

## 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 3<sup>rd</sup> ASEAN Plus and Tokushima Joint International Conference, Makassar, Indonesia, Dec 4-5<sup>th</sup>, 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 26<sup>th</sup> Innovative Research Symposium "Regenerative Medicine for Clinical Application", Tokushima, Japan, March 4<sup>th</sup>, 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 3<sup>rd</sup> ASEAN Plus and Tokushima Joint International Conference, Makassar, Indonesia, Dec 4-5<sup>th</sup>, 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 87<sup>th</sup> Annual Meeting of the Japanese Biochemical Society, Kyoto, Japan, Oct 18<sup>th</sup>, 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 18-20<sup>th</sup>, 2014. (Oral presentation)

4. Journal publication:

\* Title, journal, volume, number, paragraph, date, co-author.

(Underline the speaker.)

- ① **Adiningrat A**, Tanimura A, Miyoshi K, Yanuaryska RD, Hagita H, Horiguchi T, and Noma T.

Ctip2-mediated *Sp6* transcriptional regulation in dental epithelium-derived cells.

J Med Invest. 61: 126-136, 2014.

- 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.

J Med Invest. 61: 306-317, 2014.