= 口腔顎顔面領域の発生と再生 =

Dental and Craniofacial Morphogenesis and Tissue Regeneration

第2回　国際シンポジウム

主催：九州大学大学院　歯学研究院
「魅力ある大学院教育イニシアティブ」

共催：九州大学大学院歯学研究院リサーチプロジェクト
「口腔組織の再生・再建医療研究」
九州大学歯学研究院リサーチコア
「歯科再生医療の総合的開発」

日時：平成19年3月15日（木曜日）
10:00～17:30

場所：九州大学　医療系キャンパス内
百年記念講堂（中ホール）

Kyushu Univ. Dental Science

２００７年

「魅力ある大学院教育イニシアティブ」

第２回　国際シンポジウム

= 口腔顎顔面領域の発生と再生 =

Dental and Craniofacial Morphogenesis and Tissue Regeneration

日時　：　平成１９年３月１５日（木曜日）

１０：００～１７：３０

場所　：　九州大学　医療系キャンパス内

百年記念講堂（中ホール）

Program on March 15th, 2007

Welcome Speech

Prof. Akifumi AKAMINE, Dean, Faculty of Dental Science, Kyushu University

Special Lecture

Session 1: (Chair: Prof. Kazuaki NONAKA)

10:00-10:40

1. TGF-beta mediated Msx2 expression controls occipital somites-derived caudal region of skull development.

Yang CHAI

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

10:40-11:20

2. Differentially expressed genes detected by cDNA subtraction are implicated in mouse molar tooth germ development.

Tamotsu KIYOSHIMA

Laboratory of Oral Pathology and Medicine, Faculty of Dental Science, Kyushu University

11:20-12:00

3. Molecular Regulation of Tooth Development and Regeneration.

Irma THESLEFF

Institute of Biotechnology, University of Helsinki, Finland

Session 2: (Chair: Prof. Toshio KUKITA)

13:30-14:10

4. Mesenchymal Stem Cells: Disease and Cure.

Songtao SHI

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

14;10-14:50

5. Molecules implicated in the fate determination of injured neurons.

Hiroshi KIYAMA

Department of Anatomy & Neurobiology, Grad. Sch. of Med., Osaka City University, Japan

14:50-15:00

Break

Oral Presentation by Graduate School Students

Session 1: (Chair: H. Maeda, Y. Aida)

15:00-15:45

1. Investigating a clonal human periodontal ligament progenitor/stem cell line *in vitro* and *in vivo*.

○S. Fujii, H. Maeda, N. Wada, A.Tomokiyo and A. Akamine

Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University

2. Development of a multipotent clonal human periodontal ligament cell line.

○A. Tomokiyo[1], H. Maeda[1], S. Fujii[1], N. Wada[2], K. Shima[1], and A. Akamine[1]

[1]Department of Endodontology and Operative Dentistry, Division of Oral Rehabilitation,

[2]Division of General Oral Care, Faculty of Dental Science, Kyushu University

3. Intermittent Force Induces High RANKL Expression in Human Periodontal Ligament Cells.

○K. Nakao[1], T. Goto[2*], K.K. Gunjigake[1], T. Konoo[1], S. Kobayashi[2], and K. Yamaguchi[1]

[1]Division of Orofacial Functions and Orthodontics, and [2]Division of Anatomy, Kyushu Dental College

Session 2: (Chair: T. Fukumoto, I. Kobayashi)

15:45-16:30

4. Possible involvement of cytokines, chemokines, and chemokine receptors in the initiation and progression of Sjögren's syndrome.

○M. Moriyama, J. Hayashida, K. Hamanaka, S. Shinozaki, S. Nakamura

Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University

5. Adaptor protein SH2-B linking receptor-tyrosine kinase and Akt promotes adipocyte differentiation by regulating PPARγ mRNA levels.

○Daigo Yoshiga, Seiji Nakamura, Akihiko Yoshimura

Division of Oral and Maxillofacial Oncology Faculty of Dental Science,

Division of Molecular and Cellular Immunology, Medical Institute of Bioregulation

Kyushu University, Fukuoka, Japan Oral Presentation by Graduate School Students

6. Functional analysis of novel gene, *Clone 15* in the tooth morphogenesis.

○K. Fukiwake[1,2], T. Kiyoshima[1], I. Kobayashi[1], M. Xie[1], H. Sakai[1]

[1]Department of Oral Pathology, Faculty of Dental Science, Kyushu University, [2]Department of Fixed Prosthodontics, Faculty of Dental Science, Kyushu University

Session 3: (Chair: Y.Ayukawa, M.A. Kido)

16:30-17:15

7. CCN3/NOV inhibits BMP-2-induced osteoblast differentiation by interacting with BMP and Notch signaling pathways.

○T. Minamizato[1,2], Kei Sakamoto[1], Seiji Nakamura[2], A. Yamaguchi[1]

[1]Section of Oral Pathology, Graduate School of Tokyo Medical and Dental University.

