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Wakayama Symposium: Notch-FoxL2- α -SMA axis in eyelid levator muscle development and congenital blepharophimosis

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Abstract

This review summarizes our recent findings regarding the Notch signaling pathway in regulating normal eyelid morphogenesis and its role in the pathogenesis of human congenital blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES). We used genetic and molecular biological approaches to investigate the mechanism by which Notch1 activation controls expression of *FoxL2*, which in turn activates smooth muscle actin gene expression in periocular mesenchyma to control eyelid levator smooth muscle formation.

Keywords

blepharophimosis; eyelid; FoxL2; muscle; morphogenesis; Notch; signaling

I. Introduction

In mammals, eyelids are of paramount importance to protect the ocular surface from environmental insults. Additionally, they help to regulate the light reaching the eye, distribute the protective and optically important tear film over the cornea during blinking, and aid tear flow by their pumping action on the conjunctival and lacrimal sacs. Eyelid morphogenesis is a dynamic process involving active interactions between the epidermis and the dermis during embryonic development. To achieve eyelid closure in the fetus, a coordinative movement of the neural crest-derived periocular mesenchymal cells (POMCs) plays a pivotal role in forming the lid-specific structures, including levator smooth muscle, tarsus, and meibomian glands. In adults, the upper eyelid drapes over the eye to protect the ocular surface and help keep it moist. To enable vision, the levator muscle performs most of the work required to lift the upper eyelid out of the way. With ptosis (blepharoptosis), the eyelid droops too low and cannot be lifted enough for the eye to see.

Blepharoptosis can be classified as congenital or acquired. A more comprehensive classification is based on etiology and includes myogenic, aponeurotic, neurogenic, mechanical, traumatic, and pseudoptotic. Most commonly, congenital ptosis is myogenic

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and caused by improper development of the levator muscle. Blepharoptosis, which is a droopy upper eyelid, results from dysfunctioning of upper eyelid elevator muscles. These elevator muscles consist of the striated levator palpebrae superioris and the levator smooth muscle.¹⁻³

II. Genetic Basis of Congenital Ptosis

Genes associated with congenital ptosis include *ZFH4* and *FoxL2*. *ZFH4* codes for a zinc finger homeodomain protein that is a transcription factor expressed in both muscle and nerve tissue.⁴ However, little is known about the function of *ZFH4*, and the *Zfh4* knockout mouse model has not been available for studying the causal relationship between *Zfh4* and congenital ptosis. In contrast, mutations in *FoxL2* cause blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES) and premature ovarian failure (POF).⁵ *FoxL2*, encoding a winged-helix/forkhead domain transcription factor, is expressed mainly in the periocular mesenchymal cells during eyelid development and granulosa cells in the ovary.⁵

BPES is an autosomal dominant disorder characterized by craniofacial defects that mainly affect the development of the eyelids. There are two types of BPES. Type I consists of the four major features of blepharophimosis, ptosis, epicanthus inversus, and telecanthus plus POF, leading to infertility in women. Type II consists of only the eyelid malformations without gender preference. People with BPES are at an increased risk of developing vision problems, such as myopia, hyperopia, strabismus, and amblyopia. Mutations in the *FoxL2* coding region gene cause about 70 percent of BPES.⁶

Mice lacking *FoxL2* exhibited craniofacial anomalies, including eye-open at birth (EOB) and ovarian malformations with high rates of perinatal mortality (>50% died before 1 week).^{7, 8} These results suggest that mutations, which lead to qualitative or quantitative changes of *FoxL2*, are involved in the pathogenesis of eyelids and ovary in BPES. In the ovary, reactive oxidative stress is the major inducer for upregulating *FoxL2* to trigger the modulation of stress-related target genes, such as manganese superoxide dismutase. However, little is known about the upstream and downstream of *FoxL2* regulations during eyelid morphogenesis. Recently, we have found that Notch1 activation may serve as the upstream control of *FoxL2* expression by POMCs, which are destined for levator smooth muscle development of the eyelids.⁹

III. Notch Signaling

A. Background

The Notch signaling pathway is a central mediator of short-range intercellular communication in metazoans. Notch signaling has been shown to play a pivotal role in various cellular processes, including cell fate determination, differentiation, proliferation, apoptosis, cell-cell adhesion, and migration events through local cell-cell interactions. In ocular surface tissues, Notch signaling has been well documented to play crucial roles in homeostasis of cornea, but its specific function in eyelid morphogenesis has only been speculated.^{10,11} The cellular responses to Notch signaling are exquisite context- and dosage-dependent.

