Developmental Biology 319 (2008) 23–33

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Ectopic *Pax2* expression in chick ventral optic cup phenocopies loss of *Pax2* expression

Rachna Sehgal, Rachel Karcavich, Scott Carlson, Teri L. Belecky-Adams *

Department of Biology and Center for Regenerative Biology and Medicine, Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202, USA

ARTICLE INFO

Article history:
Received for publication 4 September 2007
Revised 28 March 2008
Accepted 28 March 2008
Available online 11 April 2008

Keywords:
Retina
Pax2
Chick
Optic Cup
Coloboma
Ectopic retina

ABSTRACT

Pax2 is essential for the development of the urogenital system, neural tube, otic vesicle, optic cup and optic tract [Dressler, G.R., Deutsch, U., et al., 1990. PAX2, a new murine paired-box-containing gene and its expression in the developing excretory system. Development 109 (4), 787–795; Nornes, H.O., Dressler, G.R., et al., 1990. Spatially and temporally restricted expression of Pax2 during murine neurogenesis. Development 109 (4), 797–809; Eccles, M.R., Wallis, L.J., et al., 1992. Expression of the PAX2 gene in human fetal kidney and Wilms' tumor. Cell Growth Differ 3 (5), 279–289]. Within the visual system, a loss-of-function leads to lack of choroid fissure closure (known as a coloboma), a loss of optic nerve astrocytes, and anomalous axonal pathfinding at the optic chiasm [Favor, J., Sandulache, R., et al., 1996. The mouse Pax2(1Neu) mutation is identical to a human PAX2 mutation in a family with renal-coloboma syndrome and results in developmental defects of the brain, ear, eye, and kidney. Proc. Natl. Acad. Sci. U. S. A. 93 (24), 13870–13875; Torres, M., Gomez-Pardo, E., et al., 1996. Pax2 contributes to inner ear patterning and optic nerve trajectory. Development 122 (11), 3381–3391]. This study is directed at determining the effects of ectopic *Pax2* expression in the chick ventral optic cup past the normal developmental period when *Pax2* is found. *In ovo* electroporation of *Pax2* into the chick ventral optic cup results in the formation of colobomas, a condition typically associated with a *loss* of *Pax2* expression. While the overexpression of *Pax2* appears to phenocopy a loss of *Pax2*, the mechanism of the failure of choroid fissure closure is associated with a cell fate switch from ventral retina and retinal pigmented epithelium (RPE) to an astrocyte fate. Further, ectopic expression of *Pax2* in RPE appears to have non-cell autonomous effects on adjacent RPE, creating an ectopic neural retina in place of the RPE.

© 2008 Elsevier Inc. All rights reserved.

Introduction

The morphogenetic events that surround the development of the vertebrate optic vesicle and optic cup are key in forming the inductive interactions that pattern the eye (reviewed in Chow and Lang, 2001). A good example of this type of event is the formation and closure of a transient gap in the ventral eye cup, known as the choroid fissure. The fissure extends through the optic cup and optic stalk and eventually closes as the two edges of the fissure undergo fusion. During its existence, this region is key for the migration of mesenchymal cells into the eye to form the vasculature that supports the lens and retina (Hughes et al., 2000). As development proceeds, the edges of the fissure fuse together to complete the ventral portion of the eye. The region of the fissure where the optic cup transitions to the optic stalk becomes the optic nerve head upon closure of the fissure (Chow and Lang, 2001). This region expresses molecules, such as cadherins, netrins, sonic hedgehog, and slits, all of which combine forces to instruct ganglion cell axons to exit the eye to form the optic nerve (Gerhardt et al., 2000; Wallace and Raff, 1999; Oster et al., 2004).

The morphogenesis of the ventral-most portions of the optic cup requires that cells remain undifferentiated until those that will make up the retinal pigmented epithelium (RPE) and neural retina have moved into place, the basal laminae that separates the two opposing lips of the choroid fissure have broken down, cells in the improper position have undergone cell death, and the two lips have fused together (Hero, 1989, 1990; Hero et al., 1991). Defects in the closure of the choroid fissure are referred to as a coloboma, a Greek word meaning "curtailed" or "mutilated". The presentation of colobomas alone in humans is a rare condition; however, colobomatous eyes frequently occur as part of syndromes that include other congenital defects, such as microphthalmia, deafness and defects in the formation of the urogenital system (Eccles and Schimmenti, 1999).

While the mechanisms that direct the choroid fissure closure are generally not well understood, a number of genetic mutations have been identified in humans, mice, and zebrafish that can give rise to colobomatous eyes (Gregory-Evans et al., 2004; Azuma et al., 2003; Barbieri et al., 2002). In particular, the loss of two transcription factors, *Pax2* and *Vax* have been associated with colobomas. *Pax2*, a member of

* Corresponding author. Fax: +1 317 274 2846.
E-mail address: tbadams@iupui.edu (T.L. Belecky-Adams).