[2]Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Graduate School of Dental Science, Kyushu University

8. Intra-arterial injected microglia engraft in the brain to rescue neuronal damage

○ Y. Hayashi[1], J. Yamada[1], Z. Wu[1], M. Sawada[2], and H. Nakanishi[1].

[1]Laboratory of Oral Aging Science, Faculty of Dental Science, Kyushu University,[2]Department of Brain Science, RIEM, Nagoya University

9. Presynaptic Inhibition of Synaptic Inputs Precedes Synaptic Stripping in Vagal Motoneurons after Axotomy.

○J. Yamada, Y. Hayashi, and H. Nakanishi

Laboratory of Oral Aging Science, Faculty of Dental Science, Kyushu University, Japan

ABSTRACTS

Special Lecture 1 (10:00-10:40)

TGF-beta mediated Msx2 expression controls occipital somites-derived caudal region of skull development.

Yang CHAI

Center for Craniofacial Molecular Biology, School of Dentistry, University of Southern California, CA, U.S.A.

Craniofacial development involves cranial neural crest (CNC) and mesoderm-derived cells. TGF-b signaling plays a critical role in instructing CNC cells to form the craniofacial skeleton. However, it is not known how TGF-b signaling regulates the fate of mesoderm-derived cells during craniofacial development. In this study, we show that occipital somites contribute to the caudal region of mammalian skull development. Conditional inactivation of Tgfbr2 in mesoderm-derived cells results in defects of the supraoccipital bone with meningoencephalocele and discontinuity of the neural arch of the C1 vertebra. At the cellular level, loss of TGF-b signaling causes decreased chondrocyte proliferation and premature differentiation of cartilage to bone. Expression of Msx2, a critical factor in the formation of the dorsoventral axis, is diminished in the Tgfbr2 mutant. Significantly, overexpression of Msx2 in Myf5-Cre;Tgfbr2flox/flox mice partially rescues supraoccipital bone development. These results suggest that the TGF-b/Msx2 signaling cascade is critical for development of the caudal region of the skull.

Special lecture 2 (10:40-11:20)

Differentially expressed genes detected by cDNA subtraction are implicated in mouse molar tooth germ development.

Tamotsu KIYOSHIMA
Laboratory of Oral Pathology and Medicine, Faculty of Dental Science, Kyushu University

Mammalian tooth development is mediated by the sequential and reciprocal epithelial-mesenchymal interactions of various molecules like other organs. In order to screen the potential genes involved in the initiation of mouse odontogenesis, we previously made cDNA subtracted libraries between mouse embryonic day (E) 10.5 and E12.0 mandibles, and found several genes to be differentially expressed in the early development stage of the mandible. We have already reported that the expressions of several genes among them were associated with the developing tooth germ. In this study, we picked up three genes, nucleolin, thymosin beta 4 (Tβ4), and a novel gene, from the libraries, and possible functions of these genes in the development of tooth germ were analyzed. Nucleolin and the novel gene mRNAs highly expressed in the E10.5 mandible, whereas Tβ4 mRNA expression was stronger in E12.0 mandible. Both nucleloin and Tβ4 expressions were seen in the odontogenic epithelial cells in the early stage, and in the inner enamel epithelial layer in the late stage. An inhibition assay using antisense phosphorothioated oligonucleotides (AS S-ODN) for each nucleloin and Tβ4 showed a marked inhibition of odontogenesis in cultured mandibles at E11.0 and/or tooth germs at E15.0. However, the histological findings after AS S-ODN showed different patterns depending on the target. A significant reduction in mRNA expressions of some genes related to them was also observed in the organ cultures after AS S-ODN. These findings suggest that nucleolin and Tβ4 play roles in the initiation, growth and differentiation of tooth germ or odontogenesis-associated genes expression. Similarly a novel gene that we tentatively called as Clone 15 has been characterized and its expression during odontogenesis in mouse embryo was examined.

