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Chapter 5

The Role of β-Catenin in Eye Development

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Abstract

The eye is a highly specialized sensory organ which originates from the diencephalon, head surface ectoderm, and neural crest-derived mesenchyme. The diencephalon laterally evaginates towards the head surface ectoderm through the extraocular mesenchyme to form the optic vesicle. When it comes into contact with the surface ectoderm, these two tissues invaginate to give rise to the double-layered optic cup and the lens vesicle. The inner layer of the optic cup later forms the neural retina, while the outer layer becomes the retinal pigment epithelium. The process is precisely controlled by a combination of extrinsic and intrinsic factors. During embryogenesis, β-catenin plays essential roles as a key mediator of Wnt/β-catenin signaling and an indispensable component in cadherin-mediated cell adhesion. Functional analysis using loss-of-function and gain-of-function mutants of *β-catenin* in mice has revealed that *β-catenin* is indispensable for the retinal pigment epithelium differentiation, lens

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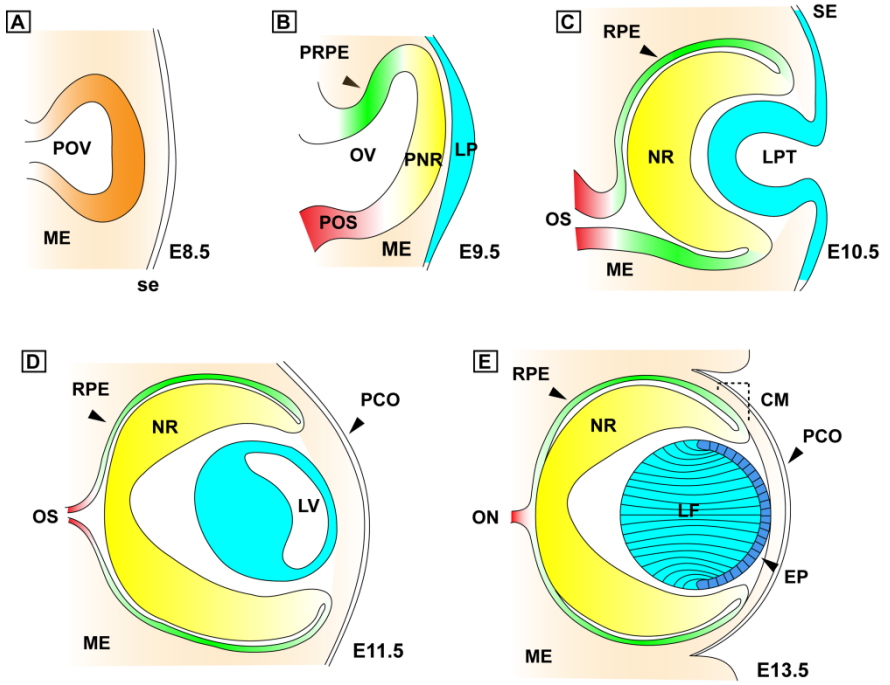
cell morphogenesis, lamination of the neural retina, and determination of the peripheral optic cup fate. This review summarizes the current knowledge about the functions of *β-catenin* in the eye development.

Overview of Vertebrate Eye Development

The first morphological indication of the eye development is formation of the optic vesicle as an evagination of the diencephalon, which expands toward the head surface ectoderm through the mesenchyme (Figure 1A) [1]. As the optic vesicle comes into close contact with the surface ectoderm, they receive signals from the adjacent tissues. In consequence, the head surface ectoderm becomes thickened to give rise to the lens placode and the optic vesicle becomes partitioned into three parts, presumptive neural retina, presumptive retinal pigment epithelium, and presumptive optic stalk (Figure 1B). The optic vesicle subsequently invaginates in coordination with the lens placode to form the double-layered optic cup (Figure 1C). The anterior rim of the optic cup, called the ciliary margin, gives rise to the ciliary body and the iris. The process of the invagination generates the optic fissure that runs from the ventral-most region of the neural retina and along the ventral aspect of the optic stalk. The mesenchymal cells migrate into the optic cup through this fissure to form the hyaloid artery, the main blood supply for the eye. The optic fissure gradually becomes closed after this migration. The lens placode further forms a hollow sphere called the lens vesicle (Figure 1D). As the lens vesicle detaches from the head surface ectoderm, the cornea begins to develop. The space between the lens vesicle and the surface ectoderm is filled by mesenchymal cells, which later condense and form the corneal endothelium and the corneal stroma, while the head surface ectoderm gives rise to the corneal epithelium [1-3].

β-Catenin Loss-of-Function Mutant and Gain-of-Function Mutant

β-catenin plays a dual role, in regulation of cell-cell adhesion and in signal transduction. It is a central component of adherens junction and is tightly associated with the intercellular domains of classical cadherin and α-catenin, which couple to the actin cytoskeleton to form adherens junctions [4, 5].



Abbreviations: E8.5: embryonic day 8.5; pov: presumptive optic vesicle; OV: optic vesicle; SE: head surface ectoderm; ME: extraocular mesenchyme; PRPE: presumptive retinal pigment epithelium; PNR: presumptive neural retina; POS: presumptive optic stalk; LP: lens placode; RPE: retinal pigment epithelium; NR: neural retina; LPT: lens pit; OS: optic stalk; ON: optic nerve; LV: lens vesicle; CM: ciliary margin; PCO: presumptive cornea; EP: lens epithelium; LF: lens fiber.

