Dental mesenchyme determines the crown size of tooth in embryogenesis

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Dental mesenchyme determines the crown size of tooth in embryogenesis

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The Doctoral Dissertation
submitted to the Department of Dentistry,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the
degree of Doctor of Philosophy

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December 2005

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December 2005

ACKNOWLEDGEMENTS

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끝으로 묵묵히 지켜 보아주신 양가 부모님과 사랑하는 아내 신 은령에게 사랑의 마음을 전하며 이 논문을 바칩니다.

2005년 12월

저자 씀

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ABSTRACT

Dental mesenchyme determines the crown size of tooth in embryogenesis

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(Directed by Professor Hee-Jin Kim)

Tooth is one of the ectodermal organs regulated by epithelial-mesenchymal interactions, and its morphogenesis conserved signaling pathways during the developmental process. The crown size of tooth is one of the most important factors for the determination of dentition and occlusion. In this study, to investigate what determines the tooth crown size, cross-recombination was carried out between mouse and rat tooth germs at cap stage. Crown size of cross-recombinant teeth between mouse epithelium and rat mesenchyme was larger than cross-recombinant teeth between rat epithelium and mouse mesenchyme. Furthermore, the cross-reaggregated teeth between mouse epithelium and rat reaggregated mesenchyme (6.0×10^4 cells) were larger than the cross-reaggregated tooth between rat epithelium and the mouse reaggregated mesenchyme (6.0×10^4 cells). These results suggested that the crown size of tooth is controlled not by the dental epithelium and the number of mesenchymal cells, but by the genetic information in mesenchymal cells. Inverification of the intracellular genetic information between mouse and rat mesenchymal cells, *Wnt5a* and *Bmp4* showed different amount of transcript between mouse and rat by RT-PCR. The exogenous WNT5A protein induced bone formation and the apoptosis of dental mesenchymal cells rather than dental epithelial cells, and subsequently resulted in shrunken tooth germ and retarded development and formation of small tooth. It is suggested that *Wnt5a* may be one of the

genes determining the tooth size.

Key words :tooth crown size, dental mesenchyme, mouse and rat, cross-recombination, cross-reaggregation, *Wnt5a*

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I. INTRODUCTION

The mammalian tooth is one of the ectodermal organs, of which the development is controlled by reciprocal interactions between the epithelium and the mesenchyme. All ectodermal organs share similar signaling molecules during early morphogenesis, but each organ undergoes its own specific pattern formation later in development[1,2].

The teeth develop from pharyngeal epithelium and the underlying neural crest-derived mesenchymal cells[3]. These neural crest cells derive from the midbrain region, and their final position in the maxillary and mandibular processes is associated with the original position of the cells in the neural crest as well as with the time when the cells leave the crest[4,5].

In the mouse tooth development, the first signal to govern tooth morphogenesis appears in a thickening (at Embryonic day 11, E11) of the oral epithelium. The thickening invaginates into the underlying mesenchyme and the mesenchymal cells condense around the bud (at E13) and during the following cap (at E14) and bell (at E16) stages. With cytodifferentiation, enamel and dentin are formed by the epithelial ameloblasts and

mesenchymal odontoblasts, respectively (Fig. 1).

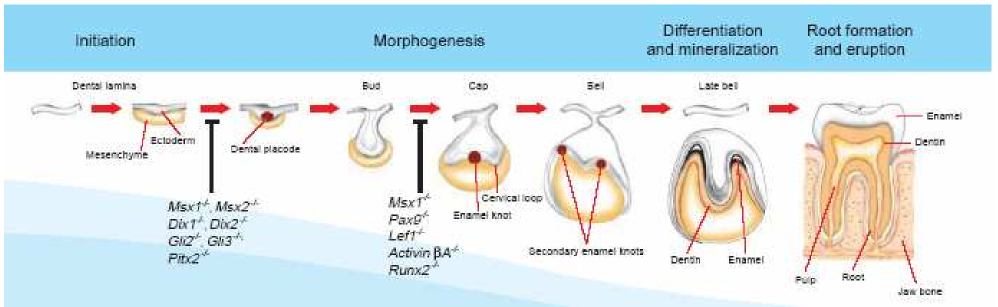

Figure 1. Tooth development in mouse

Interestingly, the development of rat tooth germs is similar to that of mouse, but the timing of development in rat tooth is about two days later than that of mouse (Fig. 2).

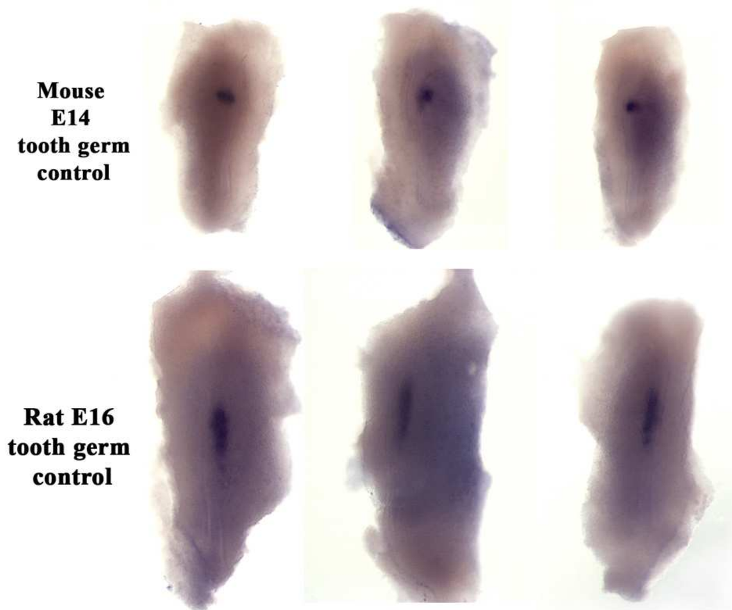

Figure 2. Molar tooth germs at cap stage taken out from the mouse E14 mandible and rat E16 mandible. The size of rat tooth germ is larger than that of mouse tooth germ.

In the later development, mouse and rat showed similar patterning of dentition and the individual molar was found almost the same patterning, but the size of each molar showed totally different (Fig. 3 and 4).

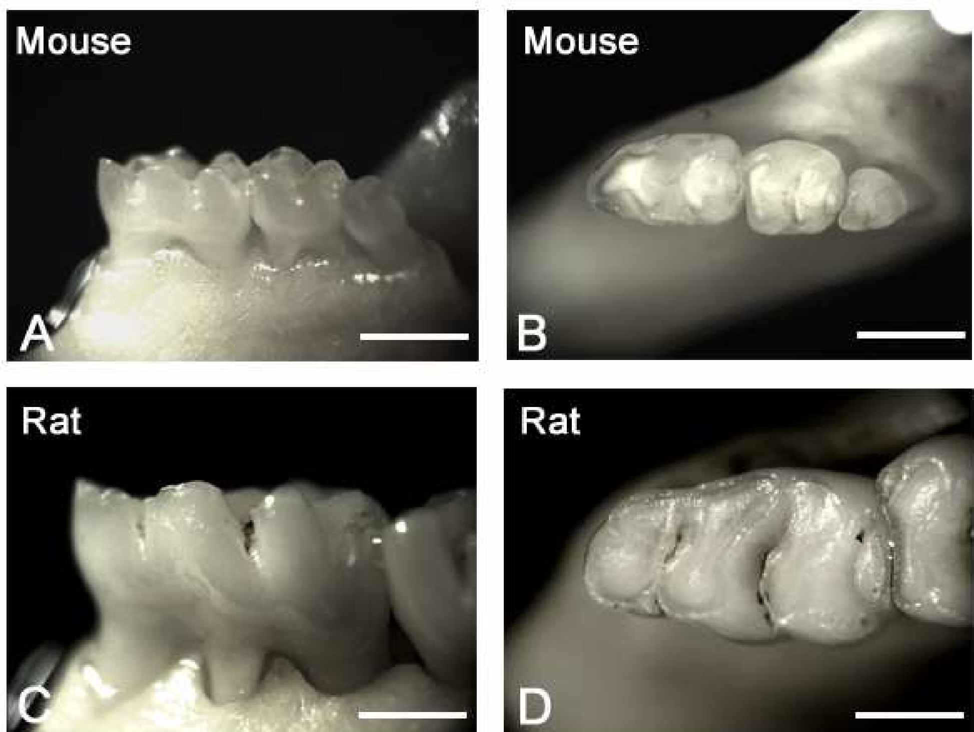

Figure 3. First molar tooth size difference between adult mouse and adult rat. The mesiodistal length of mouse first molar (A) is equal to the half of the rat first molar mesiodistal length (C). The buccolingual length of mouse first molar (B) is equal to the half of the rat first molar buccolingual length (D). However, the cusp patterning is similar between mouse first moar and rat first molar.

