Kyudai Oral Bioscience 2016

-Frontiers in Dental Research and Education in East Asia-

February 27th (Sat) 2016

Lecture Theater AB
School of Dentistry Kyushu University

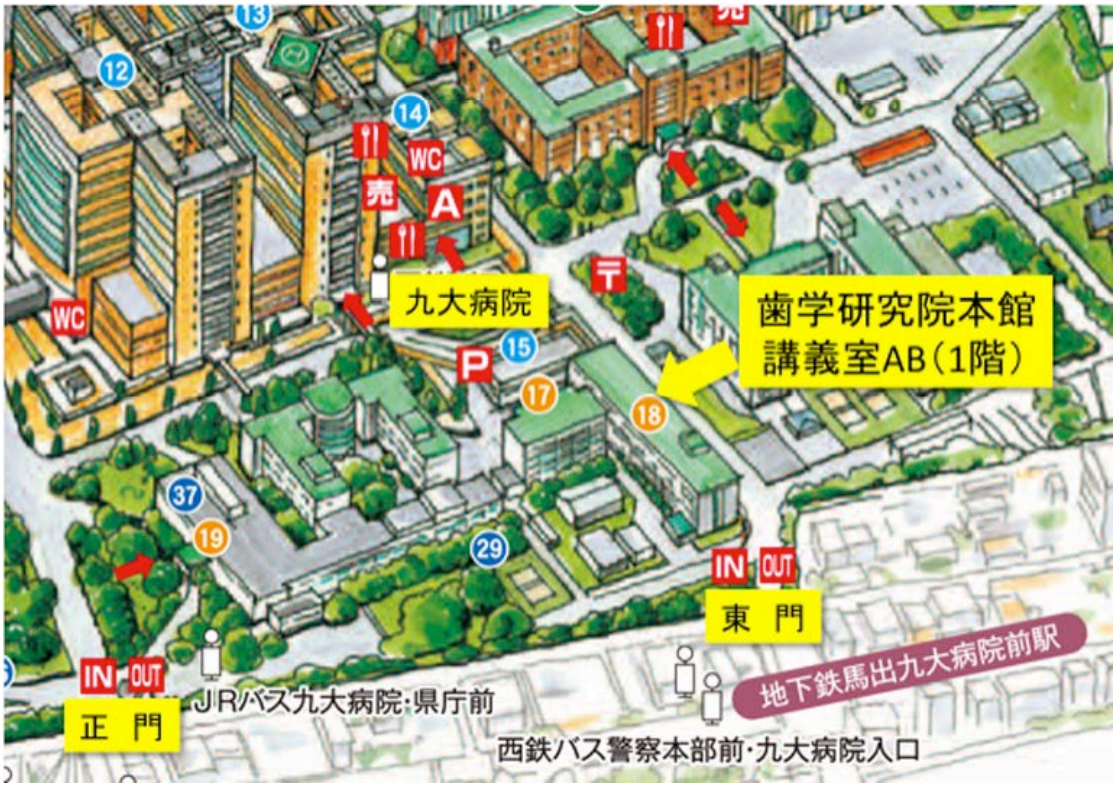
PROGRAM & ABSTRACTS

The School of Dentistry Kyushu University will celebrate its 50th Anniversary in 2017.

○会期：平成 28 年 2 月 27 日（土）　13:00～18:30

○会場：九州大学大学歯学部　講義室 AB（歯学研究院本館 1 階）
　　住所：福岡市東区馬出 1－1－1

○主催：九州大学大学院 歯学研究院



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原田 ゆか　（歯学府 1 年生）

Information for Speakers

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PROGRAM

Kyudai Oral Bioscience 2016 (KOB2016)
-Frontiers in Dental Research and Education in East Asia-

Opening Remarks
13:00～13:10
Hiroshi Nakanishi (Representative organizer of KOB)
Masato Hirata (Dean, Faculty of Dental Science, Kyushu University)

【Session 1】 *Dental Research and Education in Vietnam and Japan*
Chair person：Kazuaki Nonaka (*Section of Pediatric Dentistry*)

13:10～13:35 S1-Special Lecture 1
Ngo Thi Quynh Lan
Recent changes in dental education at the Faculty of Odonto-Stomatology, University of Medicine and Pharmacy Ho Chi Minh City, Vietnam
Faculty of Odonto-Stomatology, University of Medicine and Pharmacy Ho Chi Minh City, Vietnam

13:35～14:00 S1-Special Lecture 2
Hidefumi Maeda
How can we save a severely-damaged tooth?
Department of Endodontology and Operative Dentistry, Faculty of Dental Science, Kyushu University, Fukuoka

14:00～14:10 Coffee Break

【Session 2】 *Dental Research and Education in China and Japan*
Chair Person：Zhou Wu (*Department of Aging Science and Pharmacology*)

14:10～14:35 S2-Special Lecture 3
Wei Xian Yu
The relationship between virulence factors of *Porphyromonas gingivalis* and periodontitis
Department of Oral Biomedicine, College and Hospital of Stomatology, Jilin University

14:35～15:00 S2-Special Lecture 4
Xin Wen Zhang
Dental Education and Career Choice between China and Japan
Center of Implant Dentistry, School of Somatology, China Medical University, Shenyang, China