Figure 1. Schematic diagram of vertebrate eye development. A. Optic vesicle formation. The diencephalon envaginates toward the head surface ectoderm through the mesenchyme to give rise to the optic vesicle. B. Patterning of the optic vesicle. As the optic vesicle comes into contact with the head surface ectoderm, it becomes partitioned into three domains: a dorsal domain, a distal domain, and a proximal domain, which give rise to the retinal pigment epithelium, the neural retina, and the optic stalk, respectively. The head surface ectoderm becomes thickened to form the lens placode. C. Optic cup formation. The optic vesicle invaginates in coordination with the lens placode to form the optic cup and the lens pit. D. Lens vesicle formation. The lens pit closes to form the lens vesicle and detaches from the head surface ectoderm, which later differentiates as corneal epithelium. E. Closure of the lens vesicle. The cells locating at the posterior lens vesicle elongate anteriorly to fill the cavity and differentiate as primary lens fiber cells. The cells in the anterior part of the lens vesicle give rise to lens epithelial cells which migrate posteriorly to the equator and differentiate as secondary lens fiber cells.

As a key mediator of Wnt/β-catenin signaling, β-catenin interacts with Tcf/Lef transcription factors in the nucleus and regulates expression of Wnt target genes. [6, 7]. *β-catenin* null mutation leads to early embryonic lethality, accompanied with a failure of mesoderm generation and anterior-posterior axis formation of the mouse embryos [8, 9].

To overcome the lethality and study the function of *β-catenin* in various tissues and at later stages, conditional loss-of-function mutants of the *β-catenin* gene have been generated by flanking exons 2-6 or exons 3-6 of *β-catenin* by loxP sites [10, 11]. Cre-mediated recombination results in destruction of cell-cell adhesion and inactivation of Wnt/β-catenin signaling. In order to constitutively activate Wnt/β-catenin signaling, *β-catenin* gain-of-function mutation in which exon 3 of the *β-catenin* gene was flanked by two loxP sites has been widely used [12]. Exon 3 of the *β-catenin* gene encodes phosphorylation sites that are required for β-catenin degradation [13]. Therefore, Cre-mediated recombination results in the expression of a stabilized form of β-catenin, which leads to constitutive activation of Wnt/β-catenin signaling [12].

Recently, a novel *β-catenin* mutant mouse line strain which inactivates Wnt/β-catenin signaling but keeps cell adhesion intact has been generated [14]. β-catenin is composed of three main domains called the N-terminal, central, and C-terminal domains. The central domain consists of 12 imperfect armadillo repeats and interacts with multiple proteins, including the component of adherens junction E-cadherin [15], scaffold of the β-catenin destruction complex Axin2 [16], and transcription factor Tcf/Lef [17]. Besides that, Bcl9 interacts with the first armadillo repeat of β-catenin and functions as adaptor between β-catenin and transcriptional coactivator Pygopus. Thus, the N-terminal part of the central domain is required for the transcriptional activity of β-catenin [18-20]. The C-terminus of β-catenin acts as the main transactivation domain, which recruits multiple general transcriptional coactivators such as histone acetyl transferase CBP [21], TATA-binding protein [22], and chromatin-remodeling factor Brg-1 [23].

In order to specifically disrupt its transcriptional function without affecting its ability to form the adherens junctions, the *β-catenin* allele named *β-catenin-D164A-ΔC* was designed: the C-terminus of the *β-catenin* gene is truncated and a single amino acid is changed in order to block interaction between β-catenin and Bcl9 [14]. Therefore, it is possible to inactivate only Wnt/β-catenin signaling in specific tissues by combining the *β-catenin-*

D164A-ΔC allele and the *β-catein* loss-of-function allele with a tissue-specific Cre line [14].

Lens

The first morphological indication of the lens development is thickening of the surface ectoderm to form the lens placode (Figure 1B) [24, 25]. After formation of a hollow lens vesicle from the lens placode, the posterior portion of cells differentiates as primary lens fiber cells that elongate to fill the cavity (Figure 1E). Meanwhile, cells in the anterior part become proliferative lens epithelial cells and continue to divide throughout life. These cells migrate posteriorly to the equatorial region, called the transitional zone, and elongate and differentiate into the secondary lens fiber cells. They subsequently surround the lens nucleus formed by the primary lens fiber cells. The final step of lens differentiation is degradation of cytoplasmic organelles including the nucleus, endoplasmic reticulum, mitochondria, and Golgi apparatus within the mature lens fiber cells. This process is indispensable for the establishment and maintenance of lens transparency since these organelles scatter and absorb light, and obstruct transparency [24, 26, 27].

Several signaling pathways play important roles in the lens development [24, 26]. FGF signaling and BMP signaling are essential for the lens induction and differentiation [28-32]. Notch signaling is required for the secondary fiber cell differentiation [33-35]. Transgenic mice expressing a dominant-negative FGF receptor in the presumptive lens ectoderm show defects in formation of the lens placode and reduced expression of lens induction markers [28]. *Bmp7* is expressed in the surface ectoderm and *Bmp7* null mutation results in failure of the lens formation [29].

