International Symposium

Oral and Craniofacial Development and Diseases 2016

December 12th, 2016

Yumikura Hall

Osaka University

Program

Challenge to Intractable Oral Diseases

December 12th (Mon)

13:00- **Opening remarks** **Atsuo Amano** (Dean, Osaka University Graduate School of Dentistry)

First session: Oral presentation of young investigators

Session Chair: **Yu Usami**
Hiroshi Kurosaka

13:10- Contribution of Wnt/β-catenin-responsive cells to growth plate and articular cartilage growth
Yu Usami (Oral Pathology, Osaka Univ. Graduate School of Dentistry)

13:30- Retinoic-acid-induced cleft palate by regulating Sim2 and sonic hedgehog signaling
Qi Wang (Department of Orthodontics, Graduate School of Dentistry, Osaka University)

13:50- The Wnt/Myb pathway suppresses KIT signaling to control the timing of salivary proacinar differentiation and duct morphogenesis
Shinji Matsumoto (Departments of Molecular Biology and Biochemistry, Graduate School of Medicine, Osaka University)

14:10- Comprehensive treatment approach for the patients with cleft lip and palate during infancy and childhood in the cleft team of Osaka University Dental Hospital
Susumu Tanaka (1st Department of Oral and Maxillofacial Surgery, Osaka University Graduate School of Dentistry)

14:30- Treatment plan for velopharyngeal insufficiency of patients with the 22q11.2 deletion syndrome
Tadashi Yamanishi (Dept. of Oral and Maxillofacial Surgery, Osaka Medical Center and Research Institute for Maternal and Child Health)

14:50- Break 20min

Second session: Oral presentation of senior investigators

Session Chair: **Satoshi Wakisaka**

15:10- Runx signaling is specifically involved in the fusion between the primary palate and the secondary palate by regulating Tgfb3 signaling
Takashi Yamashiro (Department of Orthodontics, Graduate School of Dentistry, Osaka University)

15:40- Contact inhibition of locomotion via EMT by TGF-Rho signal leads to genesis of Epithelial cell rests of Malassez from Hertwig's epithelial root sheath
Hidemitsu Harada (Division of Developmental Biology and Regenerative Medicine, Department of Anatomy, Iwate Medical University, Japan)

16:10- Break 20min

Third session: Plenary lecture

Session Chair: **Takashi Yamashiro**

16:30- Craniofacial mesenchymal stem cells in bone tissue homeostasis and repair
Yang Chai (Center for Craniofacial Molecular Biology, Ostrow School of Dentistry)

17:30- **Closing remarks** **Satoshi Wakisaka** (Project Leader, Osaka University)

17:35- 18:30 **Poster session**

First session: Oral presentation of young investigators

Contribution of Wnt/β-catenin-responsive cells to growth plate and articular cartilage growth

***Yu Usami**

(Oral Pathology, Osaka Univ. Graduate School of Dentistry)

INTRODUCTION: It still remains unclear which progenitor cells contribute to cartilage development and growth. In this study, we hypothesized that Wnt/β-catenin signaling controls the progenitor cells in the epiphyses during postnatal growth, and examined location, fate and roles of Wnt/β-catenin responsive cells in the developing epiphyses.

METHODS: *Axin2CreERT2* mice that harbor *CreER* under the control of the *Axin2* promoter, a target of Wnt/□-catenin signaling, were mated with *Rosa26-ZsGreen* mice that harbor *Cre*-inducible GFP (ZsGreen, ZsG) to generate *Axin2CreERT2;ZsGreen* mice (*Axin2;ZsGreen* mice). In *Axin2;ZsGreen* mouse, tamoxifen (Tx) activates *Cre*-inducible ZsG reporter expression in the cells where Wnt/β-catenin signaling is active. For fate mapping, the *Axin2;ZsGreen* mice received Tx at postnatal day 6 (P6), and the knee joints were harvested on 3, 7, 14 or 36 days after Tx injection. The images were analyzed using a confocal microscope system.

