Kyudai Oral Bioscience

&

OBT Research Center

5th Joint International Symposium 2021

PROGRAM & ABSTRACTS

November 27-28, 2021
Zoom meeting/Lecture Room AB,
Faculty of Dental Science,
Kyushu University

KOB 2021

KYUSHU OBT

■ Date:

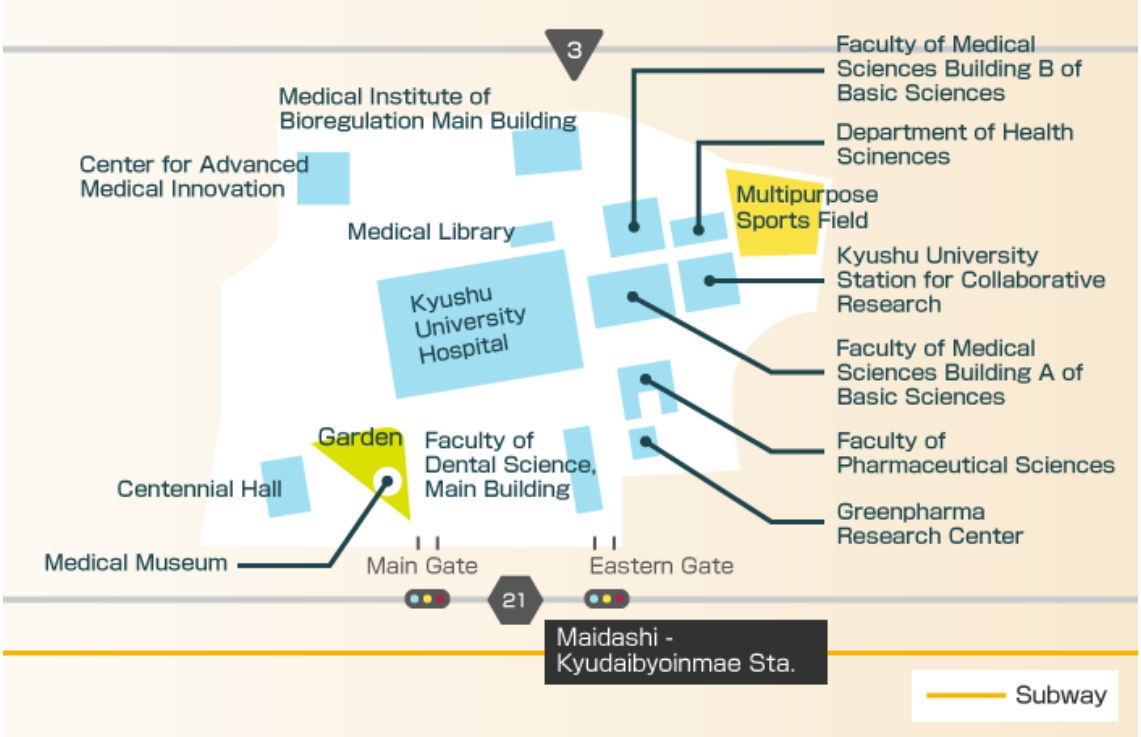
November 27-28, 2021

■ Hybrid: Zoom meeting/Lecture room AB

■ Organization

Kyudai Oral Bioscience

Oral Health・Brain Health・Total Health Research Center



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5th KOB & OBT Joint International Symposium 2021

PROGRAM

November 27 (Saturday)

Time	Title	Presenter
13:00-13:10	Opening Remark	Prof. Seiji Nakamura
Session 1	**Graduate Student's Session (1)**	**Chair: Soi Kimura**
13:10-13:25	The effects of Toll-like receptor 7 on sialadenitis	Kotono Kibe
13:25-13:40	Inhibition of c-Jun N-terminal kinase affects regeneration of periodontal tissues	Hiroshi Kaneko
13:40-13:55	Effect of carbonate content in carbonate apatite on bone remodeling	Kaai Deguchi
13:55-14:10	Relationship between anterior tongue movement and masticatory performance and pre-frailty in community-dwelling older individuals	Asuka Tani
14:10-14:20	Break	
14:20-14:35	**Award Presentation (IF and FWCI Award)**	
Session 2	**Award Lectures (IF Award winners)**	**Chair: Prof. Hidefumi Maeda**
14:35-15:00	Exosomes from TNF- α-treated human gingiva-derived MSCs enhance M2 macrophage polarization and inhibit periodontal bone loss	Dr. Yuki Nakao
15:00-15:25	YAP signaling induces PIEZO1 to promote oral squamous cell carcinoma cell proliferation	Dr. Kana Hasegawa
15:25-15:50	Human autoimmune diseases, systemic sclerosis and IgG4-related disease: latest immunological approaches into the pathogenesis	Dr. Takashi Maehara
15:50-16:10	Break	
Session 3	**KOB Special Lecture (1)**	**Chair: Prof. Eijiro Jimi**
16:10-16:50	APPLICATION OF TECHNOLOGIES IN DENTISTRY AND DENTAL RESEARCH	Dr. Kuson Tuntiwong Dr. Tharathip Kulchotirat King Mongkut's Institute of Technology, Thailand
16:50-17:00	Break	
Session 4	**OBT Special Lecture (1)**	**Chair: Dr. Masafumi Moriyama**
17:00-18:00	Specific immune response in COVID-19	Dr. Kaneko Kyushu University