15:00～15:10 Coffee Break

【Session 3】 *Understanding Patients with Mental Disorders*

Chair Person: Yoshihiro Tsukiyama (*Section of Implant and Rehabilitative Dentistry*)

15:10～15:35　S3-Special Lecture 5
Yasunari Sakai
Rare genetic variations and developmental diseases in childhood”
Department of Pediatrics, Kyushu University Hospital

15:35～16:00　S3-Special Lecture 6
Takahiro Kato
Microglia hypothesis of neuropsychiatric disorders from schizophrenia to dementia translational research
Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University

16:00～16:20　Commemorative Photo & Coffee Break

【PhD Student Session (PSS)】

Chair persons：Fumiko Takayama, Yuka Harada and Reiko Yoshimoto

16:20～16:30　PSS-1
Nguyen Thu Thuy
Salivary oxidative stress biomarkers in chronic periodontitis and acute coronary syndrome
Department of Periodontology, Faculty of Odonto-stomatology, University of Medicine and Pharmacy – Ho Chi Minh City, Ho Chi Minh City, Vietnam

16:35～16:45　PSS-2
Shohei Yoshimoto
Hyper-osmotic stress up-regulates proliferation in squamous cell carcinoma cells
Division of General Oral Clinic, Kyushu University Hospital

16:50～17:00　PSS-3
Fan Zeng
Effect of stimulated microgravity on nerve cells
School of Life Science, Beijing Institute of Technology, Beijing, China

17:05～17:15　PSS-4
Xue Li
Brazilian Green Propolis suppress the *Porphyromonas gingivalis* LPS-induced inflammatory responses in human fibroblasts by Inhibiting NF-*κ*B activation
Department of Oral Biomedicine, College and Hospital of Somatology, Jilin University, China

17:20～17:30　PSS-5
Shinichiro Yoshida
Semaphorin 3A induces odontoblastic phenotype in dental pulp stem cells
Department of Endodontology and Operative Dentistry Faculty of Dental Science, Kyushu University

17:30～17:40　Coffee Break

17:40～17:50　PSS-6
Jie Meng
IL-10 plays an essential role in anti-inflammatory effects of *Rheum tanguticum*, a traditional Tibetan medicine, in activated microglia
Department of Aging Science and Pharmacology, Faculty of Dental Science, Kyushu University

17:55～18:05　PSS-7
Masato Dekita
The critical roles of cathepsin S in *Porphyromonas gingivalis* LPS-induced $CD4^+$ T cell differentiation
Section of Orthodontics and Dentofacial Orthopedics, Faculty of Dental Science, Kyushu University

18:10～18:20　PSS-8
Chieko Arai
Nephronectin, a basement membrane protein, plays critical roles for differentiation and proliferation of dental epithelial stem cells during tooth development
Section of Orthodontics and Dentofacial Orthopedics, Faculty of Dental Science, Kyushu University

18:25～18:35　PSS-9
Soichiro Sonoda
Patient-derived dental pulp stem cells based regeneration of dentin/pulp-complex
Department of Periodontology, Faculty of Dental Science, Kyushu University

Closing Remarks
18:35～18:40

ABSTRACTS

Session 1

Dental Research and Education in Vietnam and Japan

S3-Special Lecture 1

Recent changes in dental education at the Faculty of Odonto-Stomatology, University of Medicine and Pharmacy Ho Chi Minh city, Vietnam

Prof & Dean, Ngo Thi Quynh Lan

[1]Faculty of Odonto-Stomatology, [2]University of Medicine and Pharmacy Ho Chi Minh city, Vietnam

The Faculty of Odonto-Stomatology, University of Medicine and Pharmacy, HoChiMinh city, VietNam has established since 1967, is one of the most highly ranked and oldest dental schools in Vietnam. Its mission is a leading institution in the field of Dental Education and Research through the advancement of dental knowledge and research, the education and training of dental professionals towards excellence and the promotion of oral health to the population in Vietnam. The aim of this presentation to review recent changes in dental education and to orient the revision of current dental curriculum at the Faculty in future.

The first dental curriculum was established in 1971 with a five-year program including one year for pre-dental courses, two years for basic Health Sciences and the last two years embracing all aspects of specialty in dentistry. In 1975, the curriculum was restructured into a six-year program with additional subjects (social sciences and maxillo-facial surgery) leading to the Degree of Doctor in Odonto-Stomatology. In 1979, the first formal educational reform defined the training objectives and the curriculum of the Faculty of Odonto-Stomatology. in 1989, an important reform was done according to the Guidelines of the Ministry of Education and Training in order to implement a curriculum which focused on Certificate-based teaching and Preventive and Community Dentistry. It was called 2.5 + 3.5 program. Then, it was changed 3+3 one in year 2008 and the current program is 2.5 +3.5 now.

In fact, a Mutual Recognition Arrangement (MRA) for Dental Practitioners in the ASEAN countries which will be implemented in the early of 2016 orients not only the Faculty but also the dental schools over Vietnam in challenges how to revise the dental curriculum based on the Competencies of the Dental Professions in country and region. The presentation will also focus on the issue and raise the open discussion to ask experiences from dental educational leaders in the regional integration.