RESULTS: Three days after Tx injection at P6, a cluster of ZsG-positive cells was detected in the perichondrium adjacent to growth plate, known as Ranvier's groove. These ZsG-positive cells were broadly expanded into both articular cartilage and growth plate cartilage.

DISCUSSION: We demonstrated that Wnt/β-catenin-responsive cells are located in specific areas in the epiphysis called Ranvier's groove and contribute to growth of the articular and growth plate cartilage in distinct spatio-temporal manners.

Retinoic-acid-induced cleft palate by regulating Sim2 and sonic hedgehog signaling

***Qi Wang, Takashi Yamashiro, Hiroshi Kurosaka**

(Department of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry, Osaka University)

Cleft palate is a common congenital anomaly in humans and is thought to be caused by genetic and environmental factors. Excessive intake of retinoic acid (RA) or its precursor, vitamin A, during early pregnancy is associated with increased incidence of cleft palate in offspring. However, the pathogenetic mechanism of cleft palate caused by excess RA is not fully understood. We gave all-trans RA (25 mg/kg body weight) to ICR pregnant mice by gastric intubation from embryonic day 8.5 to 10.5. We found that the distance between RA-treated embryonic palatal shelves was wider, and in those regions both of apoptotic cell death and cell proliferation increased. Moreover, tongues of RA-treated embryos exhibited malposition and disruption of the muscle patterning. Cleft palate was rescued after culture without the tongue, indicating that it might be caused by tongue malposition. Furthermore, we discovered that expression of Sonic hedgehog (Shh), and its downstream genes Ptch1 were reduced in palatal region and developing tongue of RA-treated embryos. We also observed the downregulation of single-minded homolog 2 (Sim 2) expression which is known to be associated with cleft palate. The incidence of cleft palate due to overdose RA was reduced by administration of SAG (Shh signaling agonist). Our results suggest that retinoic-acid-induced cleft palate was associated with decrease of palatal shelf proliferation and delay/failure of palatal shelf elevation caused by tongue malposition through the downregulation of Sim2 and Shh signaling pathway.

The Wnt/Myb pathway suppresses KIT signaling to control the timing of salivary proacinar differentiation and duct morphogenesis

***Shinji Matsumoto**

(Departments of Molecular Biology and Biochemistry, Graduate School of Medicine, Osaka University)

Growth factor signaling is involved in the development of various tubular organs, but how signaling regulates organ early morphogenesis and late differentiation remains to be clarified. Here, we show how Wnt signaling controls epithelial tubular branching morphogenesis and functional differentiation using the salivary gland as a model. Experiments using genetically manipulated mice and organ cultures revealed that Wnt signaling at early stage (E12-E14) of submandibular salivary gland (SMG) development inhibits end bud differentiation into proacini by suppressing KIT expression through the upregulation of the transcription factor Myb. In addition, Wnt signaling at the SMG development early stage promoted the expansion of end bud progenitor, leading to duct structure formation. In contrast, Wnt signaling reduction at a late stage (E15-E18) of SMG development promoted end bud differentiation into proacini and suppressed duct morphogenesis. These results suggest that Wnt signaling activity fine-tunes the timing of end bud morphogenesis and differentiation into proacini, in co-operation with KIT signaling during salivary gland organogenesis. In this symposium, we would like to discuss novel switching mechanisms between organ early morphogenesis and late differentiation.