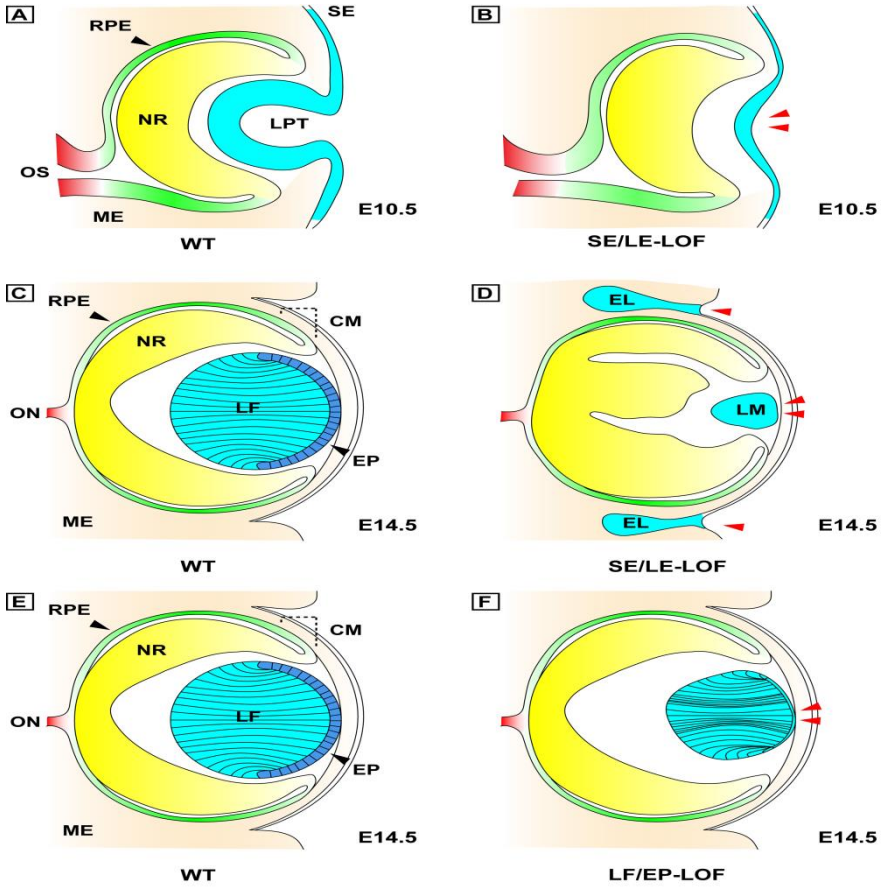
In addition to these signaling pathways, it has been shown that Wnt/β-catenin signaling plays a role in the lens development. Wnt-reporter mouse lines show that this signaling is active in the periocular surface ectoderm as well as in the lens epithelium at early stages of lens development [36-39]. Proper regulation of cell adhesion between the neighboring cells in the developing lens is indispensable for the formation of lens structures and morphogenesis [27]. The major components of cell adherens junctions such as α-catenin are abundantly expressed in the developing lens [40, 41]. Lens-specific deletion of either *E-cadherin* or *N-cadherin* suggests that *E-cadherin* is required for the lens epithelium maintenance and *N-cadherin* controls lens fiber morphogenesis, but they are not required for the lens differentiation [41].

The role of *β-catenin* at the early stage of lens development has been studied by deleting *β-catenin* in the presumptive lens vesicle and the surface ectoderm overlying the optic cup (Figures 2A-D) [37, 39]. The lens of *β-catenin* loss-of-function mutants shows severe defects in cell-cell adhesion and lens morphogenesis characterized by an abnormal expression pattern of F-actin and tight junction marker ZO-1. On the other hand, the lens induction of the *β-catenin* loss-of-function mutant is not affected since expression of the lens-specific markers such as *Prox1* and *β-crystallin* are maintained (Figures 2A, B).

Interestingly, ectopic lentoid bodies that express *Prox1* and *β-crystallin* are formed in the nasal periocular ectoderm, where Wnt/β-catenin signaling is inactivated in the *β-catenin* loss-of-function mutants (Figures 2C, D) [37, 39]. In addition, ectopic activation of Wnt/β-catenin signaling in the head surface ectoderm using *β-catenin* gain-of-function results in inhibition of the lens formation (Figures 4A, B) [38, 39]. Thus, Wnt/β-catenin signaling inhibits the lens formation and appears to suppress the lens fate in the periocular ectoderm, and appropriate activation and suppression of the signaling is required for correct patterning of the ocular tissue.

A recent study has shown that neural crest-derived TGFβ activates Wnt/β-catenin signaling to suppress the lens fate in the adjacent non-lens surface ectoderm in the chick embryo [42]. The migrating periocular neural crest cells express multiple TGFβ ligands such as *Activin-βA* and receive TGFβ signals, as evidenced by the presence of phosphorylated Smad3 [42, 43]. The non-lens surface ectoderm begins to express *Wnt2b* as it comes into contact with the neural crest cells. This coincides with the expression of Wnt target gene *Axin2* in the region, suggesting that Wnt/β-catenin signaling is activated there by Wnt2b. In addition, presumptive lens ectoderm explant experiments have shown that the neural crest induces expression of *Wnt2b* and inhibits lens specification. Furthermore, expression of *Wnt2b* is upregulated by Activin A and downregulated by TGFβ antagonist Smad7 in the same explant culture system. Combined, the neural crest-derived TGFβ activates Smad3 and Wnt/β-catenin signaling by inducing expression of *Wnt2b* in order to suppress the lens fate in the periocular ectoderm [42].

