Kyudai Oral Bioscience

&

OBT Research Center

6th Joint International Symposium 2022

PROGRAM & ABSTRACTS

October 29-30, 2022
Zoom Webinar/Lecture Room AB,
Faculty of Dental Science,
Kyushu University

KOB 2022

KYUSHU OBT

■ Date:

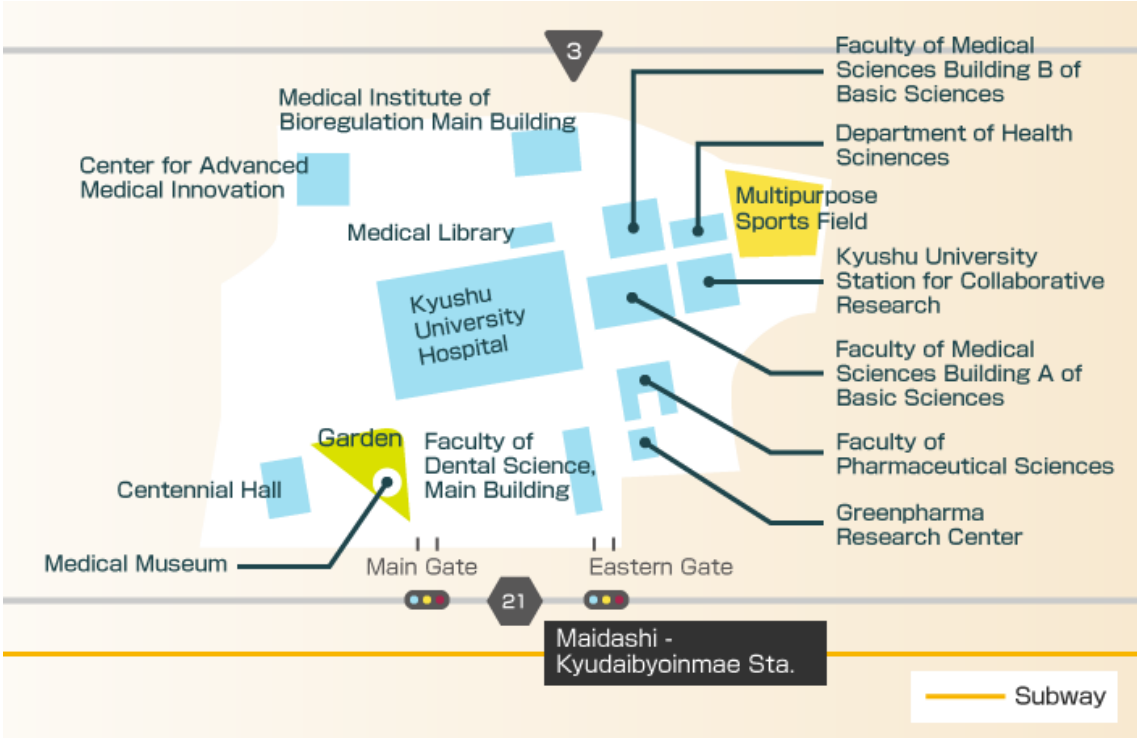
October 29-30, 2022

■ Hybrid: Zoom Webinar/Lecture room AB

■ Organization

Kyudai Oral Bioscience

Oral Health・Brain Health・Total Health Research Center



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6th KOB & OBT Joint International Symposium 2022

PROGRAM

October 29 (Saturday)