November 28 (Sunday)

Time	Title	Presenter
Session 5	**KOB Special Lecture (2) -Sharing JICA Memories and Our Success Stories-**	**Chair: Dr. Tomoyo Yasukochi**
9:00-10:00	My experience as a Kyushu University Fellow Student. A lifelong inspiration	Dr. Francisco Munoz Thomson Universidad de los Andes Dental School Santiago, Chile
10:00-10:10	Break	
Session 6	**Undergraduate Student's Session**	**Chair: Dr. Zhou Wu**
10:10-10:20	Relation between oral hypofunction and nutrient intake condition in the elderly	Kaoruko Hirata
10:20-10:30	Bring out immune ability from mouth ～Proposing from dental students～	Kouki Inoue, Saki Imamura, Ryo Hirabar
Session 7	**Graduate Student's Session (2)**	**Chair: Asuka Tani**
10:35-10:50	Exosomal miR-1260b derived from TNF-α-treated hGMSCs inhibits periodontal bone loss by targeting ATF6β-mediated regulation of ER stress	Chikako Hayashi
10:50-11:05	The role of basement membrane protein Nephronectin in tooth development	Kanji Mizuta
11:05-11:20	Soluble RANKL exacerbates menopause-associated obesity via non-canonical NF-κB signaling pathway	Kayo Mori
11:20-11:35	Physiological and Pathological Roles of Inhibitor of Differentiation 4 in the Salivary Gland	Soi Kimura
11:35-11:50	Break	
Session 8	**OBT Special Lecture (2)**	**Chair: Prof. Eijiro Jimi**
11:50-12:50	Exercising for future generations	Dr. Joji Kusuyama Tohoku University
12:50-13:00	Closing Remarks	Prof. Eijiro Jimi

Graduate Student's Session (1)

Chaired by Soi Kimura

The effects of Toll-like receptor 7 on sialadenitis

Kotono Kibe[1,2], Takuma Shibata[2], Masafumi Moriyama[1,3],
Kensuke Miyake[2], and Seiji Nakamura[1]

[1]Division of Maxillofacial Diagnostic and Surgical Sciences, Section of Oral and Maxillofacial Oncology, Faculty of Dental Science, Kyushu University
[2]Division of Innate Immunity, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo
[3]OBT Research Center, Faculty of Dental Science, Kyushu University

【Objective】
Recent studies indicate that Toll-like receptors (TLRs) play an important role in chronic sialadenitis such as Sjogren's syndrome (SS) and IgG4-related disease (IgG4-RD). Our recent study demonstrated that *human* TLR7 transgenic / *mouse* TLR7 knock-out mice showed infiltration of inflammatory cells and fibrosis by stimulation with TLR7 agonists. SLC29A3 is considered as a nucleoside transporter that suppresses the TLR7 response. We currently established SLC29A3 knock-out (SLC29A3 KO) mice, which constantly activated TLR7 signaling. In this study, we thus examined the sialadenitis dependent on TLR7 in SLC29A3 KO mice.

【Methods】
We performed (1) measurement of submandibular glands (SMGs) shape and weight, (2) measurement of saliva secretion induced by pilocarpin, (3) histopathological analysis of SMGs, (4) identification of SMGs infiltrating cells by flow cytometry analysis, (5) quantitative analysis of cytokines and chemokines of SMGs tissue lysate by proteome profiler, (6) analysis of mRNA expression of SMGs tissue by real-time PCR and (7) comparison with human samples by using wild type and SLC29A3 KO mice.

【Results】
SLC29A3 KO mice showed the significant hyposalivation and atrophy of SMGs with strong infiltration of inflammatory cells compared with wild type mice. Immunohistochemical analysis confirmed that the main components of the infiltrating cells were CD4-, CD8-, and CD19-positive cells, and CD11b- and F480-positive cells were diffusely detected throughout the SMG tissues. Flow cytometric analysis showed an increase in patrolling monocytes and classical monocytes in SLC29A3 KO mice. These phenotypes completely disappeared in SLC29A3 KO/ TLR7 KO mice. Proteome profiler was performed using SMGs tissue lysate, the expression of CXCL9, CCL5, IL16 and CXCL13 was specifically increased. Moreover, the mRNA expression levels of these chemokines were enhanced by real-time PCR using SMG tissues. Both of SS and IgG4-RD patient showed the increase in number of CD11b- positive cells in SMG tissues. Especially, SS patients showed the increased in mRNA expression levels of cDNA of CXCL9 and CCL5 by using DNA microarray.

【Conclusion】
These results suggest that TLR7 might promote monocytes/macrophages to induce infiltration of inflammatory cells via the production of CXCL9, CCL5, IL16, and CXCL13.

Inhibition of c-Jun N-terminal kinase affects regeneration of periodontal tissues

Hiroshi Kaneko[1], Daigaku Hasegawa[2], Tomohiro Itoyama[2], Shinichiro Yoshida[2], Atsushi Tomokyo[2], Sayuri Hamano[1,3], Hideki Sugii[1], Hidefumi Maeda[1,2]

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Few clinical treatments to regenerate periodontal tissue lost due to severe endodontic and periodontal disease have yet been developed. Therefore, the development of new treatment methods for the regeneration of periodontal tissue is expected. The purpose of this study was to investigate the effects of a c-Jun N-terminal kinase (JNK) inhibitor, SP600125, on the osteoblastic differentiation of human periodontal ligament stem cells (HPDLSCs) in vitro, and the function of SP600125 on the regeneration of alveolar bone in periodontal defects in vivo.