S3-Special Lecture 2

How can we save a severely-damaged tooth?

Hidefumi Maeda

Department of Endodontology & Operative Dentistry, Division of Oral Rehabilitation,
Faculty of Dental Science, Kyushu University

In Japan facing the super aging society, the government promotes the improvement of healthy life expectancy. Preservation of tooth and oral health is one of the goals. Along with this, people desire to preserve their teeth as long as possible. Periodontitis and caries had been known to be as major causes of tooth loss. A recent study in Japan, however revealed that the incidence of fracture leading to tooth extraction was almost the same as that of periodontitis and caries. To save these diseased teeth, development of novel and unique therapies is required. In this context, we have worked on the issue how the severely-damaged teeth, in particular, with deep caries and root fracture could be saved. To solve this problem, we have attempted to approach from the tissue engineering standpoint. In addition to three classical elements such as stem cells, signal molecules and scaffold, we recognize the involvement of neurogenesis and angiogenesis during tissue generation and regeneration. In this symposium, however I would like to talk about the latest topics associated with above three elements together with our data, and clinical trials. By integrating these, we believe to develop a novel dental therapy allowing for longer preservation of such damaged teeth, which will ensure the quality of life in elder people.

Session 2

Dental Research and Education in China and Japan

S3-Special Lecture 3

The relationship between virulence factors of *Porphyromonas gingivalis* and periodontitis

Wei Xian Yu[1], Yi Zhang[1], Aicaho Gao[1], Yanmin Zhou[2]

[1]Department of Oral Biomedicine, [2]Department of Dental Implantology, College and Hospital of Stomatology, Jilin University

Porphyromonas gingivalis (*Pg*) is known as a keystone pathogen that links to the periodontitis onset and progression. The bacteria may release a series of virulence factors to evade host defense mechanisms and impair the host tissue, including lipopolysaccharide (LPS), gingipains, fimbriae, capsule polysaccharide. We have focused on the pathogenicity of virulence factors of *Pg*LPS on the destruction of periodontal connective tissue and alveolar bone resorption. On the other hand, the Eph family is involved in regulating the process of osteoclast and osteoblast coordination in order to maintain bone homeostasis. Therefore, we speculate that Eph family can be involved in the restructure of alveolar bone about periodontitis. We have found that *Pg*LPS increased the expression of EphB4 while inhibiting the expression of EphrinB2. Our results demonstrate the EphB4 receptor on osteoblasts and the EphrinB2 ligand on osteoclasts may generate bidirectional anti-osteoclastogenic and pro-osteoblastogenic signaling into respective cells and potentially facilitate the transition from bone resorption to bone formation when treated with *Pg*LPS of low concentration (treated with 75 ng/ml for osteoblast and osteoclast). We have also found that *Pg*LPS increased the expression of EphA2 in both in osteoblasts and osteoclasts, decreased the expression of osteogenic-related genes and increased the expression of osteoclast-related genes. In this talk, I will review our researches data to show the important roles of Eph family in bone resorption during chronic periodontitis.

These findings may provide information on new targets for prevention and treatment of chronic periodontitis. I will also introduce the dental education of undergraduate and graduate students in our College and Hospital of Stomatology, Jilin University, I hope more the activities of education and researches will be established between our college of Stomatology, Jilin University and the faculty of dental science, Kyushu University, because one of the partner institutions of our two Universities.

S3-Special Lecture 4

Dental Education and Career Choice between China and Japan

Xin Wen Zhang[1], Xu Yan[2]

[1]Center of Implant Dentistry, School of Stomatology, China Medical University, Shenyang, China; [2] The VIP department, School of Stomatology, China Medical University, Shenyang, China.

Dependent on our dental education background in Japan as the foreign students and our dental education career in China as the teachers, we have noticed that there are large differences in dental education and career choice of dental students between China and Japan. Because of the interest, we initiated design self-answered questionnaires and investigate differences and similarities among the dental students in the two nations in 2013. We have found that from the very beginning, the motivations to choose dentistry were highly distinct. Japanese dental students mainly based on the reasons of helping others, achieving self- worth and health-care related interests. On the other hand, most of Chinese students pointed out that their choices were for the financial and prestige. Even approximate one-third of them signified that dentistry were not their original aspiration just regulated from other subjects. During the undergraduate phase, the rate of the satisfaction of teaching faculties in Japan was 3 times compared to China. More than two-thirds Chinese students wondered they wasted too much time in studying foreign language, while there is no English necessary condition for graduation in Japan Higher Education. After graduated, most of Chinese students chosen to be further educated because of the great employment pressure, while the rate of Japanese students was less. Furthermore, more than a half of the graduates prefer work in university hospitals in China, while the majority of Japanese graduates chose to be general dentists and work in dental offices.

In this talk, we will review our dental education and researches in Japan and introduce the dental education in China. I hope the information will help to realize the students' requirement of distinct cultural and social background. As foreign students, and as graduates of dental school, Kyushu University, we will do our best to work as a bridge of dental education between China and Japan.