0012-1606/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2008.03.041

paired homeobox family of transcription factors, has been associated with ocular defects in humans (Gregory-Evans et al., 2004). *Pax2* loss-of-function mutations and/or haploinsufficiency have been associated primarily with colobomas, microphthalmia, and optic nerve defects in the eye in addition to kidney, inner ear, and neural tube defects (Benetti et al., 2007; Sanyanusin et al., 1995). *Pax2* overexpression has devastating consequences in the kidney, leading to the formation of childhood tumors known as Wilms tumors (Dressler and Douglass, 1992).

Pax2 expression has been shown to be induced by the actions of several secreted factors, including bone morphogenetic protein 7 (BMP7), sonic hedgehog (SHH) and fibroblast growth factors (FGFs) (Macdonald et al., 1995; Morcillo et al., 2006; Nakamura 2001). Both BMP7 and SHH are expressed in the prechordal mesoderm underlying the ventral diencephalon and both are thought to control the identity of diencephalon (Dale et al., 1999). PAX2 is co-expressed throughout the early optic vesicle with other transcription factors such as PAX6 and CHX10 (Baumer et al., 2003). The expression of these factors becomes restricted to specific portions of the optic vesicle as, 1) inductive signals from surrounding tissues interact with the cells of optic vesicle, and 2) the cell type specific transcription factors suppress the expression of factors that induce competing cell types. At this point *Pax2* expression is restricted to the part of the optic cup destined to be ventral neural retina, RPE and optic stalk (Bovolenta et al., 1997). *Pax2* expression is exquisitely regulated in the optic cup and stalk. Expression remains in the ventral optic cup during the period in which the choroid fissure must be closed, and downregulates within the optic cup at the point when the tissue that forms the ventral retina and RPE begins to differentiate. A few stages later, *Pax2* expression becomes restricted to the cells of the optic stalk and cells that line the choroid fissure (Mansouri et al., 1996). It is generally unknown whether PAX2-positive cells at the edge of the choroid fissure undergo cell death as a result of the closure, or downregulate their expression as they take on new cell fates. Regardless, the end result is that PAX2 expressing cells are found exclusively in astrocyte precursor cells and mature astrocytes of the optic stalk/nerve and glial cells of a vascular structure peculiar to avian and reptile species, called the pecten. Here, we seek to understand the reason for the complex spatio-temporal regulation of *Pax2* during optic cup development and the consequence of a loss in *Pax2* regulation, particularly in ventral optic cup differentiation.

Previous results from our lab have shown that overexpression of the BMP binding protein, noggin, in the chick optic cup results in the loss of dorsal retinal markers and the simultaneous expansion of ventral markers, such as PAX2 (Adler and Belecky-Adams, 2002). We hypothesize that the lack of choroid fissure closure in noggin-overexpressing eyes is due to the ectopic expression of PAX2 in the ventral retina past the time at which it should be present, rather than secondary effects of noggin overexpression. Further, we propose that the mechanism of choroid fissure failure, following the ectopic expression of *Pax2* is the result of the abnormal differentiation of cells normally fated to become ventral retina and RPE, to glial cells.

Material and methods

Reagents used were as follows: phenol-chloroform, RNase A, DNase I (Invitrogen; Carlsbad, CA), isopropanol, sucrose, paraformaldehyde, EDTA, formamide, Tris, NaCl, KCl, monobasic sodium phosphate, dibasic sodium phosphate, superfrost plus slides (Fisher Scientific; Hanover Park, IL), Chaps, diethyl pyrocarbonate (Dep-C), sodium citrate-trisodium salt dehydrate, Triton X-100, Trypan-blue, Propidium iodide (Sigma; St. Louis, MO), normal Goat Serum, normal Donkey serum (Chemicon International; Temecula, CA), penicillin-streptomycin (Invitrogen; Grand Island, NY), 5-bromo-3-indolyl-phosphate (BCIP), nitro blue tetrazolium chloride (NBT) (Roche; Indianapolis, IN), blocking buffer for *in situ* hybridization (Roche, Cat# 1096176), *in situ* cell death detection kit POD, (Roche, Cat# 1684817), plasmid midi kit (Qiagen, Valencio, CA), OCT embedding compound, (Sakura; Torrance, CA), molds and aqua Polymount (Polysciences; Warrington, PA).

Materials

Eggs

White Leghorn eggs used for electroporation studies were provided by Purdue Poultry Farm (West Lafayette, IN) and the Ohio State Univeristy (Columbus, Ohio).

