Review

Iris development in vertebrates; genetic and molecular considerations

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ABSTRACT

The iris plays a key role in visual function. It regulates the amount of light entering the eye and falling on the retina and also operates in focal adjustment of closer objects. The iris is involved in circulation of the aqueous humor and hence functions in regulation of intraocular pressure. Intriguingly, iris pigmented cells possess the ability to transdifferentiate into different ocular cell types of retinal pigmented epithelium, photoreceptors and lens cells. Thus, the iris is considered a potential source for cell-replacement therapies. During embryogenesis, the iris arises from both the optic cup and the periocular mesenchyme. Its interesting mode of development includes specification of the peripheral optic cup to a non-neuronal fate, migration of cells from the surrounding periocular mesenchyme and an atypical formation of smooth muscles from the neuroectoderm. This manner of development raises some interesting general topics concerning the early patterning of the neuroectoderm, the specification and differentiation of diverse cell types and the interactions between intrinsic and extrinsic factors in the process of organogenesis. In this review, we discuss iris anatomy and development, describe major pathologies of the iris and their molecular etiology and finally summarize the recent findings on genes and signaling pathways that are involved in iris development.

Keywords: Iris, Pax6, Pitx2, FoxC1, Aniridia, Axenfeld–Rieger syndrome, Iris transdifferentiation

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1. Introduction

The iris, one of the most visually impressive and colorful organs of the human body, is aptly named after Iris, the goddess of the rainbow and the messenger for the Olympian gods. It is a thin, contractile disk that is located between the lens and the cornea and regulates the amount of light that passes through them and falls on the retina.

In the last century, iris development has been studied as a model for understanding the complex interactions between the neuroectoderm and the periocular mesenchyme, both are involved in iris morphogenesis. In the last 10 years, iris research was based on advanced technologies of molecular biology, transgenic animal studies and cell-fate tracing methods, which have unraveled some of the molecular players regulating these interactions.

2. The anatomy of the iris

The iris is made up of several different cell types: the most posterior layer, closest to the lens, is the iris pigmented
epithelium. Above these pigmented cells are the iridial muscles, and anteriorly lies the iris stroma. The iris root is attached to the ciliary body (CB) and to the corneal-sclera junction. This region is known as the iridocorneal angle. The CB shares a common embryonic origin with the iris but develops into a functionally different structure; here we discuss the CB in the context of iris development. Other aspects of CB development and physiology have been reviewed elsewhere (Beebe, 1986; Tamm et al., 1996).

3. Iris pigmented epithelium (IPE)

The IPE is composed of two cellular layers that are continuous with the pigmented and non-pigmented epithelial layers of the CB (Figs. 1E, F). Interestingly, the IPE cells are known for their outstanding plasticity; in the newt, for instance, a structurally and functionally normal lens can regenerate from the dorsal margin of the IPE (Wolff, 1895). This regeneration includes reorganization of the extracellular matrix, re-entry into the cell cycle and dedifferentiation of the dorsal IPE cells (reviewed in Tsonis et al., 2004). This fact should be viewed in light of the capability of Urodela to self-regenerate; however, recent studies have demonstrated that IPE cells of other vertebrates also possess the ability to transdifferentiate into completely different cell types. Dissociated chick IPE cells, for example, have been shown to dedifferentiate and form lentoids in culture (Kosaka et al., 1998) and in appropriate culture conditions these cells form neurospheres that may differentiate into retinal cell types. The latter finding suggests that chick IPE cells maintain progenitor/stem-cell properties and neurogenic potential, similar to the mammalian retinal stem cells of the pigmented CB (Ahmad et al., 2000; Sun et al., 2006; Tropepe et al., 2000). In mammals, iris-derived cells from rodents or primates were shown to transdifferentiate into cells expressing photoreceptor-specific markers only upon Crx/Otx2 or Crx/NeuroD viral transduction (Akagi et al., 2004, 2005; Haruta et al., 2001). Importantly, these photoreceptor-like cells showed a rod-specific electrophysiological response to light and managed to integrate into embryonic retinal explants (Akagi et al., 2005). This remarkable plasticity is already being implemented in cell-replacement therapy (reviewed in Thumann, 2001). It has been shown that subretinal transplantation of IPE cells inhibits pathologic choroidal neovascularization in rat models of retinopathy and increases the survival of photoreceptor cells (Semkova et al., 2002). Other studies have shown that IPE cells can replace the retinal pigmented epithelium (RPE) in patients with age-related macular degeneration (Thumann et al., 2000).