First, we investigated the effect of JNK inhibition on osteoblastic differentiation of HPDLSCs. SP600125 promoted Alizarin red S-positive mineralized nodule formation and the expression of osteoblast-related genes such as osterix, bone sialoprotein, and osteopontin in HPDLSCs under osteogenic conditions. Next, we clarify the intracellular signaling molecules. This inhibitor upregulated BMP2 expression and phosphorylation of Smad1/5/8 in HPDLSCs under the same conditions while it did not affect phosphorylation of Erk1/2. Furthermore, we assessed the effects of JNK inhibition on the regeneration of bone tissue by using a rat periodontal defect model. SP600125 promoted the regeneration of alveolar bone compared with that in the control at 2 weeks after its application into periodontal defects, which involved the regeneration of periodontal tissues.

This study suggests that inhibition of JNK signaling promotes osteoblastic differentiation of HPDLSCs, probably through increased expressions of BMP2 and the phosphorylation of Smad1/5/8, leading to the regeneration of periodontal tissues. Our findings may lead to the development of a novel periodontal regeneration therapy.

Effect of carbonate content in carbonate apatite on bone remodeling

Kaai Deguchi[1,2], Shunsuke Nomura[1], Akira Tsuchiya[2], Kunio Ishikawa[2] and Ichiro Takahashi[1].

[1]*Section of Orthodontics, Division of Oral Health, Growth and Development, Faculty of Dental Science, Kyushu University*

[2]*Department of Biomaterials, Faculty of Dental Science, Kyushu University*

Carbonate apatite [CO_3Ap: $Ca_{10-a}(PO_4)_{6-b}(CO_3)_c$] is an inorganic component of bone, and CO_3Ap artificial bone was approved for its clinical use in dentistry. CO_3Ap artificial bone demonstrate much higher osteoconductivity than hydroxyapatite [HAp: $Ca_{10}(PO_4)_6(OH)_2$]. A key difference between HAp and CO_3Ap is the absence and presence of CO_3 in apatitic structure. If so, content of CO_3 seems to affect bone remodeling behavior of CO_3Ap artificial bone. Therefore, in this study, feasibility to fabricate CO_3Ap with different CO_3 content was investigated along with the effects of CO_3 content on bone remodeling process. CO_3Ap granules with different CO_3 contents were prepared by a dissolution–precipitation reaction using gypsum granules as a precursor by immersing in a Na–PO_4–CO_3 mixed solution containing different concentration of CO_3. The composition of the prepared samples was analyzed using powder X-ray diffractometer and a CHN coder. The dissolution rates of the samples in acetate buffer solution of pH 5.5, which mimics the Howship's lacuna formed by osteoclasts, was quantified using an inductively coupled plasma-optical emission spectrometer. For histological study, bone defects formed in the distal femur of 18-week-old Japanese rabbits were reconstructed with CO_3Ap granules with different CO_3 contents, and their behavior was investigated by histological analysis at 4 and 8 weeks post-implantation. CO_3Ap containing larger amount of CO_3 could be fabricated by increasing the CO_3 concentration in Na–PO_4–CO_3 mixed solution. Dissolution rate of the CO_3Ap artificial bone at pH 5.5 mimicking Howship's lacuna increased with increasing the CO_3 contents in the CO_3Ap granules. Histological analysis revealed that replacement of CO_3Ap to a new bone was quicker for CO_3Ap granules with larger CO_3 content in the apatitic structure. Due to replacement of CO_3Ap to a new bone, structure of the bone defect reconstructed by CO_3Ap granules was close to the that of the bone. From these results, it was found that CO_3Ap containing different amount of CO_3 in apatitic structure was possible. CO_3Ap with higher CO_3Ap content dissolves quickly at weak acidic solution mimicking Howship's lacuna formed by osteoclasts, and quickly replaced to a new bone based on bone remodeling process.

Relationship between anterior tongue movement and masticatory performance and pre-frailty in community-dwelling older individuals

Asuka Tani[1], Shinsuke Mizutani[1,2], Saori Oku[1], Kiyomi Iyota[1], Xin Liu[3], Hiro Kishimoto[3,4], Haruhiko Kashiwazaki[1]

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[2]*OBT Research Center, Faculty of Dental Science Kyushu University, Japan*
[3]*Department of Behavior and Health Sciences, Graduate School of Human-Environment Studies, Kyushu University, Japan*
[4]*Faculty of Arts and Science, Kyushu University, Japan*

Background: Pre-frailty stage interventions can effectively prevent the need for long-term care. However, only a few studies have investigated the relationship between oral function and pre-frailty; therefore, this study aimed to examine the association between oral function and physical pre-frailty.

Methods: A total of 381 individuals (mean ± S.D., 72.6 ± 3.9 years), included in the Itoshima Frail Study, were analyzed. Physical pre-frailty was assessed by two physical indicators (fatigue and unintentional weight loss), two functional components (decreased walking speed and grip strength), and physical activity (social activity and exercise habits). The number of the remaining teeth and oral functions, tongue and lip motor function, masticatory performance, and tongue pressure were also assessed. The participants were divided into robust group (n = 255) and physical pre-frailty group (n = 126). Odds ratio (ORs) and 95% confidence interval (CIs) for physical pre-frailty were calculated using binomial logistic regression analysis.