References:

1. Xu Yan, **Xinwen Zhang**, Yong Shen, Zhe Yi, Lin Wu, Hongjun Ai. Career choice and future plan of Chinese 8-year stomatology medical doctor program students *Journal of the Chinese Medical Association.* (2015) 78: 555-561.
2. Xu Yan, **Xinwen Zhang,** Yohei Jinno, Keishu Tachibana, Jie Gao, Kiyoshi Koyano, Hongjun Ai, Yong Shen. Career choice and future design of dental students in China and Japan. *International dental journal* (2014) 64: 68-75.
3. Xu Yan, **Xinwen Zhang,** Yohei Jinno, Keishu Tachibana, Jie Gao, Kiyoshi Koyano, Yong Shen, Hongjun Ai. Comparison of attitudes towards dental education among dental students in Japan and China. *International dental journal*. (2014) 64:76-82.

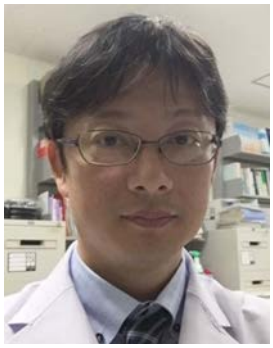
Acknowledgment

The studies are supported by National Natural Science Foundation of China to Xinwen Zhang (NO.81500858).

Session 3

Understanding Patients with Mental Disorders

S3-Special Lecture 5



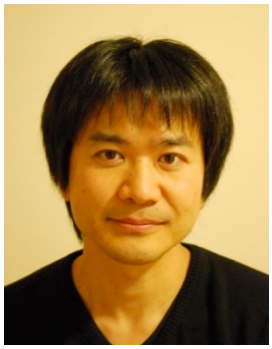
Rare genetic variations and developmental diseases in childhood

Yasunari Sakai, M.D., Ph.D.

Department of Pediatrics, Kyushu University

In the last decade, physicians have intimately collaborated with geneticists to investigate the genetic backgrounds underlying developmental disorders in childhood. In particular, whole-exome sequencing (WES) technologies enable us not only to understand the evolutional process of human genome, but it also provides the chance for identifying rare pathogenic mutations in developmentally challenged children. The latter include copy number alterations and single-nucleotide variations in individuals with autism and other forms of neurodevelopmental disorders. Notably, these genetic variations are known to occur *de novo* in probands at significantly higher rates than healthy individuals. Massive data with the WES strategies for thousands of families in the United States and Europe also revealed that hundreds of genes were likely involved in the pathogenic processes of autism and infantile-onset epilepsies. On the other hand, it remains to be discussed whether the second and third variations in affected individuals may be associated with their atypical or severe phenotypes. I herein present several cases carrying rare mutations and multiple genetic burdens that were considered to cause unusual phenotypes. Experiences from these cases support the concept that genome-wide scanning of rare variations will help identifying common therapeutic targets in future translational medicine.

S3-Special Lecture 6



Microglia hypothesis of neuropsychiatric disorders from schizophrenia to dementia - translational research

Takahiro A. Kato, MD, PhD[1, 2], Masahiro Ohgidani, PhD[1], Motoki Watabe, PhD[3], Shigenobu Kanba, MD, PhD[1]

[1]Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan ,[2]Innovation Center for Medical Redox Navigation, Kyushu University, Fukuoka, Japan, [3]Monash University, School of Business, Sunway, Malaysia

Microglia have been implicated in various neurological and psychiatric disorders in rodent and human PET and postmortem studies. However, the dynamic actions of microglia in the living human brain have not been clarified due to a lack of studies dealing with in situ microglia. We introduce a novel technique for developing induced microglia-like (iMG) cells from human peripheral blood cells. An optimized cocktail of cytokines, GM-CSF and IL-34, converted human monocytes into iMG cells within 14 days. The iMG cells have microglial characterizations; expressing markers, forming ramified morphology, and phagocytic activity with various cytokine releases. Clinical utilities were confirmed by iMG cells from a patient of Nasu-Hakola disease (Ohgidani et al. 2014). On the other hand, how microglial activities contribute to human psychosocial aspects has not been well clarified. We have recently conducted an economic game – trust game – to healthy young males with or without 4-day-treatment of minocycline, an antibiotic with microglial inhibitory effects. Interestingly, minocycline suppressed personality-oriented and/or drive-oriented social behaviors (Watabe et al. 2013). These results have proposed novel psychosocial roles of human microglia.

We believe that the iMG-technique and the pharmacological trials with minocycline promise to elucidate unresolved aspects of human microglia in various neurological and psychiatric disorders.