4. The muscles of the iris

Two different sets of involuntary muscles, the sphincter pupillae and the dilator pupillae (Fig. 1F), act in opposition to cause miosis (constriction) or mydriasis (dilation) of the pupil in response to different levels of light or during focal adjustment. The sphincter muscle is under the control of the parasympathetic nervous system and is innervated by fibers from the oculomotor nerve, which originate in the Edinger–Westphal nucleus of the midbrain. The dilator muscle is controlled by the sympathetic system and is innervated by post-ganglionic neurons from the superior cervical ganglion (Smith, 2002).

5. Iris stroma

The stroma of the iris (Figs. 1E, F) consists of cells and connective fibers that create delicate meshes, in which the blood vessels and nerves are integrated (Smith, 2002). Individual variations in iris color are typically attributed to the melanin content within the iris stroma and IPE. In albino eyes, pigment is entirely absent, in various shades of blue eyes the pigment cells are confined to the IPE, whereas in gray, brown and black eyes, pigment is also found in the cells of the stroma.

6. The iridocorneal angle

Within the angle between the cornea and the iris lies the region through which the aqueous humor constantly drains out of the eye. Aqueous humor is a transparent liquid that has a chemical composition which is slightly hypertonic to blood plasma with a much lower protein content (Kinsey, 1951). It provides nutrition for the lens and cornea and removes waste products of metabolism as both of these tissues are devoid of blood vessels. The aqueous humor is secreted continuously by the CB into the posterior chamber, flows through the pupil into the anterior chamber and drains out through the trabecular meshwork and Schlemm’s canal, located in the iridocorneal angle. Some iris pathologies involve elevation of the intraocular pressure, the major risk factor for glaucoma (Gould et al., 2004). For instance, in the pigment-dispersion syndrome, pigment granules from the IPE are deposited onto various ocular structures, including the trabecular meshwork. As a result of these obstructions, approximately 50% of patients develop increased intraocular pressure and degeneration of the optic nerve, causing permanent loss of sight (Richter et al., 1986).

It should be noted that most congenital glaucomas develop as a consequence of maldevelopment of the drainage structures themselves (Barishak et al., 1978; Fine, 1964). Indeed, in many iridial pathologies, iris malformations are also accompanied by dysgenesis of the anterior segment, including the trabecular meshwork. Good examples of this are the nail-patella or the iridogoniodygsengesis syndromes, glaucomatous conditions that include a combined dysgenesis of the iris and iridocorneal angle (Berg, 1932; Weatherill and Hart, 1969). Thus, it is reasonable to speculate that the elevation of intraocular pressure in these patients is a result of malfunction of the angle structures rather than of the iris itself. However, it is possible that in some cases the iris is directly involved in pressure elevation, due to structural changes that lead to narrowing and closure of the iridocorneal angle. This can potentially affect the drainage ability of the trabecula, as happens in acute angle-closure glaucoma, in which the angle closes and covers the trabecular meshwork and Schlemm’s canal.
7. Embryonic development of the murine iris

Formation of the complex structure of the iris is morphologically evident at mid-gestation and is completed in postnatal stages (reviewed in Cvekl and Tamm, 2004). This process is dependent on proper development of the embryonic structures from which the iris originates, the neuroectoderm and the periciliary mesenchyme. Furthermore, signals from the adjacent lens are essential triggers for iris development.