Results: Significant differences between robust and physical pre-frailty groups were observed in the maximum handgrip strength, 5-m maximum gait speed, body fat ratio of the body trunk, skeletal muscle mass, masticatory performance, oral diadochokinesis /ta/, (anterior tongue movement), and social activity. Logistic regression analysis revealed that anterior tongue movement, masticatory performance, and social activity were associated with physical pre-frailty (OR: 1.846, 95%CI: 1.060–3.215, p = 0.030; OR: 0.806, 95%CI: 0.672–0.967, p = 0.020; and OR: 2.234, 95%CI: 1.288–3.876, p = 0.004, respectively) after adjusting for confounding factors.

Conclusion: Decreased anterior tongue movement, low masticatory performance, and low social activity were associated with physical pre-frailty.

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Award Lectures

Chaired by Prof. Hidefumi Maeda

2021 年度　九大歯学優秀研究者賞　受賞者

IF 部門

基礎系　教員　　長谷川 佳那 助教

臨床系　教員　　前原 隆 助教

臨床系　教員以外　　中尾 雄紀 助教　　論文発表時：大学院生

FWCI 部門

清島 保 教授　　FWCI　1.72

竹下 徹 准教授　　FWCI　1.45

Exosomes from TNF- α-treated human gingiva-derived MSCs enhance M2 macrophage polarization and inhibit periodontal bone loss

Yuki Nakao[1], Yukari Watanabe[1], Chikako Hayashi[1], Hiroaki Yamato[1], Karen Yotsumoto[1], Terukazu Sanui[1], Takanori Shinjo[1], Takao Fukuda[1], Fusanori Nishimura[1]

[1] Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

Periodontitis is one of the most common osteolytic inflammatory diseases in human that adversely affects systemic disorders, such as diabetes. Accumulation of periodontal bacteria-associated biofilm is thought to trigger periodontitis, but is not believed to be sufficient to sustain the disease as the host immune response is critical for inflammatory tissue breakdown and disease progression. Macrophages play an important role in the immune response both during the initiation and resolution of inflammation. Macrophages are broadly classified into two phenotypes, pro-inflammatory M1 and wound-healing M2 cells. Macrophages are involved in bone homeostasis and the increased M1/M2 ratio leads to enhanced osteoclastogenesis. As M2 macrophages contribute to the tissue-remodeling process, effective M2 macrophage induction would provide favorable environment for lower inflammation and better regeneration.

We identified that gingiva-derived MSCs (GMSCs) have unique immunoregulatory capacity and secret large amounts of exosomes. Considering the facts that therapeutic effect of MSC largely depends on the paracrine efficiency of MSC and the advantages of the use of GMSCs include easier isolation from small pieces of gingival tissue (~2×2 mm^2) and rapid cell proliferation, we hypothesized that GMSC-derived exosome-based therapy could be suitable for clinical use. Moreover, recent studies indicated that appropriate preconditioning of MSC with disease-related stimuli can optimize contents of exosomes to efficiently support the repair of the tissues in particular diseases. In this context, proteins or miRNA profiles in exosomes may be influenced by the pre-treatment regimens. Therefore, optical molecular-based protocol for MSC-preconditioning needs to be investigated and established.

In this presentation, we report the therapeutic effects of TNF-α preconditioned-GMSC-derived exosomes on periodontal disease and demonstrate underlying molecular mechanisms. Our study revealed that TNF-α-enhanced exosomal CD73 expression leading to anti-inflammatory M2 macrophage polarization and exosomal miR-1260b was important negative regulator of osteoclastogenesis. Accordingly, our findings may provide a novel therapeutic strategy for patients with periodontitis and other inflammatory osteoimmune disorders.

YAP signaling induces PIEZO1 to promote oral squamous cell carcinoma cell proliferation

Kana Hasegawa, Shinsuke Fujii, Tamotsu Kiyoshima

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

It has been reported that there are few target molecules for anti-tumor therapy, such as gene mutations, in the head and neck squamous cell carcinoma. Therefore, in oral squamous cell carcinoma (OSCC), we hypothesized that abnormally activated signal transduction could be involved in tumorigenesis. Stiffness is one of the important clinical features of OSCC. In addition, recent studies showed that increased extracellular matrix stiffness promotes tumorigenesis. The Hippo pathway responds to the extracellular environment and regulates cell proliferation through the nuclear localization of major downstream effector, yes-associated protein (YAP), resulting in the induction of target gene transcription. Its pathway also has been linked to tumorigenesis. However, downstream target genes of YAP signaling in OSCC tumorigenesis remain unclear. Piezo-type mechanosensitive ion channel component 1 (PIEZO1), a mechanosensitive ion channel, is reported to be associated with organ morphogenesis, but its expression and function in OSCC tumorigenesis are unknown. Herein, we investigated the effect of YAP on the cell proliferation through PIEZO1 in OSCC cells. In OSCC cells, proliferation capabilities in suspension culture were lower than those in 2D or 3D culture. Microarray analysis and GO analysis demonstrated that several genes related to cell growth were down-regulated in suspension culture compared with those in 2D culture. The experiments using suspension culture and chromatin immunoprecipitation (ChIP) demonstrated that YAP signaling regulates PIEZO1 expression. Elevated PIEZO1 expression was required for PIEZO1 agonist-dependent Ca^{2+} influx and cell proliferation in OSCC cells. Furthermore, YAP signaling regulated OSCC cell growth through PIEZO1 expression in 3D culture. In addition, YAP was frequently expressed in the nucleus in tumor lesion where PIEZO1 and Ki-67 expression were detected. These results suggest that hyperactivated YAP signaling promotes OSCC cell proliferation through PIEZO1 expression.