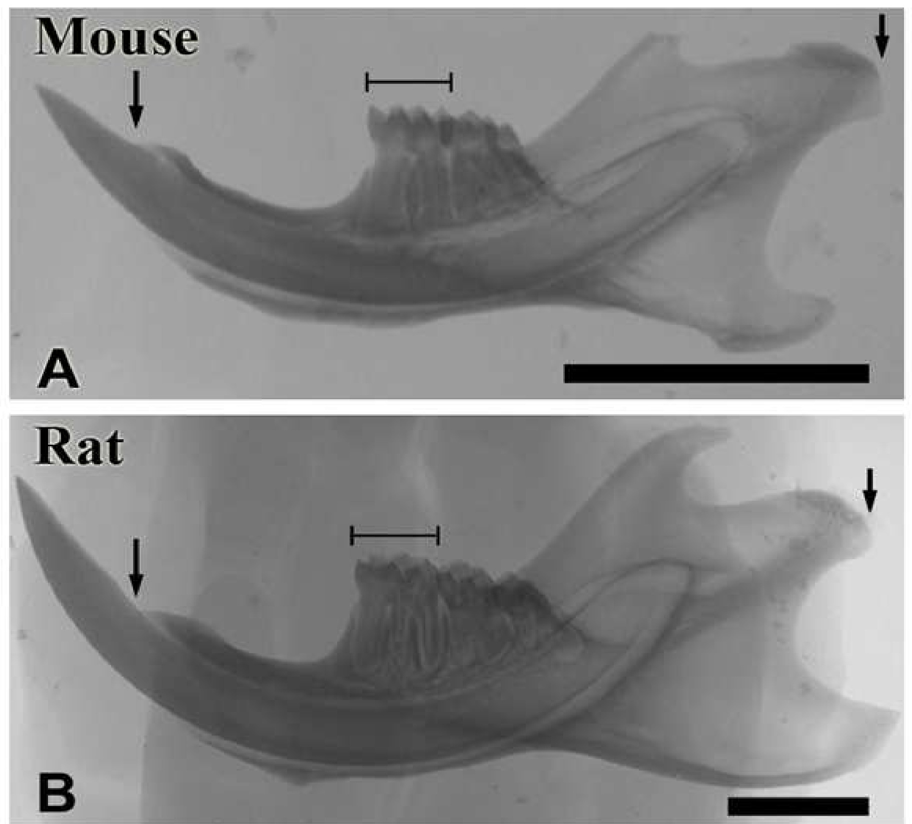

Figure 4. The same proportion of first molar both in adult mouse mandible and in adult rat mandible. The anterior-posterior length of mouse mandible corresponds to the half of rat mandible length. The mesiodistal length of first molar in mouse mandible is similar to the half of rat first molar length. Mouse and rat shows the same proportional length of first molar to the mandible.

Tooth crown size is considered as one of the most important factors for the determination of dentition and occlusion. Especially, a discrepancy

between tooth size and arch length causes orthodontic and subsequent oral functional problems[6]. Most tooth crown size studies focused on dentition of human in clinic[7-9]. Thus, the study of tooth size is of great interest to both dentists and developmental biologists in these years. In order to understand the proper factors to determine the size of tooth in genetical morphology, we introduced the recombination and reaggregation methods which were widely used in the epithelial-mesenchymal interaction researches from 1960's[10-13]. Tissue recombination studies in which epithelium and mesenchyme from different organs were cultured together indicated that in many organs the pattern of epithelial branching is regulated by mesenchymal tissue[14,15]. During the tooth development, it has been reported that the budding of the lamina marks the shifting of inductive potential of tooth formation from mandibular-arch epithelium to the mesenchyme[16]. Reaggregation system has been performed to make a tooth successfully[17,18]. All mesenchymal cells are reset to an equivalent state and have the same probability to become primordia, so it is possible to examine if the mesenchymal cell number could determine the tooth crown size with this system which has been performed to the cell number could alter the size and number of feather primordial[19]. Specially, it starts to form the transient signaling centers called primary enamel knot (EK) at bud stage and later secondary EK at the bell stage in the epithelium, which contribute to the shape of the future crown and its cusps[20,21]. Signaling molecules such as *Fgf4*, *9*, *Shh, Wnt10a, b*, and *Bmp2, 4, 7*, and transcription factors such as *Msx1, 2* and *Lef1* are expressed in the dental epithelium, especially in primary enamel knot[22-26]. In dental mesenchyme of the cap stage tooth

germs, *Fgf10, Fgf3, Fgfr1C, Bmp4, Msx1, Lef1, Wnt5a* and *β-catenin* are detected[20, 23, 24, 26-28].

It has been reported that the inductive potential of tooth formation shifts from the first pharyngeal arch epithelium to the mesenchyme at the early bud stage16 and that mesenchymal signals are necessary for the epithelial patterning and for the formation and maintenance of the epithelial compartments[29]. However, it is not evident if the dental mesenchymes, which were isolated from the tooth germs at the late bud, cap and bell stage, possess potential controlling the tooth crown size.

In this study, we applied the heterospecific recombination (cross-recombination) and the heterospecific reaggregation (cross-reaggregation) between the mouse and rat dental tissues, and compared the size of tooth crown developing from these recombinant tooth germs and reaggregated tooth germs. Furthermore, genetic differences between the mouse and rat dental tissues were investigated by RT-PCR and clarified by the implantation of exogenous protein.

II. MATERIALS AND METHODS

Animals

ICR mouse embryos at E14 and Sprague- Dawley rat (S-D rat) embryos at E16 were used in this study. Nude mice were used as hosts for tooth germ transplantation into kidney.

Tissue dissection

The lower molar tooth germs were carefully dissected from the mandibles of mouse embryos at E14 and rat embryos at E16. Dental epithelilum was removed from dental mesenchyme by means of Dispase II (Roche, Germany, 295 825) in PBS at 1.2 units/ml. E14 mouse tooth germs were incubated for 20 minutes and E16 rat tooth germs for 30 minutes in Dispase II at Room temperature RT respectively. After incubation, tooth germs were washed in a solution of Dulbecco's Modified Eagle Medium (D-MEM, Bio Whittaker, USA, 12-640F) supplemented with 10% fetal bovine serum (FBS, GIBCO, USA, 16000-044). Under a dissection microscope, the dental epithelium and dental mesenchyme were separated from each other.

Cross-recombination

Tissue recombinations between dental epithelium and dental mesenchyme were done as previously described[30]. The tooth germ composed of dental epithelium and dental mesenchyme from different species, was simplified as the cross-recombinant tooth germ. Two kinds of cross-recombinations were carried out with E14 mouse tooth germs and E16 rat tooth germs after

separation epithelium from mesenchyme respectively as follows: mouse dental epithelium was overlaid on the rat dental mesenchyme (mouse epithelium/ rat mesenchyme, M-epi / R-mes); mouse dental mesenchyme was recombined with rat dental epithelium (rat epithelium/ mouse mesenchyme, R-epi / M-mes) (Fig. 5).

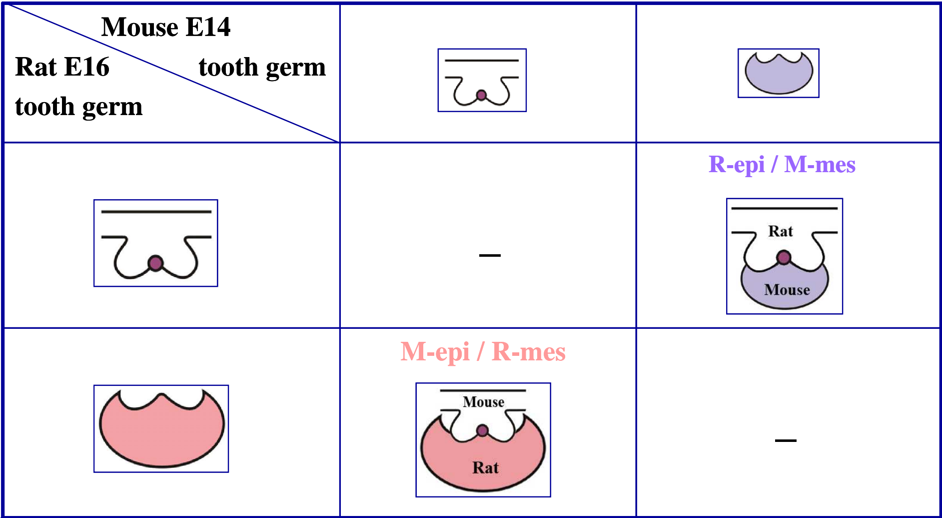

Figure 5. Cross-recombination between mouse tooth germ at E14 and rat tooth germs at E16. Cross-recombinant between rat epithelium and mouse mesenchyme corresponds to R-epi / M-mes. Cross-recombinant between mouse epithelium and rat mesenchyme corresponds to M-epi / R-mes.

