Microarray Analysis on Odontogenesis-related-genes in Mouse Dental Papillae

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Abstract: In the present study, we examined to identify down-regulated genes between pre- and post dental papillae by microarray analysis in order to identify genes involved in tooth morphogenesis.The dental papilla tissues obtained from E16, E18, P03 and P10 of ICR mice were used for microarray analysis. Microarray analysis showed that the number of up-regulated genes ranged from 860 to 2214 from E16 to P10 and was decreased following the tooth morphogenesis. On the other hand, the number of down-regulated genes ranged from 1337 to 2924, and number of down-regulated genes was the highest (2924 genes) during E18 and P3. The results suggest that the reduced or disappeared genes in dental papilla between P0 and P3 are associated with forming and calcifying the dentin of tooth crown and regeneration of tooth.

Key words: dental papilla, epithelial-mesenchymal interaction, microarray, RNA prolife

Introduction

Regenerative medicine is based on research of developmental biology and morphogenesis. One of the most important goals in dental researches is the efficient regeneration of lost teeth (Chai and Slavkin 2003; Earthman et al. 2003), and tooth regeneration is also based on researches of tooth development. However, tooth-morphogenesis is a complex process that has been characterized as a series of reciprocal epithelial-mesenchymal interactions, culminating in the differentiation of interacting tissues described above (Jernvall et al. 2000; Jernvall and Thesleff 2000), and the morphogenesis of tooth is different from that of other tissues including skin and bone, and therefore successful regeneration of tooth has not yet been established.

It has been reported that a number of growth factors, transcription factors and extracellular matrix proteins are involved in tooth development through epithelial-mesenchymal interactions, and tooth morphogenesis is the cumulative result of reiterative signaling of growth factor family members (Jernvall and Thesleff 2000). The functions were examined by using knockout and transgenic mice and experiments with antisense oligonucleotide. However, all the molecules, signal transcriptions, process and functions involved tooth morphogenesis has not been well characterized.

The interactions were researched by experiments of classical tissue recombination. Previously, tissue recombination experiments demonstrated that the dental epithelium could induce tooth formation prior to the bud stage. However, after bud stage, the dental mesenchyme takes the initiative in tooth formation away from the dental epithelium. Palmer & Lumsden (1987) demonstrated that combination of embryo 16-day (E16) enamel organ and E16 dental papilla formed teeth, while that of E16 enamel organ and post-natal 3-day (P3) pulp did not form teeth and produced dysplastic dentin with no enamel formation (Palmer and Lumsden 1987). Based on the study, we hypothesized that genes related to tooth morphogenesis were decreased or disappear between pre- and postnatal dental papilla. To test this hypothesis, investigation on gene profile in dental papilla should be compared between pre- and postnatal stage. In the present study, we examined to identify down-regulated genes between pre- and post dental papillae (dental mesenchyme) by microarray analysis in order to identify genes involved in tooth morphogenesis.

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Materials and Methods

Animals & Tissue preparation

ICR mice were obtained from Sankyo Laboratory Service Corporation (Tokyo, Japan). E16, E18, P3 and P10 mice were sacrificed in the present study. The embryonic first-molar tooth germs were dissected from the mandible under dissection microscope, and kept in RNA later RNA stabilization reagent (QIAGEN, Valencia, CA, USA) at room temperature (RT). After washed in phosphate buffered saline (PBS (-)), tooth germs was incubated in 1.2U/mg dispase I (Roche, Mannheim, Germany) for 5 min at RT to separate dental papilla from dental epithelium, and deactivated in minimum essential medium (MEM, Invitrogen, Grand Island, NY, USA) with 15 percents fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA). Under a dissecting microscope, dental papilla was isolated from tooth germ with tungsten needles, and placed in RNAlater RNA stabilization reagent on the ice. Tooth germs at P3 and P10 were dissected from the mandible, placed in dispase I and the pulpal tissue isolated as well as papilla in E16 and E18. The dental papilla and pulp tissues obtained from E16, E18, P03 and P10 were used for microarray analysis.

RNA extraction

Tissues was extracted by a modified acid-guanidinium-thiocyanate-phenol-chloroform (AGPC) method using the TRIzol RNA extracting mixture (Invitrogen) according to the manufacturer's protocol with modification (Muramatsu et al. 2004).

Microarray hybridization

Double stranded complementary DNA (cDNA) was synthesized by two-cycle target labeling method (Ohyama et al. 2000). Two hundred micrograms of total RNA were converted into first-cycle double stranded cDNA by using SuperScript Choice system (Invitrogen) with T7-Oligo (dT) primer (Affymetrix, Santa Clara, CA, USA). These samples were synthesized Biotin-labeled cDNA using Enzo BioArray HighYield RNA Transcript Labeling Kit (Enzo Diagnostics, Farmingdale, NY), and were fragmented randomly to approximately 35-200 bp with fragmented buffer. After checking fragmentation by gel electrophoresis, 20 µg of fragmented cRNA were hybridized to GeneChip® Mouse Genome 2.0 Array (Affymetrix) at 45 C for 16 h. The microarray chip was wash and stained by the Affymetrix® Fluidic Station 450 according to the Affymetrix GeneChip® protocol. The probe array that was reacted with streptavidin phycoerythrin (SAPE) of fluorescent images was scanned by Affymetrix® GeneChip® Scanner 3000.

