Kyudai Oral Bioscience 2015

—9th International Symposium—

KOB 2015

February 28th, 2015

Fukuoka Recent Hotel, Fukuoka , Japan

PROGRAM & ABSTRACTS

Kyushu University Faculty of Dental Science

〇会期：平成 27 年 2 月 28 日（土）　9:00～13:30

〇会場：福岡リーセントホテル 舞鶴の間 A（２階）：下図参照
住所：福岡市東区箱崎 2-52-1
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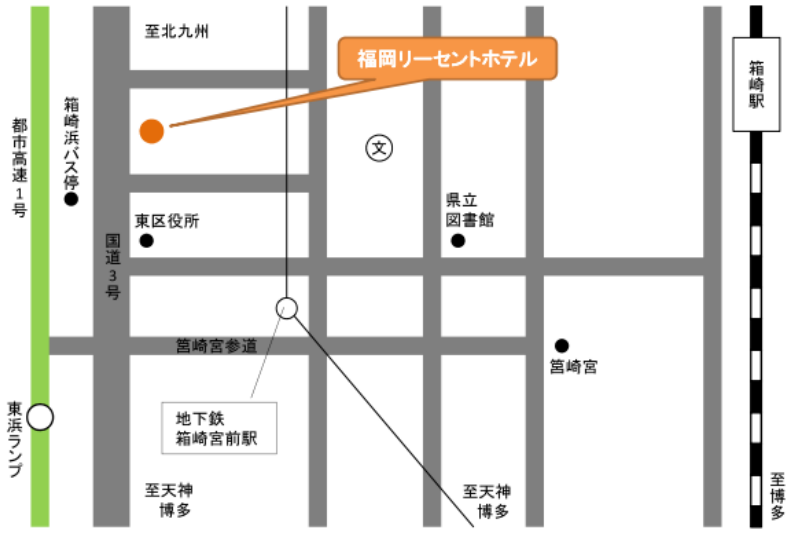
〇主催：九州大学大学院 歯学研究院

〇共催：九州大学歯学会



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PROGRAM

February 28 (Sat) *MAIZURU* Room A (2nd Floor)

Opening remarks

09:00 - 09:05 **Hiroshi Nakanishi (*vice Dean*)**
09:05 - 09:10 **Akifumi Akamine (*Dean, Faculty of Dental Science, Kyushu University*)**

■Session 1: Keynote Lecture

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

09:10 - 10:00 **Andy Y-T. Teng**
Discovery and doubt: a prologue of my quest to research and realism in life
Center for Osteoimmunology & Biotechnology Research (COBR), College of Dental Medicine, Kaohsiung Medical University and the Affiliated Hospital, Kaohsiung, Taiwan

10:00 - 10:10 **Coffee Break**

■Session 2: Student & PhD Student Session

Chairperson: **Yoko Hashimoto (*Department of Periodontology*)**
Fumiko Takayama (*Department of Aging Science and Pharmacology*)

10:10 - 10:15 **S2-1 Mizuki Abe***
Immunocytochemical detection of glucose-related protein 78 in oral squamous cell carcinoma cell lines
Laboratory of Oral Pathology, Faculty of Dental Science, Kyushu University
*(*MA is a 4th grade undergraduate student of Kyushu University School of Dentistry)*

10:17 - 10:22 **S2-2 Katsuhiro Furusho***
The expression of Netrin-1 and its receptor DCC in oral squamous cell carcinoma
Laboratory of Oral Pathology, Faculty of Dental Science, Kyushu University
*(*KF is a resident of the Section of Dentistry in Kyushu University Hospital)*

10:24 - 10:29 **S2-3 Pai-Shien Liao**
The clinical efficacy of triclosan-containing toothpaste on the anti-plaque and anti-gingivitis scores: a randomized single-blind clinical study
Center for Osteoimmunology & Biotechnology Research (COBR), College of Dental Medicine, Kaohsiung Medical University and the Affiliated Hospital, Kaohsiung, Taiwan

10:31 - 10:41 **S2-4 Hideki Sugii**

Effects of Activin A on the phenotypic properties of human periodontal ligament cells.

Department of Endodontology and Operative Dentistry, Faculty of Dental Science , Kyushu University

10:43 - 10:53 **S2-5 Kyosuke Toyoda**

Grp78 is essential for cell migration induced by amelogenin in a human periodontal ligament stem/progenitor cell line.

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

10:55 - 11:05 **S2-6 Yoko Tomita**

Strain dependent response of osteocytes in peri-implant bone

Section of Implant and Rehabilitative Dentistry, Division of Oral Rehabilitation, Faculty of Dental Science , Kyushu University

11:07 - 11:17 **S2-7 Miho Ohta**

DNA microarray analysis of salivary glands involved in IgG4-related disease.