Cross-reaggregation

Reaggregation of mesenchymal cell in tooth germ was applied as previously described[18]. The mesenchymal cell number of one rat molar tooth germ at E16 was standardized as 6.0 x 10^4. The dissociated cells were repelleted by mild centrifugation with appropriate cell number (6,500 revs/minute for 4 minutes) and allowed to reaggregate for 1 hour at 37℃

on culture insert dishes (Falcon). The required intact dental epithelium of mouse or rat was then placed on top of the relevant required reaggregated mesenchyme and the recombinant explants were cultured at 37℃ for 1 day. (Fig. 6 and 7)

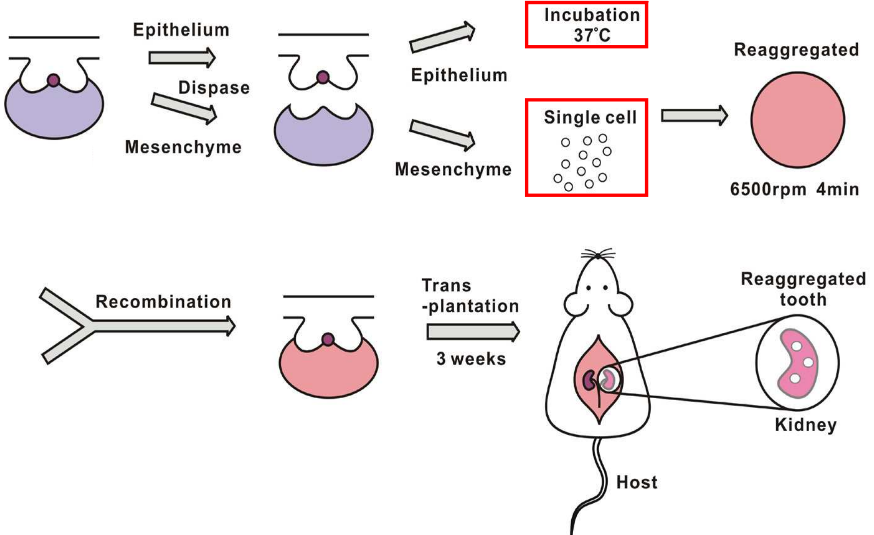

Figure 6. Reaggregation method to control the cell number of dental mesenchyme.

Kidney Capsule Transplants

Cross-recombinant explants and cross-reaggregated explants were cultured for 1 day and transplanted beneath the renal capsule of young adult nude male mice. After 3 weeks, animals were sacrificed and kidneys were dissected for gaining the calcified teeth.

Histology

The calcified teeth obtained from the kidney capsules were fixed in 4% paraformaldehyde in PBS (PFA) overnight and decalcified in the solution of

formic acid and sodium formate (1:1) for 5 days, embedded in paraffin wax, serially sectioned at a thickness of 7㎛, and stained with Hematoxylin and Eosin.

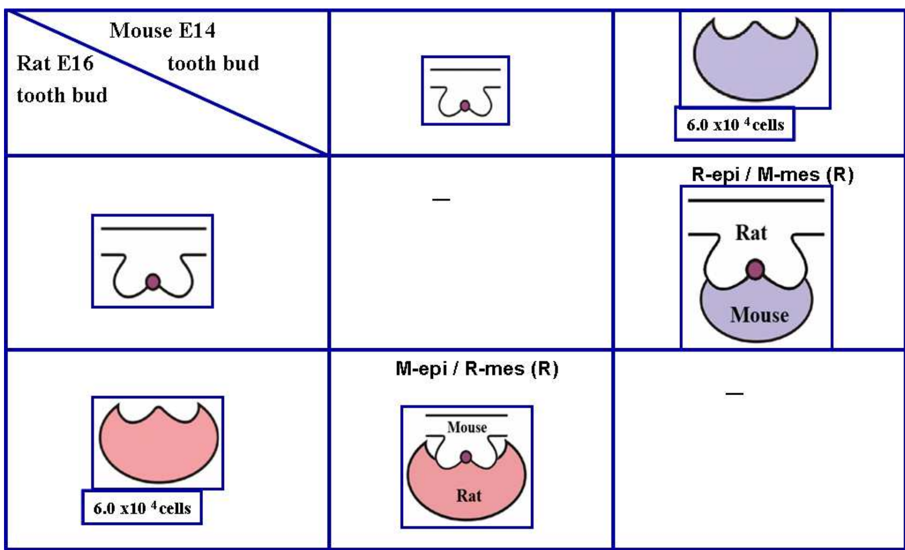

Figure 7. Cross-reaggregation between dental epithelium and reaggregated mesenchyme. Cross-reaggregated tooth germ between rat epithelium and mouse reaggregated mesenchyme corresponds to R-epi/M-mes(R). Cross-recombinant between mouse epithelium and rat mesenchyme corresponds to the M-epi/R-mes(R).

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted by the dental mesenchyme of rat and mouse respectively in TRIZOL reagent (Invitrogen, USA) with an Ultra-Turrax homogenizer (IKA, Staufen, Germany). The samples of RNA underwent reverse transcription (RT) according to the manufacturer's protocol containing MMLV reverse transcriptase enzyme (MBI fermentas Co.) The synthesis of

cDNA was carried out for 90 min at 42℃.

Primer pairs (sense and antisense) were synthesized by Sigma as follows:

for *Bmp4* (300 bp): 5'-AGGTAACGATCGGCTAATCCT-3' and 5'-CTATTTCGGGAGCAGGTGGA-3'

for *Fgfr1C* (110 bp): 5'-GGTATTTGGTCAGCAAAGCA-3' and 5'-AAGCCGTGAGGTTTCTGTTT-3'

for *Msx1* (184 bp): 5'-TCCTGGGAAAGTCTCTTCAACC-3' and 5'-GGCAGGACTTGCACAGAGAAAT-3'

for *Wnt5a* (238 bp): 5'-CCATGTCTTCCAAGTTCTTCCTA-3' and 5'-TGTACTGCATGTGGTCCTGATAC-3'

for *β-catenin* (199 bp): 5'-ACAAAAACAAGATGATGGTGTGC-3' and 5'-GTGCAGGAGTTTAACCACAACAG-3'

for *Lef1* (180 bp): 5'-TGGCAAGGTCAGCCTGTTTAT-3' and 5'-GGTGCTCCTGTTTGACCTGAG-3'

for *Fgf3* (196 bp): 5'-AGGCGGGAAGCATATGTATTGTA-3' and 5'-CTTGAGAACAGCGCCTATAGCAT-3'

for GAPDH (427 bp): 5'-GTCATCATCTCCGCCCCTTCTG-3' and 5'-ATGCCTGCTTCACCACCTTCTTG-3'.

The PCR reaction mixture was incubated at 95℃ for 10min and 30 cycles were performed at 95℃ for 1 min, (T_{An}) for 1 min, 72℃ for 1 min, and a final cycle with a prolonged elongation time of 10 min at 72℃. The primer specific annealing temperature (T_{An}) was as follows: T_{An}(*Bmp4*) = 58℃, T_{An}(*Fgf1C*) = 54℃, T_{An}(*Msx1*) = 58℃, T_{An}(*Wnt5a*) = 56℃, T_{An}(*β-catenin*) = 56℃, T_{An}(*Lef1*) = 58℃, T_{An}(*Fgf3*) = 58℃, T_{An}(GAPDH) = 62℃. The PCR products were analyzed by standard electrophoresis on 1% agarose gels

at 100V, stained with ethidiumbromide and photographed under UV illumination. The size of each PCR product was estimated by using a 100 bp DNA ladder standard (Invitrogen).

