Report of "Research Award of Oral Sciences"

Department: Molecular Biology
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Title: Demonstration of defective amelogenesis in AMI-derived rat dental epithelial (ARE) cells by co-culture system.

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

Losing teeth reduces quality of life (QOL) by compromise the ability of chewing, eating, and communication. One of the approaches to get better QOL is regeneration of tooth. In order to regenerate tooth, it is essential to understand the molecular basis for tooth development. However, the conventional *in vitro* culture system has the limitation to recapitulate the *in vivo* differentiation of dental cells. Tooth development is regulated by reciprocal interaction between epithelial and mesenchymal cells. Therefore, I established and examined a 3D culture system with the combination of epithelial and mesenchymal cells to mimic the *in vivo* condition.

As cellular materials, I applied G5 and ARE-B30 cells for dental epithelial cells and RPC-C2A cells (rat pulp derived cells) for mesenchymal cells. G5 and ARE-B30 cells are derived from wild-type and amelogenesis imperfecta (AMI) rat, respectively. AMI rat has a causative mutation of amelogenesis imperfecta in *specificity protein 6 (Sp6)*.

Epithelial cells were cultured on collagen membrane or co-cultured with RPC-C2A in separated collagen membrane, as well as conventional plastic culture for 1 to 14 days.

The expression pattern of amelogenesis-related genes was analyzed in the epithelial cells by RT-PCR in order to evaluate the effects of ECM and epithelial-mesenchymal interaction on the gene regulation.

The reciprocal expression of *Bmp2* and *Fst*, which are the regulatory molecules of ameloblast differentiation, was found. Upregulation of *Amtn* was observed in G5 in time-dependent manner. Furthermore, *Klk4* expression, but not *Mmp20*, was detected in G5 among all culture system. No expression of *Amtn, Klk4,* and *Mmp20* was found in ARE-B30.

From these results, first I obtained several interesting features due to

different culture systems and epithelial cell sources. Currently I am characterizing the SP6 involvement in these findings. Secondly, the 3D culture system that I established, could provide partly ameloblast differentiation process *in vitro*. Therefore, I would like to advance further analysis for molecular mechanism of amelogenesis in detail using this system, comparing the differential regulation of amelogenesis between G5 and ARE-B30.

2. Self-evaluation of research achievement:

   I could establish 3D co-culture system in order to dissect the effect of epithelial-ECM-mesenchymal interaction on gene expression, and distinguish different regulation of ameloblast marker gene expression between ARE-B30 and G5. I presented a poster with these results in the international symposium, Oral and Craniofacial Development and Diseases 2016, held in Osaka. Now I am preparing the manuscript with these findings.

3. Meeting presentation:

   1. The ECM-mesenchymal effects on gene expression with an *in vitro* amelogenesis imperfecta model; Oral and Craniofacial Development and Diseases 2016; Yumikura Hall, Osaka University Graduate School of Dentistry, Osaka, Japan; 12 December 2016; Arinawati DY, Miyoshi K, Tanimura A, Horiguchi T, Adiningrat A, Noma T; Poster Presentation.

4. Journal publication:

   Manuscript is under preparation.