The International Joint Symposium on "Dental and Craniofacial Morphogenesis and Tissue Regeneration" and "Oral Health Science"

国際合同シンポジウム:
「口腔組織の再生・再建医療研究」ならびに「口腔健康科学」
（文部科学省 特別教育研究経費:「口腔から QOL 向上を目指す連携研究」）

6 th, February, 2009
Centennial Hall, Kyushu University School of Medicine, Fukuoka, Japan

Program & Abstracts

▶会期: 平成 21年 2月 6日（金）

▶会場：九州大学医学部　百年講堂

〒812-8582 福岡市東区馬出 3-1-1

▶主催：九州大学大学院歯学研究院

「口腔組織の再生・再建医療」プロジェクト

「口腔健康科学」プロジェクト

文部科学省 特別教育研究経費

「口腔から QOL 向上を目指す連携研究」

連絡先：

中西　博（口腔組織の再生・再建医療プロジェクトリーダー）

二ノ宮裕三（口腔健康科学プロジェクトリーダー）

九州大学大学院歯学研究院口腔機能解析学分野

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Program

■ Opening remarks

10:00 - 10:10 Akifumi Akamine (*Dean, Faculty of Dental Science, Kyushu Univ.)*

■ Special Lectures (Dental and Craniofacial Morphogenesis and Tissue Regeneration)

Chairpersons: SL-1: Kazuaki Nonaka (*Section of Pediatric dentistry, Kyushu Univ.*)
SL-2: Hiroshi Nakanishi (*Laboratory of Oral Aging Science, Kyushu Univ.*)

SL-1. 10:10 - 11:10
Non-canonical TGF-b signaling during craniofacial morphogenesis
Yang Chai
Center for Craniofacial Molecular Biology, University of Southern California, Los Angeles, CA, USA

SL-2. 11:10 - 11:50
Glutamate signals in bone
Yukio Yoneda and Takeshi Takarada
Laboratory of Molecular Pharmacology, Kanazawa University Graduate School of Natural Science and Technology, Kanazawa, Ishikawa, Japan

▸ Lunch
11:50 - 13:00

■ Special Lectures (Oral Health Science)

Chairpersons: SL-3: Yuzo Ninomiya (*Section of Oral Neuroscience, Kyushu Univ.*)
SL-4: Seiji Nakamura (*Oral and Maxillofacial Oncology, Kyushu Univ.*)
SL-5: Yoshihisa Yamashita (*Department of Preventive Dentistry, Kyushu Univ.*)

SL-3. 13:00 - 14:00
Oral sensation and human health
Gary K. Beauchamp
Monell Chemical Senses Center, Philadelphia PA, USA

SL-4. 14:00 - 14:40
New Horizon in Oral Science: Driving Force for the Development of Mucosal Immunology
Hiroshi Kiyono
Divison of Mucosal Immunology, Dept. of Microbiology and Immunology,
The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

SL-5. 14:40 - 15:20
Prevention of vascular diseases for aging well
- Periodontal disease is a vascular disease -
Kenji Matsushita
Department of Oral Disease Research, National Institute for Longevity Sciences,
National Center for Geriatrics and Gerontology, Obu, Aichi, Japan

▸ Break
15:20 - 15:30

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■ Oral Session (Dental and Craniofacial Morphogenesis and Tissue Regeneration)

Chairpersons: OS-1 – 3: Mizuho A. Kido (*Oral Anatomy and Cell biology, Kyushu Univ.*)
OS-4 – 6: Ieyoshi Kobayashi (*Laboratory of Oral Pathology and Medicine, Kyushu Univ.*)

OS-1. 15:30 - 15:45
Increased tooth root resorption during orthodontic tooth movement in allergy model rats
Naohisa Murata[1,2], Hideki Ioi[1], Shunsuke Nakata[1] and Mizuho A. Kido[2]
Departments of [1]Orthodontics, [2]Oral Anatomy and Cell biology, Graduate School of Dental Science, Kyushu University, Fukuoka, Japan

OS-2. 15:45 - 16:00
Platelet-derived growth factors is essential for early tooth germ development
Wu Nan[1], Tsutomu Iwamoto[1], Shinya Yamamoto[3], Keigo Yoshizaki[1], Akira Sonoda[1], Kazuaki Nonaka[1] and Satoshi Fukumoto[2]
[1]Section of Pediatric dentistry, Division of Oral Health, Growth & Development Faculty of Dental Science, Kyushu University, Fukuoka, Japan, [2]Division of Pediatric Dentistry, Department of Oral Health and Development Sciences, Tohoku University Graduate School of Dentistry, Sendai, Japan, [3]Department of Pediatric Dentistry Field of Development, Medicine Course for Health Sciences, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