Bead implantation

Affigel-blue beads were incubated in BMP4 (100㎍/ml), WNT5A (1mg/ml) and phosphate buffered saline (PBS). All beads were incubated at room temperature for at least 1 hour and then carefully placed into the buccal part of E14 mouse tooth germs using fine forceps. The explants were cultured at 37℃ in a Trowell-type culture containing D-MEM with 10% FBS for 48 hours. The tooth germs implanted beads were fixed with 4% PFA after incubation for 48 hours and cryo-sectioned at a thickness of 9 ㎛, and stained with Hematoxylin and Eosin. The explants of beads implantation were transplanted into the renal capsule for three weeks as well.

Bone formation detection, apoptosis and cell proliferation assays

For the detection of the cell differentiation into osteoblasts and the bone formation, the tooth germs implanted soaked beads were fixed after culture for 48 hours in 4% PFA overnight at 4℃, embedded in Optimal Cutting Temperature (O.C.T) (Tissue-Tek, Cat. NO 4583, USA) compound using conventional methods and then cut to a thickness of 9㎛. In order to reduce nonspecific background staining due to endogenous peroxidase, slides were incubated in 3% hydrogen peroxide for 15 minutes. Sections were incubated with the rabbit polyclonal antibody against Bone Sialoprotein (BSP) (Chemicon, Cat No AB1854, USA) and mouse monoclonal antibody

against Proliferating Cell Nuclear Antigen (PCNA) (Neo Markers, Cat. NO MS-10b-P_0, USA) at 4℃ overnight. After washing for 10 mins with phosphate buffered saline (PBS), the specimens were incubated with biotinylated goat anti-mouse immunoglobulin secondary antibody and biotinylated goat anti-rabbit immunoglobulin secondary antibody for 10 minutes, followed by 10 minutes PBS wash, followed by incubation in streptavidin-peroxidase at room temperature for 10 minutes each. Finally, the antibody binding to the sections was visualized using a Diaminobenzodine tetrahydrochloride (DAB) reagent kit (Zymed, Cat No 00-2014, USA).

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (In situ Cell Death Detection Kit, POD) was used for apoptosis detection in individual cells of the histological sections of bead implanted explants. The TUNEL proceduce was carried out following the manufacturer's directions (Trevigen, USA). Engdogenous peroxidase was blocked by incubation in 3% hydrogen peroxide in PBS for 10 mintues at RT before enzymatic labelling. During the TUNEL procedure samples were washed in PBS (PH: 7.4) in distilled water. The signal conversion using Strep-HRP solution and substrate color reaction applying chromogen DAB were performed after enzymatic labelling.

III. RESULTS

Size of first molar in adult mouse and rat

Both adult mouse and rat share the same dentition including three molars and one incisor in the jaw quadrant. The mandibular first molars in both mouse and rat show seven cusps, of which the patterning is also similar in mouse and rat (Fig. 3A-D). The most distinct difference in the first molar between mouse and rat is the size of crown. The rat first molar has the larger crown than the mouse first molar. The mesiodistal length in the rat first molar is about two times of that in the mouse first molar (Fig. 3A, C), and the buccolingual length in the rat first molar is about two times of that in the mouse first molar (Fig. 3B, D).

Tooth size after transplantation into kidney

The tooth germs, which were transplanted into kidney capsule, can form calcified teeth after three weeks. However, it has not been reported if the kidney capsule, a highly dense connective tissue, can affect the size of the calcified tooth during its development. In this study, the mouse tooth germs at E14 and the rat tooth germs at E16 were transplanted into subcapsular layer of the kidney of nude mice for three weeks. As a result, the mouse tooth germs formed calcified teeth (N = 9/9), among which first molars was slightly smaller than the adult mouse first molar (Fig. 8A, B). Each rat tooth germ formed calcified teeth in kidney (N = 4/4), and the first molar among them was slightly smaller than the adult rat first molar (Fig. 8D, E). Consequently, the calcified first molar of rat developing in the kidney was about two times bigger than the mouse first molar in both mesiodistal length and buccolingual length (Fig. 8B, E). Therefore, transplantation of tooth germs

into kidney capsule can be used as a culture system for odontogenesis. The biggest tooth developing from a mouse tooth germ was considered as the mouse control tooth (Fig. 8A-C). The biggest tooth harvested from a rat presumptive E16 tooth germ was considered as the rat control tooth (Fig. 8D-F).

Cross-recombinant teeth and their size

The first molars of mouse and rat share many similarities except tooth size. To investigate the key tissue determining the tooth size developing from the cap-stage tooth germ, the mouse dental epithelium was cross-recombined with the rat dental mesenchyme, and the rat dental epithelium was cross-recombined with the mouse dental mesenchyme. The calcified teeth were formed from the cross-recombinants between mouse and rat dental tissues. The crown size of the cross-recombinant tooth between the mouse epithelium and the rat mesenchyme (M-epi / R-mes) (N = 10/10) was similar to that of the rat control tooth (Fig. 8G-I). At the same time, the cross-recombinant tooth between the rat epithelium and the mouse mesenchyme (R-epi / M-mes) (N = 8/10) showed similar crown size to that of the mouse control tooth (Fig. 8J-L). These results showed that the mesenchyme rather than the epithelium determined the tooth size in the cap-stage tooth germ and that the rat mesenchyme contributed to form the bigger tooth than the mouse mesenchyme did. However, the rat E16 mesenchyme was bigger than the mouse E14 mesenchyme at the moment of cross-recombination. This means that the cell number of the rat E16 mesenchyme is larger than that of the mouse E14 mesenchyme, because the cell size is almost the same between mouse and rat

observed under the microscope.

Mesenchymal cell number and crown size

To clarify the relationship between the mesenchymal cell number and the tooth size, the mouse and rat mesenchymal cell number was equally adjusted as a number of '6.0×10^4', which was regarded as the cell number in a rat mesenchyme at E16. The calcified teeth developed from the cross-recombinant tooth germs between the mouse epithelium and the rat reaggregated mesenchyme (N = 7/11) (M-epi / R-mes (R) in Fig. 8M-O). The size of M-epi / R-mes (R) was smaller than the rat control tooth (Fig. 8D), but bigger than the mouse control tooth (Fig. 8A) and R-epi / M-mes (Fig. 8J). The cross-recombinant tooth germs between the rat epithelium and the mouse reaggregated mesenchyme formed calcified teeth (N = 8/12) (R-epi / M-mes (R) in Fig. 8P-R), of which the crown size was similar to mouse control tooth but smaller than M-epi / R-mes (R). These results showed the mesenchymal cell number was not closely related with the size of tooth crown. The fact that the rat mesenchymal cells rather than the mouse mesenchymal cells can induce the big tooth suggests that the genetic information relating with the tooth crown size might be different between the rat mesenchymal cells and the mouse mesenchymal cells.