Special lecture 3 (11:20-12:00)

Molecular Regulation of Tooth Development and Regeneration.

Irma THESLEFF
Institute of Biotechnology, University of Helsinki, Finland

The communication between cells and tissues is a major mechanism in the regulation of embryonic development. In most organs, including the teeth, interactions between the epithelial and mesenchymal tissues are central regulators of morphogenesis. The cell and tissue interactions are mediated by secreted signaling molecules belonging to a few conserved families, and they regulate all aspects of development including growth, epithelial morphogenesis, cell differentiation and deposition of extracellular matrices. The modulation of signal activities influences the numbers, shape and size of teeth as well as the formation of the dental hard tissues, and it is believed that the evolution of the patterns of dentitions and tooth morphologies has largely resulted from the fine-tuning of signal pathways.

The number of teeth and their replacement patterns show remarkable variation in vertebrates. In non-mammalian vertebrates, such as fish and reptiles, continuous renewal of teeth is common. In most mammals tooth replacement has been reduced to the formation of only two dentitions or reduced altogether as in mice. The continuous replacement of hairs and feathers has been linked to Wnt signalling. We have shown that when Wnt signalling is activated in transgenic mice by expressing stabilized beta-catenin in the oral epithelium, the process of continuous tooth renewal is activated.

Most mammalian teeth do not grow throughout life. However, rodent incisors are characterized by the ability to grow continuously throughout the life of the animal. These teeth have a stem cell niche in their proximal ends, and our recent results indicate that the growth of mouse incisors can be dramatically altered by modulating the activities of conserved signal pathways in this niche. We showed that a complex network involving BMPs, FGFs, Activin and Follistatin regulates the proliferation as well as differentiation of epithelial stem cells in mouse incisors, and that fine-tuning of their activities may affect both growth and enamel production in the incisors

Special lecture 4 (13:30-14:10)

Mesenchymal Stem Cells: Disease and Cure.

Songtao SHI
Center for Craniofacial Molecular Biology, School of Dentistry, University of Southern California, CA, U.S.A.

Mesenchymal stem cells (MSCs) have been identified as a population of organized hierarchical postnatal stem cells with the potential to differentiate into multiple cell types. These cells were initially isolated from bone marrow, named bone marrow mesenchymal stem cells (BMMSCs), and identified by their capacity to form clonogenic adherent cell clusters with a fibroblastic morphology (CFU-F: colony-forming units-fibroblast) in vitro. Although BMMSCs are capable of multi-differentiation, it appears that they are most readily encouraged to develop towards an osteogenic pathway. When transplanted into immunocompromised mice subcutaneously, BMMSCs are capable of forming a bone/marrow organ-like structure. One of the most striking characteristics of the xenogeneic transplantation system is that BMMSCs can organize and support a hematopoietic marrow during the osteogenic process. This may represent a dynamic process whereby multipotential BMMSCs strive to re-construct the microenvironment from which they were derived. Here we demonstrated how genetic deficient of caspase-3 and beta2 integrin contributes to BMMSC-associated osteoporosis phenotype. Moreover, we show that BMMSCs possess organizing function to construct a bone/marrow organ-like system (BMOS) that contains hematopoietic progenitors of recipient origin. These hematopoietic cells expressed multiple lineages of hematopoietic cell associated markers and were able to rescue lethally irradiated mice with successful engraftment in the recipient. Importantly, BMMSC-generated BMOS could be re-transplanted to secondary recipients to provide hematopoietic cells in the circulation, suggesting a great potential of utilizing this BMOS system for stem cell therapies. In order to further explore potential of using BMMSCs for clinical therapies, we generated an mandible osteoradionecrosis (ORN) model in swine (miniature pig) and employed autologous BMMSC transplantation to cure the ORN, suggesting a novel therapeutic approach to recover ORN defects by BMMSC-generated new bone and BMMSC-organized new homeostasis microenvironment. Furthermore, we used autologous transplantation of periodontal ligament stem cells (PDLSCs), a newly identified orofacial mesenchymal stem cell population, to treat

periodontitis in swine. PDLSCs are capable of regenerating cementum and Sharpey's fibers to cure periodontitis. In summary, mesenchymal stem cells may involve in many human diseases and they are a promising stem cell population for clinical therapies.

Special lecture 5 (14:10-14:50)

Molecules implicated in the fate determination of injured neurons.