Comprehensive treatment approach for the patients with cleft lip and palate during infancy and childhood in the cleft team of Osaka University Dental Hospital

***Susumu Tanaka**

(1st Department of Oral and Maxillofacial Surgery, Osaka University Graduate School of Dentistry)

In the treatment of the patients with cleft lip and cleft palate, multi-disciplinary team approach is a key concept as advocated by many cleft teams and an early intervention and comprehensive management for the patients and their parents is required in perinatal period. Our cleft team in Osaka University Dental Hospital has encouraged closer cooperation with many maternity department or clinics outside and team staffs involving nurse specialist are routinely referred to visit hospitals or clinics immediately after the birth of a baby and give diagnosis, treatment plan, nursing care including an emotional support and instruction for feeding step by step to parents and medical staffs. To improve primary disfigurements and dysfunctions, presurgical nasoalveolar molding is begun at 2-3 weeks of age, which is followed by primary lip repair at 2-3 months of age. In palate repair, we use Furlow's method for soft palate closure at 12 months of age and vestibular flap for hard palate closure (HPC) at 18 months of age, which is more beneficial for subsequent speech outcomes managed by experienced speech therapists and maxillary growth than one-stage closure or HPC by palatal local flap from our long-term results. Secondary bone grafting for alveolar bone defects is performed prior to the eruption of canine or lateral incisor after presurgical orthodontic management by orthodontists. In our institution, iliac crest bone is harvested only in the case of BCLP and large UCLP, while chin bone harvested from mandibular symphyseal region combined with β-Tricalcium Phosphate (β-TCP) or absorbable plate is applied in UCLA and UCLP as alternative method to avoid the sutured wound and morbidity after harvesting of iliac crest bone.

Treatment plan for velopharyngeal insufficiency of patients with the 22q11.2 deletion syndrome

***Tadashi Yamanishi**

(Dept. of Oral and Maxillofacial Surgery, Osaka Medical Center and Research Institute for Maternal and Child Health**)**

The 22q11.2 deletion syndrome is a relatively rare contiguous gene syndrome with characteristic symptom of congenital heart defect, characteristic facial features, developmental delay, psychiatric disorders, cleft palate and speech difficulties. Persistent velopharyngeal insufficiency (VPI) even after surgical intervention underlies speech difficulties of the patients. However, no general consensus, so far, is established as to the treatment plan for VPI of this syndrome. In this presentation, we show the outcome of our treatment for VPI of the syndrome, and also show our current treatment direction.

So far, 126 patients were diagnosed by a clinical geneticist as the 22q11.2 deletion syndrome in our center. Of those, 54 patients visited our department for treatment of speech and/or palate problems. Twenty patients underwent palatal lengthening and muscle sling reconstruction as primary palate surgery. However, only 5 of those showed decent VP function including a patient with normal VP function and 4 patients with mild VPI. The poor outcome may be influenced by platybasia evaluated by cephalometric analysis. On the other hand, 6 out of 7 patients who received posterior pharyngeal flap as primary surgery showed normal VP function or mild VPI. It was considered that posterior pharyngeal flap would be favorable as primary palatal surgery for VPI of patients with the 22q11.2 deletion syndrome without presenting overt cleft palate.

Second session: Oral presentation of senior investigators

Runx signaling is specifically involved in the fusion between the primary palate and the secondary palate by regulating Tgfb3 signaling

***Takashi Yamashiro**

(Department of Orthodontics, Graduate School of Dentistry, Osaka University)

The mammalian palate is formed from the primary and the secondary palate and the failure in fusion at this boundary causes anteriorly-limited cleft palate. It is known that palatal fusion is regulated in different manner in the anterior-posterior axis, however, it should be still addressed more how the primary palate fuses with the fused secondary palate. *Runx1* is a member of the *Runx* family genes, which is known to be a master transcription factor for hematopoietic development. Recent studies demonstrated that *Runx* signaling is also associated with morphogenesis of various developing organs. We previously showed that Runx1 is specifically expressed at the fusing epithelium during palatogenesis and *Runx1* deficiency results in incomplete clefting only at the anterior junction between the primary and the secondary palate, but not in the junction between the palatal process in the secondary palates. In this study we further investigated the mechanisms of *Runx1*-associated palatal fusion including molecular network using epithelial specific *Runx1* knock out mice (*K14Cre;Runx1flox/flox*). Similar to our previous report, *K14Cre;Runx1flox/flox* mice exhibited anteriorly-limited cleft palate which suggests region specific association of *Runx1* during palatal fusion. Interestingly, *Tgfb3* which plays critical roles during palatal fusion showed significant downregulated only in the primary palate, but not in the secondary palate. Such spatial downregulation of Tgfb3 also accompanied the spatially specific downregulation of Mmp-13 and Fgf9, downstream targets of Tgfb3 signaling in the palate. Furthermore, epithelial apoptosis and the underlying-mesenchymal proliferation was disturbed in the mutant primary palate. In addition, reintroduced TGFB3 rescued the defects in the fusion of the mutant palate in the culture, with recovering of Mmp-13 and Fgf9 mRNA expression in the primary palate. These results suggest epithelial *Runx1* during palatogenesis regulates expression of Tgfb3 in a region specific manner that is governing anterior palatal fusion.