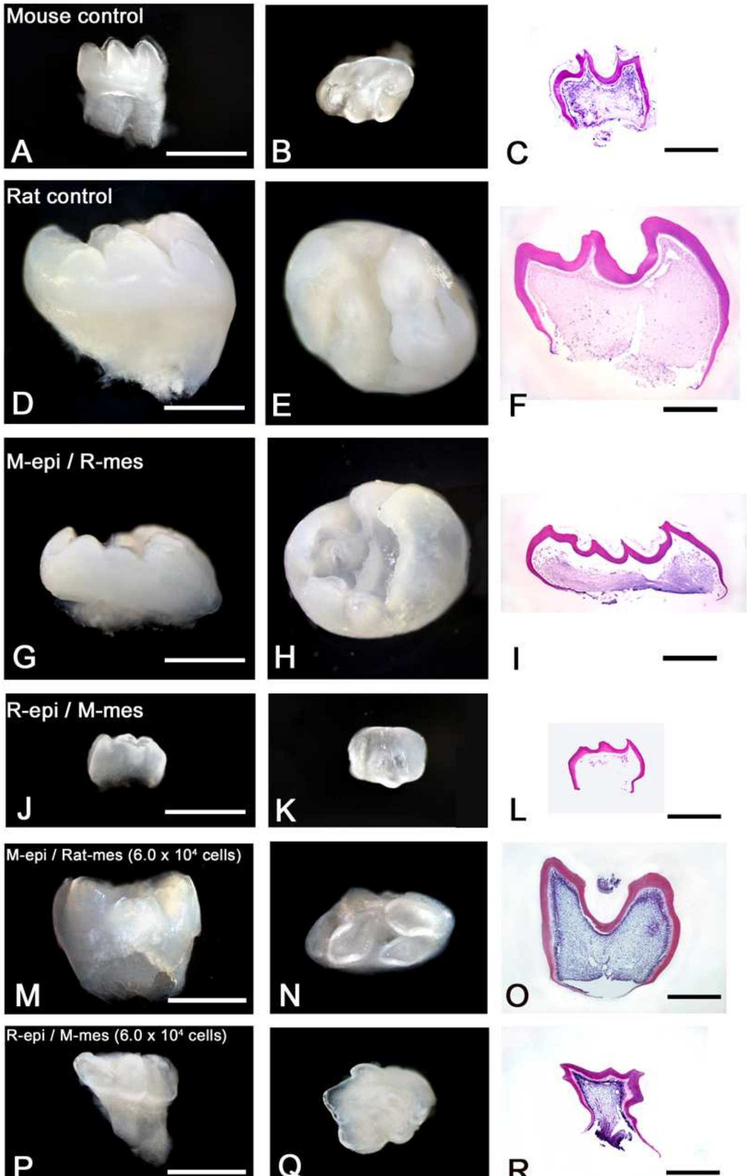

Figure 8. Diverse crown size of molar by cross-recombination and cross-reaggregation between mouse tooth germs and rat tooth germs at cap stage. (A-F) Control teeth obtained from E14 mouse tooth germs and E16 rat tooth germs that were cultured for 1 day in vitro and implanted under

kidney capsules for 3 weeks. (G-L) Cross-recombinant teeth. (M-R) Cross-reaggregated teeth. (A)Mouse control tooth showed 6 cusps. (B) An occulusal view. A section of (A) is shown in (C). (D) Control tooth with 7 cusps was detected from a presumptive rat tooth germ at E16. (E) The occlusal area showing roughly 2 times as large as mouse control tooth in both mesiodistal length and buccolingual length. A section of (D) is shown in (F). (G, H, I) The occlusal area of the M-epi / R-mes tooth was similar size to rat control tooth, which was detected to have many cusps in the occulusal view (H) and more than 3 cusps in a wax section shown in (I). (J, K, L) The R-epi / M-mes tooth showed similar size to mouse control tooth. (M, N) The M-epi / R-mes (R) reaggregated teeth was larger than of mouse control tooth. (P, Q) The R-epi / M-mes (R) was similar to the mouse control tooth. A section of (M, P) are shown in (O, R) respectively. Sale bar : 1 mm in A, B, D, E, G, H, J, K, M, N, P and Q; 500 ㎛ in C, F, I, L, O and R.

Gene expression in the dental mesenchyme of mouse and rat

To investigate the different genetic information between the mouse and rat dental mesenchyme, gene expression in both mouse and rat dental mesenchyme was examined and compared by RT-PCR using total RNA isolated from the mouse and rat dental mesenchyme. Many genes such as *Fgf3*, *Fgf10*, *Fgfr1c*, *Bmp4*, *Msx1*, *Wnt5a*, *β-catenin* and *Lef1*, which have been known to be expressed in the mouse dental mesenchyme at cap stage, were selected as candidate genes. Transcripts of all above genes except *Fgf10* were detected in both mouse and rat mesenchyme. Transcripts for *Bmp4* and *Wnt5a* showed different quantity in the dental mesenchyme between mouse and rat, while other transcripts showed similar quantity. The transcript for *Bmp4* in the rat dental mesenchyme was larger in quantity than that in mouse dental mesenchyme. On the contrary, the mouse mesenchyme showed a larger amount of the transcript for *Wnt5a* than the rat mesenchyme did (Fig. 9). These results showed that *Bmp4* and Wnt5a were different genetic information between mouse and rat.

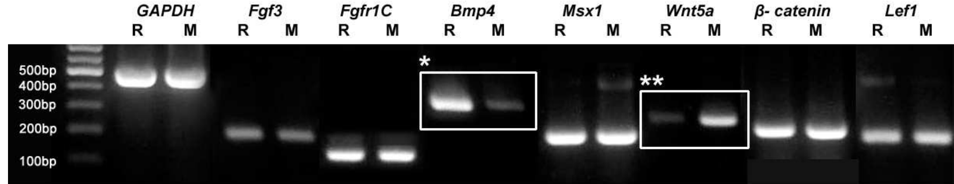

Figure 9. Different intensity of *Wnt5a* and *Bmp4* in the dental mesenchyme between mouse and rat

RT-PCR analysis: *Bmp4* transcription showed stronger intensity in the rat dental mesenchyme (asterisk), whereas *Wnt5a* transcription showed stronger

intensity in mouse dental mesenchyme than that of rat (two asterisks); *Fgf3, Fgfr1c, Msx1, β-catenin* and *lef1* were found the same intensities between mouse and rat. *GAPDH* expression level was used as a control.

Effect of BMP4 and WNT5A on the size of developing tooth germs

To investigate the effect of BMP4 and WNT5A on the size of tooth germ at cap stage, BMP4-, WNT5A- and PBS-soaked beads were implanted into the mouse E14 tooth germs respectively (Fig. 10A, C). After 48 hours in culture, no difference can be found between the BMP4-treated tooth germs (N = 16/16 data not shown) and the PBS-treated tooth germs (N= 10/10, data not shown). In contrast, the WNT5A-treated tooth germs (N = 20/22) were smaller than PBS-treated tooth germs (N = 15/15) (Fig. 10B, D). The WNT5A-treated tooth germs showed clear bone-like structure in upper view (Fig. 10D). In the section (through the line from a to a' in Fig. 10B), the PBS-treated tooth germs was at cap stage showing large dental epithelium (Black dotted line in Fig. 10I), and the WNT5A-treated tooth germs showed small dental epithelium (Black dotted line in Fig. 10J) and bone-like eosinophilic structures (arrow in Fig. 10J) in the section (through the line from b to b' in Fig. 10D). These results showed that the size of the tooth germ was decrease by the WNT5A protein.

Effect of WNT5A on the size of calcified tooth

To investigate if the WNT5A protein can affect the size of calcified tooth or not, tooth germs cultured for 48 hours with exogenous WNT5A were transplanted into kidney for tooth formation and calcification. After three weeks, WNT5A-treated tooth germs formed calcified teeth (N= 10/10). Most

of these tooth germs formed two teeth respectively (N= 8/10), of which the larger tooth was compared in crown size with the PBS-treated tooth. Two tooth germs formed the calcified teeth as big as the PBS-treated tooth (N= 11/11), while eight WNT5A-treated tooth germs formed the calcified teeth corresponding to the two thirds of PBS-treated teeth in both mesiodistal and buccolingual length (Fig. 10E, G). This result showed that not only the size of tooth germ but also the size of calcified tooth can be reduced by WNT5A protein.

Roles of *Wnt5a* in tooth development

To investigate how the WNT5A protein can reduce the size of tooth germ, the cell activities such as differentiation, apoptosis and proliferation were inquired in the cultured tooth germs with the soaked beads. Firstly, the WNT5A-treated tooth germ showed the big BSP-positive spots (arrowheads in Fig. 10L), while the PBS-treated tooth germ rarely showed the BSP-positive spots (Fig. 10K). These spots together with the bone-like structure (arrow in Fig. 10D) indicate that the cell differentiation into osteoblast was induced by the WNT5A protein. Secondly, the apoptosis were generally detected in both epithelium and mesenchyme of the PBS-treated tooth germ (Fig. 10M), while the WNT5A-treated tooth germ showed stronger positive reaction in the mesenchyme (Fig. 10N). Thirdly, the cell proliferation was not different between in the PBS-treated tooth germ and the WNT5A-treated tooth germ (Fig. 10O-P). These results indicated that *Wnt5a* might induce the differentiation into osteoblast and the apoptosis of mesenchymal cells in tooth germ, and subsequently caused the

reduction of tooth germ and calcified tooth.