Section of Oral and Maxillofacial Oncology , Kyushu University

11:17 - 11:27 **Coffee Break**

11:27 - 11:37 **S2-8 Reona Aijima**

The role of thermosensitive TRP channel in oral wound healing

Department of Molecular Cell Biology and Oral Anatomy, Graduate school of Dental Science, Kyushu University

11:39 - 11:49 **S2-9 Dan Li**

Characterization of a new target *Porphyromonas* sp. in dental caries prevention

Section of Preventive and Public Health Dentistry, Division of Oral Health, Growth and Development, Faculty of Dental Science , Kyushu University

11:51 - 12:01 **S2-10 DaGuang Wang**

Hetero-oligomerization of C2 domains of phospholipase C-related but catalytically inactive protein and synaptotagmin-1

Laboratory of Molecular and Cellular Biochemistry, Faculty of Dental Science , Kyushu University

12:03 - 12:13 **S2-11 Junjun Ni**

Cathepsin B deficiency inhibits hypoxic ischemia–induced neuronal damage through promoting the resolution of neuroinflammation by M2-like polarization of microglia/macrophages

Department of Aging Science and Pharmacology , Kyushu University

12:15 - 12:25 **S2-12 Yi Zhao**

The selection of the enteral nutritional support route in neurological intensive patients

Department of General surgery, Department of Neurosurgery, Peking Union Medical College Hospital, Beijing, China

12:27 - 12:37 **S2-13 Dong-Joon Lee**

Gastrin releasing peptide (GRP) expression and its effect to morpho-differentiation of mouse incisor

Division in Anatomy and Developmental Biology, Department of Oral Biology, Oral Science Research Center, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul, Korea

12:39 - 12:49 **S2-14 Qinghuang Tang**

The role of region-distinctive expression of Rac1 in regulating fibronectin arrangement during palatal shelf elevation

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

12:51 - 12:56 **Closing Remarks**

ABSTRACTS

Session 1: Keynote Lecture

Discovery and doubt:
a prologue of my quest to research and realism in life

Andy Y-T. Teng

Center for Osteoimmunology & Biotechnology Research (COBR),
College of Dental Medicine, Kaohsiung Medical University
and the Affiliated Hospital, Kaohsiung, Taiwan

Researchers are supposed and expected to create new knowledge and useful information in so-called discovery, as their mission on the jobs, where their outcome is uniquely situated in society and ultimately to the human history. Say: what are and have been the biggest gap(s) in knowledge of life (& our lives)? How about the fact, the factual, the real, the realism or the truth? What sort of discovery (& doubt in questions) that can, or ultimately would, be so fundamentally different or real that will lead to the fact …. truth, and thus will stun and change our futures in life?! Despite, we know better now!? Our living world (the Earth) did not exist at the time-period of The Big-Bang until about 7.9~8 billion years ago; so, if we haven't yet identified the beast in the woods at least we can detect and measure its footprints [2014-Scientific: Dark matter and energy, which we know nothing about, amount to 95% of all the matter and energy in the observable multiverse that has existed].

So, it was started in 1986 after a DDS training and mandatory military services, where my journey was beefed-up by Knowing-Nothing for a simple Quest-to-Research in Chicago, along with oral biology/pathology, then periodontology, immunology, till osteoimmunology & bone biology, etc., as I did. The factual was that a quarter of a century passed, through which a few things have been quested in the mind-sets via time (why did what I did? what value(s) defined what I did?): a) what we care for (i.e., desire for, etc.) often qualify, define and constrain what we are capable of in life (i.e., doctor & patient, treatments & diseases, and the research, too, etc.); b) what values used (in life) to measure the footprints (like the beast in the woods) determine what (& who) we are; c) life is a means to end in itself, not to achieve the purpose in life; thereafter, d) lacking the inner values (of our mindsets, in/with our beliefs) often de-ameliorate us and our life itself. Lastly, e) through the ups-and-downs, the hurtles, and all others I have learned and the not-yet-learned…, this Quest-to-Research was chosen as itself has realized the inner values of myself and the life as I have continued to proceed to further Doubts (more) and Discoveries (less) ahead of me before the next. So, in this presentation, I will share these experiences from the beginning Quest-to-Research till the next realism to come. After all, the presenter thanks you all and appreciates for the prestige and this opportunity of sharing being offered!!