Hiroshi KIYAMA
Department of Anatomy & Neurobiology, Grad. Sch. of Med., Osaka City University, Japan

CNS neurons are susceptible to nerve injury, whereas PNS neurons are relatively resistant to injury. This fate difference between CNS and PNS could be partly due to the differential expressions of molecules in response to nerve injury. Therefore the identification of molecules in response to nerve injury using PNS injury model is relevant. In order to identify therapeutic strategies for CNS injury, systematic screening of the transcriptome and proteome is an effective strategy. We have previously performed screening assays such as differential display and expressed-sequence-tag (EST) analysis, using the cDNA library from nerve-injured motor nuclei, as well as proteomic analysis. In those studies, we identified several molecules that are induced in response to peripheral nerve injury. Amongst those molecules I would like to introduce two molecules whose expression pattern and function in nerve regeneration are particularly intriguing. One is the Damage-Induced Neuronal Endopeptidase (DINE), which has been identified as a zinc metalloprotease, and as belonging to the neprilysin family[1]. Although substrates for this protease are unknown, DINE has unique features in its gene expression regulation. The expression of DINE is neuron specific and highly associated with nerve injury of both CNS and PNS neurons [2]. An analysis of the transcription mechanism therefore would provide us with a tool for gene therapy. Another molecule is a glutamate transporter EAAC1 (or EAAT3). Recently we have identified that EAAC1 has a unique action in preventing mitochondria-mediated neuronal death. This “rescue” activity does not depend on glutamate removal, but requires an interaction with a mitochondrial protein, holocytochrome-c synthetase (HCCS), which is released from the mitochondria and toxic in cytoplasm [3]. This novel mechanism actually prevents the death of the injured motor neurons of animals, whereas the failure of the expression of EAAC1 leads to slow degeneration of motor neurons. The significance of those molecules in the fate determination of injured neurons would

be discussed.

1. Kiryu-Seo S, et al, Damage induced neuronal endopeptidase (DINE) is a unique metallopeptidase expressed in response to neuronal damage and activates superoxide scavengers. ***Proc. Natl. Acad. Sci. USA*** 97(2000) 4345-4350.
2. Kato R, et al, Damage induced neuronal endopeptidase (DINE/ECEL) expression is regulated by LIF and deprivation of NGF in rat sensory ganglia after nerve injury. ***J Neurosci*** 22(2002) 9410-9418
3. Kiryu-Seo S, et al, Unique anti-apoptotic activity of EAAC1 in injured motor neurons, ***EMBO J*** 25(2006) 3411-3421

Oral presentation by Graduate School Students (15:00-17-15)

1. Investigating a clonal human periodontal ligament progenitor/stem cell line *in vitro* and *in vivo*.

○S. Fujii, H. Maeda, N. Wada, A.Tomokiyo and A. Akamine. Kyushu University, Faculty of Dental Science, Division of Oral Rehabilitation, Fukuoka, Japan

Objectives: The periodontal ligament (PDL) is a highly specialized tissue connecting the cementum with the tooth socket bone, and affects the life span of tooth. However, little is known about the precise characteristics and regenerative mechanism of human PDL, because of the absence of cell lines and specific makers, and the fact that the PDL tissue consists of heterogeneous cell populations. Therefore, to clarify the regenerative mechanism of human PDL, we aimed to establish and characterize clonal human PDL cell lines.

Methods: Normal human PDL fibroblasts (HPLF) were obtained from a healthy first premolar of one volunteer. HPLF were transfected with *SV40T-Ag gene* and *hTERT gene*, named as STPLF. The expression of SV40T-Ag and hTERT in STPLF was ascertained by RT-PCR analysis and Western blotting analysis. Twenty clonal human PDL cell lines were obtained from a limiting dilution of STPLF. Three of them, lines 1-4, 1-11, and 1-24, which were used in this study, were examined on the expression of STRO-1 and CD146 immunocytochemically, and the gene expression by RT-PCR technique. Moreover, line 1-11 was examined on the potential to differentiate into osteoblastic or adipocytic cells in vitro, and to construct PDL-like tissues in SCID mice.