Contact inhibition of locomotion via EMT by TGF-Rho signal leads to genesis of Epithelial cell rests of Malassez from Hertwig's epithelial root sheath

***Hidemitsu Harada**

(Division of Developmental Biology and Regenerative Medicine, Department of Anatomy, Iwate Medical University, Japan)

Hertwig's epithelial root sheath (HERS) is known to produce epithelial cell rests of Malassez (ERM) during root development. However, the mechanism has been controversial between epithelial-mesenchymal transition (EMT) and apoptotic theory. Here, we observed EMT and contact inhibition of locomotion (CIL) of HERS cells using live cell imaging. CIL has been known as the process through which cells move away from each other after cell-cell contact, and observed in neural crest cell migration. The imaging showed that some cells fell away from HERS and migrated into periodontal tissue, and after that, some migratory cells re-aggregated and formed epithelial colonies like ERM. In co-culture experiments of HERS and dental follicle cells, some epithelial pre-migratory cells and migratory cells expressed mesenchymal markers (N-cadherin, vimentin), and whereas non-migratory cells expressed E-cadherin. Tgf-β and ROCK inhibitors stimulated the cell migration and up-regulated the gene-expression of mesenchymal markers. In vitro organ culture, Tgf-β inhibitor and Rho activator inhibited the migration from HERS and induced the elongation of HERS. Based on these results, we coin new hypothesis that ERM develops from HERS through EMT and CIL, and that the phenomenon is not epithelial fragmentation. The observation provides insight into the new molecular mechanisms of epithelial organogenesis.

Third session: Plenary lecture

Craniofacial mesenchymal stem cells in bone tissue homeostasis and repair

Yang Chai,* Hu Zhao, Zoe Johnson, and Shery Park

(Center for Craniofacial Molecular Biology, Ostrow School of Dentistry, University of Southern California)

Bone tissue undergoes constant turnover supported by stem cells. Recent studies showed that perivascular mesenchymal stem cells (MSCs) contribute to the turnover of long bones. Craniofacial bones are flat bones derived from a different embryonic origin than the long bones. We have recently identified *Gli1+* cells within the suture mesenchyme as the major MSC population for craniofacial bones. They are not associated with vasculature, give rise to all craniofacial bones in the adult and are activated during injury repair. *Gli1+* cells are typical MSCs *in vitro*. Ablation of *Gli1+* cells leads to craniosynostosis and arrest of skull growth, indicating these cells are an indispensible stem cell population. *Twist1*$^{+/-}$ mice with craniosynostosis show reduced *Gli1+* MSCs in sutures, suggesting that craniosynostosis may result from diminished suture stem cells. Craniofacial sutures provide a unique niche for MSCs for craniofacial bone homeostasis. During calvarial bone repair, the calvarial sutures possess much stronger regeneration capability than the non-suture areas. The healing rate of the calvarial bone is inversely related to the suture-injury distance. Any injury closer to the suture heals faster. After complete removal, the sagittal suture can regenerate and restore to normal organization within 6 weeks. Gli1+ cells within the suture mesenchyme are the cellular source for the injury repair. These results suggest that calvarial bone healing is not an evenly distributed event on the calvarial surface. The suture is the origin of its capacity for regeneration. Our study has important implications for the preservation of suture structures during calvarial surgery and can also help us to design new approaches for repairing calvarial malformations and defects.