References

Watabe M*, Kato TA*, Tsuboi S, et al.: Minocycline, a microglial inhibitor, reduces ‘honey trap’ risk in human economic exchange. Scientific Reports, 3, 1685, 2013

Ohgidani M, Kato TA*, Setoyama D, et al.: Direct induction of ramified microglia-like cells from human monocytes: Dynamic microglial dysfunction in Nasu-Hakola disease. Scientific Reports, 4, 4957, 2014

Ohgidani M, Kato TA*, Kanba S: Introducing directly induced microglia-like (iMG) cells from fresh human monocytes: A novel translational research tool for psychiatric disorders. Frontiers in Cellular Neuroscience, 9, 184, 2015

PhD Student Session

PSS-1

Salivary oxidative stress biomarkers in chronic periodontitis and acute coronary syndrome

Thuy T. Nguyen[1,2], Lan Q. Ngo[3], Ananya Promsudthi[4], Rudee Surarit[2]

[1]Department of Periodontology, Faculty of Odonto-stomatology, University of Medicine and Pharmacy – Ho Chi Minh City, Ho Chi Minh City, Vietnam, [2]Department of Oral Biology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand, [3]Department of Dental Basic Sciences, Faculty of Odonto-stomatology, University of Medicine and Pharmacy – Ho Chi Minh City, Ho Chi Minh City, Vietnam, [4]Department of Oral Medicine and Periodontology, Faculty of Dentistry, Mahidol University, Bangkok, Thailand

ABSTRACT

Objectives: The study aimed at evaluating oxidative stress (OS) biomarkers in the saliva of patients with chronic periodontitis (CP) and acute coronary syndrome (ACS) and establishing their correlation to periodontal index and cardiovascular markers.

Methods: The present study enrolled 96 patients divided into four sex and age-matched groups as follows: ACSCP group (with both CP and ACS), ACS group (with ACS only), CP group (with CP only), and group of healthy controls. Plaque index (PI), gingival index, bleeding on probing, probing pocket depth (PPD), and clinical attachment loss were recorded. Cardiovascular markers included serum high sensitivity C-reactive protein and plasma fibrinogen. The levels of OS biomarkers related to lipid, DNA/RNA and protein oxidation were measured in saliva samples using ELISA and spectrophotometric assays.

Results: Salivary OS biomarkers in diseased subjects expressed statistically significant differences in levels compared to healthy controls ($p<0.05$). There were significant correlations between some OS biomarkers and periodontal parameters as well as biomarkers for cardiovascular events ($p<0.05$).

Conclusion: Salivary OS biomarkers could potentially serve as diagnostic tools for cardiovascular and/or periodontal diseases.

PSS-2

Hyper-osmotic stress up-regulates proliferation in squamous cell carcinoma cells

Shohei Yoshimoto[1], Hiromitsu Morita[2], Yoshinori Katakura[3], Miho Matsuda[1], Seiji Nakamura[4], Masato Hirata[1]

[1]Lab. of Mol. & Biochem., Fac. of Dent. Sci., Kyushu Univ., Fukuoka, Japan, [2]Sect. of General Dentistry, Dept. of General Dentistry, Fukuoka Dental College, Fukuoka, Japan, [3]Fac. of Agriculture, KyushuUniv., Fukuoka, Japan, [4]Sect. of Oral and Maxillofac. Oncol., Div. of Maxillofac. Diag. Surg. Sci., Fac. of Dent. Sci., Kyushu Univ., Fukuoka, Japan

Epidermal growth factor receptor (EGFR) is highly expressed and plays an essential role for cancer progression in oral squamous cell carcinoma. Therefore, EGFR is recognized as a molecular target in oral cancer therapy. On the other hand, tumor microenvironment is constantly inflamed, and it could be possible that the hyper-osmotic stress, given by inflammation, is involved in cancer proliferation. Here we report that hyper-osmotic stimulation to human tongue squamous cell carcinoma cells (HSC-3) triggered activation of nuclear factor of activated T-cells 5 (NFAT5), followed by subsequent activation of a regulator protein of N-glycosylation, dolichol phosphate-dependent *N*-acetylglucisamine 1-phospho-transferase (DPAGT1), thus resulting in translocation of EGFR by glycosylation from the cytosol to the plasma membrane.

EGFR were translocated to the plasma membrane by changing medium from normal (D-MEM; 300 mOsM) to hyper-osmotic condition (D-MEM plus 50 mM mannitol; 350 mOsM), as assessed by fluorescent immunocytochemistry and Western blotting analysis. Cell proliferation assays (WST-8 and BrdU uptake) revealed that the growth of HSC-3 cells was accelerated by hyper-osmotic stimulation, but suppressed by CP380736 (10 μM), an EGFR selective inhibitor or genistein (10 μM), a potent tyrosine kinase inhibitor. In addition, EGFR was accumulated in the endoplasmic reticulum assessed by immunofluorescent observation, when cells were cultured in normal osmotic condition, but with low glucose concentration. The accumulation was diminished in hyper-osmotic condition even with low glucose concentration. Furthermore, HSC-3 cells whose NFAT5 was knocked down by shRNA, showed that the events triggered by hyper-osmotic stimulation were significantly suppressed. Interestingly, expression of DPAGT1 was also decreased. Chromatin immunoprecipitation assay (ChIP assay) revealed the binding of NFAT5 to the promoter region of DPAGT1 in hyper-osmotic condition.

These results indicate that NFAT5 participates in HSC-3 cell proliferation by promoting the translocation of EGFR to the plasma membrane, triggered by glycosylation by DPAGT1 in hyper-osmotic condition.

