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Subtype-specific expression of *Fgf19* during horizontal cell development of the chicken retina

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ABSTRACT

The mechanisms underlying retinal cell diversification are crucial to proper neural development. *Fibroblast growth factor 19 (Fgf19)* is expressed by developing horizontal cells (HCs) in the chicken retina. Although there are two major HC subtypes, axon-bearing and axon-less, the precise subtype expressing *Fgf19* remains uncertain. Here we characterize *Fgf19*-expressing cells by co-labeling with antibodies against Lim1 (LIM homeodomain 1, or Lhx1), Islet1, and Prox1 (prospero-related homeobox 1) which are axon-bearing HC, axon-less HC, and pan-HC markers, respectively. We found that a subset of *Fgf19*-expressing cells was positive for Prox1 and Lim1 in the vitread neuroepithelium at embryonic day 4 (E4). By E9, the majority of *Fgf19*-expressing cells became positive for Prox1 and Lim1 prior to arrival at the prospective HC layer. In contrast, *Fgf19*-expressing cells did not overlap with the Islet1-positive population at any stage examined. These results suggest that *Fgf19* is expressed by the early migratory horizontal precursors, and later by the presumptive axon-bearing HCs.

1. Results and discussion

There are five neuronal cell-types and one glial cell type in the vertebrate retina, including ganglion cells, amacrine cells, bipolar cells, horizontal cells, photoreceptors, and Müller glia. These cells form the laminar structures and complex neural circuitry of the retina. Mammalian retinal cells are further divided into approximately 55 distinct subtypes (Masland, 2001). In the case of chicken horizontal cells (HCs), there are three morphologically distinct subtypes (Tanabe et al., 2006; Edqvist et al., 2008; Rompani and Cepko, 2008) and four immunohistochemically distinct subtypes (Fischer et al., 2007). Clarifying the extrinsic and intrinsic mechanisms underlying the genesis of retinal cell subtypes is crucial to understanding how the intricate retinal circuitry forms (Cepko et al., 1996; Livesey and Cepko, 2001).

Fibroblast Growth Factor 19 (FGF19) is a secreted factor, which binds to transmembrane FGF receptors to initiate a signal transduction cascade (Itoh and Ornitz, 2008). Chicken *Fgf19* is expressed in various organ primordia during brain, sensory organ, and somite development, as is *Fgf15*, the mouse *Fgf19* ortholog (McWhirter et al., 1997; Ladher et al., 2000; Gimeno et al., 2003; Kurose et al., 2004). In the developing retina, *Fgf19*-expressing cells are scattered in the nascent neural retina, with some eventually located on the vitread side of the neural retina, and others scattered in the neuroblastic layer as neural cell migration proceeds (Kurose et al., 2004; Francisco-Morcillo et al., 2005). When development of the three retinal nuclear layers is complete, *Fgf19* expression becomes restricted to the HC layer, abutting the presumptive outer plexiform layer. *Fgf19*-expressing cells are negative for β3-tubulin, a retinal ganglion cell marker, at embryonic day 5 (Kurose et al., 2004; Francisco-Morcillo et al., 2005). Furthermore, they do co-label with pan-HC markers Prox1 and Pax6 (Belecky-Adams et al., 1997; Dyer et al., 2003), indicating that they are a subset of retinal HCs (Kurose et al., 2004; Francisco-Morcillo et al., 2005). There are two major, morphologically distinct HC subtypes present in both birds and primates: axon-bearing and axon-less (Tanabe et al., 2006; Fischer et al., 2007; Edqvist et al., 2008; Rompani and Cepko, 2008). However, to date it is unknown which HC subtype expresses *Fgf19*. To determine the subtype of HCs expressing *Fgf19* during development, we compared expression patterns of *Fgf19 mRNA*, to the protein expression patterns of Prox1 (prospero-related homeobox 1), Lim1 (LIM homeodomain 1, or Lhx1), and Islet1 in these cells.

1.1. Subtype-specific expression of *Fgf19* in chicken HCs

We first performed double staining of fluorescence in situ hybridization and immunofluorescence using retinal sections at embryonic day 17 (E17) (Hamburger and Hamilton's stage 43;