Time	Title	Presenter
10:20–10:30	Opening Remark	Prof. Seiji Nakamura
Session 1	**Graduate Student's Session**	**Chair:** **Hu Chen** **Tsukasa Aoki**
10:30–10:45	Wnt signaling promotes tooth germ development mediated by YAP1-TGF-β signaling	Ryoko Nagano
10:45–11:00	Effect of carbonate apatite as a bone substitute from the perspective of mesenchymal stem cells	Ryosuke Takahashi
11:00–11:15	A role of SCN1A in stem cells from human exfoliated deciduous teeth	MHD Fouad Zakaria
11:15–11:30	Long-term administration of bisphosphonates enhances aversive behavioral responses to HCl	Asami Oike
11:30–11:45	Involvement of TNF-α produced by TLR8+ monocytes/macrophages in the initiation of Sjögren's syndrome	Yuka Miyahara
11:45–12:00	Extrafollicular B cell response in two different disease milieus: IgG4-relate disease and oral squamous cell carcinoma	Hu Chen
12:00–13:00	Lunch Break	
Session 2	**OBT Special Lecture (1)**	**Chair:** **Dr. Zhou Wu**
13:00–14:00	Targeting hypothalamus-habenula synapses – a novel circuitry mechanism for prevention strategy of chronic stress induced depression	Dr. Yihui Cui School of medicine, Zhejiang University, China
14:00–14:15	Break	
Session 3	**JICA Session**	**Chair:** **Dr. Tomoyo Yasukochi**
14:15–14:45	Some memorandums about International Activities of Kyushu University Faculty of Dentistry	Dr. Minoru Nakata, Professor Emeritus, Kyushu University
14:45–15:45	FLOW OF LIFE WITH AND WITHOUT KYUSHU UNIVERSITY Comparison Of The Effect Of Proanthocyanidin Surface Treatments On Shear Bond Strength Of Different Cements	Prof. Dr. A. Nehir Özden Eastern Mediterranean University, Cyprus
15:45–16:00	Break	
Session 4	**OBT Special Lecture (2)**	**Chair:** **Prof. Eijiro Jimi**
16:00–16:45	Osteoblast Lineage-Specific Cell-Surface Antigen Regulating Osteoclastogenesis and Calcification: A Possible New Player in Bone Remodeling	Dr. Tamer Badawy Cairo University, Egypt Galala University, Egypt
16:45–17:00	Break	
Session 5	**KOB Session**	**Chair:** **Prof. Eijiro Jimi**
17:00–17:20	Artifical Intelligence and Blockchain in Dentistry and Radiology: What does the future hold?	Prof. Dr. Kaan Orhan Ankara University, Turkey
17:20–17:40	Prevalence and predictors of bruxism patients with dental implants: a symptom-based diagnosis and prevention strategy	Prof. Dr. Volkan Arısan Istanbul University, Turkey
17:40–18:00	In Vitro immunomodulation of Human Periodontal Ligament Stem Cells on Dendritic Cells Phenotype and Function in Type 1 Diabetes The Association between Anti-Asthma Medications and Periodontal Health	Prof. Dr. Rola Alhabashneh Jordan University of Science and Technology (JUST), Jordan

October 30 (Sunday)

Time	Title	Presenter
9:00–9:15	**Award Presentation**	**Prof. Seiji Nakamura**
Session 6	**Award lectures**	**Chair:** **Prof. Hidefumi Maeda** **Prof. Tamotsu Kiyoshima**
9:15–9:35	RAF1–MEK/ERK pathway-dependent ARL4C expression promotes ameloblastoma cell proliferation and osteoclast formation	Dr. Shinsuke Fujii
9:35–9:55	Regeneration of glomerular metabolism and function by podocyte pyruvate kinase M2 in diabetic nephropathy	Dr. Takanori Shinjo
9:55–10:15	Targeted inhibition of EPAS1–driven IL-31 production by a small-molecule compound	Dr. Yasuhisa Kamikaseda
10:15–10:35	CD163+ M2 macrophages promote fibrosis in IgG4-related disease via TLR7/IRAK4/NF-κB signaling.	Dr. Akira Chinju
10:35–10:50	Break	
Session 7	**DDR Session**	**Chair:** **Prof. Takayoshi Yamaza**
10:10-10:20	Regulation of tooth development by Epiprofin	Dr. Takashi Nakamura Tohoku University
10:20-10:30	Strategies for functional enamel formation using hiPSCs	Prof. Han-Sung Jung Yonsei University College of Dentistry, Korea
12:50-13:00	Closing Remarks	Prof. Eijiro Jimi

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Graduate Student's Session

Chaired by Hu Chen and Tsukasa Aoki

Wnt signaling promotes tooth germ development mediated by YAP1-TGF-β signaling

Ryoko Nagano[1,2], Shinsuke Fujii[1,3], Kana Hasegawa[1], Hidefumi Maeda[2], Tamotsu Kiyoshima[1]

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Tooth germ development involves continuous and sequential steps with reciprocal interactions between odontogenic epithelium and the adjacent mesenchyme. Several growth factors, including Wnt, and their signal activation are reported to be involved in tooth germ development. Notably, genetically modified mice with odontogenic epithelium-dependent Wnt/β-catenin pathway activation showed developmental anomalies of tooth germ, indicating that the appropriate activation of Wnt/β-catenin signaling is essential for tooth germ development. However, the molecular mechanisms underlying Wnt/β-catenin-regulated tooth germ development are poorly understood.