OS-3. 16:00 - 16:15
Functional implication of thymosin beta 4 on the morphogenesis of tooth germ in the mouse lower first molar
Yukiko Ookuma[1,2], Ieyoshi Kobayashi[1], Tamotsu Kiyoshima[1], Kenogo Nagata[1], Kazuaki Nonaka[2] and Hidetaka Sakai[1]
[1]Laboratory of Oral Pathology and Medicine, Division of Maxillofacial Diagnostic and Surgical Sciences,
[2]Section of Pediatric Dentistry, Division of Oral Health, Growth and Development, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

OS-4. 16:15 - 16:30
Wnt signaling and osteoblast differentiation
Etsuko Matsuzaki[1, 2], Fumi Takahashi-Yanaga[2], Mari Kobayashi[1], Shunji Hiratsuka[1], Toshiyuki Sasaguri[2] and Katsumasa Maeda[1]
[1]Periodontology Section, Division of Oral Rehabilitation, Faculty of Dental Sciences, [2]Department of Clinical Pharmacology, Faculty of Medical Sciences, Kyushu University, Fukuoka, Japan

OS-5. 16:30 - 16:45
Development of the novel bone augmentation technique
-topical application of statin-
Yohei Jinno, Yasunori Ayukawa, Yoichiro Ogino, Ikiru Atsuta and Kiyoshi Koyano
Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

OS-6. 16:45 - 17:00
Phosphatidylserine liposomes suppress inflammatory bone destruction by phenotypic switching of macrophages and T cells
Hong Mei Ma[1], Zhou Wu[1], Toshio Kukita[2], Katsumasa Maeda[3] and Hiroshi Nakanishi[1]
[1]Laboratory of Oral Aging Science, [2]Department of Hard Tissue Regeneration Control & Science,
[3]Department of Periodontology, Faculty of Dental Sciences, Kyushu University, Fukuoka, Japan

▸ Break
17:00～17:10

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■ Oral Session (Oral Health Science)

Chairpersons: OS-7 – 9: Tazuko K. Goto (*Department of Oral and Maxillofacial Radiology, Kyushu Univ.*)
OS-10 - : Yoshihisa Yamashita (*Department of Preventive Dentistry, Kyushu Univ.*)

OS-7. 17:10 - 17:25

Detecting the taste cortical region of human using fMRI

Yuko Nakamura[1], Tazuko K. Goto[1], Kenji Tokumori[1], Takashi Yoshiura[2], Koji Kobayashi[3], Yasuhiko Nakamura[3], Hiroshi Honda[2], Yuzo Ninomiya[4] and Kazunori Yoshiura[1]
[1]Department of Oral and Maxillofacial Radiology, Faculty of Dental Science, [2]Department of Clinical Radiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, [3]Department of Medical Technology, Kyushu University Hospital, Fukuoka, Japan, [4]Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University, Fukuoka, Japan

OS-8. 17:25 - 17:40

Identification of the interaction sites for anti-sweet substances; Gymnemic acid and Grumarin

Keisuke Sanematsu[1,2], Noriatsu Shigemura[1], Masashi Jotaki[1], Seiji Nakamura[2], Toshiaki Imoto[3] and Yuzo Ninomiya[1]
[1]Section of Oral Neuroscience, and [2]Oral and Maxillofacial Oncology, Graduate School of Dental Science, Kyushu University, Fukuoka, Japan, [3]Division of Integrative Physiology, Department of Functional, Morphological and Regulatory Science, Tottori University, Yonago, Japan

OS-9. 17:40 - 17:55

Globular adiponectin induced secretion of granulocyte colony-stimulating factor in RAW 264 cells

Noriaki Kamio[1], Sumio Akifusa[1], Noboru Ymaguchi[2], Kazuaki Nonaka[2] and Yoshihisa Yamashita[1]
[1]Department of Preventive Dentistry and [2]Department of Pediatric Dentistry, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

OS-10. 17:55 - 18:10

Relationship between Oral Microbial Flora and Symptoms in Patients with Dry Mouth

Shouichi Shinozaki, Jun-nosuke Hayashida, Masafumi Moriyama, Keiko Hamanaka, Akihiko Tanaka, Haruhisa Yamamoto, Sakae Minami and Seiji Nakamura
Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

■ Concluding remarks

18:10 - 18:30 Yang Chai (*University of Southern California, CA, USA*)
Gary K. Beauchamp (*Monell Chemical Senses Center, Philadelphia PA, USA*)

Informations

Information for Chairpersons

- The chairpersons are requested to be the venue by 15 minutes before your session begins.
- Please respect punctuality for the time allocated for presentations and questions/answers in order to ensure the smooth progression of the symposium.

Information for Speakers in Special Lectures

- Language： **English**
- SL-1 and SL-3: **50 minutes** are allocated for presentation and **10 minutes** for discussion.
- SL-2, SL-4 and SL-5: **35 minutes** are allocated for presentation and **5 minutes** for discussion.