Human autoimmune diseases, systemic sclerosis and IgG4-related disease: latest immunological approaches into the pathogenesis

Takashi Maehara[1], Naoki Kaneko[1], Ryusuke Munemura[1], Yuka Murakami[1], Shiv Pillai[2], Seiji Nakamura[1]

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[2]Ragon Institute of MGH, MIT, and Harvard, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

We examine the contributions of B cells to the development and function of $CD4^+$T cells in human inflammatory disease. We study these cells and their subsets in the context of autoimmune fibrotic diseases like systemic sclerosis (SSc) and IgG4-related disease (IgG4-RD). Activated B cells and $CD4^+$T cell which likely cause organ damage and tissue fibrosis, constitute the major inflammatory cell population in these patients. We thus focused on $CD4^+$T cell subsets and the T-cell clones that are involved in the pathogenesis of these disease, by using single-cell RNA sequencing, T cell receptor repertoire analysis, and multicolor immunofluorescence staining. These latest approaches revealed prominent clonal expansions of $CD4^+$ cytotoxic T cells ($CD4^+$CTLs) in these diseases. SSc and IgG4-RD are autoimmune fibrotic disease whose pathogenesis is poorly understood and lacks effective therapies. The definitive identification of self-antigens that are recognized by expanded T cell clones that infiltrate tissues in patients with these inflammatory fibrotic diseases would strengthen the view that self-reactive T cells drive this disease process. We undertook quantitative analyses of T cell infiltrates in the skin of 35 untreated patients with early diffuse SSc and here show that $CD4^+$CTLs and $CD8^+$T cells contribute prominently to these infiltrates. We also observed an accumulation of apoptotic cells in SSc tissues, suggesting that recurring cell death may contribute to tissue damage and remodeling in this fibrotic disease. HLA-DR expressing endothelial cells were frequent targets of apoptosis in SSc. Consistent with the prominent vasculopathy seen in patients with SSc. A circulating effector population of $CD4^+$CTLs was clonally expanded in SSc patients. These data suggest that $CD4^+$CTLs may induce the apoptotic death of endothelial and other cells in SSc. Therapies should be focused on targeting these cells and studies on pathogenesis should examine the antigenic sources and mechanisms of their generation. In this session, we thus focus on the latest immunological approaches to elucidate the pathogenesis of SSc and IgG4-RD.

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KOB Special Lecture (1)

Chaired by Prof. Eijiro Jimi

APPLICATION OF TECHNOLOGIES IN DENTISTRY AND DENTAL RESEARCH

Kuson Tuntiwong[1], Tharathip Kulchotirat [1]

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Nowadays, technologies are continuously developing. Various types of technological applications were Internet of Things, Virtual and Augmented reality, Artificial Intelligence, etc. These were named as The Gaming Changing Technologies. The applications were typically based on mathematical problems, like, optimization, pattern recognition, classifications, clustering, or continuous estimation. Likewise, in dental field, these technologies had become a part of dentistry, shifted to the new perspectives of dental treatment planning and dental research. The increasing use of the Finite Element Analysis in dental research was published to solve simulation and optimization problems in CAD environment. It provided stress analysis in specific scenario and simulated stress distribution beyond the visibility of human eyes [1]. Deep learning was also used to initially detect the radiographic findings in dental images [2]. In 3D tasks, digital geometry processing was also used to analyze the error 3D scanning [3]. These are just some examples of technologies that applied to the dental field. There are a lot more problems in dentistry that waits for us to use these as analytic tools.

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OBT Special Lecture (1)

Chaired by Dr. Masafumi Moriyama

Specific immune response in COVID-19

Naoki Kaneko[1,2], Shiv Pillai[2]

[1]Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan,
[2]Ragon Institute of MGH, MIT and Harvard

Despite the rapid spread of vaccines, COVID-19 is still rampant around the world. One of the reasons is SARS-CoV2 infection does not elicit an appropriate humoral immune response and often lacks durability in humans. It is well known that proper immune memory is not induced, especially in natural infections. We demonstrate an unexpected paucity of germinal center and germinal center-related T cells and B cells in the lymphoid organs in acute COVID-19. Considering that germinal center is involved in the production of high−quality antibodies, these phenomena may explain the mechanism of limited durability against COVID-19. Furthermore, apart from broadly compromising the generation of germinal centers in lymph nodes, acute COVID-19 is also linked to attenuated CD8+ T cell activation and infiltration of the lungs, and the delayed pulmonary accumulation of CD4+T cells with a cytotoxic phenotype. These observations might help explain why SARS-CoV-2 is slowly eliminated from the lungs and why "COVID-19 long-hauler" exists who may have conditions ripe for the selection of viral variants with a transmission advantage. Many new variants of SARS-CoV2 have been reported since the first report of that, and it may be another reason why the covid pandemic has not come to an end.

