of age-related macular degeneration (Bebê et al., 2010). Importantly, in mice in which OTX2 was ablated in the retina, the re-expression specifically in RPE is sufficient to maintain photoreceptor survival (Housset et al., 2013). OTX2 overexpression in RPE cells transplanted into the eye of Royal College of Surgeon rats promotes PR survival and function (Kole et al., 2018). These are important results with potential application to photoreceptor degenerative disease such as age-related macular degeneration or retinitis pigmentosa, respectively. However, it remains unknown if the action of OTX2 is cell autonomous or non-cell autonomous. For example, OTX2 may act on the RPE cells to produce and secrete a cytokine that affects the photoreceptors to promote photoreceptor survival. Alternatively, but not necessarily mutually exclusive, the re-expressed OTX2 in RPE may be secreted and taken up by photoreceptors in a non-cell autonomous way.

Non-cell autonomous activity of HPS is based on their ability to transfer between cells. A recent study evaluated a large number of HPS and reported that secretion and transfer is a general feature (Lee et al., 2019). OTX2 is an HP that can transfer between cells (Figure 1). RGCs in the retina can take up exogenous OTX2 and the protein can then be transported down the axons to thalamic and midbrain visual structures. Transported OTX2 can even be trans synthetically conveyed to neurons in primary visual cortex where its accumulation regulates the opening and closing of the critical period for binocular vision (Sugiyama et al., 2008; Torero Ibad et al., 2011).

Since the sequences sufficient and necessary for HP secretion and internalization and thus non-cell autonomous behavior are autonomous transcriptional activity. The first clear example of HP non-cell autonomous activity in the retina was revealed using a secreted single chain antibody (scFv) against the HP PAX6. PAX6 is essential for early eye and retinal formation. Injected mRNA encoding the PAX6scFv in one-cell zebrafish embryos or the scFv itself in the blastula led to profound eye changes such as dyssymetric eyes, microphthalmia or anophthalmia, the interpretation being that the scFv sequestered and neutralized extracellular PAX6 (Lesaffre et al., 2007). These major induced defects are parallel to those in the OTX2 hypomorphic mice above.

The first example of OTX2 non-cell autonomous activity in mammalian retina was the demonstration that the protein promoted the survival of adult RGCs (Torero Ibad et al., 2011). Recall that the locus is silent in RGCs and amacrine cells, but OTX2 protein is readily detected in cells of the GCL (Sugiyama et al., 2008). Intracellular N-Methyl-D-aspartate receptor (NMDA) is an acute excitotoxic model of glaucoma in which RGCs rapidly die. We found that exogenous OTX2 completely protected against NMDA excitotoxicity and preserved visual function. Furthermore, OTX2 promoted the survival of purified adult RGCs in culture, demonstrating that the protein acted directly on RGCs.

A second example of OTX2 non-cell
autonomous activity in retinal function was revealed by the scFv strategy described above. Mice were engineered to express an OTX2scFv in parvalbumin expressing cells in the retina (Torero Ibad et al., 2020). In the mouse retina, only RGCs and amacrine cells express parvalbumin, cells that do not express OTX2. In these experiments, the goal was to express the OTX2scFv in cells in which there could be no possible interference with cell autonomous activity. Parvalbumin starts to be expressed in the mouse retina around postnatal day 11. The OTX2scFv-expressing mice showed loss of visual acuity in the absence of any electrophysiological dysfunction in either photoreceptors or bipolar cells, nor changes in retinal structure or organization. Interestingly, the ERG demonstrated a significant reduction in the oscillatory potential and an increase in the amplitude of the flicker response. Both of these electrophysiological responses arise in the inner retina largely at the level of the RGCs and amacrine cells. These results show that interfering with OTX2 non-cell autonomous signaling in the retina alters inner retinal physiological function and causes a deficit in vision, and for the first time show that an HP can modulate brain waves. Finally, a recent paper reports that exogenous OTX2 stimulates the growth of neurites from adult RGCs in culture and the regeneration of RGC axons after optic nerve crush in vivo (Torero Ibad et al., 2020). The fact that purified adult RGCs do not express OTX2 argues that the axon regenerative effect is non-cell autonomous. The level of visual acuity recovery is at best modest, however, the rate at which regenerating axons reach the optic chiasm compares favorably with other reports in the literature. It is important to note that the neuroprotective and regeneration stimulating effects of OTX2 in vivo does not require transgenesis or gene delivery at least in mice. Rather the non-cell autonomous activity of OTX2 including its ability to transfer between cells and be internalized, underlies these important beneficial effects. In summary, OTX2 is a homeo protein transcription factor that can function cell autonomously or transfer between cells and act non-cell autonomously. In addition to its paramount role during development, the continued expression of the protein in the developed retina is necessary for normal physiology. OTX2 is expressed in photoreceptors and bipolar cells and reduction in protein level leads to structural changes in the retina, ERG deficits, neuronal degeneration and vision loss. OTX2 is also expressed in the RPE and re-expression of the protein in the RPE can compensate for loss of the protein throughout the retina and promote the survival of photoreceptors. The neuroprotective activity of OTX2 also extends to adult RGCs and in this case the protein acts non-cell autonomously. Interfering with OTX2 non-autonomous signaling in the retina alters inner retinal function and leads to visual deficit, underscoring the importance of such HP signaling in normal physiology. Finally, the neuroprotective activity of OTX2 on retinal neurons and the recent report on axon regeneration in vivo lead to new therapeutic possibilities on retinal degenerative diseases such as age-related macular degeneration and glaucoma.

We thank France Maloumian of the Collège de France (Paris, France) for graphic illustration.

KLM is a coinventor for the use of OTX2 in eye disease and a cofounder of BrainEver.

This work was supported by HOMEOGEN: ERC-2013-AdG n°339379 and NeurorOptx: ANR-16-CE16-0003-02.

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Date of submission: October 10, 2020
Date of decision: November 21, 2020
Date of acceptance: December 18, 2020
Date of web publication: February 19, 2021

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Figure 1 | Schematic representation of Otx2 expression and protein localization in adult mouse retina.

Otx2 mRNA (red nuclei) is expressed in nuclei of RPE cells, photoreceptors and bipolar cells. In addition, OTX2 protein (red dots) is present in amacrine and ganglion cells. When OTX2 activity is reduced (red to orange) in hypomorph mice, there is loss of vision, abnormal ERG and global retinal cell degeneration. When non-cell autonomous OTX2 signaling is reduced, mice have reduced visual acuity, normal retinal structure and ERG function, but an abnormal inner retinal physiological activity. Figure credit: France Maloumian, Collège de France (Paris, France). ERG: Electroretinogram; RPE: retinal pigmented epithelium.

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how to cite this article: Moya KL, Ibad RT (2021)

https://doi.org/10.4103/1673-5374.308094