The first morphological evidence of eye development in vertebrates is bilateral evaginations of the forebrain that lead to the formation of the optic vesicles (Chow and Lang, 2001). These vesicles move through a layer of mesenchyme until they reach the surface ectoderm; the interaction with the surface ectoderm leads to an invagination of the vesicles into cup-like structures termed the optic cups (OCs). The peripheral margins of the OC are the embryonic source of the iris and CB (Figs. 1B, C, E), while the rest of the cup forms the neuroretina and the overlaying RPE (Figs. 1A, D).

The murine iris and CB start to develop around mid-gestation. At this stage, the adjacent retinal progenitor cells divide extensively and differentiate into the six neuronal cell types and glia composing the mature neuroretina (reviewed in Marquardt and Gruss, 2002). This neuronal differentiation progresses from the central retina toward the periphery, until it reaches the margins of the OC, which contain the non-neuronal progenitors of the iris and CB. At around embryonic day (E) 15.5, these progenitors are well distinguished from the rest of the neuroretina as they do not express typical neuronal markers (e.g., βIII-tubulin, Fig. 1D) and most of them are mitotically inactive. At around E17, the margins of the OC extend, and small subsets of cells located near the presumptive pupil start to express smooth muscle-specific markers (Davis-Silberman et al., 2005; Link and Nishi, 1998). Iris muscle development is worthy of note as it is a very rare example of ectodermal, rather than mesodermal, derived muscles (Cvekl and Tamm, 2004; Imaizumi and Kuwabara, 1971; Szili, 1901). Interestingly, in the chick, iris muscles originate from neural crest cells (Creuzet et al., 2005; Johnston et al., 1979; Nakano and Nakamura, 1985). In view of this, it would be important to re-assess the origin of mammalian iris muscles using modern techniques that can trace the fate of genetically labeled cells (Lobe et al., 1999; Zinyk et al., 1998).

At around the time of birth, the epithelia of the iris and the CB differentiate: the outer margins of the OC, which are continuous with the RPE, give rise to the anterior pigmented layers, while the inner margins, which are continuous with the neuroretina, form the posterior pigmented layer of the iris and the non-pigmented layer of the CB (Figs. 1E, F) (Beebe, 1986; Thumann, 2001).

The stroma of the iris is composed of migratory cells that move into the eye from the periciliar mesenchyme (reviewed in Cvekl and Tamm, 2004). These cells are first apparent at the angle between the future cornea and the extended margins of the OC. When the margins extend to form the iris, the mesenchymal cells proliferate and migrate along the iris epithelial layers and differentiate into the stroma. The iridocorneal structures are similarly produced from mesenchymal cells and develop mostly during postnatal life (reviewed in Cvekl and Tamm, 2004).

Much effort has been invested in tracing the embryonic origin of the periciliar mesenchyme. Historically, this mesenchyme was thought to arise from the head mesoderm (Mann, 1928). However, fate-mapping experiments performed in birds, such as chick–quail chimeras, vital dye-labeling and neural crest-specific antibodies have all demonstrated the contribution of neural crest cells to the periciliar mesenchyme (Johnston et al., 1979; Le Douarin, 1999; Noden, 1982). In mammals, this issue is still under debate. Gage and colleagues traced the movement of mesenchymal cells into the eye using a floxed reporter gene with either the Wnt1-Cre, which is specific to neural crest cells, or the aGSU-Cre, which is mesoderm-specific. Their analysis supported the hypothesis of mesodermal derivation of the iris stroma in mice (Gage et al., 2005). In contrast, Kanakubo and associates employed a different neural crest-specific Cre line, the P0-Cre, and reported that the iris stroma is composed of neural crest migratory cells (Kanakubo et al., 2006). The discrepancies between the above studies may reflect transgenic aberrations or different genetic backgrounds and need to be reconciled. Nevertheless, considering the two reports, it is most likely that the iris stroma in mice originates from both neural crest and mesoderm.