Supported by R37 DE012711 and R01 DE025221, NIDCR, NIH

Poster session

P-1

Runx signaling is specifically involved in the fusion between the primary palate and the secondary palate by regulating Tgfβ3 signaling

Safiye Esra Sarper

(Dept of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry, Osaka University)

The mammalian palate is formed from the primary and the secondary palate, the failure in fusion causes cleft palate. It is known that palatal fusion is regulated in different manner in the anterior-posterior axis, however, it should be still addressed more how the primary palate fuses with secondary palate. *Runx1* is a member of the *Runx* family genes, which is known to be a master transcription factor for hematopoietic development. We previously showed that Runx1 is specifically expressed at the fusing epithelium and *Runx1* deficiency results in cleft only at the anterior between the primary and the secondary palate. In this study we investigated the mechanisms of *Runx1*-associated palatal fusion including molecular network using epithelial specific *Runx1* knock out mice (Runx1 cKO mouse, K14 Cre Runx1$^{fl/fl}$). Similar to our previous report, Runx1 cKO mouse exhibited anteriorly-limited cleft palate which suggests region specific association of *Runx1*. In order to find out gene expression deviation we use laser microdissection and in situ hybridization. Interestingly, *Tgfβ3* showed significant downregulation only in the primary palate. Such temporospatial downregulation of Tgfβ3 also accompanied the downregulation of Mmp13 and Fgf9. There were disturbed apoptosis, persistent epithelium proliferation and periderm removal deficiency at Runx1 cKO mouse that interrupt the fusion. In addition, reintroduced TGFB3 rescued the defects in the fusion of the mutant palate in the culture. These results suggest epithelial *Runx1* regulates expression of Tgfβ3 in a region specific manner that is governing anterior palatal fusion.

P-2

Retinoic-acid-induced cleft palate by regulating Sim2 and sonic hedgehog signaling

Qi Wang, Takashi Yamashiro, Hiroshi Kurosaka

(Dept of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry, Osaka University)

Cleft palate is a common congenital anomaly in humans and is thought to be caused by genetic and environmental factors. Excessive intake of retinoic acid (RA) or its precursor, vitamin A, during early pregnancy is associated with increased incidence of cleft palate in offspring. However, the pathogenetic mechanism of cleft palate caused by excess RA is not fully understood. We gave all-trans RA (25 mg/kg body weight) to ICR pregnant mice by gastric intubation from embryonic day 8.5 to 10.5. We found that the distance between RA-treated embryonic palatal shelves was wider, and in those regions both of apoptotic cell death and cell proliferation increased. Moreover, tongues of RA-treated embryos exhibited malposition and disruption of the muscle patterning. Cleft palate was rescued after culture without the tongue, indicating that it might be caused by tongue malposition. Furthermore, we discovered that expression of Sonic hedgehog (Shh), and its downstream genes Ptch1 were reduced in palatal region and developing tongue of RA-treated embryos. We also observed the downregulation of single-minded homolog 2 (Sim 2) expression which is known to be associated with cleft palate. The incidence of cleft palate due to overdose RA was reduced by administration of SAG (Shh signaling agonist). Our results suggest that retinoic-acid-induced cleft palate was associated with decrease of palatal shelf proliferation and delay/failure of palatal shelf elevation caused by tongue malposition through the downregulation of Sim2 and Shh signaling pathway.