Keywords : EGFR, NFAT5, Hyper-osmotic

PSS-3

Effects of simulated microgravity on neuronal cells

Zeng Fan, Wang Xianghan, Wang Demei, Qing Hong*

School of Life Science, Beijing Institute of Technology, Beijing 100081, China

Microgravity is a crucial factor during space flight that affects the healthy and work efficiency of astronauts. In the present study, we investigated whether simulated microgravity could influence morphology and apoptosis of different regions of brain. Explanted tissues and primary cells were obtained from three regions of neonatal SD rats, namely hippocampus, cerebellum and cortex. After cultured for 7 days at 37 ℃, 95 % of humidity and 5 % CO_2, cultures were randomly divided into control group (Ctr) and simulated microgravity group (sMG). The sMG group was placed on clinostat for rotating and the Ctr group was cultured on the same horizontal line of the clinostat. After treated for 1 day, 7 days and 14 days for both two groups, changes of morphology and apoptosis of the three regions were detected by microscope and flow cytometry. Results showed that after treated with series of time of simulated microgravity, the surface of explanted tissues changed a lot compared to Ctr, especially the edge of tissue blocks became brighter. And the cells within explanted tissues became less. But apoptosis of primary cells of the three regions showed no difference after 1-day and 7-day rotating, while after 14 days of stimulating by simulated microgravity the apoptosis rate decreased dramatically.

Also, human glioma tissues which were offered by Xuan Wu Hospital were cut into explanted tissue blocks. The procedure was totally the same with that of SD rats and results indicated that cytoskeleton was influenced by simulated microgravity.

In conclusion, simulated microgravity had the same impact on morphology and apoptosis of the three regions of brain, which were hippocampus, cerebellum and cortex. And the cytoskeleton of glioma cells could be disorganized in the simulated microgravity environment.

*Correspondence: Qing Hong（QH) hqing@bit.edu.cn

Acknowledgment

This study was supported by the National Key Foundation for Exploring Scientific Instrument of China (2012YQ04014008, 2013YQ03059514).

PSS-4

Brazilian Green Propolis suppress the *Porphyromonas gingivalis* LPS-induced inflammatory responses in human fibroblasts by Inhibiting NF-*κ*B activation

Xue Li[1], Zhou Wu[2], Junjun Ni[2], Hiroshi Nakanishi[2], Yanmin Zhou[1]

[1] Department of Implantology, Jilin University, China, [2] Department of Aging Science and Pharmacology, Kyushu University, Japan

Periodontitis, as a chronic systemic inflammation, has been recognized positive link to cognitive impairment in Alzheimer's disease. Therefore, the management of periodontitis might be one of the approaches for preventing cognitive impairment. Fibroblasts are the resident cells in the connective tissues including gingival, which is the principal source of the extensive extracellular matrix (ECM) throughout the body. In dental area, gingival fibroblasts are consisted of oral epithelium that forming epithelium attachment for teeth as well as the dental implants during the teeth lost. Regulating of gingival fibroblasts are important for the managements of periodontitis and Peri-implantitis, because the gingival fibroblasts are involved in the procedure of inflammatory responses by the mediator production. *Porphyromonas gingivalis* (*Pg*) the keystone oral bacteria, because *Pg* and lipopolysaccharides from *Pg* (*Pg*LPS) are involved in the process of periodontitis as well as Peri-implantitis. Considering the fact that propolis, the nature production from bees, have anti-oxidative and anti-inflammatory effects, we hypothesized that propolis may have protective effects against the *Pg*LPS-induced inflammatory responses in human fibroblasts. In the present study, we firstly demonstrated the expression of Toll-like receptor 2 (TLR2), TNF-α, IL-1β and IL-6 in the gingival fibroblasts of chronic periodontitis patients. In *in vitro* studies, *Pg*LPS (1 μg/ml) increased the mean mRNA levels of TLR2, TNF-α, IL-1β and IL-6 in BJ human fibroblast cell line. The *Pg*LPS-increased the mRNA levels of TLR2 as well as TNF-α, IL-1β and IL-6 in human fibroblasts significantly decreased by pre-treatment with Bay 11-7082 (20 μM), a specific inhibitor of NF-*κ*B. Moreover, propolis significantly decreased the *Pg*LPS-increased NF-*κ*B activation as well as the expression of pro-inflammatory cytokines in human fibroblasts.

These observations provide the first evidence that propolis inhibit the *Pg*LPS-induced inflammatory responses in fibroblasts, thus suggest that propolis may be benefit for management of oral health and dental implants.

Acknowledgment

This study is partly supported by Yamada Research Grant to Zhou Wu (N0.0183), Xue Li is supported by Kyushu University Friendship Scholarships (2015-2016).

PSS-5

Semaphorin 3A induces odontoblastic phenotype in dental pulp stem cells

Shinichiro Yoshida[1], Naohisa Wada[2], Daigaku Hasegawa[3], Atsushi Tomokiyo[3], Sayuri Hamano[3], Hiromi Mitarai[1], Suguru Serita[1], Hiroyuki Mizumachi[1], Hidefumi Maeda[1, 3].

