Proteome analysis of developing mice diastema region

Young-Mi Chae1, Young-Joo Jin1, Hyung-Soo Kim2, Gi-Jeong Gwon1, Wern-Joo Sohn1,2*, Sung-Hyun Kim3, Myoung-Ok Kim4, Sanggyu Lee5, Jo-Young Suh5 & Jae-Young Kim1*

1Department of Biochemistry, School of Dentistry, IHBR, 2School of Science and Biotechnology, 3Department of Center for Laboratory Animal Resources, 4Department of Animal Science, 5Department of Periodontology, Kyungpook National University, Daegu 700-422, Korea

INTRODUCTION

Teeth develop through sequential and reciprocal interactions between oral epithelium and neural crest-derived mesenchyme (1). The first morphological sign of tooth development is a narrow band of thickened epithelium on the developing jaw primordium. The thickened epithelium progressively takes the form of the bud, cap, and bell configurations as differentiation proceeds. Subsequently, epithelial cells and mesenchymal cells differentiate into enamel-producing ameloblasts and dentin-producing odontoblasts, respectively. Based on this proteome analysis, we identified 147 up- and 173 down-regulated proteins in the diastema compared to the molar forming proteins. Based on this proteome analysis, we selected and evaluated two candidate proteins, EMERIN and RAB7A, as diastema tissue specific markers. This study provides the first list of proteins that were detected in the mouse embryonic diastema region, which will be useful to understand the mechanisms of tooth development. [BMB Reports 2012; 45(6): 337-341]

Different from humans, who have a continuous dentition of teeth, mice have only three molars and one incisor separated by a toothless region called the diastema in the hemi mandibular arch. Although tooth buds form in the embryonic diastema, they regress and do not develop into teeth. In this study, we evaluated the proteins that modulate the diastema formation through comparative analysis with molar-forming tissue by liquid chromatography-tandem mass spectroscopy (LC-MS/MS) proteome analysis. From the comparative and semi-quantitative proteome analysis, we identified 147 up- and 173 down-regulated proteins in the diastema compared to the molar forming proteins. Based on this proteome analysis, we selected and evaluated two candidate proteins, EMERIN and RAB7A, as diastema tissue specific markers. This study provides the first list of proteins that were detected in the mouse embryonic diastema region, which will be useful to understand the mechanisms of tooth development. 

Mice have only one incisor and three molars in each jaw quadrant that are divided by a toothless region, the diastema (5). Although mice lost the teeth in the diastema during evolution, the remnants of the evolutionary lost teeth are observed as transient epithelial buds in the wild-type diastema during the early stages of development. In the diastema of mice, rudimentary tooth primordia develop through the initial stages of teeth development as the remnants of evolutionary lost teeth but cease before the cap stage and regress by apoptosis (6). Previous reports have shown that there are rudimentary tooth germs, which develop into bud stage before their removal by apoptosis, in the lower diastema regions of mice at E13 (7). Peterkova et al. evaluated the apoptotic elimination of the vestigial teeth through agenesis in the diastema region and Tureckova et al. showed sporadic cell death in the diastema region (6, 8). Recently, information on apoptosis during tooth development has been reported. The question is whether proliferation and cell death are involved in the rudimentary development of teeth (9).

In previous studies, many important signaling pathways have been reported to modulate the formation of teeth in the diastema region through the repression of Shh, BMP, and Wnt signaling (10). Shh has important roles in the development of various ectodermal appendages including hair mammary glands and teeth in the diastema-forming region, it is repressed by Gas1, a known potent inhibitor. These results were confirmed by examining Gas1 mutant mice that have extra teeth in the maxilla diastema region where there would be expected agenesis of the tooth structures (11). Gas1 inhibited ectopic Ptc1, a receptor of Shh signaling, expression in the diastema suggesting that Gas1 has a role in inhibiting the activity of Shh signaling to form teeth in the diastema forming region (12).

In addition, previous reports have shown that signaling networks including Shh, Wnt, Fgf, and BMPs would produce supernumerary or ectopic teeth in the diastema region (13). Ectodin integrates BMP signaling with the Shh pathways in teeth formation. Inhibition of BMP signaling by Sostdc1 and negative feedback from Shh controls the number and patterning of the teeth (14). Modulation of these signals can rescue these vestigial tooth rudiments to develop into supernumerary diastema teeth using Fgfb treatment. A number of mutant mouse strains have been reported exhibiting supernumerary...
Proteome analysis of developing mice diastema region
Young-Mi Chae, et al.

RESULTS AND DISCUSSION
Identification of proteins in diastema-forming regions by LC-MS/MS
In this study, we evaluated the proteins that modulate the agenesis of the diastema region through comparison with molar-forming tissue by LC-MS/MS proteome analysis. At E13, the diastema- and molar-forming regions were microdissected from a developing mouse mandible, removed, and then pulverized in a homogenizer. For dissecting the mandible, we first removed the aboral and tongue parts from the mandible, and then we dissected the diastema- and molar-forming tissues under the dissecting microscope (Fig. 1A and B). Extracted proteins from the samples were resolved on a SDS-PAGE (Fig. 1C), and each sample was divided into 15 gel pieces and then subjected to in-gel digestion by trypsin. Each peptide mixture was eluted and analyzed by liquid chromatography with tandem mass spectroscopy (LC-MS/MS). The SEQUEST algorithm in the Sorcerer program was used to search the IPI mouse protein database using our MS/MS data. From data combined from two individual samples, 539 proteins were identified.

Next a comparative analysis of the proteins identified from the diastema- and molar-forming regions was performed. One hundred and forty-seven proteins had a 1.5-fold higher peptide hit number in the diastema-forming region compared to the molar-forming region (Supplement Table 1). Conversely, 173 proteins were detected with a 1.5-fold higher peptide hit number in the molar-forming region compared to the diastema-forming region (Supplement Table 2). A sub network was reconstructed using twelve proteins, which showed significant expression patterns, as the seed molecules. The sub network was statistically obvious for indirect connectivity. Ingenuity pathway analysis showed the relationships among selected differentially expressed proteins in the diastema-forming region (Fig. 2).

Up- or down-regulated proteins were classified as intracellular, membrane, nuclear, or cytoplasm proteins, based on their predicted cellular location (Fig. 3A). These proteins were also classified according to biological processes (Fig. 3B) and biological functions (Fig. 3C).