We recently demonstrated that Wnt/β-catenin signaling inhibited odontogenic epithelial cell proliferation through a reduction in Semaphorin 3A (Sema3A) expression. In tooth germ rudiments culture, Sema3A stimulation reversed Wnt/β-catenin signaling-dependent decreased cell proliferation but did not completely rescue the morphological anomalies of tooth germ, suggesting that an uncharacterized signaling pathway may be essential in Wnt/β-catenin signaling-dependent tooth germ development. Herein, an enrichment analysis using DNA microarray data revealed that Wnt/β-catenin signaling negatively regulates Yes-associated protein 1 (YAP1) and/or TGF-β signaling. In odontogenic epithelial cells and tooth germ rudiments, Wnt/β-catenin signaling reduced YAP1 expression, thereby suppressing YAP1 and TGF-β signaling sequentially. Finally, Wnt/β-catenin signaling-dependent disorganized tooth germ development, in which YAP1 signaling was suppressed, was reversed by TGF-β stimulation, possibly as a result of regulating a specific epithelial cell fate, such as stellate reticulum or outer enamel epithelium.

These results suggest that Wnt/β-catenin signaling contributes to the tooth germ development through YAP1-TGF-β signaling.

Effect of carbonate apatite as a bone substitute from the perspective of mesenchymal stem cells

Ryosuke Takahashi[1,2], Ikiru Atsuta[2], Ikue Narimatsu[1], Xiaoxu Zhang[3], Yuki Egashira[1,2], Kiyoshi Koyano[2], Yasunori Ayukawa[1]

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Objective: The use of bone substitutes has become important for safe implant treatment. Bone substitutes containing carbonate apatite (CO_3Ap), a major inorganic component of bone, have been developed in recent years. However, the detailed mechanism by which CO_3Ap acts as an effective bone substitute remains unclear. To clarify the mechanism of CO_3Ap as a bone substitute, we compared differences among CO_3Ap, hydroxyapatite (HAp), and β-tricalcium phosphate (β-TCP) by focusing on mesenchymal stem cells (MSCs) that have a role in wound healing in the oral cavity.
Methods: *In vivo* experiment: 6-week-old male Wistar rats had maxillary right first and second molars removed, and CO_3Ap, HAp, or β-TCP was inserted into the site. MSC and macrophage accumulation as well as high mobility group box 1 (HMGB1) secretion surrounding the extraction site were evaluated. *In vitro* experiment: MSCs from rats were cultured with CO_3Ap, HAp, β-TCP, or no material (control). Cell morphology, proliferative capacity, differentiation, and expression of growth factors were evaluated. Additionally, changes in the calcium concentration of the culture supernatant were measured as an influential factor.
Results: *In vivo* experiment: Healing of extraction sockets was significantly promoted in the CO_3Ap group, together with strong accumulation of MSCs and secretion of HMGB1. Significant accumulation of macrophages was observed in the β-TCP group. *In vitro* experiment: The differentiation potential and amount of calcium deposition were significantly lower in CO_3Ap and HAp groups than in control and β-TCP groups. There was no significant difference in proliferative ability between the bone substitutes. However, increases in IGF-I and VEGF were found only in the CO_3Ap group.
Conclusion: CO_3Ap-filled extraction sockets accumulate MSCs without an excessive inflammatory reaction, and MSCs cultured in the presence of CO_3Ap produce large amounts of growth factors. These results suggest that CO_3Ap as a bone substitute promotes healing of tooth extraction sockets.

Long-term administration of bisphosphonates enhances aversive behavioral responses to HCl.

Asami Oike[1,2], Shusuke Iwata[1,3], Ayaka Hirayama[1], Yurika Ono[1], Yuki Nagasato[1], Yuko Kawabata[4,5], Shingo Takai[1], Keisuke Sanematsu[1,3,6], Naohisa Wada[2], Noriatsu Shigemura[1,3].