It has been suggested that *Pax6* suppresses Wnt/β-catenin signaling in the lens surface ectoderm. *Pax6* is expressed in the presumptive lens placode at the lens induction stage, and either *Pax6* null mutation or *Pax6* inactivation in the presumptive lens placode results in a failure of the lens for mation [44-46].



Abbreviations: LOF: loss-of-function; E10.5: embryonic day 10.5; SE: head surface ectoderm; ME: extraocular mesenchyme; LPT: lens pit; RPE: retinal pigment epithelium; OS: optic stalk; NR: neural retina; LF: lens fiber; EP: lens epithelium; ON: optic nerve; LF: lens fiber; EP: lens epithelium; CM: ciliary margin; LM: lens mass; EL: ectopic lentoid body.

Figure 2. *β-catenin* loss-of-function in the developing lens. (A-D) Conditional deletion of *β-catenin* in the lens placode and periocular surface ectoderm. Although *β-catenin* loss-of-function has no obvious impact on the lens fate determination, it causes defects in lens cell adhesion and abnormal lens morphogenesis. The lens placode aberrantly invaginates and the lens pit is flattened at E10.5. At later stages, the lens is totally disorganized and the lens mass is located in the misshapen neural retina. Interestingly, ectopic lentoid bodies are formed in the periocular surface ectoderm where Wnt/β-catenin signaling is inactivated. (E, F) Conditional *β-catenin* null mutation in the lens epithelium and fiber. Lens epithelial differentiation is disrupted and lens fiber cells elongate poorly and are abnormally aligned.

It has been shown that Wnt/β-catenin signaling is ectopically active in the presumptive lens placode of *Pax6* null mutants using a Wnt reporter mouse line [38]. In addition, Pax6 directly regulates expression of Wnt inhibitors such as *Sfrp2* and *Dkk1*, suggesting that Pax6 downregulates Wnt/β-catenin signaling in the head surface ectoderm for the lens induction by controlling expression of Wnt inhibitors [38].

β-catenin is required for differentiation of lens epithelial cells as well as for lens cell adhesion at the later stages of lens development. *β-catenin* deletion in both the lens epithelial cells and the lens fiber cells results in disruption of the lens structure because of cell-cell adhesion defects (Figures 2E, F) [47]. The anterior lens epithelium is thinner and the lens fiber cells are abnormally aligned in this *β-catenin* loss-of-function mutant. Furthermore, expression of lens epithelial markers *E-cadherin* and *Pax6* is lost. The lens fiber cell marker *c-Maf* is abnormally detected in the anterior lens epithelium. The lens fiber cell differentiation is also disrupted and is characterized by poor lens fiber cell elongation and downregulation of *β-crystallin* [47]. In addition, null mutation of *Lrp6*, which encodes a coreceptor of Wnt ligands, causes microphthalmia and coloboma [36]. The anterior lens epithelium is incompletely formed in *Lrp6* null mutant as well [36]. Thus, *β-catenin* seems to regulate differentiation of the lens epithelial cells [47]. Consistent with this idea, misexpression of the stabilized form of β-catenin in the lens epithelial cells and the lens fiber cells results in the persistence of *Pax6*- and *E-cadherin*-positive lens epithelial cells in the posterior lens fiber compartment (Figures 4C, D) [48]. Besides that, neither deletion of *β-catenin* nor expression of the stabilized form of β-catenin in the differentiating lens fiber cells of the lens cortex and nucleus has any effect, indicating that β-catenin function is redundant in the lens fiber cells [47, 48]. Together, *β-catenin* plays distinct roles during the lens development. At the lens induction stage, Wnt/β-catenin signaling must be suppressed in the presumptive lens placode. Meanwhile, it should be active to inhibit the lens fate in the nasal periocular ectoderm in favor of other structures. At the early lens differentiation stage, *β-catenin* is required for the anterior lens epithelium development.

Cornea

As the lens vesicle detaches from the surface ectoderm that gives rise to the corneal epithelium, the mesenchymal cells migrate into the space between them. These cells later condense and form several layers separated from each

other by an extracellular matrix. The posterior mesenchyme cells give rise to the endothelial monolayer called the corneal endothelium. The mesenchymal cells between the corneal epithelium and the corneal endothelium differentiate as keratocytes to form the corneal stroma. The corneal epithelium is constantly renewed and maintained by corneal epithelial stem cells residing in the basal epithelium of the corneoscleral limbus, the border of the cornea and the conjunctiva [3, 49].

Cell-cell adhesion plays essential roles in the corneal development and homeostasis. For instance, N-cadherin is expressed in the corneal endothelium and is required for maintaining proper structure of the cornea [50]. Conditional deletion of the *N-cadherin* gene in the cornea results in corneal opacity. F-actin and tight junction protein ZO-1 are mislocalized, and β-catenin is downregulated in the cornea of *N-cadherin* loss-of-function mutant [50]. Transcription factor *Pax6* is also required for the corneal morphogenesis. The cornea of *Pax6* mutant is thinner and shows large gaps between corneal epithelial cells because of adhesion abnormalities [51]. The level of β-catenin, γ-catenin, and desmoglein is reduced in the cornea of the *Pax6* mutant, although the level and localization of E-cadherin are not changed and the actin cytoskeleton appears normal. Therefore, the decrease of catenins seems to be responsible for the abnormal corneal morphology in the *Pax6* mutant [51]. Although deletion of *β-catenin* in the cornea has not been studied yet, *β-catenin* is probably required for the corneal morphogenesis as a component of adherens junction.