There is a long line of evidence emphasizing the role of the lens in the development of the anterior segment structures, including the iris (reviewed in Beebe, 1986). For example, an ablation of the lens, either mechanical or by lens-specific expression of the cytotoxic diphtheria toxin A, disrupted the development of the iris, CB and cornea (Beebe and Coats, 2000; Harrington et al., 1991). In addition, lens phenotypes that are caused by mutations in lens-specific genes (e.g., FoxE3 Semina et al., 2001) or by lens-specific changes in the expression of ubiquitous genes (e.g., Taffi Flugel-Koch et al., 2002) are sometimes accompanied by anterior segment dysgenesis. Finally, it has been shown that chick lens could induce the expression of iris and CB specific markers in cultured embryonic neuroretina of mouse (Thut et al., 2001).

Another interesting evidence for this essential role of the lens in iris development came from the Astyanax mexicanus, a teleost with surface and cave forms. The cave-fish are blind: the lens vesicle is initially formed but later degenerates, and the cornea, iris and other optic tissues are absent or rudimentary. Intriguingly, transplantation of a surface-fish lens into the blind cave-fish eye stimulates the growth of the iris and cornea, suggesting that the lens secretes factors that are important for the development of the anterior segment (Yamamoto and Jeffery, 2000). However, the identity of these signaling molecules is currently unknown.

8. Molecular mechanisms regulating iris development

A review of iris development exposes two major events in its formation. The first is the central to peripheral patterning of the OC, leading to the production of an iris and CB progenitors in the OC margins. The second is the cellular migration that builds the iris stroma. Recent studies characterizing gene-expression patterns in the OC, as well as analyses of anterior
segment abnormalities, have revealed new insights into the molecular mechanisms regulating these major events in relation to morphogenesis of the iris.

The compartmentalization of the OC into neuronal/central versus non-neuronal/peripheral progenitors occurs days before the genesis of the iris and CB. This patterning is first evident by the enhanced peripheral expression of transcription factors such as the homeobox genes Meis1, Meis2, Pax6, Otx1, and the growth-arrest-specific protein Gas1 (Lee et al., 2001). Soon after, additional factors are upregulated in the OC periphery. Among them are members of signaling pathways such as the Wnt ligand Wnt2b (Liu et al., 2003a), the Notch ligand Jagged (Bao and Cepko, 1997) and the TGFβ family members Tgfβ1 and Bmp4 (Thut et al., 2001; Zhao et al., 2002). This unique molecular composition of key developmental regulators distinguishes the peripheral OC from the adjacent neuroretina and RPE. These and additional genes that are upregulated in the OC periphery are listed in Fig. 2. It could be that the expression of some of these factors implies an undifferentiated state of the OC-margin cells rather than differentiation into non-neuronal progenitors. In addition, it should be considered that this progenitor pool is common to both iris and CB and that the exact point of the commitment to either one of the two fates has not yet been determined. Taking together, it is not clear which of these factors is truly iridogenic. Nonetheless, it is most probable that some of these genes, such as Pax6, Bmp4 and Wnt2b are essential for the specification and morphogenesis of the iris, as suggested by human syndromes or functional studies.

9. Aniridia and PAX6/Pax6

Aniridia, which literally means “without iris”, was first described by Barrata in 1818. It is characterized by the complete or partial iris hypoplasia that is apparent at birth (Elsas et al., 1977). Frequently associated ocular abnormalities include optic nerve and macular hypoplasia, cataract, glaucoma, nystagmus, strabismus and corneal defects (Hittner et al., 1980; Shaw and Neel, 1960). Most cases of the aniridia are the result of heterozygous mutations in the transcription factor PAX6, a member of the PAX (Paired Box) gene family (Glaser et al., 1992; Ton et al., 1991; Walther and Gruss, 1991). To date, 309 PAX6 mutations have been described, most of them resulting in aniridia (http://Pax6.hgu.mrc.ac.uk/).

Like other members of the PAX gene family, PAX6/Pax6 is highly conserved among the Metazoan (reviewed in Gehring, 2001; Kozmik, 2005). In vertebrates, Pax6 is essential for the development of the eyes, the olfactory system, the pancreas and the central nervous system (reviewed in Simpson and Price, 2002). Most notably, Pax6 is considered a key regulator of eye development. It is invariably essential for eye formation in different species and has the intriguing capacity to induce ectopic eyes upon misexpression in flies and amphibian embryos (Chow et al., 1999; Grindley et al., 1995; Halder et al., 1995; Quiring et al., 1994). Interestingly, Pax6 in the newt is required for the proper regeneration of the lens from the IPE (Madhavan et al., 2006).