Results: STPLF obviously expressed SV40T-Ag and hTERT, and showed stable proliferation at more than 120 population doublings (PD), while HPLF stopped at 20 PD. Lines1-4, 1-11, and 1-24 expressed *RUNX2, Col I, ALP, OPN, OCN, RANKL, OPG, Scx, Periostin, Col XII,* and *α-SMA* mRNA, comparable to HPLF. Immunocytochemical data showed that CD146 was expressed in lines 1-4 and 1-11, and STRO-1 was expressed in lines 1-11 and 1-24. Lines 1-4 and 1-11 cultured in lineage-specific differentiation media differentiated into osteoblastic cells and adipocytic cell. When line 1-11 was transplanted into SCID mice with β-TCP for eight weeks, the transplant formed PDL-like tissues on the surface of β-TCP.

Conclusion: These results suggest that the clonal human PDL cell line 1-11 was derived from a progenitor/stem cell present in PDL. This clone should be a very helpful tool for studying the regeneration mechanism of human PDL and for developing new therapies for periodontitis.

2. Development of a multipotent clonal human periodontal ligament cell line.

○Atsushi Tomokiyo[1], Hidefumi Maeda[1], Shinsuke Fujii[1], Naohisa Wada[2], Kazuya Shima[1], and Akifumi Akamine[1]

[1]Department of Endodontology and Operative Dentistry, Division of Oral Rehabilitation, [2]Division of General Oral Care, Faculty of Dental Science, Kyushu University

Objectives: The development of new PDL-regenerative therapies requires the isolation of PDL stem cells. However, their characteristics are unclear because of the fact that PDL is composed of complex cell populations and the absence of somatic PDL stem cell lines. Therefore, we aimed to establish a human somatic PDL stem cell line and investigate the effects of bFGF on its osteoblastic differentiation.

Methods: A clonal human PDL cell line, designated as line 1-17, was exposed to osteoblastic, neurocytic, chondrocytic, and adipocytic induction. To investigate the regulatory mechanism of osteoblastic differentiation of line 1-17 by bFGF, it was cultured in osteoblastic differentiation medium with bFGF in various treatment lengths.

Results: Line 1-17 differentiated into osteoblastic, neurocytic, chondrocytic, and adipocytic cells, which expressed embryonic stem cell (ESC)-related pluripotency genes *OCT3/4* and *Nanog*, as well as PDL-related moleclules periostin and *scleraxis*. Continual bFGF treatment of line 1-17 in osteoblastic induction medium inhibited its calcification, whereas the delayed addition of bFGF potentiated its calcification and *ALP* and *OCN* mRNA expression. Furthermore, the addition of bFGF to a co-culture of line 1-17 with Saos2 induced calcification in line 1-17.

Discussion: Our present results suggest that line 1-17 is a PDL-committed stem cell line, that bFGF exerts biphasic (i.e., promotive and inhibitory) effects on osteoblastic differentiation of line 1-17 according to its differentiation stage, and that bFGF acts on osteoblasts to induce osteogenesis of line 1-17, possibly via osteo-inductive factors secreted by them.

3. Intermittent Force Induces High RANKL Expression in Human Periodontal Ligament Cells.

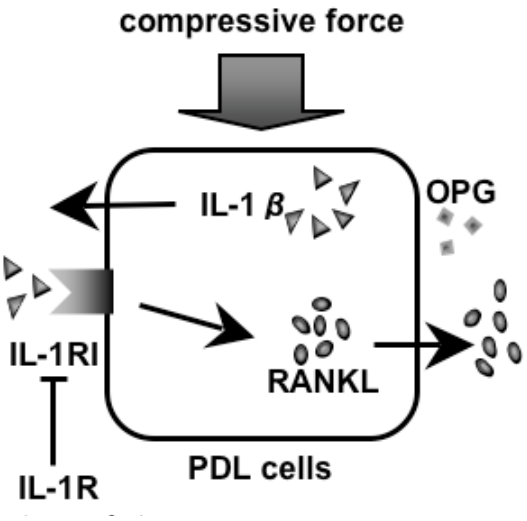
○K. Nakao[1], T. Goto[2*], K.K. Gunjigake[1], T. Konoo[1], S. Kobayashi[2], and K. Yamaguchi[1]

[1]Division of Orofacial Functions and Orthodontics, and [2]Division of Anatomy, Kyushu Dental College

In clinical orthodontics, an orthodontic force is routinely applied for months. Bone remodeling occurs during orthodontic tooth movement, including both bone resorption on the compression side and bone formation on the tensile side. Regulation of bone regeneration during orthodontic treatment is crucial for the success of orthodontic treatment. While previous studies have focused mainly on the expression of bone-related genes, such as RANKL or RUNX2, little attention has been paid to the cell damage caused by mechanical stress. This study examined the molecular mechanism of the remodeling of human periodontal ligament (PDL) cells stimulated by an intermittent force that exceeded the expected value predicted by the duration of force application compared with a continuous force.