Data analysis

The data was analyzed by GeneChip® Operating Software version 1.1 (GCOS; Affymetrix) and GeneSpring® software version 7.0 (Silicon Genetics, Redwood City, CA). The scanned images of microarray chip were converted into raw fundamental data, that contained Affymetrix ProbeSet ID, Signals, Flag value (present/absent) and p-value by GCOS (The raw expression data was scaled by GCOS), and subsequently the data were transferred to GeneSpring for normalization and statistical analysis. Gene expression data was normalized using the method of Per Chip; Normalize to 50 percentile. In brief, all expression data was normalized to 50th percentile of all values on each chip, and the data was filtered through "filtering on Expression Level of normalized intensity (data) at least 0.01" and "filtering on Flag value that was included Present value at least 1 out of 4 sample". A difference of 2-fold change was applied to select up-regulated or down-regulated genes.

Results

Microarray Analysis

Labeled cDNA probe were generated from total RNA extracts isolated from dental papilla and pulp, then hybridized with the GeneChip® mouse genome 2.0 array. We utilized cDNA microarray technology to compare multiple gene expression profiles of E16, E18, P3 and P10. We selected the genes which gene expression exceeds 2.0-fold change between the four stages of tooth development. The number of up-regulated and down-regulated genes was shown in Table 1. The number of up-regulated genes ranged from 860 to 2214 and was decreased following the tooth morphogenesis. On the other hand, the number of down-regulated genes ranged from 1337 to 2924. Number of down-regulated genes was the highest (2924 genes) during E18 and P3.

The down-regulated genes between the four stages (E16, E18, P3 and P10) were extracted using gene ontology, which was related to calcification, development and cell death by GeneSprings® software ver.7.0. These results were shown in Table2. Expression of *Adamts4*, *Lef1*, *Aldha1a2* and *Rag1* were decreased considerably (fold change >10.0) between E16 and E18. However, expression of *Plxnc1*, *Pitx2* and *Uhrf1* was reduced. There were no genes that showed over 10.0-fold change between E18 and P3. During P3 and P10, the expression of *Enam* and *Mab21l1* was abated conspicuously.

Table 1: Number of up-regulated and down-regulated genes

	up-regulated	down-regulated
E16 vs E18	2214	2134
E18 vs P03	1002	2924
P03 vs P10	860	1337

Table. 2: Down regulated genes

Down-regulated genes (Fold)

	Gene symbol	E16 vs E18	E18 vs P03	P03 vs P10
1455965_at	Adamts4	14.12		
1421299_a_at	Lef1	12.76		
1422789_at	Aldh1a2	70.80		
1450680_at	Rag1	28.76		
1457424_at	Eya1	8.08		
1450905_at	Plexinc1		8.20	
1424797_a_at	Pitx2		6.52	
1415811_at	Uhrf1		6.13	
1421369_a_at	Mab21l1			10.53
1450731_s_at	Tnfrsf21	5.76		
1417878_at	E2f1	5.28		
1424278_a_at	Birc5		5.37	

Discussion

Interaction between epithelial and mesenchymal tissues plays essential role during tooth development (Jernvall et al. 2000). However, the interaction was dynamic and complex, and therefore all the molecules, signal transcriptions, process and functions involved tooth morphogenesis has not been well characterized. We hypothesized that genes related to tooth morphogenesis were decreased or disappear in postnatal dental papilla based on the previous report (Palmer and Lumsden 1987). To test this hypothesis, gene profile in dental papilla should be compared between pre- and postnatal stage. Our results showed the number of up-regulated and down-regulated genes ranged from 860 to 2924. This is the first report to examine RNA profile in dental papilla using microarray to our knowledge. The number of up-regulated genes increased subsequent the tooth developing. These results suggest that the expression of signal molecules and interaction in dental papilla is complicated during tooth development. On the other hand, the number of down-regulated gene increased between E18 and P3. The results suggest that the reduced or disappeared genes in dental papilla between P0 and P3 are associated with forming and calcifying the dentin of tooth crown and regeneration of tooth.

We could screen more than 2,000 down-regulated genes in dental papilla between P0 and P3 in the present study. As a next step, localization and functional assay are necessary to confirm whether the genes are actually associated with tooth formation or not.

Acknowledgments

This work was supported, in part, by the Japnese Ministry of Education, Culture, Sports, Science and Technology, Grant-in-Aid No.17591926.

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