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KOB Special Lecture (2)

Chaired by Dr. Tomoyo Yasukochi

Sharing JICA Memories and Our Success Stories

The medical, research and educational fields today are facing big challenges due to the Covid 19. In the midst of these uncertainties, as part of our globalization effort our faculty set up the International office in April this year. We plan to increase our partner universities, and to recruit more international students by hosting online recruitment events.

One of the most important mission of the International office is to maintain the strong bonds and build new ones with the International students and researchers who are affiliated to us.

Our faculty welcomed and hosted 248 students from all over the world under the JICA (Japan International Cooperation Agency) Oral Health Science Education Program over a span of 23 years, from 1988 to 2010. Our International office initiated a JICA network and we also started a Facebook page and quarterly newsletter to keep the participants updated of the events at our faculty.

Starting from this year, we plan to invite JICA participants to give a presentation at our KOB/OBT symposium every year. The theme of this session would be "**Sharing JICA memories and our success stories**" and we would get presenters to share their memories with us under the JICA program, as well as how this program has created a positive impact on their lives.

You can see our Facebook page from this QR code.

My experience as a Kyushu University Fellow Student. A lifelong inspiration

Dr. Francisco Muñoz Thomson

Chair Oral and Maxillofacial Department
Universidad de los Andes Dental School
Santiago - Chile

In 2004 I was one of the 12 dentists participating in the JICA Dental Education Course. The months I got to spend as a Kyushu University fellow student introduced me to a whole new world within dentistry; I got the chance to have a broad insight of the different specialties which I had lost track of because of my total dedication to Oral and Maxillofacial Surgery.

We were exposed to state of the art technology and were inspired by top researchers and Professors who generously shared their experience. From the very first moment I had the feeling of being immerse in the centenary prestige of Kyushu University and felt privileged to be, if at least for a couple of months, part of this thriving and challenging community.

Even though it was designed to be an academic experience, it was so much more than that! Having the chance of living in a multicultural environment such us the Kyushu International Centre and spend time with fellow dental students at the Campus, I soon realized that this would be a social and personal experience that would positively impact my life.

During those months, I reassured my passion for Oral and Maxillofacial Surgery under the example of Professor Yuji Shiratsuchi and made strong friendships in the Kyushu University Karate Team. I confirmed my lifetime admiration for Japanese culture and humbly learnt the basics of the language expanding my chances to relate with more people and make new friends.

It is fair to say that this experience somehow modeled the rest of my career.

This professional and cultural experience set a new standard which I have tried to reach ever since. I came back to Chile with a clear vision of excellence and used it as an inspiration in my upcoming professional challenges with my students, then my colleagues and later on the staff members in the OMS Department which I now lead.

I traveled to Japan expecting to become a better professional and I flew back to Chile being a better person that is committed to work day after day for a better Society.

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Undergraduate Student's Session

Chaired by Dr. Zhou Wu

Relation between oral hypofunction and nutrient intake condition in the elderly

Kaoruko Hirata[1], Tomomi Ohmaru[2], Naohisa Wada[2]

[1] Kyushu University School of Dentistry,
[2] Division of General Dentistry, Kyushu University Hospital, Japan;

Japan has become a super-aging society. Roles of dentists change variety from the treatments to the improvement of oral function and prevention of frail, dementia or eldercare. However, there is a serious concern that oral hypofunction or alteration of nutrient intake is often overlooked, because of the large elderly populations.

【Hypothesis】 We hypothesized that avoidance of oral hypofunction would help to improve general health, and analyzed whether trouble of oral condition would influence oral function and nutrient intake in the elderly.

【Method】 Subjects are 27 people with more than 65 years-old, and they were the patients who visited to receive dental treatments in university hospital and agreed to be included in this study.

Measurement items were Examination of oral function (seven items), questionnaire (Mini Nutritional Assessment: MNA®), and record of food intake (Balance guide made by Ministry of Agriculture).

【Results and Discussion】 Seventeen subjects (63%) resulted to be oral hypofunction. Hypofunctions with tongue and lip motor function, tongue pressure, and occlusal force were more than 50%.

Over 90% subjects kept full support in Eichner classification when including denture support. This might influence the low percentage of masticatory hypofunction. Thus, it was important to recover masticatory area by introducing denture.

On investigation with five subjects, the amount of foods other than main dishes were relatively small, compared with standard value of similar age. Also, three of them were diagnosed as a “risk of malnutrition” or “malnutrition” in MNA examination, but there was one subject who was diagnosed as “oral hypofunction”.

There was a positive correlation between occlusal force and MNA score, but a negative correlation between occlusal force and oral hygiene. Therefore, maintenance of occlusal support can improve nutritional status. Then, it was considered that recovery of occlusal force following improvements of occlusal support or masticatory area by dental treatments could improve nutritional status.

【Conclusion】 Dentists could notice a sign of flail by evaluation of oral function and nutrient intake. Furthermore, it was suggested that dental treatment affect prevention of oral hypofunction and malnutrition.