Information for Speakers in Oral Sessions

- Language： **English**
- **12 minutes** are allocated for presentation and **3 minutes** for discussion.

Other details for Presentation

- Presentations must be done with your own PC.
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- If your presentation data contains stills, movies, graphs etc., please be sure to save them and confirm beforehand that they will play correctly.

Abstracts

SL-1.

Non-canonical TGF-b signaling during craniofacial morphogenesis

Yang Chai

Center for Craniofacial Molecular Biology, University of Southern California,
2250 Alcazar Atreet, CSA 103, Los Angeles, CA 90033, USA

TGF-b/BMP signaling plays a crucial role in an array of biological processes, including embryonic patterning, organogenesis, wound healing, and carcinogenesis. Previous studies have indicated that Smad4 serves as the central intracellular effector of TGF-b signaling. In this study, we found that p38 MAPK is specifically activated by TGF-b and functions as complementary effector in mediating Smad4-independent TGF-b signaling during craniofacial organogenesis. Unlike the Bmpr1a mutant, ablation of Smad4 in the dental epithelium does not block early tooth development and only causes defects in dental cusp patterning and root development. Inactivation of p38 MAPK signaling in the Smad4 mutant results in the arrest of tooth development at the bud stage, a phenotype identical to that of Bmpr1a mutant mice. Similarly, ablation of Smad4 in the palatal epithelium does not cause cleft palate, but blocking p38 MAPK and Smad4 together does, reproducing the phenotype of Tgfbr2 mutant mice. Thus, our study demonstrates that p38 MAPK mediated Smad4-indepenedent TGF-b signaling is a widely used mechanism in regulating embryonic organogenesis. The ability of epithelium to utilize both Smad4-dependent and independent pathways to mediate TGF-b signaling illustrates the complicated nature of the TGF-b signaling mechanism in development and disease.

Memo

SL-2.

Glutamate signals in bone

Yukio Yoneda and Takeshi Takarada

Laboratory of Molecular Pharmacology, Kanazawa University Graduate School of Natural Science and Technology

The view that L-glutamate (Glu) is an excitatory amino acid neurotransmitter in the mammalian central nervous system is prevailing on the basis of successful cloning of a number of genes encoding different signaling molecules, such as Glu receptors for signal input, Glu transporters for signal termination and vesicular Glu transporters for signal output through exocytotic release. Although little attention has been paid to an extracellular transmitter role of Glu in peripheral neuronal and non-neuronal tissues, however, we have for the first time demonstrated the presence of Glu receptors in peripheral tissues such as adrenal and pituitary glands in 1986. Subsequent molecular biological and pharmacological analyses including ours give rise to a novel function for Glu as an autocrine and/or paracrine signal mediator in bone comprised of osteoblasts, osteoclasts and osteocytes. Functional expression was found with all machineries required for glutamatergic signaling in osteoblasts, while osteoclasts were devoid of any glutamatergic signaling molecules except several transporters including the cystine/Glu antiporter. Daily intraperitoneal administration of Glu significantly prevented the loss of bone mineral density and trabecular bone in both tibia and femur of ovariectomized mice. Accordingly, Glu could play a dual role in the mechanisms underlying the maintenance of cellular homeostasis as an excitatory neurotransmitter in the CNS and as an extracellular signal mediator in peripheral autocrine and/or paracrine tissues. In particular, we would propose the possible signaling system for Glu to play a role as an extracellular signal mediator in mechanisms underlying maintenance of the cellular homeostasis in bone. An innovative and novel interdisciplinary field could be developed on “neuro-osteology” as a scientific bridge between bone and brain biology in the future.

Memo

SL-3.

Oral sensation and human health

Gary K. Beauchamp

Monell Chemical Senses Center
Philadelphia PA USA

Oral chemosensory systems (primarily taste and chemesthesis or oral chemical somatosensation) play a central role in human health. In this presentation I will first briefly describe these chemosensory systems and then provide a selected overview of a few of their roles. First and most important, these two senses are our nutrient gatekeepers, critical in determining whether a substance will be taken into the body or rejected. Thus they necessarily are involved in many of the diseases of excess such as diabetes, hypertension and obesity due to the role of taste in detecting and regulating intake of sugar, salt and perhaps fat. Disturbances of taste, particularly taste loss, which is relatively uncommon, can be devastating, putting individuals at considerable nutritional risk and making them extremely miserable. Much less work has been done on the health-related aspects of the chemesthetic system. It is generally assumed that oral irritants that engage this system arouse sensations of burn, tingly and pain in order to protect the oral cavity and subsequent stages of the digestive system from dangerous or toxic compounds. Yet, surprisingly, compounds that stimulate this system are found in common foods such as hot peppers, menthol products, wasabi, carbonated beverages and many others that humans find highly palatable. Recently we have identified an oral irritant (oleocanthal^{TM}) from extra virgin olive oil that elicits a novel throat-restricted pain that is considered a very positive sensory attribute. That this compound is also a potent anti-inflammatory with potential health benefits following long-term consumption may provide a clue as to why it is valued. Recent research identifying the mechanism by which oleocanthal elicits its burn and studies on the relation between chemical structure, burn and anti-inflammatory properties also illustrate the intimate connections between oral sensation and human health.