Wnt/β-catenin signaling is essential for corneal development in the ocular surface epithelium. β-catenin is primarily expressed at the cell membrane in the corneal epithelium and nuclear β-catenin is detected only in the basal layer of the limbal epithelium [52]. An antagonist of Wnt/β-catenin signaling, *Dkk2*, is strongly expressed in the periocular mesenchyme surrounding the optic cup at the early stage of eye development and in the epithelial layer of the cornea and limbus at postnatal stage [53, 54]. Opaque plaque is visible at the ocular surface of the *Dkk2* null mutant. Expression of *Axin2*, a general target gene of Wnt/β-catenin signaling, is highly upregulated in the conjunctiva, cornea, and limbus of *Dkk2* null mutant mice [53]. The Wnt reporter mouse line also shows that Wnt/β-catenin signaling is ectopically activated in the limbus of the mutant [54]. Furthermore, cornea epithelial cell-specific *keratin 12* and *Pax6* are absent in the corneal epithelium, while ectopic expression of epidermal markers and conjunctival markers are detected there at postnatal stage [53, 54].

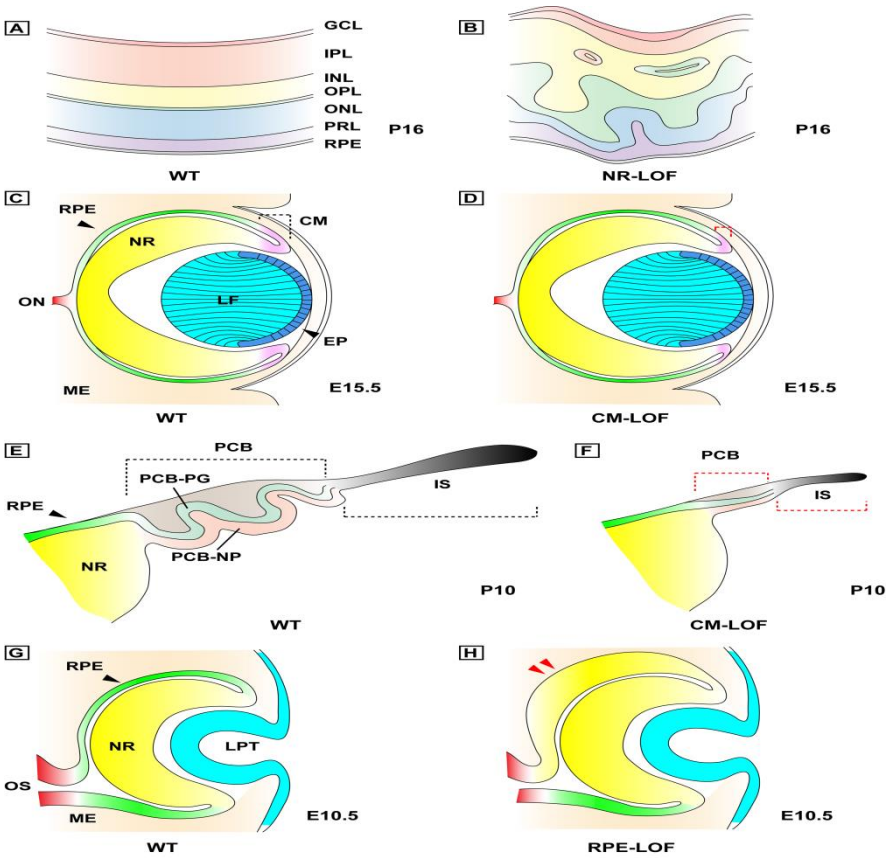
Thus, although the general corneal identity is not lost at prenatal stage, Dkk2-mediated modulation of Wnt/β-catenin signaling seems to be required for the corneal epithelium formation at postnatal stage [53,54].

Ectopic expression of the stabilized form of β-catenin in the corneal epithelium cells results in corneal neoplasia and neovascularization resembling ocular surface squamous neoplasia [55]. *β-catenin* gain-of-function mutant mice exhibit severe corneal opacity with numerous epithelial nodules, where strong nuclear β-catenin signal is detected. Furthermore, expression of the corneal epithelial markers *Pax6* and *Keratin 12* is lost, as seen in the *Dkk2* null mutant. The cornea of these mutants also shows increased cell proliferation, as evidenced by upregulation of *PCNA* and *p63*. Interestingly, conjunctiva and limbus marker *Keratin 15* is extended in the central corneal epithelium, while the expression pattern of conjunctival epithelial marker *Keratin 4* is not changed. Therefore, ectopic expression of the stabilized form of β-catenin appears to induce dedifferentiation of the corneal epithelium cells toward the limbal epithelial progenitor cells. In addition, nuclear β-catenin is abundant in the cornea of ocular surface squamous neoplasia patients, indicating that ectopic β-catenin signaling is involved in this cancer [55].