P-3

Research of medial edge epithelial cell behavior during secondary palate fusion of mouse

Aoyama gozo[1], Kurosaka Hiroshi[1], Srper Safie Esra[1], Oka Ayaka[1], Tsuchihashi Ryo[2], Yamashiro Takashi[1]

([1]Dept of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry, [2]Neurobiology Group, Div. of Biomedical Informatics, Graduate School of Medicine, Osaka University)

Failure of secondary palate fusion will lead to cleft palate, the most frequent congenital craniofacial birth defects in humans. Palate fusion process involves the removal of medial edge epithelial (MEE) cells which lies on the edge of secondary palate process. It is known that apoptosis, epithelial cell migration and epithelial-mesenchymal transition plays important role in the removal of MEE cells. However, the details that how these biological events participate in the removal of MEE cells are not fully elucidated. In this study, we cultured unpaired palatal shelves of the mice that express Green Fluorescent Protein under the promoter of keratin 14 (K14-GFP) at embryonic day 14.5 (E14.5) and used live imaging technique in order to observe the behavior of MEE cells in vivo during secondary palate fusion. We discovered that MEE cells moved dynamically to both directions towards the nasal side and the oral side. In addition, the amount distance of MEE cell movement in the nasal side was significantly longer the oral side during the removal of MEE cells. Our findings indicate specific directionality of epithelial cell migration contribute to palatal fusion by removing of MEE cells. We would also like to discuss possible molecular mechanism for MEE removal by showing the effect of Y27632 (Rho-kinase Inhibitor) during this culture method.

P-4

The ECM-mesenchymal effects on gene expression with an *in vitro* amelogenesis imperfecta model

Dian Yosi Arinawati[1], Keiko Miyoshi[2], Ayako Tanimura[2], Taigo Horiguchi[2], Arya Adiningrat[2,3], Takafumi Noma[2] (1Graduate School of Oral Sciences, Tokushima University, Tokushima

2Department of Molecular Biology, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima

3Department of Biomedical Sciences, Dental School, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia)

Tooth development is regulated by reciprocal interaction between epithelial and mesenchymal cells in direct and indirect manner. Amelogenesis imperfecta (AI) is inherited enamel defects caused by alteration of gene regulations involved in enamel formation and maturation. We have identified that Specificity protein 6 (SP6), a transcription factor, is one of the AI-causative genes using spontaneous mutant AI-rat (Muto *et al.* Orphanet J Rare Dis. 2012), which is followed by establishment of AI-derived dental epithelial cells (ARE-B30) (Adiningrat *et al.* Oral Dis. 2016). To further analyze the characteristics, we compared the amelogenesis-related gene expression between ARE-B30 and its wild type control, G5 cells, using 3D culture systems; collagen membrane (CM-6), or co-cultured with RPC-C2A (rat pulp cells) on CM-6 in separated and mixed medium, as well as conventional plastic culture.

Reciprocal expression of *Bmp2* and *Fst*, and enhancement of *Amtn* were observed in G5 by 3D co-culture system. Furthermore, G5 expressed *Klk4*, but not *Mmp20* among all culture system. In contrast, no expression of *Amtn*, *Klk4* and *Mmp20* were observed in ARE-B30. Importantly, 3D culture system could demonstrate the distinct effects of ECM and mesenchyme on gene expression in ARE-B30 compared to G5. From this study, we found AI-derived dental epithelial cells have unique responsiveness to the *in vivo* mimicking culture condition, possibly reflecting the different or aberrant cell differentiation compared to wild type control.

P-5

Hertwig's Epithelial Cells and the upper root development of molars in K14 GFP mice

Kohei Nakatsugawa, Hiroshi Kurosaka, Takashi Yamashiro

(Dept of Orthodontics and Dentofacial Orthopedics, Osaka University)