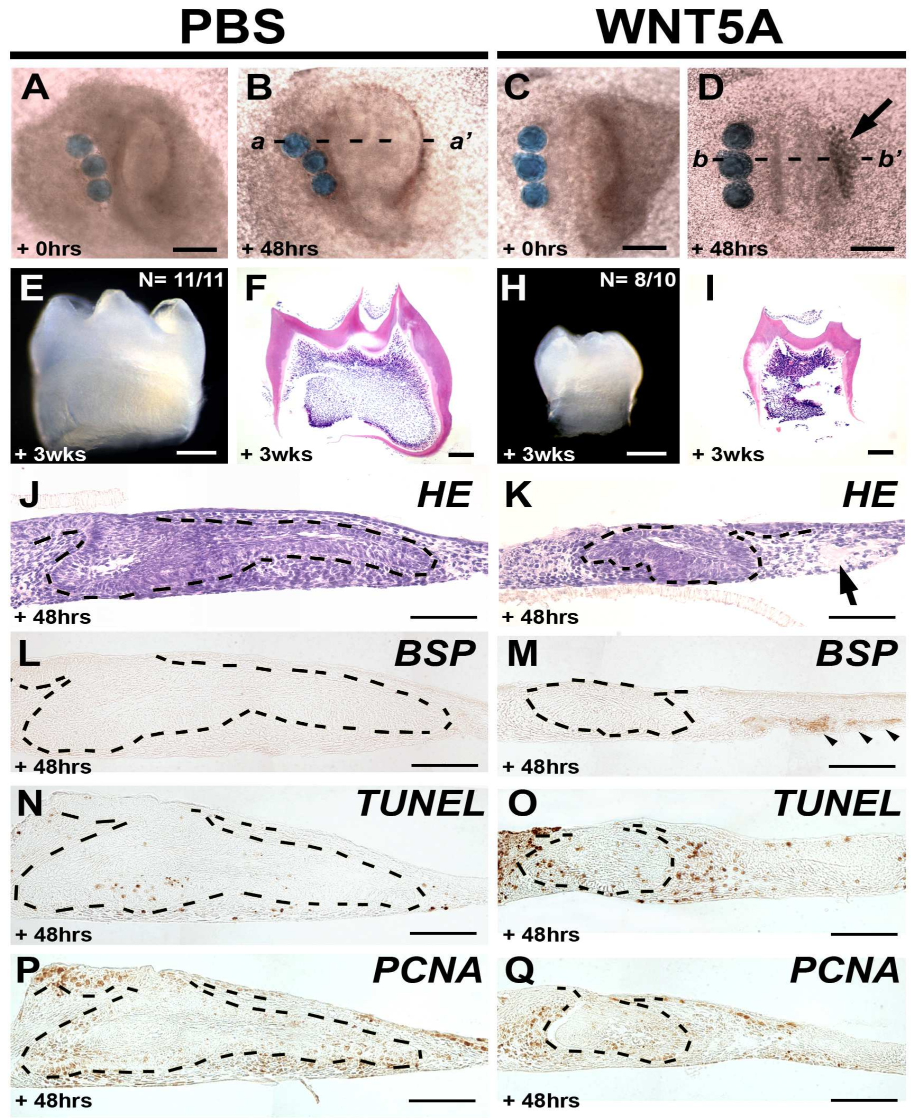

Figure 10. Tooth development from mouse tooth germ at cap stage treated with exogenous WNT5A protein

(A, B, E, F, I, K, M, O) E14 tooth germs cultured with PBS control

protein were used as a control. (C, D, G, H, J, L, N, P) E14 tooth germs incubated with WNT5A soaked beads. (A, C) Upper view of the tooth germs implanted affigel-blue beads soaked in PBS (A) and WNT5A (C) before culture. (B, D) Tooth germs with soaked beads cultured for 48 hours. (E) Teeth formed from the tooth germs cultured with PBS beads in the kidney capsule after three weeks. (G) Smaller teeth detected from the explants treated with WNT5A protein after three weeks. (F, H) Section of (E, G). (I, J) Hematoxylin and Eosin staining of frontal sections of (B, D). (I) Dental epithelium at cap stage was detected in the control after 48 hours. (J) Shrunken dental epithelium at cap stage and bone-like eosinophilic structure indicated by arrows were formed in the WNT5A-treated explants. (K, L) Bone siolaprotein (BSP) expression of frontal sections of (B, D) (L) Big BSP-positive spots were shown in the WNT5A-treated dental mesenchyme indicated by arrows. (M, N) Terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick-end labeling (TUNEL) studies on frontal sections of (B, D), which indicated the apoptosis locate in the place as visualized by dark brown. (M) TUNEL positive spots generally located in the epithelium and mesenchyme of control explants. (N) Increased labeled mesenchymal cells were observed in the WNT5A-treated groups. Black dotted line in (B, D): Section line of (I-N). Black dotted line in (I-N): Dental epithelium. Scale bar : 250 ㎛ in A-E, G; 300 ㎛ in F, H; 100 ㎛ in I-N.

Table 1. Calcified teeth from various kinds of tooth germs

Tooth germ type	Teeth/transplants
Mouse control	9/9
Rat control	4/4
Mouse epithelium/ Rat mesenchyme recombination	10/10
Rat epithelium/ Mouse mesenchyme recombination	10/10
Mouse epithelium/ Rat mesenchyme (6.0 x 10^4)	7/11
Rat epithelium/ Mouse mesenchyme (6.0 x 10^4)	8/12

Table 2. Calcified teeth after implantation of exogenous proteins and their size relative to control tooth size

TOOTH GERM TYPE	TOOTH FORMATION	TOOTH SIZE
PBS (control)	8/8	1
BMP4 beads	18/18	14/18 : about 1
		1/18 : > 1
		3/18 : about 4/5
WNT5A beads	10/11 : tooth	2/10 : about 1
	1/11 : no tooth formed	8 /10 : about 2/3

IV. DISCUSSION

It has been well known that the development of ectodermal organs including tooth is characterized by coordinated interactions between epithelium and mesenchyme. A number of tissue recombination experiments were performed to analyze the nature of epithelial-mesenchymal interactions in many different organs[31-34]. Furthermore, it has been reported that the epithelial-mesenchymal interactions occur even in heterospecific recombination between different species such as chick/mouse, quail/lizard, quail/rabbit and mouse/vole[35-40]. However, these previous studies have concentrated on the epithelial mesenchymal interaction and tooth formation, not on the tooth size.

In this study, we concentrated on the size of teeth, which can be formed by the heterospecific recombination between mouse and rat dental tissues. Mouse and rat, two species of muroid rodents, showed the similar dentition patterning in mandible and the similar cusp patterning in the individual lower first molar containing seven cusps. The most distinct difference in the first molar between mouse and rat is the size of crown. The rat first molar is two times larger than the mouse first molar both in the mesiodistal and buccolingual length.

Dental mesenchyme determines the tooth crown size

To investigate the key tissue determining the tooth size in the tooth germ at cap stage, when the dental mesenchyme has the inductive potential of tooth formation[16], the mouse dental epithelium at E14 was cross-recombined with the rat dental mesenchyme at E16, and the rat dental epithelium at E16 was cross-recombined with the mouse dental mesenchyme at E14. As a

result, the M-epi / R-mes formed the bigger teeth than the R-epi / M-mes did. This result indicates that the mesenchyme rather than the epithelium determined the tooth size in the cap-stage tooth germ and that the rat mesenchyme contributed to form the bigger tooth than the mouse mesenchyme did. Furthermore, the R-mes (R), of which the cell number is the same with that of the M-mes (R), contributed to form the bigger tooth than the M-mes (R) did. The size of the rat dental mesenchymal cells was the same size of the mouse dental mesenchymal cells. Thess results show that the size of tooth crown was determined not by the mesenchymal cell number, but by the genetic information in the mesenchymal cells.

Mouse and rat show the different amount of *Bmp4* and *Wnt5a* expression in dental mesenchyme

The different genetic information between the mouse and rat dental mesenchyme was examined and compared by RT-PCR. Among nine genes that were examined, the transcript for *Fgf10* could only be detected in the mouse dental mesenchyme. This result may be caused by the difficulty in designing the primer pair both for the mouse and rat *Fgf10*. Transcripts for *Fgf3, Fgfr1c, Bmp4, Msx1, β-catenin* and *Lef1* showed similar quantity between mouse and rat, while transcripts of *Bmp4* and *Wnt5a* showed different quantity. These results evoke the possibility that *Bmp4* and *Wnt5a* might be two genes of the genetic information, which is related with the tooth size. Furthermore, the fact that the transcript for *Bmp4* was larger in the rat mesenchyme than in mouse mesenchyme might lead to the possibility, of which *Bmp4* related with increasing the tooth size. On the other hand, the fact that the transcript for *Wnt5a* was less in the rat

mesenchyme than in the mouse mesenchyme might enhance that *Wnt5a* is related with the decreasing the tooth size.