Human PDL cells were isolated from premolars extracted for orthodontic reasons. The PDL cells were subjected to a compressive force (2.0 or 5.0 g/cm^2) for 2-4 days by increasing the amount of medium. A continuous force was applied all day or an intermittent force for 8 h per day. Total RNA was extracted from PDL cells after 2, 3, and 4 days, and we examined RANKL, OPG, and IL-1β mRNA expression using semi-quantitative RT-PCR. Cell damage was assessed by measuring the activity of lactic dehydrogenase (LDH) released from the cells.

At days 3 and 4, there was less cell damage with the intermittent force than with the continuous force. Compressed PDL cells expressed less OPG mRNA, in a force- and time-dependent manner. At day 4, the expression of RANKL and IL-1β was greater with the intermittent force. An IL-1 receptor antagonist inhibited the compressive force-induced



RANKL expression.

Based on these findings, we propose the following mechanism to explain how a compressive force induces osteoclastogenesis in PDL cells. An intermittent force promotes IL-1β expression; the released IL-1β binds to the IL-1β receptor, and this signal enhances RANKL expression, inducing osteoclastogenesis. Consequently, a weak intermittent force effectively induces expression of RANKL via IL-1β expression in human PDL cells, resulting in less overall damage.

4. **Possible involvement of cytokines, chemokines, and chemokine receptors in the initiation and progression of Sjögren's syndrome.**

○Masafumi Moriyama, Jun-nosuke Hayashida, Keiko Hamanaka, Shouichi Shinozaki
Seiji Nakamura
Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University

Objective: Sjögren's syndrome (SS) is an autoimmune disease characterized by lymphocytic infiltration into the salivary and lacrimal glands with concomitant destruction of the glandular tissue. Although the etiology and pathogenesis of SS is not fully understood, an involvement of cytokines, chemokines, and chemokine receptors in the pathogenesis of SS has been suggested. In this study, we thus addressed to clarify the involvement of that in the initiation and progression of SS

Methods: Expression of these molecules in the labial salivary glands (LSG) and peripheral blood mononuclear cells (PBMC) from 47 SS patients and 13 healthy volunteers was examined by real-time polymerase chain reaction-based method and immunohistochemical analysis.

Results: The mRNA expression of T helper 1 (Th1) and Th2 cytokines, and several chemokines and chemokine receptors in the LSG from SS patients was significantly increased in comparison with that in the PBMC from SS patients, and also with that in the LSG and PBMC from the healthy volunteers. The mRNA expression of Th2 cytokines, macrophage-derived chemokine (MDC), thymus-and activation-regulated chemokine (TARC), and CC chemokine receptor 4 (CCR4) in the LSG from SS patients was closely associated with a strong T cell infiltration in the LSG. Furthermore, MDC and TARC were immunohistochemically detected in or around ductal epithelial cells in the LSG, while CCR4 was on the infiltrating lymphocytes in the LSG.

Conclusions: These results suggest that SS might be initiated and/or maintained by

Th1/Th0 cells and thereafter progresses in association with Th2 cells accumulation via the interaction of particular chemokines and chemokine receptors.

5. Adaptor protein SH2-B linking receptor-tyrosine kinase and Akt promotes adipocyte differentiation by regulating PPARγ mRNA levels.

○Daigo Yoshiga, Seiji Nakamura, Akihiko Yoshimura

Division of Oral and Maxillofacial Oncology, Faculty of Dental Science, Kyushu University

Division of Molecular and Cellular Immunology, Medical Institute of Bioregulation, Kyushu University