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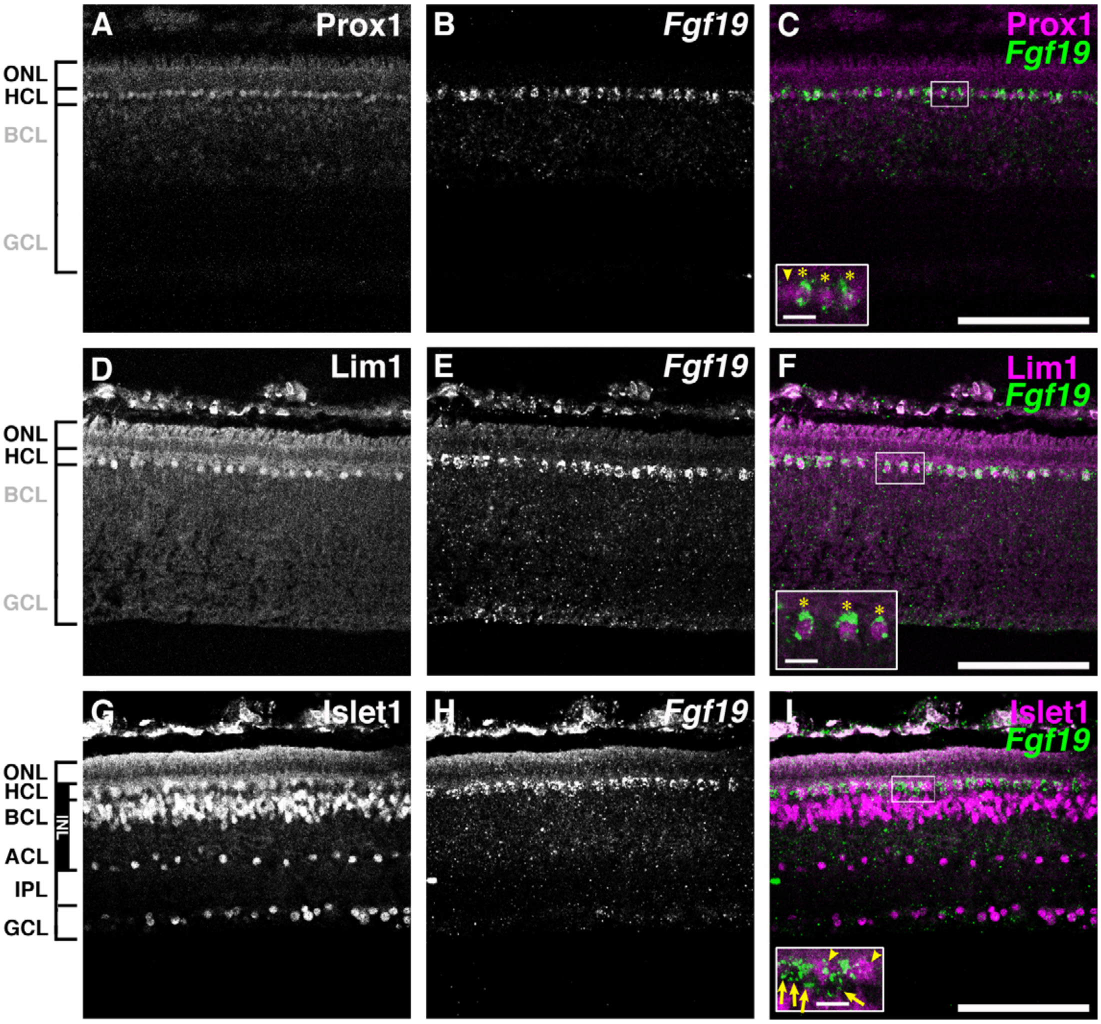

Fig. 1. *Double labeling for Fgf19 and HC subtype specific markers in stage 43 chicken retinas.* Confocal micrographs of retinal sections hybridized with *Fgf19* riboprobe and immunolabeled for Prox1, Lim1 or Islet1. Vitread side is down. (A–C) In situ hybridization for *Fgf19* (B) and immunolabeling for the pan-HC marker, Prox1 (A). Inset (C) shows Prox1+*Fgf19*− cells (arrowhead), and Prox1+*Fgf19*+ cells (asterisks). (D–F) In situ hybridization for *Fgf19* (E) and immunolabeling for the axon-bearing HC marker, Lim1 (D). Inset (F) shows all *Fgf19*+ cells are Lim1+ (asterisks). (G–I) In situ hybridization for *Fgf19* (H) and immunolabeling for axon-less HC marker, Islet1 (G). Inset (I) shows Islet1 single-positive cells (arrowheads) and *Fgf19* single-positive cells (arrows). Scale bars: 100 μm (C, F and I), 10 μm in insets. ACL, amacrine cell layer; BCL, bipolar cell layer; GCL, ganglion cell layer; HCL, horizontal cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer.

Hamburger and Hamilton, 1951) (Fig. 1). We found that *Fgf19*-expressing cells overlapped with a subset of Prox1-positive HCs (Fig. 1C, asterisks), and completely overlapped with the Lim1-positive population (Fig. 1F, asterisks). In contrast, *Fgf19*-expressing cells were complementary to Islet1-positive HCs (Fig. 1I, arrows). Thus, in the E17 retina, *Fgf19* is expressed by developing axon-bearing HC subtypes.

1.2. Time-course of Fgf19 expression during chicken retinal development

Edqvist et al. (2006) reported that Prox1-positive cells are first detected in the vitread neuroepithelium at stage 20, where they remain until stage 29. Whereas Lim1 immunoreactivity (IR) is first detected at stage 20, with the majority of Lim1+ cells aligning underneath the prospective ganglion cell layer (GCL) at stages 28–30 (Edqvist et al., 2006). They also showed that Islet1 IR is restricted to vitreally located cells beginning at stage 19. HC birthdating experiments showed that the initial phase of generation of Lim1+ HCs and Islet1+ HCs are stage 20 and stage 23, respectively (Edqvist et al., 2008). With this information in mind, we examined the time-course of *Fgf19* expression from stages 24 (E4) to 36 (E10) by co-labeling with HC immunomarkers.

At stage 24, *Fgf19*-expressing cells were scattered in the neuroepithelium, while the majority of Prox1+, Lim1+, or Islet1+ cells were detected in the vitread neuroepithelium (Fig. 2). Focusing on the cells in the vitread neuroepithelium, the Lim1-positive cells were the fewest of the four. Given that Lim1 expression in the developing retina is restricted to the prospective axon-bearing HCs, this expression pattern implies that a group of HC precursors is destined to develop as the axon-bearing subtype by the time they reach the vitread neuroepithelium during bi-directional migration (Edqvist and Hallböök, 2004; Edqvist et al., 2008). Co-labeling with *Fgf19* and Prox1 revealed that there were three cell-types: Prox1+*Fgf19*−, Prox1+*Fgf19*+, and Prox1−*Fgf19*+ cells (Fig. 2A–C; inset in Fig. 2C). Based on co-labeling studies with other markers at later stages, these are likely the presumptive axon-less HCs, axon-bearing HCs, and *Fgf19* single-positive cells, respectively. Co-labeling with *Fgf19* and Lim1 revealed that there were two cell-types: Lim1+*Fgf19*+ and Lim1−*Fgf19*+ cells (Fig. 2D–F; in-