Exogenous BMP4 protein could not change the size of tooth

It has been widely confirmed that exogenous BMP4 regulates the tissue shape during morphogenesis by increasing the apoptosis[41-45]. In the limb development, BMP4 increases the cartilage growth, chondrocyte proliferation and chondrocyte hypertrophy[45,46]. Endogenous BMPs are required to maintain cartilage growth, and exogenous BMP4 can enhance cartilage maturation and induce ectopic chondrocyte hypertrophy in the cranial base[47]. However, compared with feather and limb patterning, which can be manipulated by BMPs[45,46,48,49], normal tooth patterning was relatively robust against excess BMPs[21,50].

In this study, after the implantation of the BMP4 protein-soaked beads into the dental mesenchyme of the mouse E14 tooth germ for 48 hours, the tooth germs and the calcified teeth are not changed in size. It is suggested that exogenous BMP4 might not directly related with tooth size determination.

Exogenous WNT5A can change the size of tooth.

It is well known that Wnts, Frizzled receptors and Frizzled-related proteins (FRP) antagonists were expressed in early tooth development[26,51]. Previously, it has been suggested that teeth can be changed into the smaller teeth by treating with exogenous Mfrzb1 protein, one of the FRPs, in the mouse molar of mandibular arch at E10.5[52]. Even though Mfrzb1 is known to block many kinds of *Wnt* family genes, these previous results does not

indicate the direct relationship between *Wnts* and tooth size. Recently, it has been reported that the *Wnt5a* is related with the calcium signaling as the *β-catenin* independent pathway and *Wnt5a* signal independent of *β-catenin* affect the convergence and extension of the body axis, which refers to the extension of embryos along the anterior-posterior axis and the narrowing along the medial-lateral axis[53].

In this study, not only the size of tooth germ but also the size of calcified tooth can be reduced by exogenous WNT5A protein.

***Wnt5a* is related with the determination of tooth crown size at cap stage.**

It has been reported that *Wnt5a* is expressed in proliferative and prehypertrophic chondrocytes and is required for the first transitional event, as chondrocyte differentiation was significantly delayed in the developing long bone[54]. Furthermore, the Wnt/β-catenin pathway has been shown to regulate multiple cell properties controlling liver morphogenesis, such as growth, axial polarity determination, and apoptosis[55].

In this study, the immunohistochemical staining for BSP after WNT5A bead implantation showed previous bone formation, which suggests that WNT5A might induce bone formation and interfere with the growth of tooth germs at the cap stage. Furthermore, WNT5A, a tumor suppressor by negatively regulating B cell proliferation, induced the TUNEL-positive apoptosis in the mesenchyme. Taken together, WNT5A might be involved in cell differentiation and apoptosis during the tooth morphogenesis, suggesting that WNT5A might play an important role in the bone formation and cell death which lead to the smaller tooth formation. Based on these

information, it was considered that WNT5A may effect on the cell proliferation. While, the PCNA reaction of WNT5A-treated tooth germs showed no difference from that of PBS-treated tooth germs. This result indicated that WNT5A might not block the cell proliferation in both epithelium and mesenchyme. Several years ago, it has been reviewed that inceasing proliferation is not the way to induce growth at the organ level[56]. Taken together, WNT5A might not be a factor directly related with the cell proliferation, while WNT5A might be involved in the cell differentiation and the apoptosis during the tooth development, leading to the smaller tooth formation.

V. CONCLUSION

In conclusion, it is suggested that the crown size of tooth is not determined by the dental epithelium and the number of mesenchymal cells, but by the genetic information in mesenchymal cells. Among many genes expressed in dental mesenchyme, the expression amount of *Wnt5a* and *Bmp4* were different between mouse and rat. Especially, *Wnt5a* might be one of the genes directly related with determining the tooth size by inducing bone formation and the apoptosis of mesenchymal cells, subsequently resulting in small tooth germ and tooth.

REFERENCES

1. Hogan BL and Yingling JM. Epithelial/mesenchymal interactions and branching morphogenesis of the lung. Curr Opin Genet Dev 1998;8: 481-486.

2. Pispa J and Thesleff I. Mechanisms of ectodermal organogenesis. Dev Biol. 2003;262(2):195-205

3. Lumsden AG Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. Development. 1988;103:155-169.

4. Chai Y, Jiang X, Ito Y, Bringas P Jr, Han J, Rowitch DH, Soriano P, McMahon AP, Sucov HM. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development. 2000 ;127(8):1671-1679.

5. Graveson AC, Smith MM, Hall BK. Neural crest potential for tooth development in a urodele amphibian: developmental and evolutionary significance. Dev Biol. 1997;188(1):34-42.

6. Shimizu T, Oikawa H, Han J, Kurose E, Maeda T. Genetic analysis of crown size in the first molars using SMXA recombinant inbred mouse strains. J Dent Res. 2004;83:45-49.

7. Anderson AA. Dentition and occlusion development in African American children: mesiodistal crown diameters and tooth-size ratios of primary teeth. Pediatr Dent. 2005;27:121-128.

8. Hashim HA, Al-Ghamdi S. Tooth width and arch dimensions in normal and malocclusion samples: an odontometric study. J Contemp Dent Pract. 2005;15:36-51.

9. Adeyemi TA, Isiekwe MC. Tooth size symmetry--a comparative analysis of tooth sizes among secondary school children. Afr J Med Med Sci. 2004;33:191-194.

10. Dryburgh LC. Epigenetics of early tooth development in the mouse. J Dent Res. 1967;46:1264.

11. Koch WE. In vitro differentiation of tooth rudiments of embryonic mice. I. Transfilter interaction of embryonic incisor tissues. J Exp Zool. 1967;165:155-170.

12. Kollar EJ and Baird GR. The influence of the dental papilla on the development of tooth shape in embryonicmouse tooth germs. J Embryol Exp Morphol. 1969;21:131-148.

13. Kollar EJ and Baird GR. Tissue interactions in embryonic mouse tooth germs. II. The inductive role of the dental papilla.J Embryol Exp

Morphol. 1970;24:173-186.

14. Lawson KA. Mesenchyme specificity in rodent salivary gland development: the response of salivary epithelium to lung mesenchyme in vitro. J Embryol Exp Morphol 1974;32:469-493.

15. Sakakura T, Nishizuka Y, Dawe CJ. Mesenchyme-dependent morphogenesis and epithelium-specific cytodifferentiation in mouse mammary gland. Science. 1976;194:1439-1441.

16. Mina M, Kollar EJ. The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. Arch. Oral Biol. 1987;32:123-127.

17. Young CS, Terada S, Vacanti JP, Honda M, Bartlett JD, Yelick PC. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. J Dent Res. 2002;81:695-700.

18. Yamamoto H, Kim EJ, Cho SW,Jung HS. Analysis of tooth formation by reaggregated dental mesenchyme from mouse embryo. Journal of Electron Microscopy. 2003;52:559-566.

19. Jiang TX, Jung HS, Widelitz RB, Chuong CM. Self-organization of periodic patterns by dissociated feather mesenchymal cells and the regulation of size, number and spacing of primordial. Development.

1999;126:4997-5009.

20. Jernvall J and Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mechanisms of Development 2000;92:19-29.

21. Jernvall J and Jung HS. Genotype, phenotype, and developmental biology of molar tooth characters. Yearbook of physical anthropology 2000;43:171-190.

22. Keranen SV, Aberg T, Kettunen P, Thesleff I, Jernvall J. Association of developmental regulatory genes with the development of different molar tooth shapes in two species of rodents. Dev Genes Evol. 1998; 208:477-486.

23. Kettunen P, Thesleff I. Expression and function of FGFs-4, -8, and -9 suggest functional redundancyand repetitive use as epithelial signals during tooth morphogenesis. Developmental dynamics. 1998;211:256-268.

24. Kratochwil K, Galceran J, Tontsch S, Roth W and Grosschedl R. FGF4, a direct target of LEF1 and Wnt signaling, can rescue the arrest of tooth organogenesis in Lef1-/- mice. Genes Dev 2002;16:3173-3185.

25. Laurikkala J, Kassai Y, Pakkasjarvi L, Thesleff I, Itoh N. Identification of a secreted BMP antagonist, ectodin, integrating BMP,

FGF, and SHH signals from the tooth enamel knot. Dev Biol. 2003;1;264:91-105.

26. Sarkar L and Sharpe PT. Expression of Wnt signaling pathway genes during tooth development. Mechanisms of Development. 1999;85:197-200.

27. Aberg T, Wozney J, Thesleff I. Expression patterns of bone morphogenetic proteins (Bmps) in the developing mouse tooth suggest roles in morphogenesis and cell differentiation. Developmental dynamics. 1997;210:383-396.