Bring out immune ability from mouth

～Proposing from dental students～

Kouki Inoue, Saki Imamura, Ryo Hirabara
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Novel Coronavirus (COVID-19) is seriously damaging people's lives and economy worldwide, and immunity of people is considered as a key for preventing the virus infection and aggravation of the infection. As dental students, the future health-care professionals, we eager to seek the ways to overcome the virus infection from the perspective of oral medicine. During our research lecture, we set up a project and have made a proposal as "maintain and improve immunity from the mouth." based on the literature search. In the present presentation, we firstly review the impacts of oral conditions (periodontal disease, etc.) on systemic immunity. In our constructed proposals, we will introduce how to enhance immunity by nutrition intake from the mouth and how to enhance immunity by improving quantity and quality of saliva in the mouth. We hope our proposal will contribute to the prevention of infection and the spread of infection.

5th KOB & OBT Joint International Symposium 2021

Graduate Student's Session

Chaired by Asuka Tani

Exosomal miR-1260b derived from TNF-α-treated hGMSCs inhibits periodontal bone loss by targeting ATF6β-mediated regulation of ER stress

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Objectives: Human MSCs from gingiva (hGMSCs) are easier to isolate, and appear to secret higher amounts of exosome than other MSCs. We recently demonstrated that exosome from TNF-α treated GMSCs increased the level of miR-1260b and inhibited periodontal bone loss（Nakao Y, *et al*., *Acta Biomater*, 2021）. We further screened novel miR-1260b targeting genes by database analysis and found that it could be associated with ER stress by targeting ATF6β. It has reported that ER stress-related genes were up-regulated in periodontal tissue（Yamada H, et al. *J Periodontal Res*., 2002）. In this study, we investigated the therapeutic effect of miR-1260b in periodontal disease by targeting ATF6β-mediated regulation of ER stress.

Materials and Methods: Human periodontal ligament cells (hPDLCs) were transfected with miR-1260b mimic to validate miR-1260b-mediated inhibition of ATF6β. The effect of miR-1260b on inflammatory bone loss were examined using mouse ligature-induced periodontitis model under an institutionally approved animal research protocol (Kyushu University, #A21-131-0). The expression levels of ATF6β in mice were compared by qRT-PCR and immunohistochemistry, and alveolar bone loss was analyzed by Micro CT. To validate the effect of miR-1260b on osteoclastogenesis, miR-1260b mimic transfected THP-1 cells and PBMC were stimulated with M-CSF and RANKL for osteoclast differentiation and the number of TRAP-positive cells were counted.

Results: Transfection of miR-1260b mimic inhibited ATF6β expression and knock down of ATF6β decreased expression of RANKL mRNA in hPDLCs. Increased expression of ATF6β was observed in the ligated periodontal tissue and local injection miR-1260b mimic decreased periodontal bone resorption in mice. The number of TRAP positive cells were decreased in miR-1260b mimic transfected THP-1 and PBMC - differentiated osteoclasts.

Conclusion: miR-1260b inhibited osteoclastogenesis and periodontal bone loss by targeting ATF6β-mediated regulation of ER stress. miR-1260b may be a target for miRNA mimic-based treatment against inflammation-induced bone loss diseases such as osteoporosis and rheumatoid arthritis.

The role of basement membrane protein Nephronectin in tooth development

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Tooth development is initiated by epithelial-mesenchymal interactions. The basement membrane between the epithelium and mesenchyme is an essential scaffold that regulates various signals. We focused on Nephronectin (NPNT), which is specifically localized in the basement membrane during epithelial-mesenchymal interaction. NPNT has an EGF-like repeats domain on the N-terminus and an RGD domain, known as an integrin binding domain, on the C-terminus. Previously we reported that NPNT plays critical roles in dental epithelial stem cell differentiation through regulation of SOX2 expression via the EGF signaling pathway through its EGF-like repeats domain.

In this study, we analyzed the function of RGD domain of NPNT using Npnt-ΔEGF and Npnt-ΔRGD expression vectors, lacking the EGF-like repeats and the RGD domain of NPNT. Npnt-FL and Npnt-ΔEGF overexpression in dental epithelial cells (M3H1) resulted in increased expression of ameloblastin (Ambn), which is a differentiation marker for ameloblasts. However, overexpression of Npnt-ΔRGD did not alter the expression level of Ambn in M3H1 cells. These data suggest that the RGD domain of NPNT may play an important role for ameloblast differentiation.

In addition, we revealed that BMP2 decreased the mRNA level of Egfr, so that the signaling of EGF-like repeats domain is downregulated. We also confirmed that the functional domain of NPNT was switched to the RGD domain in M3H1 cells with reduced EGFR expression.

In conclusion, NPNT regulates dental epithelial cell proliferation by using the EGF-like repeats domain during morphogenesis stage, followed by switching to the function of RGD domain in differentiation stage. NPNT plays critical role for tooth development by using the two functional domains as the extracellular scaffold.

Soluble RANKL exacerbates menopause-associated obesity via non-canonical NF-κB signaling pathway

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[Objectives] It is well known that obesity is commonly associated with chronic low-grade systemic inflammation. Immune cells infiltrated in obese adipose tissues produce proinflammatory cytokines that can exacerbate insulin resistance, leading to metabolic disorders such as type 2 diabetes and dyslipidemia. Rapid decrease of estrogen due to menopause makes women more susceptible to visceral obesity. However, the precise mechanism of menopause-induced obesity is still unclear. It has been reported that circulating levels of receptor activator of NF-κB ligand (RANKL) is increased in both postmenopausal women and mice models of menopause. RANKL activates both canonical and non-canonical pathways of NF-κB. In this study, we investigated the potential role of RANKL-induced NF-κB activation, specifically focusing on non-canonical pathway, in inducing systemic chronic inflammation and menopause-related lipid accumulation.