Memo

SL-4.

New Horizon in Oral Science:
Driving Force for the Development of Mucosal Immunology

Hiroshi Kiyono

Division of Mucosal Immunology, Department of Mucosal Immunology
The Institute of Medical Science, The University of Tokyo

Oral cavity is the entry of the mucosal immune system (MIS) which is continuously exposed to infinite antigenic challenges in handling its day-to-day duties. MIS is the first line of immunological defense against invasion of pathogens and hence induces a prompt and robust immune response in order to avoid invasion of infectious microorganisms. At same time, it also expose to an enormous number and volume of innocuous and/or instructive antigens which need to be appropriately 'ignored'. Mounting an immunologically harmonized response therefore represents a key decision-making process of active and/or quiescent responses for the host. The tissues covered by mucosal surfaces including oral and nasal cavities, aero-digestive tract, reproductive tract, middle year and eye represent a complex immunological network structured to execute the immunologically harmonized regulation of the two opposite immune responses. Although the area of mucosal immunology is now highly recognized as one of major streams in the area of medical and life science, it should be noted that oral science, for example the efforts provided in the development of caries vaccine in early 1970 triggered the initiation for the concept of the mucosal immune system. Since oral cavity is an immunologically unique organ consisting with both mucosal and systemic immune systems, the molecular and cellular understanding of systematic immune net work in oral cavity will contribute to open up new strategy for the control of infectious and immunological diseases.

Memo

SL-5.

Prevention of vascular diseases for aging well
- Periodontal disease is a vascular disease -

Kenji Matsushita, D.D.S. Ph.D.

Director, Department of Oral Disease Research, National Institute for Longevity Sciences, National Center for Geriatrics and Gerontology, Obu, Aichi, Japan

Many changes in the vasculature, hemostasis and endothelium occur during aging. It is important for aging well to prevent those changes. Nitric oxide (NO) is a second messenger with diverse roles in the cardiovascular system, such as maintaining continuous vasodilator tone and regulating local perfusion and systemic blood pressure. Impaired endothelial-dependent (NO mediated) vasorelaxation is observed in aging, atherosclerosis, and hypertension. NO is also inhibiting thrombosis and limiting vascular inflammation. One mechanism by which NO modulates such disparate physiological processes is by regulating protein trafficking within cells. NO inhibits exocytosis of endothelial granules which would otherwise trigger inflammation. NO also blocks platelet secretion of granules that would otherwise activate thrombosis. NO decreases granule trafficking from the Golgi to the plasma membrane by targeting a key component of the exocytic machinery, N-ethylmaleimide sensitive factor (NSF). NO regulation of vesicle trafficking is a molecular mechanism that explains some of the cardiovascular effects of NO, and may be of broad physiological significance. Excessive endothelial exocytosis may play a role in inflammatory and thrombotic disorders such as atherosclerosis, acute coronary syndromes, myocardial infarction, and deep vein thrombosis. Patients with defective NO synthesis—the hallmark of endothelial dysfunction—may be at increased risk for acquiring atherosclerosis and coronary events partly because less NO synthesis enables more exocytosis in response to vascular injury. Drugs directed at the exocytic machinery of endothelial cells may be useful in decreasing coagulation and vascular inflammation. Peptides that target NSF prolong bleeding time and decrease inflammation in mice. The exocytic machinery may be a novel drug target for vascular diseases.

Periodontal disease is a disorder that affects about 80% of Japanese adults. Although it is considered as an infectious disease, the importance of systemic factors and environmental factors, such as aging and lifestyle habits, in the etiology of periodontal disease has not been clarified. The increasing hypercoagulability observed with aging may account for the higher incidence of thrombotic cardiovascular disorders. Not only impairments of vasculature and gingival tissue by aging and accumulation of lifestyle habit but also evocation of vascular inflammation and blood coagulation are also involved in the etiology of periodontitis. In addition, gingipains, which are trypsin-like proteinases produced by *P. gingivalis,* activate several blood coagulation factors, augment exocytosis of endothelial granules, induce expressions of proinflammatory cytokines and matrix metalloproteases, and then evoke vascular inflammation and blood coagulation. The cooperative relationship among aging, lifestyle habits, and periodontopathic bacteria is important for considering periodontal disease as a vascular disease.

Memo

OS-1.