[1]Department of Endodontology and Operative Dentistry, Faculty of Dental Science, Kyushu University, [2]Division of General Dentistry, Kyushu University Hospital, and [3]Department of Endodontology, Kyushu University Hospital

Exisiting pulp capping materials possess low potentials to accelerate the biological activities of dental pulp cells, and have a limited capacity to reconstruct dentin/pulp complexes. Therefore, the development of more effective therapeutic agents for direct pulp capping has been anticipated. Here, we investigated the effects of Semaphorin 3A (Sema3A) on various functions of human dental pulp stem cells (DPSCs) in vitro and reparative dentin formation in a rat dental pulp exposure model in vivo.

The expression of Sema3A and its receptors in human DPSCs was examined by semi-quantitative RT-PCR, immunofluorescence staining and western blotting analysis. The effects of Sema3A on cell migration, chemotaxis, proliferation and odontoblastic differentiation of DPSCs were examined by scratch wound healing assay, transwell assay, WST-1 proliferation assay, quantitative RT-PCR and Alizarin Red S staining, respectively. The effect of Sema3A application by direct pulp capping on reparative dentin formation was assessed using a rat pulp exposure model. Nuclear accumulation of β-catenin, the expression of *FARP2* gene and activated Rac1 in Sema3A-treated DPSCs were examined by immunofluorescence staining, western blotting analysis, quantitative RT-PCR and Rac1 pull-down assay, respectively.

Sema3A and its receptors were expressed in rat dental pulp tissues and human DPSCs. Sema3A promoted cell migration, chemotaxis, proliferation and odontoblastic differentiation of human DPSCs. In a rat pulp exposure model, Sema3A facilitates reparative dentin formation with dentin tubules and well-aligned odontoblast-like cell layer after direct pulp capping treatment. Exogenous application of Sema3A to human DPSCs increased nuclear accumulation of β-catenin, and also upregulated the expression levels of *FARP2* gene and activated Rac1.

Sema3A plays crucial roles in various events associated with dentin regeneration, probably via canonical Wnt/β-catenin signaling. Although further study is needed, Sema3A might be an alternative agent for direct pulp capping.

PSS-6

IL-10 plays an essential role in anti-inflammatory effects of *Rheum tanguticum,* a traditional Tibetan medicine, in activated microglia

Jie Meng[1], Aiqin Zhu[2], Hiroshi Nakanishi[1], Zhou Wu[1]

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Over the past decade, numerous lines of evidence have strongly suggested that chronic neuron-inflammation characterized by the release of pro-inflammatory mediators from hyper-activated microglia plays a critical role in the pathogenesis of cognitive dysfunction associated with Alzheimer's disease (AD). Basic researches provide substantial evidence that the development of agents that can regulate microglial activation has been suggested as one potential strategy for the treatment or prevention of cognitive dysfunction associated with AD. *Rheum tanguticum* Maxim. ex Balf., a traditional Tibetan medicine, is known to exhibit various bioactivities, including anti-bacteria, anti-inflammatory and anti-oxidant activities. Therefore, *Rheum tanguticum* may have the potential to be used as pharmacological therapeutic agent in treatment with AD. In this study, we have thus attempted to elucidate effects of *Rheum tanguticum* on pro-inflammatory effects in activated microglia. *Rheum tanguticum* significantly inhibited the mRNA expression of pro-inflammatory molecules, including interleukin-1β, tumor necrosis factors-α and inducible nitric oxide synthase in microglia after treatment with chromogranin A, an endogenous microglial activator. This anti-inflammatory effect of *Rheum tanguticum* was suppressed by the inhibition of the NF-κB and STAT-1 activation pathways. Interestingly, *Rheum tanguticum* significantly increased the mRNA expression of interleukin-10 (IL-10), an anti-inflammatory cytokine with important roles in preventing inflammation, even in non-stimulated microglia. Furthermore, *Rheum tanguticum* significantly increased the mRNA expression of myocyte enhancer factor 2D (MEF2D), a potential transcription factor of *IL-10* gene, in microglia.

These results suggest that *Rheum tanguticum* has anti-inflammatory effects in activated microglia through increased expression of IL-10.

Key words: microglia, *Rheum tanguticum*, anti-inflammatory effects, IL-10, MEF2D

PSS-7

The critical roles of cathepsin S in *Porphyromonas gingivalis* LPS-induced $CD4^+$ T cell differentiation

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【**Background and Objective**】There is accumulating evidence that periodontitis is exacerbating risk factor for numerous of diseases, including Alzheimer's disease. Low-grade chronic systemic inflammatory responses associated with periodontitis may activate primed or senescent microglia, which subsequently provoke an exaggerated neuroinflammation, which may lead to the cognitive impairment. It is considered that oral bacteria inducing periodontal disease and their virulence factors can amplify the host immune responses after entering systemic circulation. *Porphyromonas gingivalis* (*Pg*) is well known as a keystone bacterium in periodontitis because it mainly responsible for the onset and progression of periodontitis. Therefore, we hypothesize that lipopolysaccharide from *Pg* (*Pg*LPS) may amplify the host immune responses in the spleen, the largest lymphatic organ, because we have recently reported that the spleen is involved in the amplification of abnormal chronic pain through cathepsin S (CatS)-dependent differentiation of $CD4^+$ helper T cells (Zhang et al., J. Neurosci., 2014). In the present study, we have thus attempted to clarify possible roles of CatS in *Pg*LPS-induced immune responses in the spleen.