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Drug-induced taste disorders cause malnutrition and reduce the quality of life, but little is known about the molecular mechanism. One of taste disorders is known adverse effects of bisphosphonates, which are administered as anti-osteoporotic drugs or anti-bone-resorptive and temporary. Therefore, the present study evaluated the effects of risedronate (a bisphosphonate) on taste bud cells. Bisphosphonates inhibit farnesyl diphosphate synthase (FDPS), which is a key enzyme in the mevalonate pathway that mediates cholesterol synthesis. Expression analyses revealed that FDPS was present in a subset of mouse taste bud cells, especially type III sour-sensitive taste cells. Behavioral analyses (short-term lick tests) revealed that mice administered risedronate for 28 days exhibited a significant reduction in their preference for sour tastant (HCl) but not for other taste solutions (salty; NaCl, KCl, sweet; sucrose, bitter; quinine HCl, and umami; monopotassium glutamate) compared to controls. In contrast, taste nerve (the chorda tympani nerve and the glossopharyngeal nerve) responses showed no significant differences in responses to all taste substances. On the other hand, the taste buds of mice administered risedronate for 28 days exhibited significantly higher mRNA expression of FDPS and significantly lower mRNA expression of desmoglein-2, an integral component of desmosomes. Taken together, these findings suggest that risedronate may interact directly with FDPS to inhibit the mevalonate pathway in type III taste cells, thereby affecting the structure of desmosomes in the plasma membrane related with paracellular permeability to H^+ and/or Cl^- in the taste buds. These findings provide new insights into the mechanisms of FDPS in taste bud cells by which bisphosphonates may cause taste-like disorders.

Involvement of TNF-α produced by TLR8^{+} monocytes/macrophages in the initiation of Sjögren's syndrome

Yuka Miyahara[1], Masafumi Moriyama[1,2], Mizuki Sakamoto[1], Hu Chen[1], Naoki Kaneko[1], Kazuki Kai[1], Noriko Kakizoe-Ishiguro[1], Akira Chinju[1], Kotono Okabe-Kibe[1], Kenichi Ogata[1], Takashi Maehara[1], and Seiji Nakamura[1]

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Backgrounds: Sjogren's syndrome (SS) is an autoimmune disease characterized by lymphocytic infiltration in the salivary and lacrimal glands with concomitant autoantibody production. Although the pathogenic involvement of adaptive immune responses mediated by CD4^{+} T helper cells in SS is well established, recent reports have highlighted the innate immune responses underlying in the initiation of SS. In this study, we thus focused on Toll-like receptors (TLRs), which are important for innate immunity.

Methods: Gene expression was analyzed by DNA microarrays in labial salivary glands (LSGs) from 3 patients with SS and 3 patients with mucocele as healthy control (HC). Differentially expressed genes of TLR family were validated by real-time PCR and immunohistochemical staining in LSGs from patient with SS (n=32) and HC (n=18). Next, the human monocyte cell lines, U937, were stimulated by TLR8 agonist (R848) and then measured the cytokine concentration of culture supernatant by ELISA.

Results: In SS, four genes of TLR family (TLR1, TLR7-9) were overexpressed by DNA microarray. PCR validated significantly higher expression of TLR7 and TLR8 in SS compared with HC. Double immunofluorescence staining showed that TLR7^{+} and TLR8^{+} cells were mainly colocalized with CD123^{+} plasmacytoid dendritic cells (pDCs) and CD68^{+} monocytes/macrophages in LSGs, respectively. Several studies have already shown the involvement of pDCs in the pathogenesis of SS via TLR7/IFN-α signaling, while the association of TLR8 with SS is not still unclear. Recently, it was shown that TNF-α produced by TLR8 signaling triggered Th1-induced inflammation in other autoimmune disease. After in vitro stimulation with TLR8 agonist, U937 increased supernatant TNF-α concentration. Moreover, supernatant TNF-α concentration was distinctly increased in TLR8-overexpression U937, while was significantly decreased in TLR8-knockout U937.

Conclusion: Our current data suggest that monocytes/macrophages might be involved in the initiation of SS by the production of TNF-α via TLR8 signaling.

Extrafollicular B cell response in two different disease milieus: IgG4-relate disease and oral squamous cell carcinoma.