Brief biography:

Andy Y-T. Teng is currently a Professor and Director at the Center for Osteo-immunology & Biotechnology Research (COBR), College of Dental Medicine, Kaohsiung Medical University (KMU), Kaohsiung, Taiwan, and also at the Lab. of Microbial Immunity, Div. of Periodontology, Eastman Institute for Oral Health (EIOH), University of Rochester, Rochester, USA. He has held a DDS from KMU, Taiwan and a DMD from University of Rochester, New York, USA, along with MS of Experimental Pathology from Loyola University of Chicago, USA, and a PhD. of Immunology from University of Toronto, Canada, through which Teng acquired his full-time academic faculty appointments and career for over 24 years in North America.

His specialties lie on Periodontology, Pathology and General Dentistry for over 20 years, with much focused research interests on the immune-regulation of T-&-bone cells' interactions & cytokine biology, experimental models of infectious diseases and molecular cell biology, PCR-based diagnostics, and has been involved in the editorial services of several scientific journals (i.e., JDR, etc.) and for over 40 scientific & professional journals world-wide in the last twenty years.

Prof. Teng has received several international research awards such as the Career-Scientists Award of Ontario, Canada, International Research Prize of 2003 by the International Congress of Bone and Mineral Tissue Research, Austria, and 2006-IADR/GSK International -"Innovation Oral Care Research Award". Also, Teng has involved in pioneering the field of osteo-immunology and been working with colleagues on the molecular & cellular mechanisms of periodontal diseases, arthritis and osteoporosis as the future therapies for clinical applications.

Session 2:
Student & PhD Student Session

S2-1

Immunocytochemical detection of glucose-related protein 78 in oral squamous cell carcinoma cell lines

Mizuki Abe*, Kengo Nagata, Akiko Jinno, Tamotsu Kiyoshima

Laboratory of Oral Pathology, Faculty of Dental Science, Kyushu University
*(*MA is a 4th grade undergraduate student of Kyushu University School of Dentistry)*

S2-2

The expression of Netrin-1 and its receptor DCC in oral squamous cell carcinoma

Katsuhiro Furusho*, Hiroko Wada, Yurie Mikami, Tamotsu Kiyoshima

Laboratory of Oral Pathology, Faculty of Dental Science, Kyushu University
*(*KF is a resident of the Section of Dentistry in Kyushu University Hospital)*

S2-3

The clinical efficacy of triclosan-containing toothpaste on the anti-plaque and anti-gingivitis scores: a randomized single-blind clinical study

Pai- Shien Liao*[1] & Andy Y-T. Teng[1]

[1.] Center for Osteoimmunology & Biotechnology Research (COBR), College of Dental Medicine, Kaohsiung Medical University and the Affiliated Hospital, Kaohsiung, Taiwan

Background: Triclosan has been shown to mediate certain anti-microbial effects and modulation of the immune responses. To assess its clinical efficacy, we rationalized and designed a single-blinded randomization clinical study based on its effects. **Methods:** Sixty male and female subjects with gingivitis and a minimal of 17 teeth present, who have met the inclusion and exclusion criteria, are recruited and consented as well, based on IRB protocols with approval. Subjects are examined at baseline for the oral indexes including Loe & Silness (**GI**) and a plaque index (Turesky modified Quigley & Hein (**PI**). Subjects are informed of possible adverse reactions that they could experience, such as oral irritations. Then, all subjects are blinded and randomly subjected to receive a Tricosan-containing toothpaste or another tooth-paste containing separate control ingredients without Triclosan. All subjects are instructed with standardized protocol to brush their teeth twice daily for 6 weeks. At the end of 6 weeks, an exit-examination is carried out again for GI & PI, through which a two factor ANOVA will be used to determine if the differences between the above treatments are significant and the differences are considered significant if a 95% confidence level is achieved. **Summary**: The present study is ongoing without the results yet; however, it is anticipated that certain tooth-paste ingredients like triclosan, in its technology under test, is to manifest certain anti-microbial effects in human subjects with gingivitis for at least a short-term 6-week effect. **Note**: The study has been funded by Center for Osteoimmunology & Biotechnology Res (COBR), College of Dental Medicine, Kaohsiung Medical University, and a pilot fund from the Colgate-Palmolive Co., Global & Taiwan divisions (S102023).

S2-4

Effects of Activin A on the phenotypic properties of human periodontal ligament cells

Hideki Sugii[1], Hidefumi Maeda[2], Atsushi Tomokiyo[2], Naohisa Wada[2], Satoshi Monnouchi[1], Daigaku Hasegawa[1], Sayuri Hamano[1], Asuka Yuda[1], Shinichiro Yoshida[1], Suguru Serita[1], Hiroyuki Mizumachi[1],Hiromi Mitarai[1], Akifumi Akamine[1,2].