【**Methods and Results** 】In the *in vivo* experiments, a significant hypertrophy of the spleen was found in the wild-type (female DBA mice with12-week old), but not in $CatS^{-/-}$ mice, after systemic treatment with *Pg*LPS (100 μg/mg/day, i.p.) for 7 consecutive days. Rather surprisingly, $CD11c^+$ dendritic cells (CDs), which expressed IL-6, were significantly increased in the spleen of wild-type, but not $CatS^{-/-}$, mice. In the i*n vitro* experiments, *Pg*LPS (1 μg/ml, 24 hs) induced the IL-6 production in DCs acutely isolated from the spleen of wild-type, but not $CatS^{-/-}$, mice. The IL-6 production after treatment with *Pg*LPS was completely suppressed by a pretreatment with Z-Phe-Leu-COCHO (1μM), a selective inhibitor of CatS.

【**Conclusion**】These observations indicate that CatS plays a critical role in the differentiation of Th17 cell in the spleen following chronic systemic treatment with *Pg*LPS. Further studies are needed to elucidate the precise mechanism underlying CatS-mediated IL-6 production in DCs, because IL-6 is the necessary factor for the differentiation of Th17 cells.

Key words: *Porphyromonas gingivalis*, LPS, cathepsin S, dendritic cells, IL-6, Th17 cells

PSS-8

Nephronectin, a basement membrane protein, plays critical roles for differentiation and proliferation of dental epithelial stem cells during tooth development.

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Tooth morphogenesis is initiated by reciprocal interactions between epithelial and mesenchymal cells. Basement membrane (BM) lying between epithelia and mesenchyme plays important roles for those signaling interactions. To identify the functions of BM in tooth development, we focused on an extracellular matrix protein, Nephronectin (Npnt), which is highly expressed in organs of which development is largely dependent upon epithelial-mesenchymal interactions.

In RT-qPCR assay, Npnt is highly expressed in tooth, lung and kidney during their embryonic morphogenesis, especially, strongly during the early stages of tooth morphogenesis. It is reported that Npnt deficient mice showed kidney agenesis, suggesting that Npnt is also important for tooth development. As we expected, the size of tooth germ was significantly smaller than that of the controls in presence of Npnt siRNA in organ culture system. Immunohistochemistry showed that Npnt localized in BM in E11, E13 and E14 molar tooth germ. Near to the cervical loop of P1 incisor, immunostaining of Npnt showed inversed expression pattern of Sox2, which is dental epithelial stem cell marker. Consistently, number of Sox2 positive (Sox2+) cells was significantly reduced by overexpression of Npnt, while the proliferation increased *in vitro*.

These results indicated that Npnt localizes in BM, and regulates the differentiation and proliferation of SOX2+ dental epithelial cell during tooth development.

PSS-9

Patient-derived dental pulp stem cells based regeneration of dentin/pulp-complex

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Since dental pulp tissue with irreversible pulpitis lacks self-regenarative ability, it is usually completely removed and replaced by artificial materials. The teeth that received pulpectomy lose their physiological bioactivity, including strength, sensitivity and immune defense, and many cases ultimately require extraction because of fractures or secondary caries. Therefore reconstruction of dentin/pulp-complex is considered as an ideal treatment for pulpectomized teeth. Autologous transplantation of human dental pulp stem cells (DPSCs) is promising treatment to reconstruct dentin/pulp-complex because of their capacity to regenerate dentin. Unfortunately, it is very limited opportunity to isolate healthy DPSCs from patients. Therefore, irreversible pulpitis-derived dental pulp stem cells (IP-DPSCs) are considered to be useful resource for autologous DPSCs based dentin/pulp regenerative therapy. We succeeded in isolating IP-DPSCs from ectomized dental pulp tissues which was diagnosed as irreversible pulpitis. IP-DPSCs expressed lower stem cell properties, immunosuppressive functions and dentin/pulp regenerative capacity in comparison with healthy DPSCs.

In this study we attempted to improve these failure functions of IP-DPSCs to enable dentin/pulp-complex regeneration. In vitro pre-treatment with interferon-gamma (IFN-γ) improved *in vivo* dentin/pulp regeneration of IP-DPSCs when they were implanted with hydroxyapatite/tricalcium phosphate carrier under the dorsal skin of immunocompromised mice. Moreover, pre-treatment with IFN-γ improved immunosuppressive function of IP-DPSCs. To confirm whether IP-DPSCs can be applied dentin/pulp regenerative therapy, we transplanted in vitro IFN-γ pre-treated IP-DPSCs into root canal of human extracted teeth and transplanted them with hydroxyapatite/tricalcium phosphate carrier under the dorsal skin of immunocompromised mice. After 8 weeks, transplanted tissues showed dentin/pulp-complex like structure. IFN-γ pre-treated IP-DPSCs expressed enhanced capacity to form newly dentin like structure on the surface of human dentin.

These findings suggest that IFN-γ pre-treatment is effective method to improve impaired function of IP-DPSCs. Moreover, IP-DPSCs are promising resource for DPSCs based dentin/pulp-complex regenerative therapy.