Electroporation Constructs: Plasmid expressing chick *Pax2* was a kind gift from Harukazu Nakamura, Tohoku University, Japan (Okafuji et al., 1999), *β-gal* expression vector was a kind gift from Ruben Adler (Toy et al., 2002). The coding sequence of GFP (BD Biosciences; Palo Alto, CA) was driven by the cytomegalovirus promoter (CMV).

In situ hybridization probes

cRaldh3 (Suzuki et al., 2000) a gift from Amasaharu Noda (National Inst. Basic Biology, Okazaki Japan), *Tbx5* (described as *cTbx5*) a gift from Katherine Yutzey (Cincinnati Children's Hospital Medical Center, Cincinnati, OH) and *Bmp4* was a kind gift from Thomas M. Jessell (Columbia University, New York).

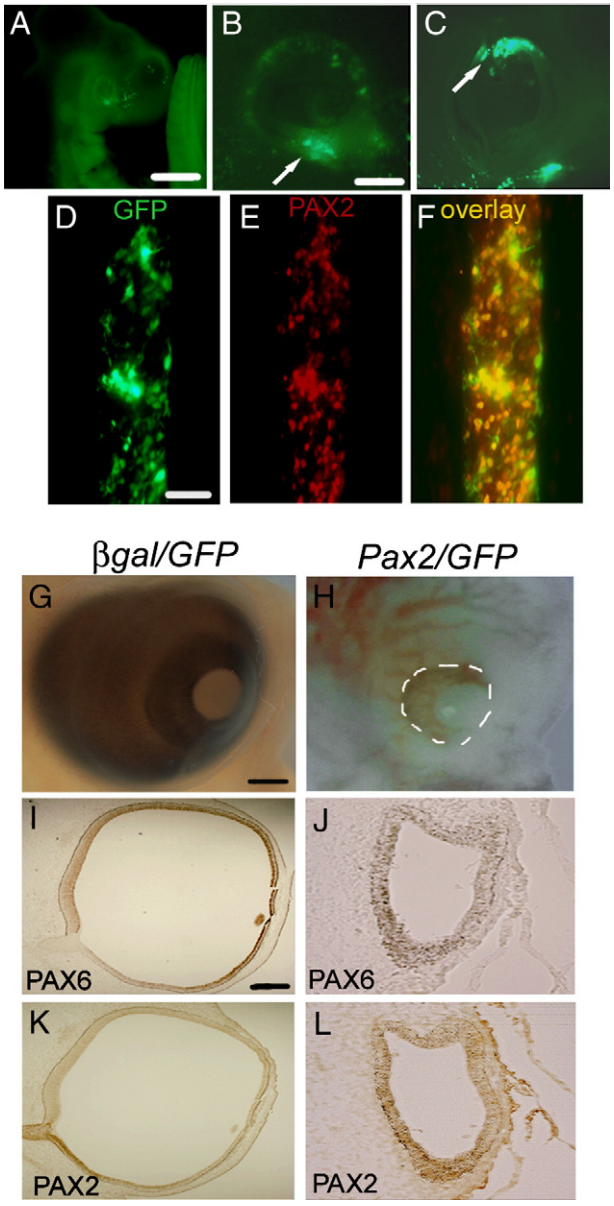

Fig. 1. (A–F) Co-expression of GFP with *β-gal* and *Pax2*. E3 chick eyes (HH 13–15) were electroporated with β-gal/GFP (10:1) and analyzed at E4. (A, B) GFP is expressed throughout the optic cup, but expression predominates in the ventral portion (arrow). (C) Shows GFP expression in dorsal optic cup (arrow). (D, E) Stage 10 chicks were electroporated with *Pax2/GFP* (10:1) and sections through forebrain were labeled with GFP (D) and PAX2 (E). (F) Electroporated cells co-express *GFP* and *Pax2*. Scale bar (500 μm) in panel A; scale bar (50 μm) in panel B applies to (B, C); scale bar (50 μm) in panel D applies to panels D–F. (G, H) Embryos electroporated at stage 10 were allowed to develop until E6. Embryos electroporated with *β-gal/GFP* developed normally (G) and showed the typical PAX6 expression patterns throughout the retina (I) and PAX2 expression in the optic nerve (K). In comparison, *Pax2/GFP* electroporated embryos developed microphthalmia (H; white dashes) in which the optic vesicle-like structure that developed showed very little PAX6 expression (J) and ectopic PAX2 expression (L). Scale bar (50 μm) in panel G applies to panels G, H; scale bar (500 μm) in panel I applies to panels I-L.

Download English Version:

https://daneshyari.com/en/article/2174731

Download Persian Version:

https://daneshyari.com/article/2174731

Daneshyari.com