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

Activin A (ACTA) that is a member of the TGF-β superfamily and a dimer of inhibinβa contributes to tissue healing. It was reported that ACTA exerts an inhibitory effect on osteoblastic differentiation and mineralization. However, the expression and function of ACTA in human periodontal ligament cells (HPDLCs) have not been reported. Thus, we aimed to analyze the biological effects of ACTA on HPDLCs.

HPDLCs were isolated from healthy third molars. HPDLCs were cultured with or without ACTA in the presence or absence of $CaCl_2$ that was used for inducing osteoblastic differentiation. The cells were then subjected to Alizarin red S staining and von Kossa staining. Gene expressions were evaluated by quantitative RT-PCR, and protein expressions were investigated by immunofluorescence (IF) and immunochemical (IC) analyses. Chemotaxis, migration and proliferation of HPDLCs treated by ACTA were also examined using modified Boyden Chamber assay, scratch wound healing assay and WST-1 proliferation assay, respectively.

IC analysis with an anti-ACTA antibody revealed positive staining in HPDLCs and rat PDL tissue. When rat PDL tissue was experimentally damaged, IF results showed that positive reactions against both anti-ACTA and anti-IL-1β antibodies were up-regulated around the damaged sites where co-localized detection was seen. ACTA promoted chemotaxis, migration and proliferation of HPDLCs. Quantitative RT-PCR data showed that this protein induced the expression of genes related to fibroblastic differentiation, but inhibited the osteoblast-related gene expression in HPDLCs. ACTA furthermore inhibited mineralization of $CaCl_2$-treated HPDLCs.

Our results suggested that ACTA was expressed in the entire PDL tissue at low levels while its expression was promoted during the early phase of damaged PDL tissue healing. In addition, ACTA could promote proliferation, migration, chemotaxis and fibroblastic differentiation of PDL cells whereas inhibited their osteoblastic differentiation. This protein might have the role to prevent ectopic PDL tissue ossification by blocking osteoblastic differentiation of PDL cells. Therefore, ACTA could be applied as a therapeutic factor that promotes healing and regenerating of PDL tissue damaged by disease, trauma or surgical reconstruction.

S2-5

Grp78 is essential for cell migration induced by amelogenin in a human periodontal ligament stem/progenitor cell line.

Kyosuke Toyoda, Takao Fukuda, Terukaze Sanui, Kensuke Yamamichi, Ryo Atomura, Urara Tanaka, and Fusanori Nishimura.

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

Objective

The major component of enamel matrix proteins, amelogenin is a potential bioactive molecule for periodontal regeneration, although the downstream target molecules and/or signaling are still unknown. Recently, we performed proteomics analysis to elucidate amelogenin-interacting networks. We identified Grp78 as a new amelogenin binding protein, and the biological interaction of amelogenin with this molecule enhanced cell proliferation in osteoblastic cells (Fukuda et al, 2013). In this study, since specific migration and proliferation of periodontal ligament cells (PDL) play key role in successful periodontal regeneration, we evaluated the biological interaction between amelogenin and Grp78, and its effect on cellular responses in periodontal ligament (PDL) cells.

Method

An established multipotent clonal human PDL stem/progenitor cell line (PDLSCs), 1-17, was used. GST pull down assay and confocal co-localization were performed to validate the binding of recombinant amelogenin (rM180:10μg/mL) with endogenous Grp78. Grp78 was overexpressed or knock-downed in PDLSCs for further characterization of these molecular interactions to observe gene expression profiles (microarray, pathway, and heatmap analysis), cell motility (wound healing and boyden chamber assay), cell viability (WST-8, Ki-67 positive staining), Rho signal transductions (Rho family activation), and morphological changes (confocal microscopy).

Result

Internalization of rM180 via binding to the cell surface Grp78 was observed in PDLSCs. Microarray analysis demonstrated that rM180 activates TGF-β pathway, and Grp78 altered the expression of several cell migration related genes. Overexpression of Grp78 enhanced rM180-induced cell migration and adhesion without affecting cell proliferation, while silencing of Grp78 diminished these activities. Binding of rM180 with Grp78 promoted lamellipodia formation, and the simultaneous activation of Rac1 was observed.

Discussion

The results suggested that amelogenin binding with Grp78 is involved in the regulation of cell migration, moreover, the Rac1 activation and lamellipodia formation are critical steps for amelogenin-induced cell migration.