B. Findings in a Transgenic Mouse Model

To elucidate Notch function in eyelid development, we generated a doxycycline (Dox)-inducible transgenic mouse strain, designated as *Kera-rtTA/tetO-Cre/R26^{flxed}N1-ICD* (hereafter abbreviated as *KR/TC/R26^{fN1-ICD}*) which harbored the three following transgenes:

1. *Kera-rtTA (KR)*: *Keratocan (Kera)* promoter-driven reverse tetracycline trans-activator;
2. *tet-O-Cre (TC)*: Cre recombinase under the control of a tetracycline-responsive promoter element (*TRE*; *tet-O*); and
3. *Rosa26^{flxedN1-ICD} (R26^{flN1-ICD})*: two LoxP sites flanked Notch 1-intracellular domain (*N1-ICD*) under the control of mouse *Rosa26* gene locus.

We showed that POMC^{N1-ICD} in Dox-treated *KR/TC/R26^{flN1-ICD}* triple transgenic mice were viable and fertile, but exhibited craniofacial defects in ears and eyelid morphogenesis during embryonic development and incomplete eyelid opening postnatally. We observed that Dox-treated *KR/TC/R26^{flN1-ICD}* mice showed delayed eyelid closure at E15.5 and exhibited partially open eyelid without secondary exposure keratitis at birth. In addition, the eyelid re-opening, which normally occurs at post-natal day 12, was delayed and the lid never fully opened throughout life in these mice. These phenotypes vividly resemble type II BPES in humans (Figure 1).

We further showed that POMC^{N1-ICD} effects resulted in a dramatic downregulation of FoxL2 expression and absence of α -SMA-positive levator smooth muscle in the eyelids. Therefore, the Dox-treated *KR/TC/R26^{flN1-ICD}* triple transgenic mice can mimic *FoxL2* conditional knockdown in POMC^{N1-ICD}. Our data suggest that a physiologically low level of Notch has a critical role in controlling proper FoxL2 expression in the POMCs, which is essential for eyelid levator muscle formation and normal eyelid development. In contrast, aberrantly sustained Notch activation mediated through recombination signal binding protein for immunoglobulin kappa J region protein and mastermind-like protein-1 (**RBPJ–MAML-1**) complex inhibits FoxL2 expression and impairs levator muscle formation, leading to eyelid malformation and BPES-like phenotypes (Figure 2). Thus, *KR/TC/R26^{flN1-ICD}* mouse strain can serve as a novel disease model for studying eyelid morphogenesis and the pathogenesis of human BPES type II.

IV. Summary

It remains unknown how the Notch activity is tightly controlled in vivo to ensure appropriate output during eyelid morphogenesis. It has been recently shown that Notch activation is regulated by positive and negative signals. The Hippo pathway promotes notch signaling¹²; on the other hand, Nemo-like kinase (**NLK**) suppresses Notch signaling by interfering with formation of the Notch transcriptional complex.¹³ It will be interesting to investigate whether Notch activation is under the control of Hippo and/or NLK for eyelid morphogenesis.

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A. **B.** **Figure 1.**

POMCN1-ICD caused blepharophimosis in mice, resembling congenital BPES in human. A. Eyelid defects in *KR/TC/R26^{fN1-ICD}* mouse family. Agouti mother (first generation-F1, at P60) and two (second generation-F2, at P15). B. Eyelid defects in a BPES family. Father (first generation-I) and two children (second generation-II). (Reprinted from Crisponi L, et al. with permission of Nature Publishing Group.)

Fig.2.

A proposed model depicts POMCN1-ICD effects in the mouse to impair eyelid levator muscle formation via down-regulation of FoxL2, leading to BPES-like malformations.