Wnt/β-catenin signaling regulates proliferation of corneal stem cells in the limbal basal epithelium. Although β-catenin is primarily present in the cytoskeleton, nuclear localization of β-catenin is detected in a small subset of cells in the limbal basal epithelium where corneal stem cells reside [52]. The limbus also expresses Wnt signaling components such as *Wnt2*, *Wnt11*, *Dkk1*, and *Sfrp5*. Activation of Wnt/β-catenin signaling increases proliferation of primary corneal stem cell culture [52]. Consistently, expression of the stabilized form of β-catenin in the corneal epithelium results in upregulation of cell proliferative markers [55], suggesting that Wnt/β-catenin signaling regulates proliferation of corneal stem cells in the limbal basal epithelium [52].

Neural Retina

The inner layer of the optic cup gives rise to the presumptive neural retina, which is composed of multipotent retinal progenitor cells (Figure 1C). The pool of the retinal progenitor cells expands by precisely regulated proliferation and subsequently generates one type of glia, called Müller glia, and six major types of neurons, retinal ganglion cells, amacrine cells, rod photoreceptor cells, cone photoreceptor cells, horizontal cells, and bipolar cells [56, 57].



Abbreviations: LOF: loss-of-function; P16: postnatal day 16; E15.5: embryonic day 15.5; GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; PRL: photoreceptor layer; ME: extraocular mesenchyme; LPT: lens pit; RPE: retinal pigment epithelium; OS: optic stalk; NR: neural retina; LF: lens fiber; EP: lens epithelium; ON: optic nerve; CM: ciliary margin; PCB: presumptive ciliary body; PCB-PG: presumptive pigmented epithelium of the ciliary body; PCB-NP: presumptive non-pigmented epithelium of the ciliary body; IS: iris.

Figure 3. *β-catenin* loss-of-function in the developing retina. (A, B) Elimination of *β-catenin* in the central neural retina. The laminar structure is severely disrupted due to the failure of retinal cell adhesion, although all retinal cell types are generated. (C-F) Conditional deletion of *β-catenin* in the peripheral retina. Inactivation of β-catenin causes reduction in the folds of the ciliary body, as well as in the size of the ciliary margin, ciliary body and iris. (G, H) In activation of Wnt/β-catenin signaling in the retinal pigment epithelium. The retinal pigment epithelium of the mutant hyperproliferates and is transdifferentiated into the neural retina.

The cell bodies of these neurons are arranged into three main cellular layers which are interconnected with two synaptic layers and positioned as follows: the ganglion cell layer contains retinal ganglion cells and dispatched amacrine cells, the inner nuclear layer is composed of amacrine cells, bipolar cells, and horizontal cells and the outer nuclear layer consists of cone and rod photoreceptor cells [56, 57].

β-catenin is localized to the cell membrane at the early stage of retinal development. As the retinal development proceeds, a high level of β-catenin is detected in the ganglion cell layer and the inner plexiform layer [58, 59]. *β-catenin* deletion in the central neural retina results in disruption of the retinal laminar structure and abnormal migration of differentiated retinal cells, while all retinal cell types are generated (Figures 3A, B). Thus, β-catenin mediates retinal lamination by maintaining normal cell-cell adhesion during the neural retinal development, but β-catenin is not required for the neurogenesis [59]. Consistent with this, deletion of *α-catenin* in the developing neural retina also results in disorganization of the neural retina [60]. In addition, ectopic activation of β-catenin signaling results in impaired retinal development both *in vitro* and *in vivo* [59, 61].

It has been indicated that Wnt/β-catenin signaling contributes to the retinal regeneration. It is well established that all types of neurons are regenerated after damage in cold blooded vertebrates [62]. Several reports show that avian and rodent retinae can also generate a limited number of neurons after neurotoxic injury *in vivo*. A limited number of Müller glial cells reenter the cell cycle, proliferate, dedifferentiate into retinal progenitors, and subsequently differentiate as neurons [63-65]. Interestingly, retinal damage induces nuclear accumulation of β-catenin and Wnt reporter activity in mice [66]. Furthermore, Wnt3a treatment promotes proliferation and dedifferentiation of Müller glial cells after retinal injury, while inhibition of Wnt/β-catenin signaling by Dkk1 treatment attenuates the retinal regeneration [66].