S2-6

Strain dependent response of osteocytes in peri-implant bone

Yoko Tomita[1], Yasuko Moriyama[1], Yasunori Ayukawa[1], Kosaku Kurata[2], Takanobu Fukunaga[2], Hiroshi Takamatsu[2], Kiyoshi Koyano[1]

[1] Section of Implant and Rehabilitative Dentistry, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University

[2] Department of Mechanical Engineering, Faculty of Engineering, Kyushu University

Osteocytes are thought to be mechanosensors and responsible for orchestrating bone remodeling process. However, little is known about how osteocytes react to mechanical stress received from dental implant. The purpose of this study was to investigate the effects of strain-induced biochemical response of osteocytes in vitro.

We established two types of loading apparatuses, in which MLO-Y4 osteocyte cell line was three-dimensionally cultured, subjected to the application of cyclic mechanical stress in physiological strain for 24 hours. One could simultaneously apply five kinds of (from 0.095% to 1.22%) stretching strains and the other could reciprocate titanium plate, which imitated dental implant screw shape, in the cell-embedded gel. Cyclic physiological strain applied by both devices induced the cell death of MLO-Y4. Real-time RT-PCR analyses showed that the mechanical strain increased RANKL/OPG ratio and suppressed sclerostin expression in a magnitude-dependent manner. Connexin43 expression increased in the middle strain magnitude. The conditioned medium obtained from low magnitude cyclic stretch culture increased ALP activity of bone marrow cell culture.

These studies suggest that osteocytes may respond to strain from implant and coordinate peri-implant bone remodeling.

S2-7

DNA microarray analysis of salivary glands involved in IgG4-related disease.

Miho Ohta, Masafumi Moriyama, Akihiko Tanaka, Takashi Maehara, Sachiko Furukawa, Shintaro Kawano, Jun-Nosuke Hayashida, and Seiji Nakamura

Section of Oral and Maxillofacial Oncology, Kyushu University

Objectives. IgG4-related disease (IgG4-RD) is a systemic disease characterized by infiltration of IgG4-positive plasma cells with severe fibrosis in multiple target organs. Our previous reports demonstrated that helper T type 2 (Th2) cells are involved in IgG4 production of IgG4-RD. Moreover, Watanabe et al. reported that abnormal innate immune responses might enhance Th2 immune responses and the immunopathogenesis of IgG4-RD. In this study, we thus addressed to identify the disease-associated genes, especially innate immune molecules, using DNA microarray.

Methods. Gene expression was analyzed by DNA microarray in submandibular gland (SMG) from patients with IgG4-RD (n=3) and healthy controls (n=3). Differentially expressed genes (DEGs) between the two groups were identified, and gene-annotation enrichment analysis was performed by using Gene Ontology (GO) annotation. Validation of the results was performed by quantitative PCR and immunohistochemistry using salivary glands from IgG4-RD (n=7), Sjögren's syndrome (n=10), and healthy controls (n=10).

Results. Gene expression patterns in IgG4-RD and healthy controls were quite different with each other in hierarchical clustering as well as principal component analysis. In IgG4-RD, 450 up-regulated genes and 732 down-regulated genes were identified as DEGs (adjust p-value <0.01). GO term analysis indicated that the up-regulated genes of DEGs in IgG4-RD encoded proteins that function in T/B cell activation, and chemotaxis. PCR validated significantly higher expression of "macrophage receptor with collagenous structure (MARCO)" in IgG4-RD than that in controls (p<0.001). MARCO belongs to Class A scavenger receptor and expressed on "alternatively activated (M2) macrophages" which is activated by Th2 cytokines can induce wound healing and fibrosis. Immunohistochemical analysis confirmed that the number and the frequency of M2 macrophages in IgG4-RD were significantly higher than those in the other groups.

Conclusion. MARCO was identified as a disease-associated gene and might be critically important in elucidating the relationship between innate immunity and the pathogenesis of IgG4-RD.

S2-8

The role of thermosensitive TRP channel in oral wound healing

Reona Aijima[1,2,3], Bing Wang[1], Tomoka Takao[1], Hiroshi Mihara[4], Makiko Kashio[4], Yasuyoshi Ohsaki[1], Jing-Qi Zhang[1], Sadahiko Masuko[2], Makoto Tominaga[4], Mizuho A. Kido[1]

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[2]Department of Oral and Maxillofacial Surgery, Faculty of Medicine, Saga University
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Oral cavity, the entrance to the alimentary tract, experiences drastic changes of temperature, chemical or mechanical stimuli, compared with other tissues. Because of this, oral mucosa is susceptible to injury, but it is known to provide faster wound healing than the skin and less scar formation. However, the molecular bases that regulate this wound healing are still unclear.