It is well known that tooth root formation is initiated by the development of Hertwig's epithelial root sheath (HERS). In this study, we investigated the process of molar roots development using the mice that express Green Fluorescent Protein (GFP) under the promoter of keratin 14 (K14-GFP). At first, we assessed the morphogenesis of epithelial diaphragm (ED) of upper first molar in vivo by dissecting out the molar from P1 to P3 and capturing picture from the bottom of the molars. We discovered that mesial palatal ED(MPED) and distal palatal ED(DPED) elongate to buccal side,while buccal ED(BuED) elongate to palatal side. In order to investigate the mechanism of ED elongation, we cultured the upper first molar in vitro from the stage of P1 to P3. The velocity of ED elongation during tooth root development was analyzed by comparing the length of ED between in vitro and in vivo by measuring the length of ED during the time course of root development. From these results, we discovered that the velocity of ED elongation in vitro was about half as much in vivo. We are now trying to utilize this culture method in order to further understand the molecular mechanism of ED elongation by supplementing some of the drugs which target specific signaling pathway such as LiCl (inhibitor of GSK3). We would also like to discuss the results from these experiments in present study.

P-6

Histological analysis of periodontal tissue in Col-GFP mice

Tsugumi Ueda , Tomoaki Iwayama, Shinya Muarkami

(Dept of Periodontology, Graduate School of Dentistry, Osaka University)

The periodontal ligament (PDL) plays important roles in maintaining homeostasis of periodontal tissue including alveolar bone, cementum and gingiva. PDL is a non-calcified tissue, where type I collagen is a main component, and *Col1a1* is the most highly expressed gene in PDL. However, there has been little discussion about the collagen metabolism and precise localization of fibrogenic cells in PDL. Traditional staining for collagen protein cannot fully explain which cells are producing the matrix. In this study, we characterized the expression and localization of *Col1a1* in periodontal tissue using Col-GFP mice that express GFP reporter gene under the *Col1a1* promoter and its transcriptional regulatory elements. The periodontal tissue sections of 6 to 8-week-old Col-GFP mice were histologically analyzed using the fluorescent immunostaining. The strong GFP signals were detected throughout PDL. Interestingly, not only osteoblasts and cementoblasts but also most of the cells that make up PDL were strongly positive for GFP, suggesting PDL cells are continuously producing collagen at high level. Conversely, the laminin$^+$ vascular endothelial cells in PDL were GFP negative. In the pulp tissue, GFP was expressed strongly in odontoblasts, and their cellular processes were extended through the dentinal tubules. In the gingival tissue, the epithelial cells showed GFP negative, and underneath fibroblasts were clearly differentiated from epithelial cells by GFP signal. These results suggest that the Col-GFP mouse is a useful model to clarify the collagen metabolism in PDL.

P-7

Role of TGF-β super family signaling in the mechanism of tooth formation in adult mice

Aiko Machiya[1,2], Satoshi Ohte[1], Sho Tsukamoto[1], Noriko Sekine[1], Eijiro Jimi[3], Naoto Suda[2], Takenobu Katagiri[1]

([1]Division of Pathophysiology, Research Center for Genomic Medicine, Saitama Med. Univ. [2]Division of Orthodontics, Meikai Univ. School of Dentistry, [3]Division of Molecular Signaling and Biochemistry, Kyushu Dental College)

Members of the transforming growth factor-β (TGF-β) family play important roles in epithelial-mesenchymal interaction during tooth development. The members are divided in two classes (BMP and TGF-β/Activin) based on the downstream intracellular signaling. Bone morphogenetic proteins (BMPs) activate transcription factors Smad1/5, although TGF-β and Activin activate Smad2/3. The roles of the TGF-β super family members in tooth formation have been studied using embryonic dental tissues. In the present study, however, we examined them in adult mice using incisors, which are continuously growing teeth in rodents even after birth. Quantitative RT-PCR analysis revealed that mRNA levels of the TGF-β super family ligands (BMP2/4, TGF-β1/2/3 and Activin A) and Smads (Smad1 through Smad9) were higher in incisor than in those of other tissues examined, including calvaria, femur and skeletal muscle. Because Smad4 is a common co-activator of both BMP and TGF-β/Activin pathways, we analyzed the localization of Smad4 using a specific antibody. High levels of Smad4 were detected in odontoblasts, pulp, ameloblasts and surrounding dental epithelial lineage cells. Moreover, immunohistochemical analysis using antibodies specific for phosphorylated Smad1/5/9 and Smad2/3 indicated that both of which were activated in not only undifferentiating cells but also differentiating cells in incisors. These findings suggest that the TGF-β super family signaling through Smad proteins is involved in the regulation of incisor formation not only in embryonic but also in adult stage.