Human aniridia, or the parallel phenotype in mice, designated “small eye” (Sey) (Baulmann et al., 2002; Hill et al., 1992;
Pax6 is essential for onset of differentiation of the iris muscles, and later on for proper development of the sphincter. Finally, a non-cell-autonomous effect of Pax6 dosage reduction in the OC is evidenced by the maldevelopment of iris stroma in the α-Cre;Pax6<sup>flox/−</sup> mutants. The latter finding is in agreement with the observation that the correct dosage of Pax6 is essential for the distribution and integration of neural crest cells into various ocular tissues (Kanakubo et al., 2006).

Pax6 involvement in cell migration to the iris seems to be non-cell-autonomous as its expression is mostly restricted to the iris epithelia and musculature (Figs. 1E, F). Pax6 may regulate the expression of molecules required for the guidance or adhesion of the migratory cells to the developing iris. Pax6 has indeed been implicated in the regulation of cell-adhesion molecules, including N-cadherin in the lens (van Raamsdonk and Tilghman, 2000) and R-cadherin in the brain (Stoykova et al., 1997). However, the expression of other cell-adhesion molecules, including NCAM, L1, β-integrin and HNK1, was recently reported to be unchanged in Sey mice (Kanakubo et al., 2006).

Interestingly, the expression of the retinoic acid-synthesizing enzyme Raldh3 was found to be reduced in Sey rats (Suzuki et al., 2000). Retinoic acid is secreted from the retina, RPE and cornea and appears to function in a paracrine manner on the morphogenesis of the periorcular mesenchyme-derived structures (Matt et al., 2005). Raldh1/Raldh3 are upregulated in the OC periphery (Fig. 2 and Matt et al., 2005) and the double-mutant mice (Raldh1<sup>−/−</sup>;Raldh3<sup>−/−</sup>) display several malformations of the anterior segment structures, including the iris stroma. The association between Pax6 and Raldh3 suggests that abnormal retinoic acid signaling might explain at least part of the iris defect observed in α-Cre;Pax6<sup>flox/−</sup> mice.

10. Axenfeld–Rieger syndrome, PITX2/Pitx2 and FOXC1/FoxC1

As the iris stroma consists of migratory cells, hypoplasia of the iris is a rather frequent feature of genetic disorders that involve cell-migration failure, such as in Axenfeld–Rieger syndrome (ARS).
ARS is a genetically heterogeneous, autosomal dominant disorder that is characterized by anterior segment defects, glaucoma and other extraocular anomalies (Rieger, 1935; Shields, 1983). Iris malformation includes stromal hypoplasia, distorted or displaced pupils and extra holes in the iris, symptoms that are referred to as Rieger anomaly. When iris strands bridge the iridocorneal angle to the trabecular meshwork, patients are considered to have Axenfeld anomaly (Axenfeld, 1920). Shields proposed that an arrest in the development of neural crest-derived tissues in the anterior segment causes the ocular features of ARS. This leads to retention of primordial endothelial tissue on the iris and across the anterior chamber angle, which produces the iridic changes (Shields, 1983).

Mutations in two main genes have been described in ARS: the bicoid-like homeobox gene PITX2 (Semia et al., 1996) and in the forkhead/winged helix transcription factor FOXC1 (Mears et al., 1998). In addition, there has been one report of a complete form of ARS resulting from a small deletion on 11p13, where the PAX6 gene resides (Riise et al., 2001). However, as the expression pattern of PAX6 cannot explain the extraocular symptoms of ARS, it is possible that other putative genes, linked to the same region, were also involved. Furthermore, as 40% of ARS patients were not diagnosed with mutations in PITX2, FOXC1 or PAX6, the potential for discovering new ARS genes has not yet been fulfilled (reviewed in Hjalt and Semina, 2005).