28. Kettunen P, Karavanova I, Thesleff I. Responsiveness of developing dental tissues to fibroblast growth factors: expression of splicing alternatives of FGFR1, -2, -3, and of FGFR4; and stimulation of cell proliferation by FGF-2, -4, -8, and -9. Developmental genetics. 1998;22:374-385.

29. Mustonen T, TummersM, Mikami T, Itoh N, Zhang N, Gridley T, Thesleff I. Lunatic fringe, FGF, and BMP regulate the Notch pathway during epithelial morphogenesis of teeth. Dev Biol. 2002;248:281-293.

30. Vainio S, Karavanova I, Jowett A, Thesleff I. Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. Cell. 1993;8;75:45-58.

31. Grobstein C. Tissue disaggregation in relation to determination and stability of cell type. Ann N Y Acad Sci. 1955;60:1095-1097.

32. Wessells NK. Mammalian lung development: interactions in formation and morphogenesis of tracheal buds. J Exp Zool. 1970;175:455-466.

33. Rush AJ. Cognitive therapy of depression: rationale, techniques, and efficacy. Psychiatr Clin North Am. 1983;6:105-127.

34. Saxen L, Sariola H. Early organogenesis of the kidney. Pediatr Nephrol. 1987;1:385-392.

35. Arechaga J, Karcher-Djuricic V, Ruch JV. Neutral morphogenetic activity of epithelium in heterologous tissue recombinations. Differentiation. 1983;25:142-147.

36. Fuenzalida M, Lemus R, Romero S, Fernandez-Valencia R, Lemus D. Behavior of rabbit dental tissues in heterospecific association with embryonic quail ectoderm. J Exp Zool. 1990;256:264-272.

37. Lemus D. Contributions of heterospecific tissue recombinations to odontogenesis. Int J Dev Biol. 1995;39:291-297.

38. Keranen SV, Kettunen P, Aberg T, Thesleff I, Jernvall J. Gene expression patterns associated with suppression of odontogenesis in

mouse and vole diastema regions. Dev Genes Evol. 1999;209(8):495-506.

39. Wang YH, Upholt WB, Sharpe PT, Kollar EJ, Mina M. Odontogenic epithelium induces similar molecular responses in chick and mouse mandibular mesenchyme. Dev Dyn. 1998;213:386-397.

40. Lemus D, Coloma L, Fuenzalida M, Illanes J, Paz de la Vega Y, Ondarza A, Blanquez MJ. Odontogenesis and amelogenesis in interacting lizard-quail tissue combinations. J Morphol. 1986;189(2):121-129.

41. Miyazaki Y, Oshima K, Fogo A, Ichikawa I. Evidence that bone morphogenetic protein4 has multiple biological functions during kidney and urinary tract development. Kidney Int. 2003;63:835-844.

42. Liu W, Sun X, Braut A, Mishina Y, Behringer RR, Mina M, Martin JF. Distinct functions for Bmp signaling in lip and palate fusion in mice. Development. 2005;132:1453-1461.

43. Liu W, Selever J, Wang D, Lu MF, Moses KA, Schwarts RJ, Martin JF. Bmp4 signaling is required for outflow-tract septation and branchial-arch artery remodeling. Proc Natl Acad Sci USA. 2004;101:4489-4494.

44. Nie XG. Differential expression of Bmp2, Bmp4 and Bmp3 in embryonic development of mouse anterior and posterior palate. Chin Med

J (Engl). 2005;118:1710-1716

45. Merino R, Rodriguez-Leon J, Macias D, Ganan Y, Economides AN, Hurle JM. The BMP antagonist Gremlin regulates outgrowth, chondrogenesis and programmed cell death in the developing limb. Development. 1999;126:5515-5522.

46. Selever J, Liu W, Lu MF, Behringer RR, Martin JF. Bmp4 in limb bud mesoderm regulates digit pattern by controlling AER development. Dev Biol. 2004;276:268-279.

47. Shum L, Wang X, Kane AA, Nuckolls GH. BMP4 promotes chondrocyte proliferation and hypertrophy in the endochondral cranial base.Int J Dev Biol. 2003;47:423-431.

48. Jung HS, Francis-West PH, Widelitz RB, Jiang TX, Ting-Berreth S, Tickle C, Wolpert L, Chuong CM. Local inhibitory action of BMPs and their relationships with activators in feather formation: implications for periodic patterning. Dev Biol. 1998;196:11-23.

49. Noramly S, Morgan BA. BMPs mediate lateral inhibition at successive stages in feather tract development. Development. 1998;125:3775-3787.

50. Kassai Y, Munne P, Hotta Y, Penttila E, Kavanagh K, Ohbayashi N, Takada S, Thesleff I, Jernvall J, Itoh N.Regulation of mammalian tooth

cusp patterning by ectodin. Science. 2005;309:2067-2070.

51. Dassule HR, McMahon AP. Analysis of epithelial-mesenchymal interactions in the initial morphogenesis of the mammalian tooth. Dev Biol. 1998;202:215-227.

52. Sarkar L and Sharpe P. T. Inhibition of Wnt signaling by exogenous Mfrzb1 protein affects molar tooth size. J Dent Res. 2000;79:920-925.

53. Kohn AD and Moon RT. Wnt and calcium signaling: β-catenin-independent pathways. Cell Calcium 2005;38:439-446.

54. Chimal-Monroy J, Montero JA, Ganan Y, Macias D, Garcia-Porrero JA, Hurle JM. Comparative analysis of the expression and regulation of Wnt5a, Fz4, and Frzb1 during digit formation and in micromass cultures. Dev Dyn. 2002 Jul;224(3):314-20.

55. Suksaweang S, Lin CM, Jiang TX, Hughes MW, Widelitz RB, Chuong CM. Morphogenesis of chicken liver: identification of localized growth zones and the role of beta-catenin/Wnt in size regulation. Dev Biol. 2004 Feb 1;266(1):109-22.

56. Su TT, O'Farrell PH. Size control: cell proliferation does not equal growth. Curr Biol. 1998 Sep 24;8(19):R687-9.

ABSTRACT(IN KOREAN)

발생과정 중 치아의 간엽이 치아머리의 크기에 미치는 영향에 대한 연구

치아는 상피와 간엽조직간의 상호작용에 의해서 조절되는 외배엽성기원의 기관 중 하나이다. 치아의 형태는 여러 가지 신호전달물질들의 전달체계에 의해 형성된다. 치아형태의 중요한 요소로서, 치아머리의 크기는 치열을 결정하고, 교합을 결정한다. 이번 연구에서는 치아머리의 크기를 결정하는 주요인자를 알아내기위해, 흰쥐와 쥐에서 모자시기의 치배조직을 떼어낸 다음, 이 조직의 상피와 간엽을 서로 교차결합 (cross-recombination) 시키는 방법을 도입하였다. 실험결과, 흰쥐의 간엽을 이용한 치아보다, 쥐의 치아간엽을 이용한 치아가 더 크다는 것을 알 수 있었다. 또한 재응집 (reaggregation)법으로써, 흰쥐의 치아간엽 세포수와 쥐의 치아간엽 세포수를 동일하게 (6.0×10^4 cells) 하였을 경우에도 쥐의 치아간엽이 더 큰 치아를 형성하였다. 이러한 사실을 바탕으로 치아머리의 크기는 치아상피가 아닌 치아간엽에 의해 결정됨을 알 수 있었다. 흰쥐의 치아간엽과 쥐의 치아간엽은 동일한 유전자를 동일한 양만큼 발현하고 있음을 확인할 수 있었는데, *Bmp4*와 *Wnt5a*는 그 발현 정도에서 차이를 보였다. 또한, BMP4 단백질을 치배에 처리하였을 때는 치배와 치아의 크기가 변하지 않은 반면, WNT5A 단백질을 처리하였을 때는 치배와 치아의 크기가 작아지는 것을 확인할 수 있었다. WNT5A 단백질은 치배 간엽조직에서 분화를 촉진시키고, 세포사멸을 유도하는 작용으로 치배를 작게 만들어 결과적으로 치아의 크기 또한 정상보다 작게 만드는 것임을 알 수 있었다. 이것으로 보아 *Wnt5a* 유전자는 치아의 크기를 결정하는데 중요한 역할을 하는 유전자로 생각되어진다.

핵심되는 말 : 치아머리 크기, 치아간엽, 흰쥐와 쥐, 이종간 교차결합, 이종간 재응집법, *Wnt5a*