Increased tooth root resorption during orthodontic tooth movement in allergy model rats

[1,2]Naohisa Murata, [1]Hideki Ioi, [1]Shunsuke Nakata, [2]Mizuho A. Kido

Departments of [1]Orthodontics, [2]Oral Anatomy and Cell biology, Graduate School of Dental Science, Kyushu University.

In the course of orthodontic treatment, tooth root resorption represents an unwanted sequela. However, the onset and progression of root resorption mechanisms has not been elucidated. In addition to the factors related to orthodontic treatment (e.g. the duration of treatment or the magnitude of force applied), the patient-related factors (e.g. abnormalities of root morphology or systemic factors) are also considered to be a risk. Recently we demonstrated using an epidemiologic approach that orthodontic patients who suffered from an allergic disease such as asthma showed an increased incident rate of root resorption.

The aim of this study is to determine whether a systemic allergic disease has an adverse effect on orthodontic tooth resorption. We used Brown-Norway (BN) rats, which are known as high IgE producers after sensitization that develop late airway response after antigen challenges. Six week old male BN rats were actively sensitized by a single shot of ovalbumin and aluminium hydroxide followed by a second shot of ovalbumin after seven days. Seven days after the second sensitization, a stretched closed-coil spring as an orthodontic appliance was inserted between the maxillary left first molar and the incisors moving the molar mesially with a force of 0.5N. After 7 or 14 days with the orthodontic appliance, the animals were fixed and were processed for tartrate-resistant acid phospatase (TRAP) histochemistry. The control animal group was also applied with an orthodontic appliance received no antigen challenge. The pressure zone of the disto-palatal root of the first molar was analysed. The area of root resortpion lacuna was wide and deep and the number of odontoclasts and osteoclasts in the resorption lacuna was significantly elevated in the experimental group compared with that of the control group. Our results suggest that a systemic allergy is one of the risk factors for root resorption in orthodontic treatment.

OS-2.

Platelet-derived growth factors is essential for early tooth germ development

Wu Nan[1], Tsutomu Iwamoto[1], Shinya Yamamoto[3], Keigo Yoshizaki[1], Akira Sonoda[1], Kazuaki Nonaka[1], Satoshi Fukumoto[2]

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2. Division of Pediatric Dentistry, Department of Oral Health and Development Sciences, Tohoku University Graduate School of Dentistry
3. Department of Pediatric Dentistry Field of Development, Medicine Course for Health Sciences, Kagoshima University Graduate School of Medical and Dental Sciences

Tooth development is a complex process that results from a series of sequential and reciprocal interactions between epithelial and mesenchymal cells. Platelet-derived growth factor (PDGFs) are known as paracrine growth factors that mediate epithelial-mesenchymal interactions in organogenesis. In tooth development, PDGF receptor (PDGFR)-α signaling pathway has been found to be important for tooth cusp formation. However, the mechanism and function of PDGFs in odontogenesis remain unclear. We found that PDGF-A, PDGF-B, PDGFR α and PDGFR β mRNA were expressed from initial stage of tooth development by RT-PCR. Immunofluorescence also revealed PDGFs expression pattern. PDGF-A and PDGF-B were expressed in both dental epithelium and mesenchyme. PDGFR α was mainly expressed in dental mesenchyme. Although PDGFR β was also mainly expressed in dental mesenchyme, its expression was also observed in the enamel knot, which is a signaling center of the tooth that provides positional information for tooth morphogenesis and regulates the formation of tooth cusps. To analyze the function of PDGFs, we established tooth organ culture system using the lower first molar tooth germ from E14.5 ICR mouse embryo. Interestingly, in the presence of PDGF-A, 60% tooth germ became bigger than the control ones after 5 days culture. Further, PDGF-A accelerated cusp formation. On the other hand, treatment of AG17, a PDGF receptor tyrosine kinase inhibitor, suppressed the growth and cusp formation of tooth germ. AG17 also inhibited BrdU positive cells numbers in tooth germ. Thus, our results indicate that PDGFs are necessary for initial tooth development, and suggest that PDGF-A regulate tooth size and cusp formation through controlling cell proliferation.

Key word: PDGF, AG17, proliferation, tooth, mouse.

OS-3.

Functional implication of thymosin beta 4 on the morphogenesis of tooth germ in the mouse lower first molar

Yukiko Ookuma[1,2], Ieyoshi Kobayashi[1], Tamotsu Kiyoshima[1], Kenogo Nagata[1], Kazuaki Nonaka[2] and Hidetaka Sakai[1*]

1. *Laboratory of Oral Pathology and Medicine, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan*
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** Research supervisor.*

Tooth development is mediated through sequential and reciprocal epithelial-mesenchymal interactions. There have been many reports regarding the expression of various kinds of genes which were related in tooth morphogenesis, however the precise signaling pathway has been still unclear. We previously detected that thymosin beta 4 (Tb4) was a highly expressed gene in embryonic day 12.0 (E12.0) mouse mandible. In this study, we analyzed the functional implications of Tb4 in the mouse first molar development, and focused on the effects of Tb4 on the expressions of *matrix metalloproteinase (Mmp),* odontogenic genes and *nucleolin (Ncl)* by using odontogenic cell lines.

Expressions of the Tb4 mRNA in developing tooth germ were detected by an *in situ* hybridization. At E10.5 and E12.0, the expression of the Tb4 mRNA was predominantly observed in the presumptive dental epithelium. The Tb4 mRNA was expressed in the enamel organ without primary enamel knot at E15.0. At E18.0, the intense expression was observed in the inner enamel epithelium. The expression pattern of Tb4 protein was corresponded to that of *in situ* mRNA expression in the tooth germ. On the basis of theseexpression patterns of Tb4, we performed inhibition assay by using Tb4 antisense phosphorothioated oligonucleotide (AS S-ODN) in cultured E11.0 mouse mandibles or E15.0 tooth germs. In addition, we analyzed the mRNA expression of *Mmp-2/-9,* odontogenic genes and *Ncl* by using quantitative real-time PCR in cultured odontogenic cell treated with Tb4 AS S-ODN. As a result, development of the tooth germ in the organ cultured mandibles at E11.0 was significantly suppressed by the treatment with the Tb4 AS S-ODN. Enamel and dentin layering formation was also slightly inhibited in cultured E15.0 tooth germ treated with Tb4 AS S-ODN. These results indicate that Tb4 plays important roles in the morphogenesis of the developing tooth germ. The analysis of real-time PCR in the cultured odontogenic cell revealed that the expression of *Mmp-2/-9,* odontogenic genes and *Ncl* mRNAs was markedly suppressed in those cells treated with Tb4 AS S-ODN. These vitro data may indicate that Tb4 is related to the direct or indirect regulation of transcriptions of *Mmp-2/-9,* odontogenic genes, *Ncl*.

The present results demonstrate that Tb4 play important roles in the morphogenesis of the tooth germ by regulating the expressions of *Mmp-2/-9,* odontogenic genes and *Ncl*.

OS-4.

Wnt signaling and osteoblast differentiation

Etsuko Matsuzaki*[1, 2], Fumi Takahashi-Yanaga[2], Mari Kobayashi[1], Shunji Hiratsuka[1], Toshiyuki Sasaguri[2], Katsumasa Maeda[1]

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Differentiation-inducing factor-1 (DIF-1), a morphogen of *Dictyostelium*, inhibits cell proliferation and induces cell differentiation in several mammalian cells. We previously reported that DIF-1 activated glycogen synthase kinase-3b, resulting in the degradation of b-catenin and the inhibition of Wnt/b-catenin signaling pathway. As this pathway has been shown to be involved in bone biology, we investigated the effects of DIF-1 as a negative regulator of this signaling pathway on SaOS-2 and MC3T3-E1, an osteosarcoma cell line widely used as a model system for osteoblastic cells and murine osteoblast-like cell line, respectively. We found that DIF-1 suppressed the b-catenin protein amount and the activity of the reporter gene containing T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) consensus binding sites, indicating that DIF-1 inhibited Wnt/b-catenin signaling. DIF-1 also suppressed the expression of osteoblast differentiation markers, including alkaline phosphatase (ALP), core binding factor a1 and osteocalcin, in a time-dependent manner. Subsequently, we analyzed the effect of DIF-1 on ALP promoter activity to clarify the mechanism by which DIF-1 suppressed the ALP expression and found that DIF-1 significantly reduced the promoter activity through the TCF/LEF binding site (-1023/-1017 bp). These results suggest that DIF-1 inhibits ALP promoter activity through the suppression of Wnt/b-catenin signaling pathway.

Next we examined the effect of anti-Dkk1 antibody and Wnt-3a to investigate the effect of Wnt/b-catenin signaling activator on osteoblast differentiation. We found that activation of the Wnt/b-catenin signaling pathway increased osteoblast mineralization and ALP activity. Therefore, activator of the Wnt/b-catenin signaling pathway might nave a therapeutic value to induce the bone regeneration.

OS-5.

Development of the novel bone augmentation technique
-topical application of statin-

Yohei Jinno, Yasunori Ayukawa, Yoichiro Ogino, Ikiru Atsuta, Kiyoshi Koyano

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

Objectives:
Statins are the drugs for the treatment of hyperlipidemia, and their osteogenic effects have also been reported. The purpose of this study is to confirm the effectiveness of the locally applied α-TCP-collagen gel composite with fluvastatin for the vertical bone augmentation.
Methods:
Fifty-four 10-week-old female rats were used. The composite with (experimental group) or without (control group) 1.0 weight % fluvastatin were percutaneously injected into supra-parietal region. One, 2, and 4 weeks after the injection, animals were sacrificed and then serum osteocalcin (OC), alkaline phosphatase (ALP), and tartrate-resistant acid phosphatase (TRAP) were measured. Both histological and histomorphometrical analyses were also performed.
The test pieces for the analysis of release profile were produced according to the size of injected composite. They were placed into 5ml of saline at 37°C. Once a day, release profiles were analyzed by UV spectrometry for 4 weeks.
Results:
In the experimental group, bone thickness was significantly thicker than that in control group.
During the period of observation, there were no significant differences in both ALP and TRAP activities between groups. However, expression level of OC in the experimental group was significantly higher (ANOVA, $P<0.01$).
The release amount of fluvastatin from the test piece was maximum at the first day of the experimental period. From that day, release of fluvastatin continued during the period of observation.
Discussion:
The release profile of fluvastatin from this composite was similar to that estimated by the histological data in our previous study. This may result in the increase of both bone thickness and the serum amount of OC.
Conclusion:
This composite was a potent material for the local delivery of statin to achieve vertical bone augmentation.

OS-6.

Phosphatidylserine liposomes suppress inflammatory bone destruction by phenotypic switching of macrophages and T cells

Hong Mei Ma[1], Zhou Wu[1], Toshio Kukita[2], Katsumasa Maeda[3],
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We recently found that posphatidylserine-containing liposomes (PS liposomes) inhibit the osteoclast differentiation after phagocytozed by osteoclast precursors. Therefore, PS liposomes can be implicated as a potential new pharmaceutical intervention against the inflammatory bone destruction. However, how PS liposomes regulate immune cells infiltration and involve inflammatory bone destruction remain to be clarified. In the present study, we assess this issue using inflammatory bone destruction model, adjuvant arthritic (AA) rats. During acute stage of AA rats, the infiltrated macrophages and helper T cells induced mRNA expression of interleukin (IL)-1b, IL-23, IL-17, transforming growth factor (TGF)-b1 and IL-10 in ankle joints. During chronic stage of AA rats, mRNA expression of receptor activator of nuclear factor-kB (NF-kB) ligand (RANKL) and RANK increased significantly in ankle joints, and consistently manifested bone destruction. Systemic treatment with PS liposomes significantly increased mRNA levels of IL-10 and TGF-bı. In contrast, PS liposomes significantly decreased those of IL-1b, IL-23 and IL-17 without affecting the infiltration of both macrophages and helper T cells during acute stage of AA rats. Furthermore, PS liposomes also decreased mRNA expression significantly in both RANKL and RANK during chronic stage of AA rats. Moreover, PS liposomes markedly reduced the bone destruction. Therefore, consisting with the inhibitory effect on osteoclast differentiation, PS liposomes may also switch macrophages and T cells from pro- to anti-inflammatory phenotype in turn to inhibit inflammatory bone destruction.

OS-7.

Detecting the taste cortical region of human using fMRI

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Objectives: fMRI studies of taste tend to decrease specificity of the results by head movements associated with swallowing. The purpose of this study was to detect the human taste cortical regions by fMRI using our new system that spreads taste stimuli on the dorsal surface of the tongue as widely as possible and also prevents swallowing.
Methods: The delivery system of taste solution consisted of an intra-oral and an extra-oral device. The intra-oral device was assembled with four solution-inlet tubes and an outlet tube that were attached to an individual mouthpiece. The outlet tube was connected to a continuous suction apparatus. Healthy volunteers (10 males and 10 females, 19-28 years) participated in this study. The taste stimuli were sweet (0.5 M sucrose), salty (0.1M sodium chloride), and a tasteless control solution containing the main ionic components of saliva (25 mM KCl plus 2.5 mM $NaHCO_3$). All images were acquired with a whole-body 3.0-T MRI scanner. Image processing and data analysis were performed using the Statistical Parametric Mapping 5 software package.
Results: This device delivered taste solution on most of the dorsum part of tongue and prevented the swallowing. The head motions of all subjects were less than 2.2 mm in translation and less than 3.0 degrees in rotation in all sessions. As the result of brain mapping, activated area by sweet and salty taste stimulus located on the putative human primary taste cortices (uncorrected $P < 0.001$). These areas were confined and similar to each other.
Conclusion: We detect the human taste cortical regions by fMRI using our new system. This device could stimulate the subject's tongue as widely as possible under constant conditions, and suppressed each subject's head movements sufficiently.
This system will make it possible to perform precise functional brain mapping with various taste substances. (300words)

OS-8.

Identification of the interaction sites for anti-sweet substances; Gymnemic acid and Grumarin

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Gymnemic acid (GA) and Gurmarin (Gur) are a triterpen glycoside and a polypeptide that are isolated from the plant *Gymnema sylvestre*, respectively. These chemical compounds are known to selectively suppress taste responses to various sweet substances without affecting responses to salty, sour and bitter substances. Sweet suppressing effect of GA is specific to humans and chimpanzees, but not to rodents, whereas Gurmarin inhibits the responses to sweet compounds in rodents, but not in humans. It has also been shown that the sweet-suppressing effects of GA and Gur are diminished by rinsing the tongue with γ-cyclodextrin (CD) and β-CD, respectively.

In order to examine whether GA and Gur directly interact with T1r2+T1r3, we used the sweet receptor T1r2+T1r3 assay in transiently transfected HEK293 cells. Similar to previous studies in humans and mice, GA (0.1 mg/ml) inhibited the $[Ca^{2+}]_i$ responses of cells heterologously expressing hT1r2+hT1r3 to SC45647, saccharin and D-tryptophan. The effect of GA rapidly disappeared after rinsing the cells with 1% γ-CD. The mouse pair (mT1r2+mT1r3) was not sensitive to GA. Gur (30 μg/ml) suppressed the responses of mT1r2+mT1r3-expressing HEK293 cells to various sweetners. The effect of Gur disappeared after application of β-CD. The human pair (hT1r2+hT1r3) was not sensitive to Gur. To identify the interaction sites for GA and Gur, we examined the responses of the mouse/human chimeras of T1r2 and T1r3. The results suggest that the sensitivity to GA depends mainly on the transmembrane region of human T1r3 and Gur interacts with the extracellular domain of mouse T1r3.

OS-9.

Globular adiponectin induced secretion of granulocyte colony-stimulating factor in RAW 264 cells

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Adiponectin, an adipocyte-derived cytokine, affects a number of physiological processes, including immune function and inflammation. Adiponectin may be present as full-length adiponectin (fAd) or converted to globular domain adiponectin (gAd) by leukocyte elastase, which cleaves the protein's N-terminal collagen-like domain. An earlier study demonstrated that the biological activity of gAd was much stronger than that of fAd. We investigated whether globular adiponectin (gAd) affects the expression of inflammation-related genes in murine macrophages (RAW 264 cells). Using DNA microarray analysis, we identified 707 genes with significantly elevated expression levels after 60 min of exposure to 20 μg/ml gAd. Of those genes, granulocyte colony-stimulating factor (G-CSF) showed the most prominent change in expression after 60 min of exposure (104.0 times). The gAd-induced secretion of G-CSF increased in a time- and dose-dependent manner. U0126 (MEK1/2 inhibitor) and PD98059 (MEK1 inhibitor) reduced gAd-induced G-CSF mRNA expression by 64.5 ± 14.0 and 44.2 ± 1.5%, respectively, and they reduced gAd-induced G-CSF protein production by 80.0 ± 2.4 and 46.3 ± 11.9%, respectively. gAd induced the phosphorylation of MEK1/2 and ERK1/2 in RAW 264 cells. In addition, the gAd-induced phosphorylation of MEK1/2 and ERK1/2 was dramatically reduced by PD98059 and U0126, respectively. Collectively, these results suggest that MEK1/2-ERK1/2 signaling is involved in the adiponectin-induced secretion of G-CSF.

OS-10.

Relationship between Oral Microbial Flora and Symptoms in Patients with Dry Mouth

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【Objective】

Dry mouth is characterized by decreased salivary secretion caused by various systemic factors and side effects of internal drugs. The patients with dry mouth frequently suffer from dental caries, periodontal diseases and oral mucosal diseases. It has been reported that the oral diseases were closely associated with changes in oral microbial flora. In this study, we thus addressed to clarify the relationship between the oral microbial flora and symptoms in patients with dry mouth.

【Materials and Methods】

Thirty-nine patients with dry mouth (28 patients with Sjögren's syndrome, 11 patients with drug-induced or neurotical dry mouth) and 15 healthy controls were studied. Samples were obtained from water rinsed in their mouths for 30 seconds. We examined clinical findings including stimulated whole salivary flow rate (SWS), unstimulated whole salivary flow rate (UWS), number of teeth, and presence of oral mucosal diseases. Oral microbial flora was examined by using terminal restriction fragment length polymorphism. The number of *Candida* species was measured after cultivated on CHROMager *Candida*. *Candida* species were identified by restriction fragment length polymorphism after DNA extraction.

【Results】

In comparison with those in the healthy controls, the patients with dry mouth show the following significant results. The SWS, the UWS, and the number of teeth were decreased. The presence of oral mucosal diseases in dry mouth was increased. In the microbial analyses, *Actinomyces* species and *Villonella* species in dry mouth were more frequently identified. The number of *Candida* species was increased.

【Conclusion】

There results thus suggest that dry mouth induced by hyposalivation closely associated with dental and oral mucosal diseases, and also with changes in oral microbial flora.