Adipocyte differentiation is regulated by insulin and IGF-I, which transmit signals by activating their receptor tyrosine kinase. SH2-B is an adaptor protein containing pleckstrin homology (PH) and Src homology 2 (SH2) domains that have been implicated in insulin and IGF-I receptor signaling. In this study, we found a strong link between SH2-B levels and adipogenesis. The fat mass and expression of adipogenic genes including peroxisome proliferator-activated receptor g (PPARg) were reduced in white adipose tissue of SH2-B$^{-/-}$ mice. Reduced adipocyte differentiation of SH2-B-deficient mouse embryonic fibroblasts (MEFs) was observed in response to insulin and dexamethasone, while retroviral SH2-B overexpression enhanced differentiation of 3T3-L1 preadipocytes to adipocytes. SH2-B overexpression enhanced mRNA level of PPARg in 3T3-L1 cells, while PPARg levels were reduced in SH2-B deficient MEFs in response to insulin. SH2-B-mediated upregulation of PPARg mRNA was blocked by a PI3-kinase inhibitor, but not by a MEK inhibitor. Insulin-induced Akt activation and the phosphorylation of forkhead transcription factor (FKHR/Foxo1), a negative regulator of PPARg transcription, were upregulated by SH2-B overexpression, but reduced in SH2-B-deficeint MEFs. These data indicate that SH2-B is a key regulator of adipogenesis both *in vivo* and *in vitro* by regulating the insulin / IGF-I - receptor - Akt - Foxo1 - PPARg pathway.

6. Functional analysis of novel gene, *Clone 15* in the tooth morphogenesis.

○Keiko Fukiwake[1,2], Tamotsu Kiyoshima[1], Ieyoshi Kobayashi[1], Ming Xie[1], Hidetaka Sakai [1],

[1]Laboratory of Oral Pathology and Medicine, Faculty of Dental Science, Kyushu University

[2]Department of Fixed Prosthodontics, Faculty of Dental Science, Kyushu University

Objective: We previously performed cDNA subtraction between the embryonic day 10.5 (E10.5) and E12.0 mouse mandibles to detect factors which are related to the early stage of tooth morphogenesis, and found differentially expressed genes in each mandible. The expressions of some genes were apparently associated with the developing tooth germ. *Clone 15* was a novel gene of them. The function of this gene was not yet been elucidated. The aim of this study is to identify and clarify the functional implication of this gene in the tooth morphogenesis.

Experimental procedures & Results: At first, we confirmed the sequence of this gene and performed structure analysis according with the deduced amino acid sequence. *Clone15* gene belongs to the immunoglobulin superfamily and showed significant homology to DEAL (DCC et al).

Next, we generated anti-Cl.15 polyclonal antibody, and tried to prove an existence of this protein. Immunoblotting revealed this protein to be seen in E10.5 mouse mandible, and highly glycosylated. Furthermore, we examined expression pattern of Cl.15 protein by immunohistochemistry, and compare with that of *Clone.15* mRNA by *in situ* hybridization in developing mouse tooth germ (first molar tooth). As a result, strong signals of both protein and mRNA were expressed at early stage of embryonic days, especially at E10.5. The expression of Clone15 protein and *Cl.15* mRNA in tooth germ was getting weakly day by day. While, in other organs, signals were seen in nervous systems during embryonic days, these expression patterns were very similar to DEAL.

Conclusion: *Clone 15* actually existed as a functional gene that encoded Clone 15 protein, and appeared at early stage of tooth morphogenesis. Therefore, *Clone15* may play important roles to guide a thickening of epidermis as a start point of tooth morphogenesis. Because *Clone 15* was also observed at nervous systems, it was

suggested that *Clone 15* has some relation to functions of DEAL.

7. CCN3/NOV inhibits BMP-2-induced osteoblast differentiation by interacting with BMP and Notch signaling pathways.

○T. Minamizato[1,2], Kei Sakamoto[1], Seiji Nakamura[2], A. Yamaguchi[1]
[1]Section of Oral Pathology, Graduate School of Tokyo Medical and Dental University.
[2]Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Graduate School of Dental Science, Kyushu University

We elucidate the role of NOV/CCN3, a member of the CCN family proteins, in osteoblast differentiation using MC3T3-E1 osteoblastic cells. Transduction with *CCN3* adenovirus (*AdCCN3*) alone induced no apparent changes in the expression of osteoblast-related markers, whereas cotransduction with *BMP-2* adenovirus (*AdBMP-2*) and *AdCCN3* significantly inhibited the *AdBMP-2*-induced mRNA expression of *Runx2*, *osterix*, *ALP,* and *osteocalcin*. Immunoprecipitation-western analysis revealed that CCN3 associated with BMP-2. Compared to transduction with *AdBMP-2* alone, cotransduction with *AdBMP-2* and *AdCCN3* attenuated the expression of phosphorylated Smad1/5/8 and the mRNA for *Id1*, *Id2*, and *Id3*. Transduction with *AdCCN3* stimulated the expression of cleaved Notch1, the mRNA expression of *Hes1* and *Hey1*/*Hesr1*, and the promoter activities of *Hes1* and *Hey1*. The inhibitory effects of CCN3 on the expression of BMP-2-induced osteoblast-related markers were nullified in *Hey1*-deficient osteoblastic cells. These results indicate that CCN3 exerts inhibitory effects on BMP-2-induced osteoblast differentiation by its involvement of the BMP and Notch signaling pathways.