[Material and methods] We used ovariectomized mice as a surgical model of menopause (OVX) using WT and Alymphoplasia (*aly/aly*) mice, and fed a high-fat, high-sucrose diet to induce obesity. *aly/aly* is a naturally occurring strain with a mutation in nuclear factor-kappa B inducing kinase (NIK), and therefore cannot activate the non-canonical NF-kB pathway. Model of postmenopausal obesity was generated by ovariectomy and subsequent high-fat and high-sucrose diet feeding.

[Results] We confirmed that RANKL stimulation induced *Tnfa* expression via non-canonical NF-κB pathway in bone marrow cells. In *aly/aly* mouse model of menopause-induced obesity, all the deleterious effects of ovariectomy in wild-type mice such as adipocyte hypertrophy with increased macrophage infiltration in the adipose tissue accompanied by increased mRNA expression of inflammatory cytokines, hepatic lipid accumulation, and glucose intolerance caused by systemic insulin resistance.

[Conclusion] Our results suggest that the elevation of serum RANKL levels contributes to the development of glucose intolerance, insulin resistance, and obesity in menopausal women through the activation of NF-κB non-canonical pathway.

Physiological and Pathological Roles of Inhibitor of Differentiation 4 in the Salivary Gland

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Inhibitor of differentiation 4 (Id4) is a dominant negative transcriptional regulator of basic helix-loop-helix (bHLH) transcription factors that lack the basic DNA binding domain, but have an intact HLH domain. It regulates the function of various ubiquitously expressed or tissue-specific bHLH transcription factors as well as many non-bHLH proteins with different affinities. The molecular mechanism responsible for the unique role of Id4 as compared to other Id proteins still remains largely unexplored.

Recently, we identified Id4 as a molecule involved in the pathogenesis of IgG4-related diseases (IgG4RD) from the results of a comprehensive miRNA-mRNA pairing analysis focusing on patient serum miRNA and intracellular mRNA in normal salivary epithelial cells. IgG4-related disease is a disease in which infiltration of IgG4-positive plasma cells into various organs including salivary glands and hyper-IgG4emia are observed; however, its pathogenic mechanism is still unknown. In fact, Id4 expression at the protein level in the salivary glands of IgG4RD was significantly decreased compared with that in normal tissue. This indicated that Id4 may be involved in the pathophysiology of salivary gland diseases.

Thus, we further investigated the physiological roles of Id4 in the salivary gland by using Id4-deficient mice and RSMG-1 rat salivary gland epithelial cell line. In the cell culture experiment, the expression level of Id4 was decreased in RSMG-1 treated with Activin A, but its expression level returned to normal level after 72 h. From histological analysis, PAS-positive granules were more abundant in the acinar lumen of Id4-deficient submandibular gland than in that of wild-type littermates, and abnormal expression of various differentiation markers was observed in Id4-deficient salivary glands.

From these results, it was suggested that Id4 plays an important role in the development and cell differentiation of the salivary gland, and decrease in its expression affects the pathophysiology of various salivary gland diseases including IgG4-related diseases.

5th KOB & OBT Joint International Symposium 2021

OBT Special Lecture (2)

Chaired by Prof. Eijiro Jimi

Exercising for future generations

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The global prevalence of type 2 diabetes is expected to rise to 630 million by 2045, resulting in a worldwide health care crisis, which will require drastic intervention. Recently, we and other groups studies have reported that maternal obesity and type 2 diabetes increase the risk of diabetes in offspring, despite healthy offspring lifestyle. Therefore, determining an effective means to reduce the transmission of metabolic disease from mother to offspring, as well as understanding the mechanisms for these effects, will have invaluable impact on health care policy. We establish that maternal exercise improves the metabolic health of offspring through enhanced liver function, leading to improved glucose tolerance. We are the first to discover that placenta-derived superoxide dismutase 3 (SOD3) mediates the transmission of the favorable effects of maternal exercise to offspring. The mechanism for this effect involves SOD3 functioning as a signaling protein, activating DNA demethylation at the promoter of key glucose metabolic genes in the liver, which increases expression of these genes and improves liver function. Finally, these discoveries bear the clinical relevance; our animal work is recapitulated by our human data, showing that SOD3 is upregulated in the serum and placenta from highly physically active pregnant women. We demonstrated that maternal exercise is a promising method as a "preemptive medical treatment" to prevent the transmission obesity and diabetes from the current generation to the next generation. We also identified that placenta-derived proteins are important factors that transmit the beneficial effects of exercise to offspring. The placental–fetal system is another hotspot for offspring developmental programming, because it has a major role during the critical windows of prenatal offspring development. This talk summarizes our latest finding about the exercise-nutrition crosstalk pathway and our hypothesis that the placenta is a transducer that conveys maternal information to their children. Identifying the mediating factors and signaling pathways that connect exercise stimuli to these phenotypic and developmental outcomes is essential for human translation, and given the ever-expanding issues of global obesity, these issues will only become more relevant in the future.