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ABSTRACT OF DISSERTATION

Title	Ctip2-mediated *Sp6* transcriptional regulation in dental epithelium-derived cells 歯原性上皮細胞における Ctip2 による *Sp6* 遺伝子の転写制御
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Tooth development relies on sequential and reciprocal interaction between the oral ectoderm and the underlying mesenchyme, and is regulated by a complex genetic cascade. This transcriptional cascade is regulated by the spatiotemporal activation and deactivation of transcription factors. The specificity proteins 6 (Sp6) and chicken ovalbumin upstream promoter transcription factor-interacting protein 2 (Ctip2) were identified as key transcription factors required for tooth development in loss-of-function studies. Also, Ctip2 has been reported to bind to the *Sp6* promoter *in vivo*. However, its role in *Sp6* expression remains unclear. Therefore, the aim of this study is to understand the molecular basis for the role of Ctip2 in Sp6 regulation in dental epithelial cells.

In this study, I investigated *Sp6* transcriptional regulation by Ctip2. Immunohistochemistry showed that both Sp6 and Ctip2 were co-localized in the nucleus of ameloblasts in rat mandibular incisors at postnatal day 1. This result supports the possible interaction between these two molecules.

Then, I assumed that Ctip2 regulate *Sp6* transcriptional activity via GGCCGG motif in the proximal region of the first promoter. Unfortunately by *in silico* analysis, GGCCGG motif could not be found in this proximal region. However, I found another consensus motif AGCCAG in this proximal region of the first promoter, and also both AGCCAG and GGCCGG motifs in the second promoter of *Sp6*. Based on this *in silico* analysis, I utilized several *Sp6* promoter constructs, named A - E regions, to examine the molecular basis of Ctip2 in *Sp6* transcriptional activity. Interestingly, I observed the suppressive effect of Ctip2 in *Sp6* transcriptional activity via D-region of *Sp6* second promoter containing GGCCGG motif, but not from the *Sp6* first promoter. Moreover, I also observed the different effect between Ctip2 isoforms, in which Ctip2-short showed the stronger suppressive effect

compare to Ctip2-long.

In protein-DNA binding analysis by ChIP-PCR, I confirmed the direct binding of Ctip2 isoforms in both the first and second *Sp6* promoter. These findings indicated that Ctip2 binds directly to *Sp6* first and second promoters, but Ctip2 regulates *Sp6* gene expression through the *Sp6* second promoter region.

In conclusion, my study demonstrated the molecular linkage between Ctip2 and *Sp6* transcriptional activity in the dental epithelial derived cells. However, there are some discrepancies between our *in vitro* condition and the previous *in vivo* condition. Further investigations are required for better understanding the molecular basis underlying tooth development regulated by Ctip2 and Sp6.