8. Intra-arterial injected microglia engraft in the brain to rescue neuronal damage.

○ Yoshinori Hayashi[1], Jun Yamada[1], Zhou Wu[1], Makoto Sawada[2], and Hiroshi Nakanishi[1]

[1]Laboratory of Oral Aging Science, Faculty of Dental Science, Kyushu University, Japan
[2]Department of Brain Science, RIEM, Nagoya University, Japan

There is accumulating evidence that transplanted bone marrow cells recruit to the brain parenchyma as microglia to ameriolate neuronal damage. In the present study, we have thus attempted to elucidate the effects of the intra-arterial injection of microglia on the global ischemia-induced functional and morphological deficits of hippocampal CA1 neurons. When PKH26-labeled immortalized microglial cells, GMIR1, were injected into the subclavian artery, these exogenous microglia were found to accumulate in the hippocampus at 24 h after ischemia. In hippocampal slices prepared from medium-injected rats subjected to ischemia 48 h earlier, synaptic dysfunctions including a significant reduction of synaptic responses and a marked reduction of long-term potentiation (LTP) of the CA3–CA1 Schaffer collateral synapses were observed. At this stage, however, neither significant neuronal degeneration nor gliosis was observed in the hippocampus. At 96 h after ischemia, there was a total loss of the synaptic activity and a marked neuronal death in the CA1 subfield. In contrast, the basal synaptic transmission and LTP of the CA3–CA1 synapses were well preserved after ischemia in the slices prepared from the microglia-injected animals. We also found the microglial-conditioned medium (MCM) to significantly increase the frequency of the spontaneous postsynaptic currents of CA1 neurons without affecting the amplitude, thus indicating that MCM increased the provability of the neurotransmitter release. The protective effect of the intra-arterial injected microglia against the ischemia-induced neuronal degeneration in the hippocampus was substantiated by immunohistochemical and immunoblot analyses. Furthermore, the arterial-injected microglia prevented the ischemia-induced decline of the brain-derived neurotrophic factor (BDNF) levels in CA1 neurons. These observations strongly suggest that the intra-arterial injected microglia engraft in the brain to rescue neuronal damage.

9. Presynaptic Inhibition of Synaptic Inputs Precedes Synaptic Stripping in Vagal Motoneurons after Axotomy.

○Jun Yamada, Yoshinori Hayashi, and Hiroshi Nakanishi

Laboratory of Oral Aging Science, Faculty of Dental Science, Kyushu University, Japan

Microglia are representatives of the resident mononuclear phagocyte population within the central nervous system. They secrete soluble factors including neurotrophins to support neuronal survival and functions. There is accumulating evidence that activated microglia also protect neurons by physically removing synaptic inputs. Following an axotomy, microglia are rapidly activated and then transform into a deramified form, and they thereafter proliferate, adhere to regenerating motoneurons, and spread on their surface. This phenomenon is now known as “synapic stripping”. It is believed that activated microglia spread on the motoneurons engage in the displacement of detached afferent synaptic boutons from the surface of a regenerating neuron. It is widely believed that synaptic stripping plays an important role in neuronal survival and axonal regeneration of injured motoneurons. However, little information is available for the detailed pathological significance of synaptic stripping. In the present study, we have first attempted to examine whether changes in synaptic inputs after an axotomy are correlated with synaptic stripping in the dorsal motoneurons of vagas (DMV) of rats. At 2 days after an axotomy, there was a significant decrease in frequencies of both spontaneous EPSCs and IPSCs recorded from DMV without significant changes in their amplitudes. However, synaptic stripping was not evident at this stage. Furthermore, high-K^+ condition restored frequencies of both spontaneous EPSCs and IPSCs to the control levels. These results strongly suggest that presynaptic inhibition of synaptic inputs precedes synaptic stripping in DMV after an axotomy. Therefore, it is reasonable to consider that some soluble factors inhibit release of glutamate and/or GABA by the presynaptic mechanism. ATP was found to be one of potential candidates for such soluble factors because PPADS, a P2 receptor antagonist, recovered the frequency of spontaneous EPSCs recorded from the axotomized DMV to the control level.