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Report of "Research Award of Oral Sciences"

Major: Oral Sciences
Grade: 4th
Department: Molecular Biology
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Title: Involvement of Sp6 in the stage-specific gene regulation of amelogenesis

1. Aim of research and results obtained (Approximately 400 words):

Tooth loss has significant impact on the health and quality of life. Regeneration of tooth is an ideal treatment for tooth lost in the future. However, to achieve this goal, the molecular mechanism of tooth development needs to be clarified. To address this question, we have established the Amelogenesis imperfecta rat (AMI) derived epithelial cell-line, ARE-B30 and wild type cells, G5. ARE-B30 has two-base insertional mutation in the coding region of *Specificity protein* (*Sp6*) gene, resulting the truncated form of the third zinc finger of Sp6 protein. Using these cells, the *in vitro* culture systems were established mimicking the *in vivo* cellular composition. Based on my previous comparative gene expression study, the early and late stage-specific gene expression of ameloblast differentiation markers was detected in G5, but not in ARE-B30 (Arinawati *et al.*, *J Biosci Bioeng,* 125(4):479-489, 2018). These findings suggested that Sp6 might play the dual roles in cell fate decision and control of maturation stage during amelogenesis. To prove this hypothesis, I focus on the role of Sp6 in the stage-specific regulation of amelogenesis-related genes; *Ngfr; Klk4* and *Amtn*.

To assess the causative link between Sp6 and the stage-specific gene expression, I performed two functional analyses to see the effect on the gene expression. First is loss-of-function analysis; Mithramycin A, GC-box inhibitor

and Sp6 siRNA were applied to G5 culture, and second is gain-of-function analysis; *Sp6* expression vector was introduced into ARE-B30 and G5 by transient transfection.

Loss-of-function study revealed the specific reduction of *Amtn* and *Klk4* expression, but no reduction of *Ngfr*. *Amtn* expression was decreased by Sp6 siRNA, while *Klk4* expression was decreased by mithramycin A but not by Sp6 siRNA. Gain-of-function study showed the overexpression of Sp6 could not restore the gene expression of *Ngfr*; *Amtn*, and *Klk4* in ARE-B30. These results suggested that not only Sp6, but also other regulatory mechanism(s) may control the stage-specific gene expression.

Both genetic and epigenetic regulations are reported in controlling cell fate decision. Therefore, I examined the restoration of gene expression in ARE-B30 by DNA methylation inhibitor (5-aza-2-deoxycitidine, 5AC) and histone deacetylation inhibitor (Valproic acid, VPA) treatment. The results showed that 5AC only restored *Klk4* expression, and VPA only restored *Ngfr* expression, while both 5AC and VPA could not restored *Amtn* expression in ARE-B30 cells. Next, to examine Sp6 function under open chromatin condition, I introduced *Sp6* under 5AC or VPA treatment in G5 and ARE-B30. Further analysis is required to prove the function of Sp6 in the distinct amelogenesis step.

2. Self-evaluation of research achievement:

From this study I found several important molecular mechanisms in amelogenesis. First, I could detect the Sp6 involvement on the stage-specific regulation of amelogenesis-related genes by loss-of-function study of Sp6, which may directly regulate *Amtn* expression and indirectly control *Klk4* expression. In addition, other Sp family member(s) may regulate *Klk4* expression. I also could find that epigenetic regulation for early stage marker expression (*Ngfr*), and for late stage marker expression (*Klk4*) in ARE-B30 cells. Rescue experiment combined 5AC/VPA treatment is under investigation to elucidate the molecular link between Sp6 and epigenetic regulation on the gene expression. I hope my

study will provide us a better understanding of the molecular basis for amelogenesis.

3. Meeting presentation:
   1) Demonstration of defective amelogenesis using an *in vitro* amelogenesis imperfecta model; The 257th Tokushima Medical Association Meeting; Tokushima Physician Hall, Tokushima, Japan; 5 August 2018; Arinawati DY, Miyoshi K, Horiguchi T, Hagita H, Noma T; Poster Presentation.

   2) Involvement of Sp6 in the stage-specific gene regulation of amelogenesis; The 60th Annual Meeting of the Japanese Society of Oral Biology; Kyushu University Auditorium Hospital, Fukuoka, Japan; 5-7 September 2018; Arinawati DY, Miyoshi K, Horiguchi T, Noma T; Oral Presentation.

   3) Perturbation of gene regulation in an *in vitro* amelogenesis imperfecta model; The 91st Annual Meeting of the Japanese Biochemical Society; Kyoto International Conference Center, Kyoto, Japan; 24-26 September 2018; Arinawati DY, Miyoshi K, Horiguchi T, Noma T; Poster Presentation.

4. Journal publication:
   In preparation