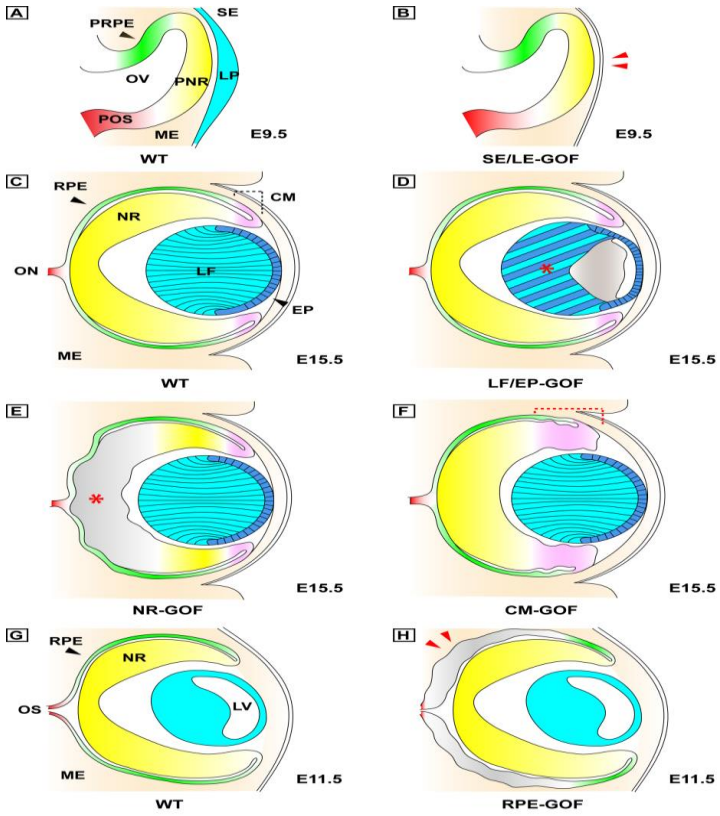
Ciliary Margin

The anterior rims of the optic cup, called the ciliary margin, generate the iris and the ciliary body. They are composed of the nonpigmented inner and the pigmented outer layer, which extends from the neural retina and the retinal pigment epithelium, respectively. The periocular mesenchyme contributes to the formation of the connective tissues and ciliary muscles [1]. In fish and

some amphibians, the multipotent and self-renewing retinal progenitors are located in the ciliary marginal zone. These cells can generate all types of neurons and glial cells and add new retinal cells to the peripheral retina throughout life. The ciliary marginal zone cells in the chick retina generate a subset of retinal cells. Although the peripheral retina lacks the proliferative ciliary marginal zone-like region in mammals, the retinal progenitor cells can be isolated from the pigmented layer of the ciliary body [62, 67, 68].

β-catenin plays essential roles in the ciliary margin development. Wnt/β-catenin signaling components such as *Wnt2b*, *Lef1*, and *Frizzled 4* are expressed in the developing ciliary margin [69]. It has been shown that Wnt/β-catenin signaling is active in the developing peripheral retina while it is inactive in the central retina using Wnt/β-catenin signaling reporters in mice, chicks, and frogs [69-71]. Conditional deletion of the *β-catenin* gene in the peripheral retina results in defects in retinal lamination, probably because of abnormal localization of adherens junction components [59]. In addition, development of the ciliary margin and ciliary body is attenuated, as evidenced by a decrease in size of the ciliary body and downregulation of the ciliary margin markers *Otx1* and *Msx1* (Figures 3C-F) [72]. On the contrary, ectopic activation of Wnt/β-catenin signaling by overexpression of the stabilized form of β-catenin in the peripheral retina leads to an expansion of peripheral retina at the expense of neural retina, characterized by increased expression of the ciliary margin markers and downregulation of the neural retinal markers (Figures 4C, F) [72].

Similarly, gain-of-function and loss-of-function analysis in the chick embryo also suggests that Wnt/β-catenin signaling is required for the determination of the identity of peripheral eye [70]. The retina which expresses constitutively active β-catenin by use of *in ovo* electroporation, is unusually thin and folded and similar to the morphology of the ciliary body and iris. Furthermore, expression of the retinal progenitor marker and retinal neuronal markers is lost, while ectopic expression of the ciliary body markers and ciliary/iris epithelial markers is detected in the thin and folded retina. Conversely, expression of dominant-negative Lef1, a fusion protein of Lef1 with transcriptional repressor Engrailed, interferes with the peripheral eye development, including iris hypoplasia [70]. Additionally, overexpression of *Wnt2b*, which is usually expressed in the ciliary margin in the chick embryo, also shows similar phenotypes, although the phenotype is weaker and less penetrant [70].



Abbreviations: GOF: gain-of-function; E9.5: embryonic day 9.5; OV: optic vesicle; SE: head surface ectoderm; ME: extraocular mesenchyme; PRPE: presumptive retinal pigment epithelium; PNR: presumptive neural retina; POS: presumptive optic stalk; LP: lens placode; RPE: retinal pigment epithelium; NR: neural retina; OS: optic stalk; ON: optic nerve; LV: lens vesicle; CM: ciliary margin; EP: lens epithelium; LF: lens fiber.

Figure 4. *β-catenin* gain-of-function in the developing eye. (A, B) Ectopic activation of Wnt/β-catenin signaling in the lens placode and periocular surface ectoderm. Lens development is suppressed and lens placode formation fails. (C, D) Expression of the stabilized form of β-catenin in the lens epithelium and fiber. Lens fiber cell differentiation is disrupted in the mutant. The anterior fiber cell compartment forms a large space containing degenerated cells and the posterior compartment is abnormally populated by numerous epithelial-like cells. E. Over expression of the stabilized form of β-catenin in the central neural retina. The central neural retina loses the neural retinal identity and its structure is disrupted. F. Targeted expression of the constitutively active form of β-catenin in the peripheral retina. The ciliary margin expands towards the neural retina. (G, H) Aberrant activation of Wnt/β-catenin signaling in the retinal pigment epithelium. Retinal pigment epithelium development is interrupted in the proximal part of the retinal pigment epithelium, in which Wnt/β-catenin signaling is ectopically activated.