Recently, there has been accumulating evidence that thermosensitive transient receptor potential (TRP) channels contribute to not only environmental sensors but also physiological functions. We found TRP vanilloid 3 (TRPV3), a thermosensitive Ca^{2+}-permeable channel activated by warm temperatures (above 33°C), was functionally expressed in oral epithelia. Using a molar tooth extraction model, TRPV3-deficient (TRPV3KO) mice showed delayed wound closures compared with those in wild-type (WT) mice. Furthermore, prominent up-regulation of TRPV3 mRNA was found in the wounded tissues at an early stage of wounding. TRPV3 agonist stimulation or temperature above 33°C to primary cultured oral epithelial cells enhanced the number of proliferating cells. In addition, the activation of TRPV3 increased phosphorylation of epidermal growth factor receptor (EGFR) in the cells from WT, which was not found in the cells from TRPV3KO.

These results suggest TRPV3 in oral epithelia promotes the proliferation of oral epithelial cells and contributes to rapid wound repair via EGFR phosphorylation. Our findings suggest that TRPV3 activation could be a potential therapeutic target for wound healing in skin and oral mucosa.

Aijima R, Wang B, Takao T, Mihara H, Kashio M, Ohsaki Y, Zhang JQ, Mizuno A, Suzuki M, Yamashita Y, Masuko S, Goto M, Tominaga M, Kido MA. FASEB J. 2015 29(1):182-92.

S2-9

Characterization of a new target *Porphyromonas* sp. in dental caries prevention

Dan Li, Yukie Shibata, Toru Takeshita, Yoshihisa Yamashita

Section of Preventive and Public Health Dentistry, Division of Oral Health, Growth and Development, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

Dental caries forms by cariogenic virulent bacteria through complex interactions among oral microbiota. Although the causative agents of dental caries have been extensively studied, little attention was given to complex and forming process of healthy plaque. Our previous study with pyrosequencing analysis indicated that the prevalence ratio of an oral taxonomic unit (OTU) assigned to genus *Porphyromonas* showed a higher tendency in plaque of caries-free subjects than in that of caries-experienced ones during a 7-day in vivo experiment. The OTU presented a colonization mode similar to a middle colonizer, *Fusobacterium* species and was significantly more predominant in saliva of caries-free subjects than in that of caries-experienced ones.

In the present study, in order to further confirm the results of the pyrosequencing analysis, quantitative real time PCR and clone library were performed. The results of quantitative real time PCR of saliva and day-7 plaque were consistent with that of the pyrosequencing analysis. Clone library analysis revealed that an oral clone CW034 resembler which belong to *Porphyromonas* sp. HOT-279 was identified as the predominant species in caries free subjects of both in saliva and day-7 plaque. We isolated a bacterial strain resembling an oral clone CW034 from human saliva and characterized its biological property. It was weakly saccharolytic and did not form pigmented colonies, being different from *Porphyromonas gingivalis*, a periodontal pathogen, in general. Based on characterized its property, we proposed to name it *Porphyromonas pasteri* KUFDS01. *Fusobacterium* species are considered important “bridge” organisms that coaggregate initial, early, and late colonizers, and contribute to an increase in microbial diversity in plaque development. We compared the ability of coaggregation of *Fusobacterium nucleatum* ATCC 10953 and *P. pasteri* KUFDS01, and *P. pasteri* KUFDS01 and *F. nucleatum* ATCC 10953 differed in coaggregation ability.

These results suggested *P. pasteri* KUFDS01 is a new target to elucidate the mechanism of plaque biofilm formation. To investigate ‘healthy plaque’ is a novel viewpoint and meaningful for examining the progress of dental caries.

S2-10

Hetero-oligomerization of C2 domains of phospholipase C-related but catalytically inactive protein and synaptotagmin-1

DaGuang Wang[1], Hiroshi Takeuchi[2], Jing Gao[1], Zhao Zhang[1,3], Masato Hirata[1]

[1]Laboratory of Molecular and Cellular Biochemistry, Faculty of Dental Science, Kyushu University,
[2]Division of Applied Pharmacology, Kyushu Dental University,
[3]Stomatological Hospital of Hebei Medical University,

