論文の内容の要旨

Functional analysis of Ripply2, a suppressor of Tbx6, in mouse somitogenesis

（マウスの体節形成における Tbx6 抑制因子 Ripply2 の機能解析）

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Introduction

Somites are transient reiterated structures formed by paraxial mesoderm, which bud off from the anterior end of the presomitic mesoderm (PSM) in every 2 hours in mice (Fig.1). During mouse somitogenesis, the anterior limit of Tbx6 protein expression is an important factor for the accurate somite segmentation. This anterior limit is defined by Mesp2, which transcription is induced by Tbx6 but in turn promotes the Tbx6 degradation at the segmentation boundary of newly forming somite. In previous studies, *Ripply2*, a Mesp2 target, is proposed to be involved in the Mesp2-mediated Tbx6 degradation, since *Ripply2*-null mouse exhibits an anterior expansion of the Tbx6 expression domain, resemble the *Mesp2*-null phenotype (Fig. 2). Although Ripply2 plays a role in the suppression of Tbx6, it is unclear whether Ripply2 acts independently of Mesp2. The molecular mechanism of Tbx6 degradation is also still unknown.

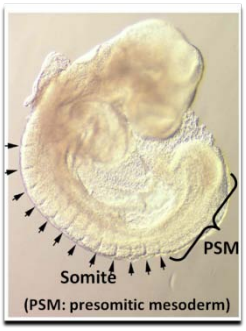

Fig.1 Somites in a mouse embryo

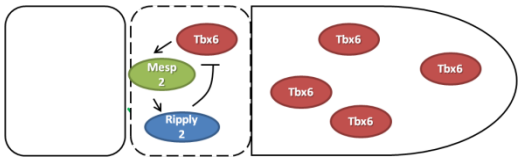

Fig.2 Expected cascade in somitogenesis

Result

1. Effect of Ripply2 over-expression (OE) in somitogenesis

I generated *Ripply2* over-expressing transgenic (TG) mice controlled by *Ripply2* enhancer -promoter. These mice exhibited vertebra formation defects (Fig.3).

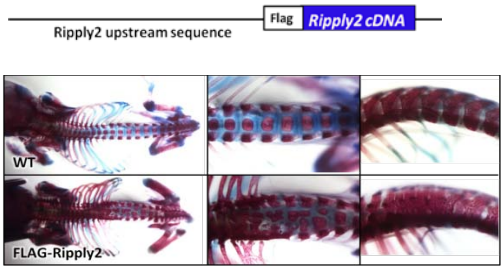

Fig.3 Transgenic mouse exhibited caudalized phenotype

Analysis of somite specific genes expression suggested that over-expressed *Ripply2* significantly down regulated *Mesp2* expression and affected rostro-caudal patterning. Since the caudalized phenotype was also observed in *Mesp2*-null embryos, Ripply2 probably influenced Tbx6-dependant *Mesp2* expression. To clarify this issue, I decided to examine dynamics of Tbx6, Mesp2 and Ripply2 protein expression. For this purpose, I generated anti-mouse Ripply2 antibody, and investigated changes of three factors within different time phases. Immunohistochemical experiments showed down regulation of Tbx6 and Mesp2 protein in the Ripply2-OE embryo compared to WT embryos at the same time phase. Quick downregulation of Tbx6 by Ripply2 must contribute to the temporally shorter Mesp2 expression, which may lead to the caudalized phenotype as seen in *Mesp2*-null embryos. Therefore, this result supported the predicted cascade, in which Tbx6 is degraded via Ripply2 function.

2. Segmental border is established by Ripply2independent of Mesp2

Since the endogenous expression patterns of Ripply2 and Mesp2 were similar, I cannot exclude the possibility that Ripply2 and Mesp2 works in concert to conduct this suppression. To test this possibility, I generated a $Mesp2^{Ripply2/Ripply2}$ mouse by replacing *Mesp2* with *Ripply2*. I found that the anterior limit of Tbx6 expression domain, which indicates the segmental border, can be established by Ripply2 without the function of Mesp2 (Fig. 4). However, segmented somites were never

observed in *Mesp2*$^{Ripply2/Ripply2}$ embryo, suggesting that Mesp2 is required for the following somite segregation, which is a different event from positioning of segmental border.

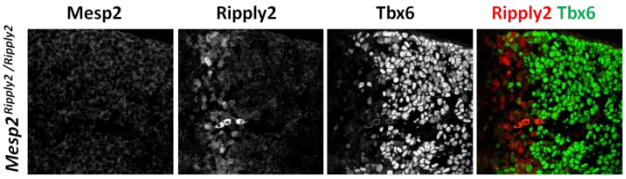

Fig.4 Ripply2 alone is sufficient for establishing Tbx6 anterior limit

3. Tbx6 degradation induced by ectopic expression of Ripply2 in the PSM

To obtain more direct evidence that Ripply2 is the factor that directly degrades Tbx6 protein independently of Mesp2, I generated transgenic mice in which Ripply2 can be conditionally induced via Cre-mediated recombination. When Ripply2 was induced ectopically in the PSM, embryos lost their posterior somites. Interestingly, Sox2 positive neural tubes were formed instead of Tbx6-expressing PSM at bilateral domains, which resemble the *Tbx6*-null phenotype (Fig.5). These results provide evidences that Ripply2 suppresses Tbx6 independent of Mesp2 expression.

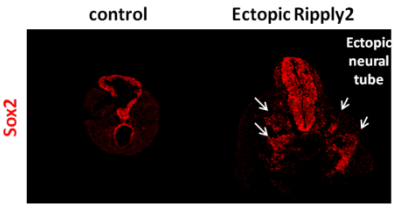

Fig.5 Down-regulation of Tbx6 by Ripply2 GOF leads to lower Mesp2 expression, which contributed to caudalized phenotype.

Conclusion

① Ripply2-OE mouse confirmed Tbx6-Mesp2-Ripply2 cascade, and demonstrated that Ripply2 dosage is important for normal somitogenesis.

② The anterior limit of Tbx6 expression domain, which indicates the segmental border, can be established by Ripply2 without the function of Mesp2

③ Ectopic Ripply2 expression in the PSM induced ectopic neural tubes as similar to *Tbx6*-null embryos, indicating that Ripply2 can directly destabilize Tbx6 protein independently of Mesp2.