PITX2/Pitx2 is a member of the PITX homeobox gene family that is involved in the determination of left–right asymmetry. It plays a pivotal role in the normal development of the heart, lung, brain, eyes and craniofacial structures in vertebrates (Gage and Camper, 1997; Gage et al., 1999; Kitamura et al., 1999; Lin et al., 1999; Logan et al., 1998; Lu et al., 1999; Yoshioka et al., 1998). PITX2/Pitx2 encodes three isoforms in mice and four in humans (PITX2a-d (Cox et al., 2002)). These isoforms partially overlap in their expression patterns, and at least few of Pitx2 targets are regulated by distinct isoforms (Cox et al., 2002; Kitamura et al., 1999; Yu et al., 2001).

Heterozygous mutations in human PITX2 result in ARS (Semia et al., 1996). The ocular phenotype of these patients correlates with the normal expression of Pitx2 in the murine developing eye: it is first expressed in the pericocular mesenchyme (Fig. 2) and then in the mesenchymal cells that migrate to the corneal and iris stroma and to the iridocorneal angle (Hjalt et al., 2000; Lu et al., 1999; Semina et al., 1996). This expression pattern implies that Pitx2 is involved in the migration of pericocular mesenchyme cells into the eye. Indeed, both cell-culture and in vivo studies have provided evidence for this view. For example, expression of Pitx2a in HeLa cells induces actin-myosin reorganization, which leads to increased cell spreading, suppression of cell migration and strengthening of cell–cell adhesion (Wei and Adelstein, 2002). Fate-mapping studies with a Pitx2-Cre recombinase knock-in allele revealed that Pitx2 functions to regulate local cell movement during development of the heart and craniofacial structures (Liu et al., 2002, 2003b).

Interestingly, Pitx2 is expressed in both the neural crest and the mesoderm-derived precursors of the pericocular mesenchyme. To gain an insight into Pitx2’s role in the neural crest-derived mesenchyme, Evans and Gage generated neural crest-specific Pitx2 knockout mice (Pitx2-ncko Evans and Gage, 2005). The ocular phenotype of the Pitx2-ncko mice showed a variety of symptoms in structures derived from the neural crest, including complete absence of the sclera and the corneal endothelium and stroma. Interestingly, optic stalk development was aborted suggesting that Pitx2 regulates the expression of factors within the pericocular mesenchyme that are required for the development of the adjacent optic nerve (Evans and Gage, 2005). The severe developmental ocular abnormality of the Pitx2-ncko mutants has thus precluded the analysis of the iris phenotype resulting from neural crest inactivation of Pitx2. The use of temporally controlled somatic mutagenesis (Hayashi and McMahon, 2002) for the functional study of Pitx2 is expected to reveal its roles in iris development.

FOXC1/FoxC1 is a forkhead/winged helix type of transcription factor. It is expressed in many embryonic tissues, including prechondrogenic mesenchyme, pericellular mesenchyme, meninges, endothelial cells and kidney. Homozygous null mice die at birth exhibiting phenotype of hydrocephalus, multiple skeletal abnormalities and eye defects (Kume et al., 1998). Heterozygous mutations in FOXC1 result in ARS; mutations in the murine homologous gene lead to similar ocular abnormalities, including iris hypoplasia with severely eccentric pupils, corneal opacities, small or absent Schlemm’s channel and aberrantly developed trabecular meshwork (Kidson et al., 1999; Smith et al., 2000). Interestingly, normal development of the murine anterior segment requires an additional member of the forkhead/winged helix family together with FoxC1 as the ocular phenotype of FoxC1−/−;FoxC2−/− mice is broader than that of either single heterozygote. The role of FOXC2 in human eye development is currently unknown (Smith et al., 2000).