P-8

Lineage analysis of nestin positive cells in periodontal tissue

Tomoaki Iwayama[1,2], Tsugumi Ueda[2], Satoshi Wakisaka[1], Shinya Muarkami[2] (1Department of Oral Anatomy and developmental biology, 2Department of Periodontology, Graduate School of Dentistry, Osaka University)

Recent evidence indicates that each adult tissue has both quiescent and active stem cell subpopulations. In periodontal tissue, periodontal ligament (PDL) plays an important role to maintain tissue homeostasis, and contains a mesenchymal stem cell (MSC) /progenitor population. Previously we have found that isolated nestin$^+$ cells are an MSC/progenitor population *in vitro*, and nestin$^+$ cells in 6 to 8-week-old PDL are pericytes adjacent to capillaries. In this study, we utilized various age of Nes-GFP; Nes-Cre; R26-tdTomato double or triple transgenic mice to identify localization of nestin$^+$ cells and their progenies in developing PDL of the first maxillary molar as well as aged PDL up to 1-year-old. At postnatal day 3, GFP$^+$ cells were found in the outer enamel epithelium and odontoblast layer. In dental follicle and dental papilla, similar to their localization in adult PDL, they were perivascular cells. Lineage analysis of this age showed an expression pattern of tdTomato had some discrepancy with that of GFP, and were mainly vascular related cells. At postnatal day 24, localization of GFP$^+$ and tdTomato$^+$ cells were largely overlapped, and their cellular bodies were wrapped by vascular basement membrane, suggesting they became a pericyte population even before completion of root formation. Histological analysis of 6-month-old and a-year-old Nes-Cre; R26-tdTomato mice showed tdTomato$^+$ cells kept their cell type as pericytes. These data suggest that Nes-Cre lineage cells and Nes-GFP cells would not contribute to the development of PDL, and these cells stay perivascular area as quiescent cells in adult PDL.

P-9

The role of *Runx1* in salivary gland development

Hitomi Ono[1], Sarper Safiye[2], Hiroshi Kurosaka[2], Takayoshi Sakai[1], Takashi Yamashiro[2]

([1]Dept of Oral-facial Disorders, [2]Dept of Orthodontics and Dentofacial Orthopedics, Osaka Univ)

The physiological roles of sexual hormones and their influence on organ morphogenetic differences have not yet been elucidated in detail. Salivary glands have important functions in maintaining oral health. Sex hormones have been suggested to play a role in salivation; however, the underlying molecular mechanisms currently remain unclear. Among the components of the *Cbfb* complex, mouse genetic studies revealed that *Runx1* is essential for hematopoiesis. Here we address dominant roles of *Runx1* in androgen-dependent sexual dimorphism in the salivary glands. By using *Runx1* conditional knockout (cKO) mice, we herein demonstrate that epithelial *Runx1* genes are involved in the postnatal development of sexual dimorphism. And, circulating testosterone levels were not affected in the cKO mice, indicating that the duct phenotypes could be direct effect of epithelial *Runx1* deficiently. Such salivary gland phenotypes are not evident in the female cKO salivary gland or in prepubertal cKO salivary gland at P17. On the other hand, saliva flow rates were decreased in the cKO male mice. In the cKO male salivary gland, expression of Aquaporin 5 (AQP5), the main water channel protein, were deviated in immunohistostaining. Aberrant shape of AQP5-immunoreactive protein were abundantly stuck in the cytoplasm of the cKO acinus endoplasmic reticulum thus *Runx1-Cbfb* signaling showed various phenotypes in salivary glands and it involved in the secondary sex characteristic of the sexual dimorphism in salivary gland.