Report of "Research Award of Oral Sciences"

Department: Molecular Biology
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Title: Isolation and characterization of ARE-clones derived from amelogenesis imperfecta rat as an *in vitro* disease model

1. Aim of research and results obtained

SP6 is a zinc-finger type transcription factor that is expressed in the developing ectodermal tissues like tooth. It was previously reported that the disruption of the third zinc finger domain in the C-terminus of SP6 protein exhibits tooth-specific defect in amelogenesis imperfecta (AI) rat (AMI). AMI shows no systemic defects as shown in the *Sp6* knockout mouse. In order to further elucidate the molecular basis of AI induced by *Sp6* mutation, I isolated and characterized AMI-derived epithelial cells (ARE).

Step 1)

To confirm the difference of gene expressions in dental epithelial cells between wild type and AMI, microarray analysis was performed using total RNA isolated from rat molars of wild-type rat and AMI. I observed the differential genes expression pattern.

Step2)

To investigate the effects of *Sp6* mutation in AI phenotype, dental epithelial cell clones were isolated from incisors of AMI. Each clone was grouped according to their morphological similarity and gene expression profile of SP6-target genes. Two ARE clones were selected based on the gene expression profiles and further analyzed. Nuclear localization of both wild-type (SP6WT) and mutant-type SP6 (SP6AMI) were examined by immunocytochemistry using transfectants with both wild and mutant *Sp6* expression plasmids in both dental and non-dental epithelial cells (COS-7 and G5). As a result, nuclear localization of SP6WT and SP6AMI was similar in both cells.

Step3)

The transcriptional activity mediated by both SP6WT and SP6AMI was examined by *Luc*-reporter assay using *Rock1* gene promoter which is a downstream target gene of SP6 in G5, ARE and COS-7 cells. DNA-binding activity of both SP6WT and SP6AMI was analysed by chromatin immuno-precipitation (ChIP) assay in G5, ARE, and COS-7 cells. The analysis of *Rock1* promoter activity revealed that both SP6WT and SP6AMI could enhance the *Rock1* promoter activity, but SP6AMI exhibited weaker transcriptional enhancement compared to SP6WT. In contrast, any enhancement of *Rock1* promoter activity with co-transfection of either SP6WT or SP6AMI was not detected in ARE and COS-7 cells. However, both SP6WT and SP6AMI could bind to the *Rock1* promoter in all cells. From these results, disruption of the third zinc-finger domain of SP6 affected SP6-mediated transcriptional activity regardless of its DNA binding activity.

In conclusion, I proposed AMI-derived cells (ARE clones) as a good *in vitro* disease model.

2. Self-evaluation of research achievement:

My research data suggested that the C-terminal region of SP6 plays the critical roles in *Rock1* promoter regulation. Moreover, cell type specificity also controls SP6 activity. Further precise molecular mechanisms of SP6 function to the target genes in dental epithelial cells were required for better understanding.

3. Meeting presentation:

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

Adiningrat A, Tanimura A, Miyoshi K, Hagita H, Yanuaryska RD, Arinawati DY, Horiguchi T, and Noma T.
Isolation and characterization of dental epithelial cells derived from amelogenesis imperfecta rat as an *in vitro* disease model.
The 57th Japanese Association for Oral Biology Annual Meeting, Niigata, 11-13th September, 2015. (Oral Presentation)

4. Journal publication:

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

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.

③ **Adiningrat A,** Tanimura A, Miyoshi K, Hagita H, Yanuaryska RD, Arinawati DY, Horiguchi T, Noma T.
Isolation and characterization of ARE-clones derived from amelogenesis imperfecta rat.
Oral Dis. 2016: 132-139, 2016.