Common to all of the ocular structures affected in FoxC1 and FoxC2 heterozygous mice is their derivation from the pericocular mesenchyme, where these two genes are mainly expressed (Fig. 2). Thus, it is reasonable to speculate that this phenotype results from a primary defect in the migration or differentiation of the mesenchymal cells. In particular, it may be the outcome of aberrant extracellular matrix synthesis or organization as mutant eyes show abnormally thin collagen bundles (Smith et al., 2000).

Co-expression of FoxC1 and Pitx2 in the pericocular mesenchyme, together with the similar phenotypes of their mutants, implies a functional interaction between the two transcription factors. Indeed, Pitx2a and FoxC1 were recently found to interact in common protein complex on the chromatin (Berry et al., 2006). This type of protein interaction may explain the strict dosage sensitivity for both transcription factors as cell-culture studies demonstrate that changes in the stoichiometry of these two factors lead to an alteration in their transcriptional activity. The next challenge will be to identify the direct targets of Pitx2, FoxC1 and FoxC2, which mediates their roles in the formation of the anterior segment of the eye. Interestingly, the expressions of FoxC1 and Pitx2 appeared to be reduced in Raldh1/Raldh3 null mice. Possibly, this finding could potentially correlate Pitx2, FoxC1 and Pax6 since Raldh3 expression was found to be reduced in Sey rats, as noted above (Suzuki et al., 2000).
11. Signal transduction pathways involved in iris development

In general, organogenesis is regulated by a relatively small number of evolutionarily conserved signaling pathways. Here we describe components of two signaling pathways, BMP and Wnt, which have been recognized as participants in the development of the iris and CB.

12. The bone morphogenetic proteins (BMPs)

The BMPs are growth factors that belong to the TGFβ superfamily. These secreted factors have been shown to be essential for cell differentiation and morphogenesis of numerous tissues during embryonic development (Zhao, 2003).

In the eye, Bmp4 and Bmp7 are first expressed in the lens and optic vesicle and then in the dorsal OC (Zhao et al., 2002). At around mid-gestation, the expression of Bmp4 and Bmp7 and the putative downstream target, the transcription factor Msx1, is restricted to the OC margins, and later to the developing CB and the adult iris (Jensen, 2005; Monaghan et al., 1991; Zhao et al., 2002). Bmp receptors, including all six type-I receptors, ActR-II and the alternatively spliced long version of Bmpr-II are expressed throughout the eye (Obata et al., 1999). Knockout studies have shown that Bmp4 and Bmp7 are essential for early morphogenesis of the eye, but due to the lethality of these mice, information regarding the later roles of Bmp4 could not be provided (Furuta and Hogan, 1998; Wawersik et al., 2005). To circumvent this, Zhao and colleagues generated mice expressing the BMP inhibitor Noggin in an ectopic manner, suppressing both Bmp4 and Bmp7 expression in the developing CB and iris. This inhibition of BMP signaling resulted in a complete loss of the CB. The iris of these transgene mice was also malformed, with thinning of the epithelial layers and reduction in size of the smooth muscles (Zhao et al., 2002).

Direct evidence of Bmp4 role in iris development came from the phenotype of the Bmp4−/− mice (Chang et al., 2001). As in the cases of Pax6 and FoxC1/2 heterozygous mice, the reduction in Bmp4 levels resulted in hypoplasia of the iris. The iris phenotype of the Bmp4−/− mice consisted of large, irregularly shaped and often eccentric pupils. Abnormalities were also observed in the iridocorneal angle, including a small or absent Schlemm’s canal and a hypoplastic trabecular meshwork. Subsequently, these defects lead to elevated intraocular pressure, which implicate BMP4 as a candidate contributor to congenital ocular syndromes in humans such as ARS and other glaucomas.

The absence of detectable Bmp4 expression in the drainage structures suggests that Bmp4 produced by the CB or iris acts as a growth and differentiation factor during angle development. It should be noted that, in contrast to mice, RT-PCR experiments clearly showed that, in humans, BMP4 is expressed in the trabecular meshwork (Wordinger et al., 2002). This implies that BMP4’s mode of action is slightly different in human and might involve some aspects of autocrine activity, at least in the trabecular meshwork.