Retinal Pigment Epithelium

The retinal pigment epithelium, a single layer of pigmented cells, is derived from the outer layer of the optic cup, while the inner layer forms the neural retina (Figure 1C). Signals from adjacent tissues are essential for the specification of the neural retina and the retinal pigment epithelium within the optic vesicle. The distal part of the optic vesicle exchanges signals with the head surface ectoderm to give rise to the neural retina. Meanwhile, the dorsal optic vesicle receives signals from the extraocular mesenchyme to differentiate into the retinal pigment epithelium [2, 73]. Chick optic vesicle explant culture experiments have shown that the presumptive retinal pigment epithelium loses expression of the retinal pigment epithelial markers such as *Wnt2b* and *Mitf* and ectopically expresses the neural retina-specific markers in the absence of extracellular mesenchyme. Further experiments show that TGFβ family member. Activin, which is expressed in the extraocular mesenchyme, can substitute for it [74]. In addition to signals from the extraocular mesenchyme, transcription factors *Mitf* and *Otx1*/*Otx2* are important for the retinal pigment epithelium. The retinal pigment epithelium of *Mitf* null mutant mice remains unpigmented, hyperproliferates, and differentiates as the neural retina [75]. The retinal pigment epithelium of *Otx1*/*Otx2*-deficient mice is also replaced by the neural retina-like tissue [76]. Furthermore, Mitf and Otx2 regulate expression of melanogenic enzymes such as *Tyr* [77-80].

Wnt/β-catenin signaling plays an essential role in the retinal pigment epithelial development [81, 82]. This signaling is active in the presumptive retinal pigment epithelium already at the optic vesicle stage and subsequently is restricted to the peripheral retinal pigment epithelium at the optic cup stage [69, 81, 82]. In addition, *Wnt2b* is strongly expressed in the retinal pigment epithelium [69,70,74]. Conditional deletion of *β-catenin* in the developing retinal pigment epithelium results in severe ocular defects. The retinal pigment epithelium, a single layer of cuboidal cells, is hypopigmented and hypercellular [81, 82]. At later stages, bridge-like structures are observed between the neural retina and the hypocellular retinal pigment epithelium. The laminar structure of the neural retina is also disrupted [82]. These defects observed at later stages are observed in mice with ablated retinal pigment epithelium [83]. In addition, expression of the retinal pigmented epithelium markers *Mitf* and *Otx2* is downregulated. As seen in *Mitf* null mutant or *Otx1*/*Otx2*deficient mutant, the retinal pigment epithelium of *β-catenin* loss-of-function mutant is transdifferentiated into the neural retina, as evidenced by ectopic expression of the neural retinal markers *Chx10* and *Rax* (Figures 3G,

H) [81, 82]. Neurogenesis occurs normally in the transdifferentiated retinal pigment epithelium, indicating that *β-catenin* is not required for this process, as seen in *β-catenin* conditional knockout mutants in the neural retina [82]. Consistently, inactivation of Wnt/β-catenin signaling in explant cultures of the chick optic vesicle results in transdifferentiation of the retinal pigment epithelium into the neural tissue [84].

Interestingly, ectopic activation of Wnt/β-catenin signaling in the entire retinal pigment epithelium also results in disruption of the retinal pigment epithelium patterning. The peripheral retinal pigment epithelium remains normal, while the central part where Wnt/β-catenin signaling is ectopically active becomes hypercellular and loses expression of the retinal pigment epithelium markers *Mitf* and *Otx2*. In contrast to *β-catenin* loss-of-function mutant, the retinal pigment epithelium is not transdifferentiated to the neural retina (Figures 4G, H) [81]. Thus, the activity of Wnt/β-catenin signaling should be spatially and temporally regulated during the retinal pigment epithelium development.

Expression of *Mitf* and *Otx2* is directly regulated by Wnt/β-catenin signaling. The retinal pigment epithelium-specific *Mitf-D* and *Otx2* enhancers contain Tcf/Lef binding sites. β-catenin activates *Mitf-D* and *Otx2* enhancers *in vitro* and it also present on their enhancers *in vivo* [81, 82]. Furthermore, misexpression of *Otx2* and *β-catenin* in the presumptive chick neural retina promotes the retinal pigment epithelium fates, although ectopic expression of *Otx2* or *β-catenin* alone is not sufficient. Thus, *Otx2* and *β-catenin* seem to cooperate in the retinal pigment epithelium differentiation [84]. Together, Wnt/β-catenin signaling is required for maintaining the cell fate of the retinal pigment epithelium by regulating expression of *Mitf* and *Otx2*.

Conclusion

The vertebrate eye originates from the neuroectoderm of the diencephalon, the head surface ectoderm, and the neural crest-derived mesenchyme. During the eye development, a series of cellular interactions occur to determine the fate of the prospective ocular tissue.

Transcription factors, extracellular signaling molecules, and cytoskeletal proteins control this complex process. The activity of Wnt/β-catenin signaling is precisely controlled during the eye development and misregulation of the

signaling results in ocular malformations due to the defects in the process of cell fate determination and differentiation.

The key mediator of this signaling β-catenin has another important role as a component of adherens junctions and controls morphogenesis in multiple ocular tissues during the eye development. Further research is necessary to clarify the mechanisms by which *β-catenin* integrates into the genetic regulatory networks controlling the eye development.

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