Report of “Research Award of Oral Sciences”

Department: Department of Molecular Biology
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Title: Switching mechanism of Sp6 transcriptional regulation by Ctip2 in dental epithelial cells

1. Aim of research and the results obtained (Approximately 800 words):

The cell-tissue based regenerative therapy is one of the alternative treatments to regain high QOL after losing tooth. In order to regenerate tooth, understanding the molecular basis for tooth development is essentially required.

One of the critical molecules involves in tooth development is Sp6. Also Ctip2 has been known to be one of the possible upstream regulators for Sp6 in the context-dependent manner according to our previous achievements. Then it is required to understand the specific role of Ctip2 for controlling dynamic Sp6 regulation during tooth development, especially ameloblast differentiation.

To understand the specific role of Ctip2 in Sp6 regulation, first I tried to identify the Ctip2 responsible element with luc assay using several motif-mutated of Sp6 promoter. Unfortunately, it was difficult to recover the suppressive effect from both Ctip2 isoform. Then I tried to screen the Ctip2 responsible element(s) by narrowed ChIP-PCR primer sets of 4 divided regions, (e-1), (e), (e+1), and (e+2) within Sp6 promoter and the result suggested that Ctip2 could strongly bind to the (e) and (e+1) regions compared to the (e-1) and (e+2) regions. Next, I utilized the (e) region which contains more predicted binding-motifs compared to (e+1) as an oligo DNA probes for EMSA assay using both Ctip2 and Sp6-promoter co-transfected COS-7 cells to confirm the specific binding. From this data, I could get the positive signal for the specific binding of Ctip2 to the (e) region of Sp6 promoter harbouring six predicted binding GC motifs, which suggested that Ctip2 specifically binds to this indicated region and promoter context dependent regulation. The finding was reported in The 3rd ASEAN Plus and Tokushima Joint International Conference, Makassar, Indonesia, Dec 4-5th, 2014.

2. Self-evaluation of research achievement:

Before checking the effect of Ctip2 modification on Sp6 promoter activity, it
was crucial for further detailed analysis of Sp6 promoter itself. This time, I could narrow the responsible region of Ctip2 binding to Sp6 promoter down to six GC-containing motifs and confirmed the binding by EMSA. In conclusion, I could confirm a Ctip2 responsible region within Sp6 promoter.

Furthermore, based on this achievement, I have started to characterize the effect of mutated-Sp6 protein on amelogenesis, in which in vitro disease model has been established from the amelogenesis imperfecta (AMI) rat. Now, I am trying to confirm the molecular based-function of this third zinc finger domain of Sp6 to the transcriptional activation of downstream-target genes using AMI-derived cells.

3. Meeting presentation:
   * Title, conference, venue, date, co-author, presentation (oral/poster).
     (Underline the speaker.)

1. **Adiningrat A.** Miyoshi K, Yanuaryska RD, Hagita H, Horiguchi T, Tanimura A and Noma T:
   Establishment and characterization of dental epithelial cells derived from amelogenesis imperfecta (AMI) rat
   The 26th Innovative Research Symposium "Regenerative Medicine for Clinical Application", Tokushima, Japan, March 4th, 2015. (Oral Presentation)

2. **Adiningrat A.** Tanimura A, Miyoshi K, Yanuaryska RD, Hagita H, Horiguchi T and Noma T:
   Ctip2 Regulation of Tooth Development via Sp6 Gene Expression
   The 3rd ASEAN Plus and Tokushima Joint International Conference, Makassar, Indonesia, Dec 4-5th, 2014. (Oral presentation)

3. **Adiningrat A.** Miyoshi K, Yanuaryska RD, Hagita H, Horiguchi T, Tanimura A and Noma T:
   Establishment and characterization of dental epithelial cells derived from amelogenesis imperfecta rat
   The 87th Annual Meeting of the Japanese Biochemical Society, Kyoto, Japan, Oct 18th, 2014. (Poster presentation)

4. **Adiningrat A.** Tanimura A, Miyoshi K, Yanuaryska RD, Horiguchi T, Hagita H and Noma T:
Role of Ctip2 in Sp6 regulation in dental epithelial-derived cells
Biosciences Retreat Meeting, Shodoshima, Japan, Sep 18-20th, 2014. (Oral presentation)

4. Journal publication:
   * Title, journal, volume, number, paragraph, date, co-author.
     (Underline the speaker.)
   ② Yanuaryska RD, Miyoshi K, Adiningrat A, Horiguchi T, Tanimura A, Hagita H, and Noma T.
     Sp6 regulation of Rock1 promoter activity in dental epithelial cells.