Hu Chen[1], Naoki Kaneko[1], Yuka Miyahara[1], Masafumi Moriyama[1,2], Seiji Nakamura[1]

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[2] *OBT Research Center, Faculty of Dental Science, Kyushu University, Fukuoka, Japan.*

Backgrounds: Autoantibody production is one of the most characteristic features of autoimmune diseases. Recent studies have also shown that autoantibodies are generated by specific B cells in several types of cancer. These autoantibody-producing B cells might have pivotal roles in the pathogenesis of autoimmune diseases and cancers, which have potential as a therapeutic target. However, very little is known about how to produce the autoantibodies and those cell origins. We here showed the expansion of a distinct specific subset of $IgD^{-}CD27^{-}$ double negative (DN) B cells and the potential ability to produce autoantibodies.
Methods: Flow cytometry was used to investigate the DN B cells in the blood from patients with IgG4-related disease (IgG4-RD), oral squamous cell carcinoma (OSCC) and healthy control. Then DN B cells were investigated in the FFPE tissue specimens using multi-color immunofluorescence. The submandibular glands from patients with IgG4-RD and chronic sialadenitis, and the resected tongue from a patient with OSCC were dissociated into single-cell suspensions, and then immune cells were selected for downstream single-cell RNA and BCR repertoire sequencing.
Results: $IgD^{-}CD27^{-}CXCR5^{-}CD11c^{-}$ DN3 B cells, one subset of DN B cells, were increased in blood and tissue in patients with IgG4-RD and OSCC. The single-cell RNA sequencing data showed that DN3 B cells had low expression or absence of genes involved in germinal center response, but high expression of antibody-secreting genes. BCR repertoire sequencing revealed that DN3 B cells were clonally expanded in IgG4-RD and OSCC compared with disease control, and DN3 B cells contain low frequency of somatic hypermutations compared with antibody secreting cells in both diseases.
Discussion: We identify DN3 B cells as a distinct B cell subset that accumulates in both blood and tissues in patients with IgG4-RD and OSCC. In both diseases, the gene expression pattern of DN3 B cells indicates that these specific B cells are synthesizing antibodies and generated by extrafollicular responses with the low frequency of somatic hypermutations. DN3 B cells are likely to have the potential to produce autoantibodies and might contribute to the pathogenesis of autoimmune diseases and cancers.

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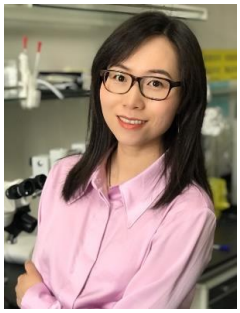
OBT Special Lecture (1)

Chaired by Dr. Zhou Wu

Yihui CUI

Principal investigator, Doctoral supervisor, School of medicine, Zhejiang University



Dr. Yihui Cui received her Ph.D. in Neurobiology from University of Paris 6. She was then a postdoctoral fellow at Medical School of Zhejiang University before she was appointed as a Principal investigator at the Center of Neuroscience. Dr. Cui has been a member of "Hundred Talents Program" at Zhejiang University since 2019. She has been focusing on the molecular and circuitry mechanisms of chronic stress induced depression and made important findings (Nature, 2018a; Nature, 2018b; Trends in Neurosciences, 2019; Neuron, 2022).

Main Publications:

1. Hypothalamus-habenula potentiation encodes chronic stress experience and drives depression onset. Zheng Z[#], Guo C[#], Li M, Yang L, Liu P, Zhang X, Liu Y, Guo X, Cao S, Dong Y, Zhang C, Chen C, Xu J, Hu H, and **Cui Y***. ***Neuron.*** 2022. (Cover)
2. Region-specific anti-Hebbian plasticity subtend distinct learning strategies in the striatum. Perez S[#], **Cui Y[#]**, Vignoud G[#], Perrin E, Mendes A, Zheng Z, Touboul J* and Venance L*. ***Cell report.*** 2022
3. Circuits and functions of the lateral habenula in health and in disease. Hu H, **Cui Y**, Yang Y. ***Nat Rev Neurosci.*** 2020.
4. Lateral Habenular Burst Firing as a Target of the Rapid Antidepressant Effects of Ketamine. **Cui Y**, Hu S and Hu H*. ***Trends in Neurosciences*** 2019 (Cover)
5. Decoding depression: insights from glial and ketamine regulation of neuronal burst firing in lateral habenula. **Cui Y**, Yang Y, Dong Y, Hu H*. ***Cold Spring Harb Symp Quant Biol***, 2019.
6. Astroglial Kir4. 1 in the lateral habenula drives neuronal bursts in depression. **Cui Y**, