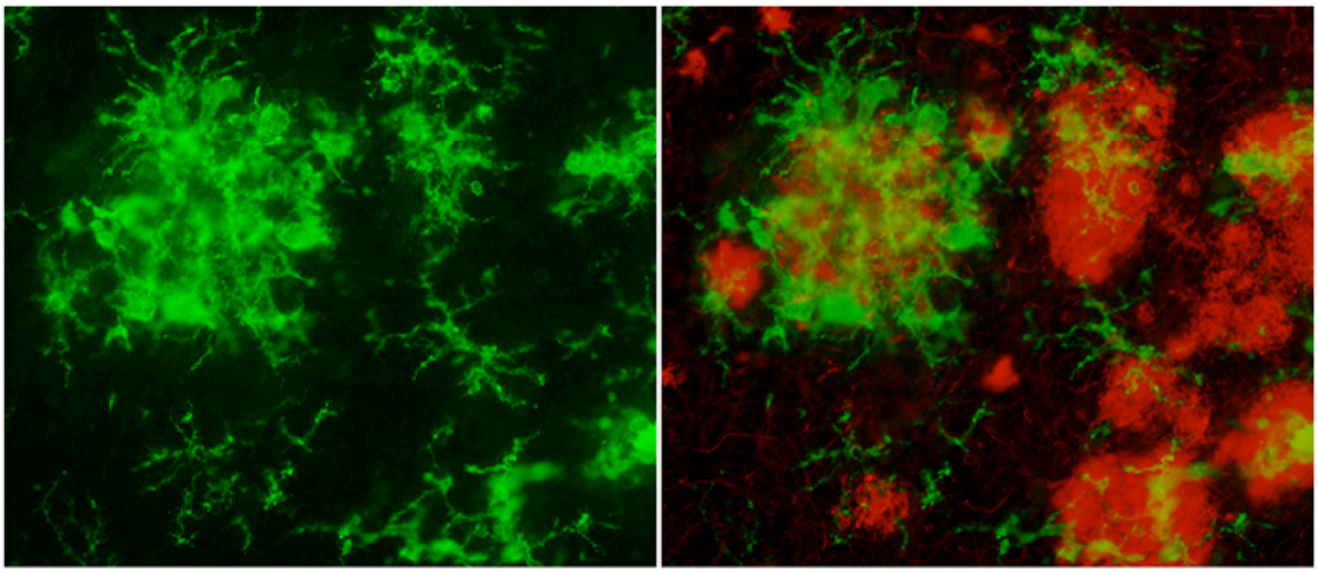
Kyudai Oral Bioscience 2014

—8th International Symposium —

February 28th - March 1st, 2014

Fukuoka Recent Hotel, Fukuoka, Japan

PROGRAM & ABSTRACTS



Kyushu University Faculty of Dental Science

○会期：平成 26 年 2 月 28 日～ 3 月1日
2 月 28日（金）13:00～19:00、懇親会 19:00～21:00
3 月　1日（土）　9:00～16:45

○会場：福岡リーセントホテル 舞鶴の間 BC（２階）：下図参照
住所：福岡市東区箱崎 2-52-1
電話：092 - 641 - 7741

○主催：九州大学大学院　歯学研究院
歯学研究院研究プロジェクト
「口腔組織の再生・再建医療」、「口腔健康科学」
文部科学省　特別教育研究経費

「歯学連携ネットワークによる口腔からＱＯＬ向上を目指す研究」

連絡先：　中西　博
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表紙写真:アルツハイマー病患者脳の老人斑に集積するミクログリア
老人斑（赤色蛍光:Aβ）、ミクログリア（緑色光:CR3/43）
Cover illustration: Accumulation of microglia (green: CR3/43)
surrounding senile plaques (red: Aβ).
From Wu et al. Neurobiol Aging, 34:2715-2725, 2013.

Information for Speakers

■ *Presentation Instruments*

・Presentations are restricted to computer presentations using your own personal computer.

・Please use a computer running Windows XP, Windows Vista, Windows 7 or Machintosh OS X or later and fitted with an external monitor output terminal.

・All speakers are also requested to bring the data of your presentation on a USB or a CD-ROM. Please mark your name and the session number (e.g. S1-1) on the file. Please make sure that virus check is executed beforehand.

■ *Making Presentations*

・For the speakers in S1-1～S1-6, please bring your own personal computer to the Computer Operating Desk (on the left hand side facing toward inside the presentation venue) at 12:40.

・For the speaker in Special Lecture, please bring your own personal computer to the Computer Operating Desk at 16:20 (after Session 1).

・For the speakers in S3-1～S3-6, please bring your own personal computer to the Computer Operating Desk at 8:40.

・For the speakers in Session 4-1～4-6, please bring your own personal computer to the Computer Operating Desk at 13:10.

・All speakers are requested to operate the computer by themselves.

・Your cooperation in finishing your presentation within the allotted time is appreciated.

・After your presentation, please reclaim your computer from the Computer Operating Desk.

PROGRAM

February 28 (Fri) *MAIZURU* Room BC (2nd Floor)

Opening remarks

13:00 - 13:05 Hiroshi Nakanishi (*vice Dean*)
13:05 - 13:10 Akifumi Akamine (*Dean, Faculty of Dental Science, Kyushu University*)

■Session 1: Dental and Craniofacial Morphogenesis and Tissue Regeneration

Chairperson: **Takayoshi Yamaza (*Department of Molecular Cell Biology and Oral Anatomy*)**

13:10 - 13:40 S1-1 Hiroshi Egusa
Department of Fixed Prosthodontics, Osaka University Graduate School of Dentistry, Osaka, Japan

13:40 - 14:10 S1-2 Keishi Otsu
Division of Developmental Biology & Regenerative Medicine, Department of Anatomy, Iwate Medical University, Iwate, Japan

14:10 - 14:40 S1-3 Kentaro Akiyama
Department of Oral Rehabilitation and Regenerative Medicine, Okayama University Graduate School of Medicine and Dentistry and Pharmaceutical Sciences, Okayama, Japan

14:40 - 14:50 Coffee Break

Chairperson: **Kazuaki Nonaka (*Department of Pediatric Dentistry*)**

14:50 - 15:20 S1-4 Han-Sun Jung
Department of Oral Biology, College of Dentistry, Yonsei University, Seoul, Korea

15:20 - 15:50 S1-5 Takayoshi Yamaza
Department of Molecular Cell Biology and Oral Anatomy, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

15:50 - 16:20 S1-6 Fang Jingxian
Southern Medical University, Guangdong Provincial Stomatological Hospital, China

16:20 - 16:30 Break

■Special Lecture

***Chairperson*: Hiroshi Nakanishi (*Department of Aging Science and Pharmacology*)**

16:30 - 17:00 SL Hidetaka Sakai
Laboratory of Oral Pathology, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

17:00 - 17:10 Coffee Break

■ Session 2: PhD Student Session

***Chairpersons*: Xinwen Zhang (D4) & Shingo Takai (D3)**

17:10 - 17:20 S2-1 Junko Obata
Section of Preventive and Public Health Dentistry
17:20 - 17:30 S2-2 Daigaku Hasegawa
Department of Endodontology and Operative Dentistry
17:30 - 17:40 S2-3 Yoko Hashimoto
Department of Periodontology
17:40 - 17:50 S2-4 Junjun Ni
Department of Aging Science and Pharmacology
17:50 - 18:00 S2-5 Makiko Kihara
Laboratory of Oral Pathology

18:00 - 18:10 Break

18:10 - 18:20 S2-6 Takahito Otani
Laboratory Molecular and Cellular Biochemistry
18:20 - 18:30 S2-7 Ryosuke Kondo
Section of Implant and Rehabilitative Dentistry
18:30 - 18:40 S2-8 Misa Shin
Section of Oral Neuroscience
18:40 - 18:50 S2-9 Shinsuke Ieda
Section of Oral and Maxillofacial Oncology
18:50 - 19:00 S2-10 Khairul Anuar Shariff
Department of Biomaterials

19:00 - 21:00 Getting Together Party
***CRYSTAL* Room (2nd Floor)**

March 1 (Sat) *MAIZURU* Room BC (2nd Floor)

■Session 3: Oral-Systemic Medicine

***Chairperson*: Seiji Nakamura (*Section of Oral and Maxillofacial Oncology*)**

9:00 - 9:30 S3-1 Kazuhisa Yamazaki
Division of Oral Science for Health Care, Niigata University Graduate School of Medical and Dental Sciences,Niigata, Japan

9:30 - 10:00 S3-2 Naozumi Ishimaru
Department of Oral Molecular Pathology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan

10:00 - 10:30 S3-3 Masafumi Moriyama
Section of Oral and Maxillofacial Oncology, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

10:30 - 10:40 Coffee Break

***Chairperson*: Fusanori Nishimura (*Department of Periodontology*)**

10:40 - 11:10 S3-4 Fusanori Nishimura
Department of Periodontology, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

11:10 - 11:40 S3-5 Andy Y-T. Teng
College of Dental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

11:40 - 12:10 S3-6 Rangsini Mahanonda
Department of Periodontology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

12:10 - 13:30 Lunch
For the Invited Speakers: Restaurant *SARAFURU* (1st Floor)

■Session 4: Oral Health Science

***Chairperson*: Hiroshi Nakanishi (*Department of Aging Science and Pharmacology*)**

13:30 - 14:00 S4-1 Kenji Matsushita
National Center for Geriatrics and Gerontology, Aichi, Japan

14:00 - 14:30 S4-2 Zhou Wu
Department of Aging Science and Pharmacology, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

14:30 - 15:00 S4-3 Aiqin Zhu
Institution of Geriatric Qinghai Provincial Hospital, Qinghai, China

15:00 - 15:10 Coffee Break

***Chairperson*: Yoshihisa Yamashita (*Section of Preventive and Public Health Dentistry*)**

15:10 - 15:45 S4-4 Mariko Naito
Division of Microbiology and Oral Infection, Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

15:45 - 16:20 S4-5 Atsuo Amano
Department of Preventive Dentistry, Osaka University Graduate School of Dentistry, Osaka, Japan

16:20 - 16:40 S4-6 Michiko Furuta
Section of Preventive and Public Health Dentistry, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

16:40 - 16:45 Closing Remarks

ABSTRACTS

Session 1

Dental and Craniofacial Morphogenesis and Tissue Regeneration

S1-1

Application of iPS Cells in Bone Biology

Hiroshi Egusa

Department of Fixed Prosthodontics
Osaka University Graduate School of Dentistry, Osaka, Japan

Induced pluripotent stem (iPS) cells can be generated through the reprogramming of somatic cells from different tissues by forced expression of defined exogenous factors. These iPS cells efficiently generated from accessible tissues have the potential to be used for various clinical applications. The oral gingiva is an easily obtainable tissue for dentists, and cells can be isolated from patients with minimal discomfort. We successfully generated iPS cells from adult mouse or human gingival fibroblasts via transduction of the Yamanaka factors without c-Myc oncogene. Gingival fibroblasts demonstrate a higher reprogramming efficiency than the skin fibroblasts which have been conventionally used for the generation of iPS cells. These iPS cells were capable of osteogenic differentiation, which could form new bone in the animal models. The generation of iPS cells from the gingiva is expected to provide a breakthrough, especially in the dental sciences, because it offers a promising method for the facile production of pluripotent stem cells by dental researchers. In this presentation, generation and basic aspects of osteogenic capacity of the gingiva-derived iPS cells will be discussed, with an emphasis on potential applications of the iPS cell technologies to bone biology and bioengineering.

S1-2

Differentiation of induced Pluripotent Stem Cells into Odontogenic Lineage for Tooth Regeneration

Keishi Otsu, Mika Sakano, Naoki Fujiwara, and Hidemitsu Harada

Division of Developmental Biology & Regenerative Medicine, Department of Anatomy, Iwate Medical University, Iwate, Japan

The studies of tissue regeneration have been dramatically developed with the advancement of stem cell biology in the last decade. Induced pluripotent stem (iPS) cells are prepared by returning the differentiated somatic cells of the patients to the undifferentiated state by introduction of several genes. This technique allows us to obtain pluripotent stem cells without the use of embryonic cells to overcome the rejection and ethical problem. In this presentation, we will introduce our recent approach to investigate the potential of iPS cells for tooth regeneration. We developed an efficient culture protocol to induce neural crest like cells (NCLC) from mouse iPS cells. In recombination cultures between NCLC and mouse dental epithelium, NCLC exhibited a gene expression pattern involving dental mesenchymal cells. Some NCLC also expressed odontoblasts marker, dentin sialoprotein. Further, the recombinants formed the calcified tooth germ structure without teratomas formation after transplantation under kidney capsule. The conditioned medium of mouse dental epithelium enhanced the differentiation of NCLC into odontoblasts. These results suggest that iPS cells will be promising cell sources for the tooth regeneration and become a powerful research tool for tooth development studies.

S1-3

MSCs Based Immunotherapy

Kentaro Akiyama

Department of Oral Rehabilitation and Regenerative Medicine, Okayama University Graduate School of Medicine and Dentistry and Pharmaceutical Sciences, Okayama, Japan

Mesenchymal Stem Cells (MSCs) are a population of hierarchical postnatal stem cell with the potential to differentiate into mesodermal lineage-derived cells such as osteoblasts, chondrocytes, and adipocytes. Therefore, numerous numbers of MSCs based tissue engineering studies are performed. More recently, immunomodulation property has been considered as an important factor to assess MSC's character. In fact, the interaction between immune system such as T cells and MSCs are gradually discovered with accumulation of some evidence during last decade. Until now, we found disorder of MSCs contribute to the development of a variety of diseases including Systemic Lupus Erythematosus (SLE), Systemic Sclerosis (SS). Thus, systemically injected allogeneic MSCs could influence host immune system and lead the establishment of immunotolerance in diseases.

Although MSCs based immunotherapy become one of promising therapy for refractory immune disease, the detailed mechanism was not fully understood. In this time, I would like to share with you about our new findings; a new mechanism about MSC-induced T cell apoptosis through Fas/FasL signaling is required for MSC-mediated therapeutic effects in SS and experimental colitis.

S1-4

Adhesion Junctions and Cellular Architecture Regulate Epithelial Morphogenesis in Response to FGF Signaling

Han-Sung Jung

Department of Oral Biology, College of Dentistry,
Yonsei University, Seoul, Korea

Number of mutations has elucidated specifically to affect morphogenesis by changing processes like cell division, cell fate and differentiation. We learned how different variables affect arrangements and shapes of cells, and in turn contribute to organ morphogenesis. Diversity of tooth shape is a used model to facilitate learning organ morphogenesis. We compared two species of rodents, gerbils and mice, which display different dental patterns. Our present results reveal that sharp cusps in mouse and the flat loph in gerbils due to the invagination of inner dental epithelium in intercuspal region occurs only in mouse. The expression of F-actin goes along with inner dental epithelium in gerbil and is discontinuous in mouse. The stratum intermedium-expressing E-cadherin and β-catenin are dense in gerbil and rare in mouse. Fibronectin accumulates in the basal lamina in the invaginated region in mouse but not in gerbil. Inhibition of Rac1 in gerbil is able to convert the tooth shape from lophs to cusps. Conversely, both Inhibition of RhoA by Y-27632 and inhibition of fibronectin by anti-fibronectin have the ability to convert the tooth shape from cusps to lophs. Furthermore, the Fgf4 expression pattern resembles a flat sheet along the inner enamel epithelium in gerbil and is restrict to secondary enamel knots in mouse. Treatment with SU5402 in gerbil increases the expression of E-cadherin and cytoplasm β-catenin in inner dental epithelium, increase apical contrition, and leads to the invagination of inner dental epithelium. Together, these results demonstrate for the first time that the RhoA/Rac1 pathway controls the invagination of the inner dental epithelium in response to FGF signaling. It is conceivable that the RhoA/Rac1 pathway influences epithelial invagination through its effect on actin polymerization dynamics, E-cadherin integrity and fibronectin accumulated.

S1-5

Dental Stem Cell based Translational Medicine

Takayoshi Yamaza

Department of Molecular Cell Biology and Oral Anatomy,
Faculty of Dental Science, Kyushu University, Fukuoka, Japan

Recent stem cell technology has developed a new stem cell polpulation, dental stem cells. Dental stem cells are isolated from oral tissues including dental pulp, periodontal ligament, gingiva, and jaw bones and share mesenchymal stem cell characteristics; high cell proliferation capacity and multipotency into several lineaged cells, such as odontoblasts, cementoblasts, osteoblasts, chondrocytes, ligament cells, adipocytes, neural cells, endothelial cells, hepatocytes, and immunomodulatory property to regulate B- and T-lymphocytes, macropages, dendritic cells and natural killer cells. Therefore, numerous attention has been focused on dental stem cells for tissue engineering and cell-based therapy. Among dental stem cells, we have focused on the regenerative properties of stem cells from human exfoliated deciduous teeth (SHED) and challenged to the translational application. In this presentation, we will introduce basic characteristics and potential of dental stem cells, discuss the potency of dental stem cells to regenerative medicine.

S1-6

Zebrafish as Model for Craniofacial Research

Fang Jingxian

Southern Medical University,
Guangdong Provincial Stomatological Hospital, China

Zebrafish had been widely used as a scientific research model nowadays, because of high fecundity, low maintenance cost, transparency and clear genetic background. In order to establish an effective craniofacial research model and to translate our previous Miller syndrome research from human to animal, Tupel long fin has been pharmacologically inhibited by leflunomide and brequinar (two DHODH inhibitors) for 5 days. Growth and behavior changes have been investigated. Results suggested that after inhibition, growth retardation occurred, some bone and cartilage are missing, and behavior has been changed as well.

Special Lecture

SL

Induction of Dental Epithelial Cell Differentiation Marker Gene Expression in Non-odontogenic Human Keratinocytes by Transfection with Thymosin beta 4

Hidetaka Sakai

Laboratory of Oral Pathology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

Previous studies have shown that the recombination of cells liberated from developing tooth germs develop into teeth. However, it is difficult to use human developing tooth germ as a source of cells because of ethical issues. Previous studies have reported that thymosin beta 4 (Tmsb4x) is closely related to the initiation and development of the tooth germ. We herein attempted to establish odontogenic epithelial cells from non-odontogenic HaCaT cells by transfection with TMSB4X. TMSB4X-transfected cells formed nodules that were positive for Alizarin-red S (ALZ) and von Kossa staining (calcium phosphate deposits) when cultured in calcification-inducing medium. Three selected clones showing larger amounts of calcium deposits than the other clones, expressed PITX2, Cytokeratin 14, and Sonic Hedgehog. The upregulation of odontogenesis-related genes, such as runt-related transcription factor 2 (RUNX2), Amelogenin (AMELX), Ameloblastin (AMBN) and Enamelin (ENAM) was also detected. These proteins were immunohistochemically observed in nodules positive for the ALZ and von Kossa staining. RUNX2-positive selected TMSB4X-transfected cells implanted into the dorsal subcutaneous tissue of nude mice formed matrix deposits. Immunohistochemically, AMELX, AMBN and ENAM were observed in the matrix deposits. This study demonstrated the possibility of induction of dental epithelial cell differentiation marker gene expression in non-odontogenic HaCaT cells by TMSB4X.

Session 2
PhD Student Session

S2-1

Identification of Microbiota in Carious Dentin Lesions using 16S rRNA gene Sequencing Analysis

Junko Obata[1,2], Toru Takeshita[1], Yukie Shibata[1], Masako Unemori[2], Akifumi Akamine[2], Yoshihisa Yamashita[1]

[1]Section of Preventive and Public Health Dentistry, Division of Oral Health, Growth and Development, [2]Department of Endodontology and Operative Dentistry, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

Molecular analysis recently revealed the combined predominance of *Lactobacillus* and *Prevotella* in dentinal caries lesions, whereas mutans streptococci had long been assumed to be the main pathogens responsible for human dental caries. This seeming contradiction should be explored in different ethnicities and races. In the present study, the bacterial communities in the carious dentin of Japanese subjects were analyzed comprehensively using next-generation sequencing. Carious dentin lesion samples were collected from 32 subjects aged 4–76 years, and bacterial DNA was extracted. The 16S rRNA genes, amplified from the extracted DNA with universal primers, were sequenced with a pyrosequencer to generate 92,520 reads consisting of 297 oral taxons in the Human Oral Microbiome Database and 1,858 species-level OTUs. The bacterial composition was classified into clusters I, II, and III according to the relative abundance (high, middle, and low) of *Lactobacillus*. The bacterial composition in cluster II was further classified into two subclusters: IIA, composed of relatively high proportions of *Olsenella* and *Propionibacterium*, and IIB, subdominated by heterogeneous genera. The bacterial communities in cluster III were characterized by the predominance of *Atopobium* (cluster IIIA), *Prevotella* (cluster IIIB), and *Propionibacterium* and *Streptococcus* or *Actinomyces* (cluster IIIC). Clusters IIA, IIIA, and IIIC, mainly related to *Atopobium* and *Propionibacterium*, were novel combinations of microbiota in carious dentin lesions and may be characteristic of the Japanese population. Clone library analysis revealed that *Atopobium* sp. HOT-416 and *P. acidifaciens* were specific species associated with dentinal caries among these genera in a Japanese population.

S2-2

The Effects of Wnt5a on Human Periodontal Ligament Cells

Daigaku Hasegawa[1], Naohisa Wada[2], Hidefumi Maeda[2], Shinichiro Yoshida[1], Satoshi Monnouchi[1], Katsuaki Koori[2], Sayuri Hamano[1], Hiromi Mitarai[1] and Akifumi Akamine[1,2]

[1]Department of Endodontology and Operative Dentistry, Faculty of Dental Science, Kyushu University
[2]Department of Endodontology, Kyushu University Hospital

Wnt5a, a member of the noncanonical Wnt proteins, is known to play important roles in the developmental process of various organs and postnatal cell functions. However, little is known about the effects of Wnt5a on human periodontal ligament cells. In this study, we examined the localization and the potential functions of Wnt5a in periodontal ligament (PDL) tissue and cells.
Immunohistochemical analysis revealed that Wnt5a was expressed specifically and predominantly in rat PDL tissue. Semi-quantitative RT-PCR and Western blotting analysis demonstrated that human PDL cells (HPDLCs) expressed Wnt5a and its receptors, Ror2, Fzd2, Fzd4 and Fzd5. In addition, the expression of Wnt5a and its receptors in HPDLCs was up-regulated by exposure to mechanical stress.

Wnt5a stimulation significantly enhanced proliferation and migration of HPDLCs. Wnt5a suppressed osteoblastic differentiation of HPDLCs cultured in osteogenic induction medium, while Wnt5a significantly up-regulated PDL-related gene expression and collagen production in HPDLCs. Inhibition assays using siRNA for periostin and neutralizing antibody for TGFβ1 suppressed the Wnt5a-induced PDL-related gene expression and collagen production in HPDLCs.
In conclusion, Wnt5a that is present in the PDL tissue and up-regulated by mechanical stress promoted PDL-related gene expression and collagen production in human PDL cells through TGFβ1-mediated up-regulation of periostin expression. Therefore, Wnt5a may be involved in the mechanisms to maintain the homeostasis of PDL tissue.

S2-3

The Molecular Mechanism of Sphingosine-1-phosphate Signaling on Adipocyte Differentiation

Yoko Hashimoto, Etsuko Matsuzaki, Katsumasa Higashi, Aiko Takano, Fusanori Nishimura

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

Sphingosine-1-phosphate (S1P) is a well-known signaling sphingolipid and an important bioactive lipid mediator. Signaling through cell surface G-protein-coupled receptors (GPCRs), termed $S1P_1$-$S1P_5$, mediates most of the biological effects of S1P. Mesenchymal stem cell (MSC) can differentiate into multiple cell types including adipocytes, osteoblasts, chondrocytes, and smooth muscle cells. Particularly, the adipogenic differentiation program is well-orchestrated, which is triggered by binding of adipogenic hormones, such as insulin and glucocorticoids, to GPCRs, leading to induce cAMP accumulation and PKA activity, followed by the transcriptional activation of CCAAT/enhancer binding protein β (C/EBPβ) and peroxisome proliferator-activated receptor γ (PPARγ). These master adipogenic transcription factors regulate their target genes such as fatty acid binding protein 4 (FABP4).

It has been reported that S1P inhibits cAMP accumulation through GPCR ($S1P_1$) in cardiomyocytes. However, the effect of signaling pathway relating to S1P on MSC differentiation into adipocyte is yet unknown. In this study, we investigated the molecular action of S1P signaling pathway on adipocyte differentiation of MSC using C3H10T1/2 cells.

During adipogenic differentiation of C3H10T1/2 cells, S1P inhibited the relative amounts of PPARγ, and FABP4 both at mRNA and protein levels in a time- and dose-dependent manner. S1P also decreased the formation of lipid droplets, suggesting that S1P inhibits adipogenic differentiation of MSC. We next examined the effect of Rosiglitazone, the directional PPARγ agonist, on adipogenic differentiation to see whether S1P action targeted to PPARγ. Although S1P did not affect the expression levels of C/EBPβ and PPARγ, S1P suppressed FABP4 mRNA and protein expression, indicating that the target molecule of S1P is present on upstream of PPARγ.

Furthermore, increased expression of C/EBPβ mRNA during adipogenic differentiation was diminished by addition of S1P as well as by inhibition of PKA. These results suggest that S1P plays a negative role on adipogenic differentiation of MSC, probably by interacting with an upstream molecule of PKA.

S2-4

Microglial Cathepsin B Contributes to Hypoxic/Ischemic Brain Injury through Promotion of M1 Polarization

Junjun Ni, Zhou Wu, Ryo Okada, Hiroshi Nakanishi

Department of Aging Science and Pharmacology, Kyushu University

It is widely accepted that activated microglia exert dual functions: pro-inflammatory (M1) and anti-inflammatory (M2) functions. The *in vivo* status of microglia is regulated microglial polarity between two states. However, the mechanisms regulating microglial polarity remain unclear. Here, we provide evidence that cathepsin B (CatB), a typical cysteine lysosomal protease, is an M1-amplifier in microglia.

Neonatal wild-type mice subjected to hypoxic/ischemia (H/I) showed an extensive brain injury. CatB was significantly increased exclusively in microglia after H/I. It was also noted that the mean expression levels of both polarization markers for M1 (IL-1β, iNOS and TNF-α) and M2 (arginase1, IL-4, YM1 and IL-10) in microglia isolated from H/I-subjected wild-type mouse brains were significantly increased after H/I. On the other hand, in neonatal CatB-deficient mice, H/I-induced brain injury was significantly reduced. Although microglia in CatB-deficient mice showed activated morphology, the mean expression levels of M1 polarization markers in microglia isolated from CatB-deficient mouse brains were significantly lower than those of wild-type mice.

These observations suggest that CatB promotes microglial polarization into M1, thus CatB deficiency reduces H/I-induced brain injury in neonatal mice. Therefore, CatB-specific inhibitors may represent a useful new strategy for treating ischemic brain injury.

S2-5

Itm2a Expression during the Tooth Germ Development

Makiko Kihara[1, 2], Tamotsu Kiyoshima[1], Kengo Nagata[1], Hiroko Wada[1],Hiroaki Fujiwara[1], Kana Hasegawa[1, 3], Hirotaka Someya[1, 4], Ichiro Takahashi[2] and Hidetaka Sakai[1]

[1] Laboratory of Oral Pathology, [2]Section of Orthodontics and Dentofacial Orthopedics, [3]Department of Endodontology and Operative Dentistry, [4]Section of Implant and Rehabilitative Dentistry, Faculty of Dental Science, Kyushu University

Itm2a is a type II transmembrane protein belonging to the BRICHOS superfamily. Our previous study reported that Itm2a was one of genes highly expressed in murine mandible on embryonic day 12.0 (E12.0) compared with that on E10.5. Thus far, however, the expression pattern and intracellular localization of Itm2a mRNA and protein have not yet been fully elucidated. We investigated the temporospatial expression pattern of Itm2a mRNA and protein in the developing lower first molar of the murine embryos and neonates, and the subcellular localization of Itm2a in murine dental epithelial (mDE6) cells. The *in situ* and protein signals of Itm2a were first observed in outer and inner enamel epithelia at cap stage. At the bell stage, these signals of Itm2a were mainly detected in the inner enamel epithelium of the enamel organ. After the initiation of the matrix formation, Itm2a *in situ* and protein signals expressed in ameloblasts and odontoblasts. Itm2a showed a punctate pattern in the cytoplasm of the mDE6 cells. The perinuclear-localized Itm2a colocalized with the Golgi apparatus marker, GM130, frequently. Colocalization of Itm2a with the endosome marker, clathrin, near the Golgi apparatus, was observed in part. A tiny amount of Itm2a overlapped with lysosomes and endoplasmic reticulum. In cytoplasm, there was minimal or no overlap between the Itm2a-EGFP signals with the other organelle markers for endoplasmic reticulum, endosome, lysosome and mitochondria used in this study. After stimulation by BMP2, nuclear translocations of Itm2a were observed, but not EGF. These findings suggest that Itm2a may participate in cell differentiation during the initiation of tooth germ and the targeting of proteins associated with enamel and dentin matrices in the secretory pathway.

S2-6

Osteocalcin Signaling in Adipocyte Differentiation and Functional Regulation

Takahito Otani[1], Hiroshi Takeuchi[2], Akiko Mizokami[1], Koki Nagano[1],Jing Gao[1] and Masato Hirata[1]

[1]Laboratory of Molecular and Cellular Biochemistry, Faculty of Dental Science, Kyushu University,
[2]Division of Applied Pharmacology, Kyushu Dental University

Osteocalcin is a noncollagenous bone matrix protein regulating hydroxyapatite size and shape through its vitamin K-dependent γ-carboxylated form (GlaOC). On the other hand, a small portion of osteocalcin molecules remain uncarboxylated. This uncarboxylated form of osteocalcin (ucOC) has recently been reported to play a key role in energy metabolism. In the present research, we examined the effects of ucOC on adipose tissue using 3T3-L1 adipocytes and epididymal white adipose tissue (eWAT) of mice. We initially identified the expression of Gprc6a which is a putative ucOC receptor in 3T3-L1 adipocytes by RT-PCR. We then examined the effect of OC on the production and secretion of adiponectin from adipocytes, regulating the metabolism of lipids and glucose. ucOC, but not GlaOC promoted the phosphorylation of ERK and the expression of a peroxisome proliferator-activated receptor γ (PPARγ) which is a master regulator of adipogenesis, and then the expression of adiponectin was also upregulated by ucOC in 3T3-L1 adipocytes. To investigate the implication of Gprc6a in these phenomena, we inhibited the expression of this receptor by siRNA, and validated a reduction of the phosphorylation of ERK and the expressions of adiponectin and PPARγ induced by ucOC. In addition, the expressions of adiponectin and PPARγ were inhibited by both MEK and PKA inhibitors. Also, ucOC increased the intracellular cyclic AMP concentration, and dibutyryl cyclic AMP, a cell permeable cyclic AMP analog mimicked the effects of ucOC in 3T3-L1 adipocytes. The phosphorylation of CREB was also promoted by ucOC, but inhibited by both MEK and PKA inhibitors, as a result of transactivation by PKA via MEK activation. Furthermore, we identified that Rap1 connected ERK and PKA pathways by detecting Rap1 activation in ucOC signaling.

As in vivo experiments, mice were treated orally with ucOC at 10 μg/kg 3 times a week for 10 weeks since 3 weeks of the age. We found that eWAT of mice administered with ucOC promoted the phosphorylation of ERK or the expressions of PPARγ and adiponectin.

These results suggest that ucOC via Gprc6a increases the intracellular cyclic AMP concentration, leading PKA activation and then transactivation of ERK via Rap1 activation, followed by the expressions of PPARγ and adiponectin.

S2-7

Effect of Systemically Transplanted Mesenchymal Stem Cells on Epithelial Sealing around Dental Implants

Ryosuke Kondo[1], Ikiru Atsuta[1], Yasunori Ayukawa[1], Takayoshi Yamaza[2], Yuri Matsuura[1], Kiyoshi Koyano[1]

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Mesenchymal stem cells (MSCs) are an attractive cell source in regenerative medicine because of their multi-differentiation capacity and regulatory functions, specifically at sites of inflammation. Here, we investigated the effect of systemic MSC transplantation on peri-implant epithelial sealing. MSCs were isolated from the bone marrow of donor rats and expanded in culture. Right maxillary first molars were extracted from experimental rats and replaced with experimental titanium implants. After 24h, MSCs were systemically injected into recipient rats via the tail vein. The injected MSCs accumulated at the injured gingival mucosa, following which peri-implants epithelium (PIE) formed from the oral sulcular epithelium earlier than control rats. Furthermore, in the MSC-implant group, a laminin-332 (Ln)-positive layer was detected along the interface of the PIE-implant at 4 weeks. Conversely, Ln was only observed in the apical region and connective tissue region in the control group. At 16 weeks, the MSC-implant group exhibited markedly less PIE down-growth than the control group. We also identified an increase in the number of attached oral epithelial cells (OECs) upon direct or indirect co-culture with MSCs, and OEC apoptosis increased in co-culture with MSCs. Systemic MSC transplantation may be effective for enhancing epithelial sealing around titanium implants and reducing apical PIE down-growth.

S2-8

Involvement of CCK in Normal Gustatory Responses to Bitter Compounds

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Taste receptor cells detect chemicals in the oral cavity and transmit the information to taste nerve fibers. However, the molecular mechanism for the taste signal transmission has not fully been clarified. ATP is shown to be released from type Ⅱ taste cells (sweet, bitter and umami taste cell) and may serve as a neurotransmitter between receptor cells and primary afferent nerve fibers (Finger et al., 2005).

Recent studies demonstrated that bitter receptors (T2Rs) are expressed in the gut endocrine cells and CCK is secreted from the endocrine cells by bitter taste stimuli. Also in the taste tissue, CCK is shown to be expressed in a subset of taste cells which co-express gustducin, an alpha G-protein, involved in signal transduction for bitter, sweet and umami tastes. Therefore, we hypothesized that CCK may also function as a transmitter and/or a modulator in taste buds.

Here we tested potential expression of CCK, CCKR (A, B) in taste bud cells and the geniculate ganglion of wild-type mice. To elucidate the function of CCK in the gustatory signaling, we next compared CT nerve responses of wild-type, CCKAR-KO, CCKBR-KO and CCKR-WKO mice. Additionally, we examined potential changes of CT nerve activities in response to direct stimulation of circulating CCK injected from the femoral vein to CT nerve fibers.

As a result, we found that CCK and CCKR (A, B) are expressed in a subset of the taste cells and cell bodies of the geniculate ganglion. Furthermore, it is found that the i.v. injection of CCK increased activities of CT nerve in a dose-dependent manner.

Collectively, these findings suggest that CCK may be involved in the normal signal transmission for bitter taste from taste cells to nerve fibers.

S2-9

Exhaustive Analysis of Fungal Populations from Patients with Oral Candidiasis

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Objectives: There is increasing interest in oral hygiene of elderly people, who subject to various oral mucosal diseases such as oral candidiasis. Several studies reported that oral candidiasis is closely associated with the changes in oral fungal flora and caused primarily by Candida species in the oral mucosa. Identification of fungal flora at the species level may be useful to initiate an early and appropriate treatment of those patients. Conventional methods used by fungal culture are time-consuming and not always conclusive. In contract, molecular genetic analysis of internal transcribed spacer (ITS) regions of ribosomal DNA is rapid, reproducible and easy to be performed. We thus investigated the fungal flora of patients with oral candidiasis and its change before/after treatments for oral candidiasis by using this method.

Methods: Twenty-seven patients with oral candidiasis and 23 with healthy controls were studied. Fungal DNA of oral swabs taken from tongue and saliva were extracted and were examined by length heterogeneity polymerization chain reaction (LH-PCR) and nucleotide sequence analyses of the ITS1-5.8S rRNA-ITS2 region.

Results: As the results of LH-PCR, fungi in patients with oral candidiasis showed increased diversity and abundance compared with controls. *Candida albicans* was dominant in both patients and controls. Furthermore, 54 fungal populations were detected in patients with oral candidiasis and 47 in controls. Out of them, 39 populations were common and 15 were specific to patients and 8 were specific to controls. With regard to previously-unidentified fungi, the nucleotide sequence analysis identified some novel fungal populations. In addition, these fungi have reduced after treatment for oral candidiasis.

Conclusion: These results suggest that particular unidentified fungi or fungal flora might be related to the pathogenesis of oral candidiasis. Further studies including nucleotide sequence are needed to figure out and characterize virulent fungal species.

S2-10

Interconnected Porous Calcium Phosphate Forming Cement consisting of α-TCP Foam Granules

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Interconnected porous calcium phosphate forming cement is thought to be an ideal material as artificial bone substitute and scaffold for bone tissue regeneration, since its interconnected pores provide space that enables cells' growth and penetration into it. In this study, the feasibility to fabricate interconnected porous calcium phosphate forming cement was evaluated based on the setting reaction of α-tricalcium phosphate (α-TCP) foam granules. α-TCP foam granules were prepared using polyurethane foam as a template. When α-TCP foam granules were exposed with calcium phosphate acidic solution, α-TCP foam granular was found to set at room temperature. Interconnected porous structure was kept after the setting reaction. X-ray diffraction analysis revealed that dicalcium phosphate dihydrate (DCPD) was formed at the surface of α-TCP foam granular. SEM observation revealed that dicalcium phosphate dihydrate provides bridge between α-TCP foam granular. It is concluded that these methods may be useful to fabricate interconnected porous calcium phosphate for bone tissue regeneration.

Session 3
Oral-Systemic Medicine

S3-1

Periodontitis and Systemic Disease -New Insights into the Mechanisms-

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Periodontitis has been implicated as a risk factor for metabolic disorders such as type 2 diabetes, atherosclerotic vascular diseases, and non-alcoholic fatty liver disease. Hypothesized underlying mechanisms by which periodontitis affect these diseases include a disseminated bacteremia from the periodontal plaque bacteria and/or elevated circulating inflammatory cytokines that are produced locally in the inflamed gingival tissues. However, there is no direct evidence that either a periodontal bacteria-associated bacteremia and/or periodontitis tissue-derived inflammatory cytokines are involved in the course of these systemic metabolic diseases. Recent evidence suggests that the gut microbiome plays an important role not only in metabolic homeostasis but also in immune and inflammatory responses. High-fat diet-induced alterations of the gut microbiome result in increased gut permeability and subsequent metabolic endotoxemia, which induces an inflammatory response in various tissues. Because the saliva of patients with periodontitis contains oral bacteria, including periodontopathic bacteria, which can be swallowed into the gastrointestinal tract, the composition of the gut microbiota could be influenced by these periodontopathic bacteria. We hypothesize that this phenomenon can induces changes in the gut microbiota leading to a metabolic endotoxemia and an increase in the incidence and progression of metabolic disorders. To test this hypothesis, C57BL/6 mice were orally administered *Porphyromonas gingivalis (P. gingivalis),* a representative periodontopathic bacteria. Changes in the gut microbiota were analyzed by meta-analysis of 16S rRNA gene expression, insulin and glucose intolerance, and by levels of tissue inflammation. The results demonstrated that *P. gingivalis* administration significantly increased the proportion of the phylum Bacteroidetes in the ileum whereas the phylum Firmicutes was decreased. Operational taxonomy unit (OTU)-based bacterial diversity analysis revealed that the population belonging to Bacteroidales was significantly elevated in *P. gingivalis*-administered mice compared with sham-administered mice. Interestingly, this change coincided with increases in insulin resistance, adipose tissue and liver inflammation, and elevated levels of serum inflammatory markers. Furthermore, in *P. gingivalis*-administered mice, blood endotoxin levels tended to be higher, whereas gene expression of tight junction proteins in the ileum was significantly decreased compared with sham-administered mice. These results provide a new paradigm for the interrelationship between periodontal diseases and systemic diseases.

S3-2

Molecular Pathogenesis of Sjögren's Syndrome

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Sjögren's syndrome (SS) is an autoimmune disease targeting exocrine glands such as the salivary and lacrimal glands, causing patients with SS to experience dryness of the mouth and dry eyes. SS is characterized by a 9:1 ratio of women to men, and almost all SS patients are postmenopausal women. We have demonstrated that estrogen deficiency caused by ovariectomy exacerbates autoimmune lesions in a murine model of SS. In addition, we reported that retinoblastoma-associated protein 48 (RbAp48) induces tissue-specific apoptosis in the salivary and lacrimal glands of SS mice depending on the level of estrogen deficiency. Moreover, we observed that transgenic expression of RbAp48 in exocrine glands causes the development of autoimmune exocrinopathy, resembling SS. Although postmenopausal estrogen deficiency triggers the breakdown of immune tolerance and induces autoimmune disease, multiple direct or indirect factors that are affected by the change in estrogen level impede efforts to understand the molecular mechanisms involved in autoimmunity.

Aromatase is a converting enzyme from androgens to estrogens. In the present study, we used female aromatase gene knockout (ArKO) mice as a model of estrogen deficiency to investigate the molecular mechanism that underlies the onset and development of autoimmunity. Histological analyses showed that inflammatory lesions in the lacrimal and salivary glands of ArKO mice increased with age. Adoptive transfer of spleen cells from ArKO mice into recombination activating gene 2 knockout mice failed to induce the autoimmune lesions. Expression of mRNA encoding proinflammatory cytokines and monocyte chemotactic protein-1 (MCP-1) increased in white adipose tissue (WAT) of ArKO mice and was significantly higher than that in wild-type mice. Moreover, an increased number of inflammatory M1-macrophages was observed in WAT and salivary glands of ArKO mice. Furthermore, the autoimmune lesions in a murine model of Sjögren's syndrome were exacerbated by administration of an aromatase inhibitor. These results suggest that adiposity in the target organ may disrupt peripheral immune tolerance leading to the development of autoimmunity.

S3-3

T Helper Subsets in Sjögren's Syndrome and IgG4-related Dacryoadenitis and Sialoadenitis, so-called Mikulicz's Disease

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IgG4-related disease (IgG4-RD) is a systemic disease characterized by the elevation of serum IgG4 and infiltration of IgG4-positive plasma cells in multiple target organs, including pancreas, kidney, biliary tract, lung, lymph node and salivary gland. Until just a decade ago, Mikulicz's disease (MD) has been considered a subtype of Sjögren's syndrome (SS) based on histopathological similarities. However, it is now recognized that MD is an IgG4-RD distinguishable from SS and called as IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS). Regarding immunological aspects, it is generally accepted that CD4þ T helper (Th) cells play a crucial role in the pathogenesis of SS. Since it is well known that IgG4 is induced by Th2 cytokines such as IL-4 and IL-13, IgG4-DS is speculated to be a unique inflammatory disorder characterized by Th2 immune reactions. However, the involvement of Th cells in the pathogenesis of IgG4-DS remains to be clarified. Exploring the role of Th cell subsets in IgG4-DS is a highly promising field of investigation. In this study, we focus on the selective localization and respective functions of Th cell subsets and discuss the differences between SS and IgG4-DS to clarify the pathogenic mechanisms of these diseases.

S3-4

Cellular and Molecular Pathogenesis of Periodontal Microinflammation

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In the past decade, periodontal disease has been recognized as not merely a local infectious disease, but as chronic, subclinical, inflammatory disease for the host. Such micro-inflammation has been proposed to influence insulin sensitivity known as insulin resistance, atherosclerotic changes, and even renal dysfunction. Thus, it is very important to elucidate molecular mechanisms as to why local periodontal inflammation is amplified to the level of influencing our overall systemic health. Recently, macrophages have been suggested to infiltrate into adipose tissues, and to interact with adipocytes, thereby exacerbating adipose tissue inflammation. Furthermore, both cell types appear to express toll-like receptor-4 (TLR4), and oxLDL has been found to act as endogenous ligand for TLR4. Based on these findings, we hypothesized that, in cases of infectious diseases such as severe periodontal disease and gut infection, classical exogenous ligand for TLR4 may further exacerbate inflammatory responses in adipose tissue, thereby contributing to the induction of many unwanted side effects such as insulin resistance. To prove this, we established co-culture system between adipocytes and macrophages and stimulated these cells with bacterial lipopolysaccharide (LPS). We found that stimulation of the cells with LPS markedly up-regulated inflammatory gene expression in adipocytes as well as protein productions from co-cultures. Some of these effects were confirmed in vivo model by using both genetically induced and environmentally induced obese model mice as well. Furthermore, we recently obtained important findings as to how host responses at local inflammation caused by local irritants extended to adipose tissues via circulation by using gene knockout mouse model. Current understanding on these mechanisms will be discussed in the context of developing new “order-made” diagnostic strategies to avoid such unwanted side effects.

S3-5

Intriguing Myeloid DC Precursor and CD4$^+$T-cell Interactions as a Model to Study the Innate-vs.-Adaptive Immunity associated with Osteoclastogenesis and Bone Loss

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Background: Dendritic cells (DC) are innate immune effectors, critically involved in regulating T-cell immunity. DC/T-cell interactions play a pivotal role in the inflammatory conditions, such as destructive bone disorders like rheumatoid arthritis and periodontitis. Inflammation-induced bone loss is a well-established osteo-immune phenomenon, whereby the frequency and activity of osteoclasts (OC) become elevated under inflammatory conditions in response to various challenges including signaling of osteotropic cytokines. Besides being professional antigen presenting cells, DC share common precursors with OC, which infiltrate bony tissues during inflammation. RANKL/RANK-OPG triad is known to be the central paradigm involving in regulating the osteoclastogenesis & bone remodeling. **Methods:** My lab has developed a working model, with which immature myeloid dendritic cell precursors (being CD11c$^+$ MHC-II$^{-/lo}$ CD11b$^-$ F4/80$^-$ CD31$^-$ Ly-6C$^-$ CT-R$^-$ Cath-K$^-$) can act like OC precursors/OCp (called: DDOC) in response to RANKL plus inflammatory stimuli vs. activation signals (Blood 2009, Infect Immun 2009, Periodontology 2000, 2010). Herein, the cellular & molecular interactions between DDOC/T-cells (e.g., Treg & Th17 cells) and osteotropic cytokines (i.e., TGF-β, IL-17, *etc*.) that modulate the innate-vs.-adaptive immune responses for osteoclastogenesis are dissected and discussed. **Results:** The resulting data showed that: **i)** neutralization of TGF-β activity by Mab (or si-RNA) abolished DDOC development in co-cultures ($p<0.03$) and *in-vivo* post-adoptive transfer ($p<0.02$), based on TRAP & resorptive-pit assays; **ii)** transfection of TGFβ-RII lentiviral vectors containing knock-down sh-RNA into CD11c$^+$DDOC yielded a significant inhibition of bone resorption and osteoclatogenesis *in-vitro* & via post-adoptive transfer ($p<0.05$); **iii)** addition of Foxp3$^+$CD4$^+$Treg cells into CD11c$^+$DDOC with sonicated *Aggregatibactor actinomycetemcomitans-Aa* Ag in co-cultures resulted in strongly reduced TRAP expression and osteoclastogenesis vs. bone resorption *in-vitro,* & the calvarias in NOD/SCID mice, respectively ($p<0.05$), where such effects were significantly abolished by IL-17 administered ($p<0.05$); **iv)** while TRAF6-signaling was essential for RANKL-mediated DDOC

development for osteoclastogensis, TGF-β signals rescued the phenotype detected in DDOC transduced by TRAF6-KO vector (p=0.037); whereas such defected phenotype was more significantly rescued in IL-17-treated co-cultures (or by Th17 cells), as compared to that of TGF-β treatment (p<0.03). **Conclusion:** our findings strongly suggest that: **i)** the immature $CD11c^{+}$DC precursor and $CD4^{+}$T-cell interactions are intriguing, which may serve as a unique model to investigate specific-links of the innate-adaptive immunity associated with osteoclastogenesis and bone loss for bone remodeling. In addition, cytokine TGF-β & IL-17 (or Th17 cells) can interact with RANKL via TRAF6 or unclear pathways in DDOC-OCp & OC to modulate osteoclastogenesis, whose signaling interactions may be further defined, depending on which key environmental factors are in action. Project supported by NIH-DE015786, USA; National Health Research Institute EX101-S34199N & National Science Committee NSC-101-2314-B-037-050-MY3, Taiwan.

Keyword 1: ***myeloid* CD11c^{+}dendritic cell-osteoclast (DDOC) & osteoclastogenesis**
Keyword 2: **T-cells/Treg-Th17 cells**
Keyword 3: **Innate-adaptive immunity**
Keyword 4: ***RANKL-RANK/OPG & cytokine signaling***

S3-6

α-Defensin-induced MxA Expression in Healthy Human Periodontal Tissue

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Although periodontal tissue is continually challenged by microbial plaque, it is generally maintained in a healthy state. To understand the basis for this, we investigated innate antiviral immunity in human periodontal tissue. The expression of mRNA encoding different antiviral proteins, myxovirus resistance A (MxA), protein kinase R (PKR), oligoadenylate synthetase (OAS), and secretory leukocyte protease inhibitor (SLPI) were detected in both healthy tissue and that with periodontitis. Immunostaining data consistently showed higher MxA protein expression in the epithelial layer of healthy gingiva as compared with tissue with periodontitis. Human MxA is thought to be induced by type I and III IFNs but neither cytokine type was detected in healthy periodontal tissues. Treatment *in vitro* of primary human gingival epithelial cells (HGECs) with α-defensins, but not with the antimicrobial peptides β-defensins or LL-37, led to MxA protein expression. α-defensin was also detected in healthy periodontal tissue. In addition, MxA in α-defensin-treated HGECs was associated with protection against avian influenza H5N1infection and silencing of the MxA gene using MxA-targeted-siRNA abolished this antiviral activity. To our knowledge, this is the first study to uncover a novel pathway of human MxA induction, which is initiated by an endogenous antimicrobial peptide, namely α-defensin. This pathway may play an important role in the first line of antiviral defense in periodontal tissue.

Session 4
Oral Health Science

S4-1

Periodontal Disease as a Possible Risk Factor for Alzheimer's Disease

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Periodontitis is a localized infectious disease caused by oral bacteria, such as *Porphyromonas gingivalis*. Recently, associations between periodontitis and systemic diseases such as diabetes and atherosclerosis have been demonstrated. It has also been reported that bacterial infections might contribute to the onset and progression of Alzheimer's disease (AD); however, evidence is sparse regarding the causative relationship. In this study, we used a transgenic mouse model of AD to investigate whether periodontitis, evoked by *P. gingivalis,* modulates the pathological features of this neurodegenerative disease. We found that cognitive functions were significantly impaired in J20 mice inoculated with *P. gingivalis* compared to those in control J20 mice. Additionally, brain levels of Aβ1-40 and Aβ1-42 were higher in inoculated J20 mice than in control J20 mice. Aβ deposition in the hippocampus and cortex was also significantly greater in inoculated J20 mice than in control J20 mice. Furthermore, brain levels of proinflammatory cytokines, including IL-1β and TNF-α, were higher in inoculated mice than in control mice. These results suggest that periodontitis, evoked by *P. gingivalis,* may exacerbate brain amyloid deposition and trigger brain inflammation, leading to enhanced cognitive impairment. Possible mechanisms by which periodontitis modifies AD are 1) proinflammatory molecules such as proinflammatory cytokines and bacterial toxins (e.g., lipopolysaccharides) originating in the oral cavity might affect the brain via systemic circulation and/or neural pathways and 2) periodontopathic bacteria, including *P.g*., invade the brain and directly affect the pathogenesis of AD. Periodontal infections are treatable, and treatment of those infections may be effective for preventing and delaying the progression of AD.

S4-2

Systemic *Porphyromonas gingivalis* LPS Induces Microglial Activation and Amyloid β Accumulation in the Brain

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Chronic systemic inflammation can influence the brain functions through induction of neuroinflammation. Recently, accumulating evidence suggests a possible link between periodontitis and Alzheimer disease (AD). For example, the severity of periodontitis in elderly people has a positive relationship with the cognitive impairments. Furthermore, LPS from *Porphyromonas gingivalis* (*P. gingivalis*) has been detected in the brain of AD patients. However, a link between periodontitis and AD remains hypothetical.

Microglia, brain-resident mononuclear phagocytes, are well accepted as the primary cells that respond to peripheral inflammatory stimulation. It is also known that microglia are the main cellular source of oxidation products and inflammatory molecules in the brain during aging. Recently, we have found that amyloid β can induce interleukin-1β secretion from microglia isolated from the brains of aged mice, but not young mice, explaining the reason why senescence is an important factor for AD (Wu et al., Neurobiol Aging, 2013). Furthermore, systemic inflammation caused by adjuvant arthritis promotes the phenotypic changes in microglia to induce exaggerated neuroinflammation in middle-aged rats, but not in young rats, resulting in deficits of the hippocampal long-term potentiation, a cellular basis of learning and memory (Liu et al., Neuroscience, 2012). These observations suggest that chronic systemic inflammation accelerates "microglia aging", which enables microglia to be hypersensitive to secondary responses in the brain (Nakanishi & Wu, Behav Brain Res, 2009). Therefore, both senescence and chronic systemic inflammation are considered to be potent accelerators of "microglia aging".

In this symposium, I will review our "microglia aging" hypothesis, and introduce our recent works on systemic *P. gingivalis* LPS-induced microglial activation and amyloid β accumulation in the brain. I will also discuss a possible link between periodontitis and AD associated with cognitive impairments. I believe that better understanding of "microglial aging" will help to develop strategies for preventing the cognitive impairments during aging and aging-related neurodegenerative diseases such as AD.

S4-3

Effects of Nature Materials on Alzheimer's disease

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By the year 2030, roughly 20% of the population will be over 65 years of age in the world. The cognitive impairment is coming to the worldly problem with the mean life expectancy continual increasing. Recently, much attention has been paid to the association of hypoxia with cognitive impairments, because the brain is highly vulnerable to hypoxic stress due to its high oxygen requirement. The negative effect of hypoxia on cognitive function has been well described. However, less has been shown regarding cognitive dysfunction resulting from hypoxia produced by exposure to different altitudes. People living in Qinghai-Tibet Plateau experience chronic hypoxia at high altitude. We have found that healthy individuals in the Qinghai-Tibetan Plateau are excursions with mild cognitive impairment (MCI), which is frequently seen as a prodromal stage of Alzheimer's disease (AD). Current medical researches on the cognitive impairments pay a special attention on nature materials, because their traditional usages. Ratanasampil (RNSP), one of the most important Tibetan medicines with 70 components, is used to treat cerebrovascular diseases such as cerebral hemorrhage, cerebral infarction. In addition, propolis is a resinous substance produced by honeybees as a defense against intruders, which has been used as therapeutic properties since ancient times. In this talk, I will introduce our previous studies that RNSP improves learning and memory in a mouse model of AD (Tg2576), and that RNSP also improves the cognitive functions in mild-to-moderate AD patients. Furthermore, propolis improves the cognitive functions in patients of MCI at high altitude, because timely intervention MCI may effectively prevents the processing of AD. We will discuss the working mechanism of the nature materials on the cognitive impairments and give an idea of nature materials may benefit to improve the learning and memory ability of elderly people for preventing cognitive impairments.

S4-4

The Impact of Genomics on Research in Periodontal B acteria

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Progress in technology always brings us a great impact. In these days, the development and spread of the next generation sequencing platform have made the whole genome analysis to be a general experiment. In periodontal pathogens, complete whole genome sequence has been determined from 6 major periodontal bacteria. Our group has been determined the complete whole genome sequence of *Porphyromonas gingivalis* ATCC 33277, type strain, and carried out a genomic comparison with other strains of *Por. gingivalis*. About 20 % of total genes were strain specific ones. They might be closely associated with difference in virulence among *Por. gingivalis* strains. Whole genome comparison also identified the specific features of *Por. gingivalis*. The extensive genomic rearrangements were observed among strains. Many of which have been induced by various mobile genetic elements, such as insertion sequence, conjugative transposon (CTn) and transposon. CTnPg1, identified in *Por. gingivalis* ATCC 33277, is the first intact CTn reported for genus *Porphyromonas*. CTnPg1 can transfer to another strain of *Por. gingivalis*, and also can transfer to the closely related genera *Bacteroides* and *Prevotella*. Several oral anaerobic bacteria have CTnPg1-like CTns. Those suggested the CTnPg1 family is widely distributed in oral and intestinal anaerobic bacteria and plays an important role in horizontal gene transfer among those bacteria and can contribute to genetic diversity. Whole genome sequence shows that *Por. gingivalis* has no well-known secretion systems, type I to VI. The comparison of the whole genome sequences among close related bacteria, we found out a novel secretion system, type IX secretion system (T9SS), in *P. gingivalis* that translocates virulence factors across the outer membrane. T9SS also found in other periodontal pathogen, *Pre. intermedia* and *Tannerella forsythia.* We expected that T9SS might be a good target for new periodontal drug development.

S4-5

Unique Strategic System of *Porphyromonas gingivalis* in Periodontitis

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Gingival epithelial cells function as an innate host defense system to prevent intrusion by periodontal bacteria. Nevertheless, *Porphyromonas gingivalis*, the most well-known periodontal pathogen, can enter gingival epithelial cells and pass through the epithelial barrier into deeper tissues. *P. gingivalis* fimbriae specifically interact with α5β1-integrin of epithelial cells. The bacterium is then subsequently captured by cellular pseudopodia, which enables invagination through the endosomal pathway. Following passage through the epithelial barrier, intracellular *P. gingivalis* impairs integrin-related signaling molecules, paxillin, and focal adhesion kinase, which disables cellular migration and proliferation. Thereafter some intracellular bacteria are sorted to lytic compartments, including autolysosomes and late endosomes/lysosomes, while a considerable number of the remaining organisms are sorted to recycling endosomes, leading to bacterial exit from infected cells to neighboring cells, a mechanism of cell-to-cell spreading in periodontal tissue.

Most Gram-negative bacteria including *P. gingivalis* produce outer membrane vesicles (MVs) which are natural vehicles, or bacterial “bombs,” for directed intercellular transport of bacterial virulence factors into host cells and tissues. *P. gingivalis*-MVs retain a full complement of outer membrane constituents including LPS, gingipains and fimbriae. Although *P. gingivalis*-MVs are suspected to invade various host cells, it is absolutely unknown how *P. gingivalis*-MVs invade the cells. We analyzed the invasion of host cells and cellular impairment by *P. gingivalis*-MVs. The remarkable strategies used by *P. gingivalis* to destruct periodontal tissues will be introduced.

S4-6

Gender-specific Associations of Periodontal Disease with Serum Antibody Titer against *Porphyromonas gingivalis* and Systemic Inflammation

Michiko Furuta

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It has been reported that serum antibody titer against *Porphyromonas gingivalis* (*P. gingivalis*) and systemic inflammation are elevated in individuals with periodontal disease. However, it remains unclear whether they lead to periodontal and systemic deterioration in each gender, as periodontal and systemic status is influenced by gender. The present study investigates the gender-specific probable effects of titer against *P. gingivalis* and systemic inflammation on periodontal disease or systemic health status in a longitudinal study. At two time points over an 8-year period (in 2003 and 2011), 414 individuals (mean age, 58.1 ± 11.0 years, 296 males and 118 females) were surveyed. 81.2% of males with periodontal disease in 2003 and 50.0% of females with periodontal disease in 2003 persisted periodontal disease 8 years later. Among periodontally healthy males in 2003, 25.7% developed periodontal disease 8 years later, while for females, 21.2% developed periodontal disease 8 years later. Poisson regression analyses showed that persistence of periodontal disease 8 years later in females was significantly associated with antibody titer against *P. gingivalis* (prevalence ratio, PR 2.58; 95% confidence interval, CI: 1.05–6.35) and hsCRP (PR 3.59; 95% CI: 1.36–9.51). Levels of hsCRP were significantly related to development of periodontal disease 8 years later in periodontally healthy females in 2003 (PR 3.56; 95% CI: 1.53– 8.29). Males did not have a significant association among titer against *P. gingivalis*, systemic inflammation and periodontal disease. These findings suggest that immune response to *P. gingivalis* infection and systemic inflammation showed a stronger association with periodontal disease in females than in males.


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