13. Wnt pathway

The Wnt signaling pathway regulates a wide range of developmental processes such as proliferation, cell migration, axon guidance and cell-fate determination (Clevers, 2006). Activity of the canonical Wnt signaling results in the stabilization of the cytoplasmic β-catenin which is then associated with the LeF/TCF-type of transcription factors to regulate the expression of downstream targets. The Wnt pathway has been implicated in retinal development with multiple roles (Kubo et al., 2003, 2005; reviewed in Van Raay and Vetter, 2004).

Recent studies have demonstrated a novel role for the canonical Wnt pathway in the specification of retinal progenitors to the non-neuronal fates of the anterior structures, the iris and CB. This proposed function was based on several observations: firstly, some of the Wnt pathway components are highly expressed in the developing iris and CB (Fig. 2) and in the adult structures (Jasoni et al., 1999; Kubo et al., 2003; Liu et al., 2003a). Second, transgenic reporters designed for the detection of canonical Wnt signaling demonstrate activity of this pathway in the iris and CB progenitors in both chick and mouse embryos (Cho and Cepko, 2006; Liu et al., 2003a). Finally, direct evidence for the role of Wnt signaling in the formation of the anterior structures was obtained by recent loss- and gain-of-function studies in avian embryos (Cho and Cepko, 2006; Kubo et al., 2003). Forced expression of Wnt2b or constitutively active β-catenin in the retina caused a dramatic change in the specification of proximal retinal progenitor cells that adopt non-neuronal fate. Coinciding with these findings, blockage of Wnt signaling by expressing dominant-negative Lef1 resulted in an inhibition of the expression of peripheral markers such as Collagen-9 and Bmp7 and consequently led to an iris hypoplasia with a severe reduction in muscle size (Cho and Cepko, 2006). Together, these findings suggest an autonomous function for the Wnt pathway in the specification of the iris and CB progenitors. It should be noted that, although Wnt signaling is fundamental for the development of the iris and CB, it is probably not sufficient to execute their full developmental program as the expression of Pax6 was downregulated following forced expression of β-catenin (Cho and Cepko, 2006).

Co-expression of BMP and Wnt family members in the OC periphery suggests a possible interaction of these two pathways in the development of the iris and CB. The convergence of the two pathways could come about via the regulation of common target genes such as the Msx1 and Msx2. These transcription factors have been shown to be downstream of both pathways (Foerst-Potts et al., 1997; Furuta and Hogan, 1998; Hussein et al., 2003; Willert et al., 2002; Zhao et al., 2002) are expressed in the OC margins and may participate in the initial acquisition of the non-neuronal fate of the progenitor cells.

Furthermore, it should be considered that Wnts and BMPs may have paracrine effect on the pericocular mesenchyme and the morphogenesis of the derivative structures. Supporting this notion are the findings that Pitx2 transcription is modulated by the two pathways in other developmental systems (Kioussi et al., 2002; St Amand et al., 2000).
14. Future prospects

Work done in the last few years has revealed genes that are involved in the specification of non-neuronal structures originating from the OC periphery. One of the emerging questions is what the conversion points of the different signaling pathways are and how these pathways interact with the tissue-specific genes. Another intriguing question concerns the cross-talk between the OC periphery and the adjacent periocular mesenchyme and how each influences the other’s morphogenesis. The third question concerns the partitioning of the non-neuronal progenitors of the OC periphery to CB and iris fates. The latter issue is also interesting in terms of the regeneration properties of the adult CB and iris epithelia. As already mentioned, the mammalian CB pigmented cells are considered retinal stem cells as they form neurospheres and can give rise to multiple retinal cell types (Ahmad et al., 2000; Tropepe et al., 2000). The adjacent IPE is recognized to possess the capacity to transdifferentiate to lens and RPE cell types (Haruta et al., 2001; Tropepe et al., 2000) but its neurogenic potential has not yet been demonstrated in mammals. Learning the possible differences between these two adjacent epithelia may shed light on the mechanisms that maintain regeneration and stem-cell properties in the adult.

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