The C2 domain is a protein module often found in molecules that regulate exocytosis. C2 domains mediate interactions between the parental molecule and Ca^{2+}, phospholipids, and proteins. Although various molecules have been shown to interact with several C2 domains, no interactions between the C2 domains from different molecules have yet been reported. In the present study, we identified direct interactions between the C2 domain of PRIP (phospholipase C-related but catalytically inactive protein) and the C2 domains of other molecules. Among the C2 domains examined, those of synaptotagmin-1 (Syt1-C2A and Syt1-C2B) and phospholipase C δ-1 bound to the C2 domain of PRIP. We investigated the interactions between the C2 domain of PRIP (PRIP-C2) with Syt1-C2A and Syt1-C2B, and the mode of binding of each was Ca^{2+}-dependent and -independent, respectively. We further demonstrated that the Ca^{2+} dependence of the interaction between PRIP-C2 and Syt1-C2A was attributed to Ca^{2+} binding with Syt1-C2A, but not PRIP-C2, using a series of mutants prepared from both C2 domains. We previously reported that the interaction between PRIP-C2 and the membrane fusion machinery suggested a critical role for PRIP in exocytosis; therefore, the results of the present study further support the importance of PRIP-C2 in the inhibitory function of PRIP in regulating exocytosis.

S2-11

Cathepsin B deficiency inhibits hypoxic ischemia–induced neuronal damage through promoting the resolution of neuroinflammation by M2-like polarization of microglia/macrophages

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It is widely accepted that microglia/macrophages acquire distinct functional phenotypes: classically (M1) or alternatively activated (M2) microglia/macrophages. M1- and M2-like microglia/macrophages respectively dominate in the chronic and acute states of neuroinflammation. In spite of clinical importance, little is known about the mechanisms underlying phenotypic shift of microglia/macrophages. Here, we provide the first evidence that cathepsin B (CatB), a typical cysteine lysosomal protease, can work as a phenotype switch of microglia/macrophages along the M1-M2 phenotypic continuum. Neonatal wild-type mice subjected to hypoxic/ischemia (HI) injury showed an extensive brain injury. CatB was significantly increased exclusively in microglia/macrophages after HI injury. It was also noted that microglia/macrophages showed early and persistent M1-like polarization followed by transient M2-like polarization after HI injury. In neonatal CatB-/- mice, however, HI-induced brain injury was significantly reduced. Furthermore, CatB-deficient microglia/macrophages showed only the early and transient M2-like, but not M1-like, polarization. When conditioned medium from M1-polarized microglia (M1-CM) was prepared in the presence of CA-074Me, a specific inhibitor of CatB, the neuronal death of primary cultured hippocampal neurons induced by M1-CM was significantly declined. Furthermore, CA-074Me prevented activation of nuclear factor-κB (NF-κB) through inhibiting autophagic degradation of IκBα in microglia following oxygen glucose deprivation. These observations suggest that CatB is a potential phenotype switch for promoting M1-like polarization of microglia/macrophages through prolonged activation of NF-κB, thus inhibition of CatB activity, either through genetic deletion a pharmacological inhibitor, ameliorates HI-induced brain injury via promoting M2-like polarization of microglia/macrophages.

Keywords: microglia/macrophage, phenotype switch, hypoxic-ischemia

S2-12

The selection of the enteral nutritional support route in neurological intensive patients

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Background:
Neurological patients suffered from swallow disorders in acute or chronic neurological diseases such as skull fracture, stroke, and motor neuron disease and so on, have more nutritional risks, complications and mortality. Early and adequate nutritional support is thought to improve neurological outcome and decrease morbidity. The selection of the enteral tube feeding route by oral or nasal gastric tube (NGT) or percutaneous endoscopic gastrostomy (PEG) for long-term feeding issues is controversy.

Objective:

1. To compare the influence of normal format meal and non-normal format meal to the nutritional condition, complication and prognosis of patients with a nasogastric tube suffering from neurological diseases.
2. Retrospective analysis of the influence of PEG treatment to gender ratio, distribution of etiology, turnover, reason of death, and complications of the patients.
3. Compare incidence rate of nutrition risk, nutrition index and complications between patients whose GCS was less than 8 and those whose GCS not less than 8.

Methods:

1. Admitted patients who have swallowing disorder and with nutritional risk were treated with NGT therapy, being divided by two groups (group A and Group B, each group contains 9 patients). Patients in group A were treated with normal format meal which was given 1125kcal/d, with 48g protein per day in the first 3d, and given 1500kcal/d, with 64g protein per day after 3d, while those in Group B were treated with non-normal format meal which was given less than 1125kcal/d, with no more than 32g protein per day in the first 3d, and given less than or more than 1500kcal/d, with more than or less than 64g protein per day after 3d. Nutrition index, immune index, and complications of three periods (before intubation, 1w after intubation and 2w after intubation) were observed.
2. Retrospective analysis of 72 cases of PEG treatment of neurological patients was performed, in which patients' gender ratio, distribution of etiology, turnover, reason of death, complications were analyzed.
3. Divide the 32 admitted patients into 2 groups, one is whose GCS was less than 8, and the other is whose GCS was not less than 8. Compare incidence rate of nutrition risk as soon as they came to hospital, and nutrition index and complications when they came to hospital and a week later between patients whose GCS was less than 8 and those whose GCS not less than 8.

