

## 論文の内容の要旨

論文題目 BMP signaling regulates retinal cell differentiation and morphogenesis  
in mouse developing retina  
(マウス網膜発生後期におけるBMPシグナルの機能解析)

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### **Abstract**

The vertebrate neural retina is a light sensitive tissue that lines the inner surface of the eye. The neural retina is organized into a laminar structure that is comprised of six types of neurons and glial cells, Müller glia and astrocytes. In the mice, these major retinal cell types are generated from a common population of multipotent retinal progenitor cells between embryonic day (E) 10 and postnatal day (P) 11, in a conserved temporal order. The progression of retinal neurogenesis and retinal cell fate specification are controlled by intrinsic cues, such as transcription factors, as well as by extrinsic signals.

It has been known that bone morphogenetic protein (BMP) signaling controls various processes of early retinal development including maintenance of D-V patterning, expansion of retinal progenitor cells and apoptosis. However, the involvement of BMP signaling in later retinal development, including retinal cell differentiation and maturation is still unclear. Therefore, I focused on the function of BMP signaling in late phase development in mice retina.

To unveil this issue, I first confirmed the expression of BMP-2, -4, -7, BMP receptor-Ia (BMPR-Ia), -Ib and -II in the developing mice retina by RT-qPCR. All molecules expressed in the retina during not only embryonic stages but also postnatal stages. Furthermore, active BMP signaling was detected in retinal progenitor cells,

ganglion cells, amacrine cells, horizontal cells, bipolar cells and Müller glia by immunostaining.

I next performed gain- and loss of function analysis of BMP signaling by the introduction of constitutively active (ca) and dominant negative forms of BMPRs. It revealed that BMP signaling promotes the differentiation of bipolar cells and Müller glia at the expense of rod photoreceptors. Furthermore, BMP signaling induced the expression of one of basic-helix-loop-helix transcriptional repressors, Hey2. To confirm the effect of BMP-Hey2 pathway on the retinal development, I next performed rescue of the differentiation of the above-mentioned retinal cell types by co-transduction of shHey2 together with caBMPRs. As a result, suppression of Hey2 partly reversed the phenotype in caBMPRs expressed retina. Because Notch signaling target gene Hey2 was regulated by BMP signaling, I examined the crosstalk between BMP signaling and Notch signaling. Manipulation of BMP signaling and Notch signaling in explant retina showed that these two signal pathway had redundant manner and partly depended on each other.

To further confirm the function of BMP signaling in retinal cell differentiation, I analyzed the retinal development in both *Smad4<sup>fx/fx</sup>;ROSA26-CreER<sup>T2</sup>* mice and *Smad4<sup>fx/fx</sup>;Dkk3-Cre* mice. As a result, *Smad4<sup>fx/fx</sup>;ROSA26-CreER<sup>T2</sup>* mice showed that the number of bipolar cells and Müller glia were decreased. This result emphasized above conclusion that BMP signaling regulated the differentiation of bipolar cells and Müller glia. Furthermore, *Smad4<sup>fx/fx</sup>;Dkk3-Cre* mice showed that the number of various retinal cell types were decreased.

Lastly, Morphological analysis of Müller glia revealed that the extension of Müller glial process is also regulated by BMP signaling. Application of inhibitor of BMPRs, LDN193189 suppressed the process extension of Müller glia after 14 days culture from E17. I furthermore found that this regulation in Müller glial process

extension was independent of Hey2 by performing the experiment by the introduction of shHey2.

As a conclusion, activation of BMP signaling was maintained in postnatal mice retina, and BMP signaling plays important roles in morphogenesis of retina and retinal cell differentiation during not only early stages but also later stages.