Result:

1.As soon as they came to hospital, the serum Creatinine of Group A is 72.0(55.0-82.5), of Group B is 50.3(32.5-63.7), with p=0.027(p<0.05); 1w later, the CHOL of Group A is 3.05(2.88-3.90), of Group B is 3.98(3.83-4.48), which is significant with p=0.031(p<0.05). 2w later, the BUN of Group A is 6.89(5.32-7.34), of Group B is 4.33(3.11-5.51), with p=0.009(<0.05); the serum Creatinine of Group A is 56.0(42.5-66.0), of Group B is 37.5(30.4-51.0), p=0.047(<0.05). However, the incidence of other immune index and complications does not have statistical significance.

2. The average age of patients in the research is 73.1±15.5 yrs old, male to female 1.77:1. Those above 80 were in the largest number 29(40.3%) were mainly suffering from cerebral thromboembolism 35(48.6%) and Motor neuron disease 21(29.2%). The total number of patients are 72, with 63 of whom (87.5%) improved, 9 of whom died (12.5%) while most of death cases of 7 (77.8%) were due to pneumonia. 65 patients were found with complications which contains 34 aspirated pneumonia (47.2%) and 51 swallowing difficulty (70.8%).

3. Among the admitted 32 patients, there are 3 patients of GCS less than 8 who had the complications (33.3%), 6 patients without any complication(66.7%), none of patients whose GCS was not less than 8 points had any complications, p<0.05. At the time of admission, great significance was found among the patients from two groups, p=0.032 (<0.05). The calf girth of patients of GCS<8 is (36.0±1.1) cm , and is (34.8±1.3) cm of GCS≥8, with p=0.032(<0.05).

Conclusion: Treating patients suffering from neurological diseases with nutritional risk with normal-format meal can significantly ameliorate their index of BUN and serum Creatinine, however cannot lower the incidence of complications, there are no significant difference among other nutritional indicators. PEG has more meanings for old age patients, pneumonia is the chief cause for bad prognosis. Patients whose GCS is less than 8 have more probability in nutrition risk and complications than those whose GCS is not less than 8.

S2-13

Gastrin releasing peptide (GRP) expression and its effect to morpho-differentiation of mouse incisor

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The gastrin-releasing peptide (*GRP*), and its receptor (*GRPR*) are widely distributed in mammalian peripheral tissues and in the central nervous system. Effects of *GRP* and its receptor on the production and release of cytokines are described both in animal models and humans with inflammatory diseases. Moreover, *GRP* is a major growth factor in many types of human cancers. The *GRPR* is overexpressed in different malignancies and its activation stimulates tumor growth. And *GRP* oncogenic signaling pathway was turned out relatively detailed. Thus, *GRPR* antagonists have been developed as candidate anticancer agents and shown to display anti-proliferative activity in experimental cancer models. Recent studies of *GRP* and *GRPR* are concentrated on gastrointestinal tract, pulmonary region and central nervous system. However, the function of *GRP* and *GRPR* is still poorly revealed in the oro-facial region.

The GRPR is G-protein coupled receptor and 7-transmembrane glycosylated receptor that activates the phospholipase C signaling pathway. And the GRP regulates mobilization of calcium from intracelluar stores. We suggest that *GRP* can be one of the factors which affect regulation of calcification during tooth development based on these previous studies.

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S2-14

The role of region-distinctive expression of Rac1 in regulating fibronectin arrangement during palatal shelf elevation

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Palatal shelf elevation is a crucial process in palate development, contributed by various factors. Disturbances in any factor during this process result in cleft palate. Prior to palatal shelf elevation, started from E12.5, higher Rac1 expression level in bend region and increased mesenchymal cell density in the bend and groove regions of mid-palatal shelf were observed. The high cell densities in the bend and groove regions correlated with a ring-like arrangement of fibronectin (FN), whereas low cell density in middle region correlated with a fibrillar FN arrangement. Rac1 overexpression altered the fibrillar FN arrangement in the middle region to the ring-like arrangement. This alteration was sufficient to induce the failure of palatal shelf elevation and, ultimately leading to cleft palate. Furthermore, inhibition of FN delayed palatal shelf elevation. Our results indicate that the regional expression of Rac1 played an impressive role in palatal shelf elevation, by regulating the arrangement of FN within the palatal shelf.

Key words: Rac1, Fibronectin, Palatal shelf, Elevation, Cleft palate


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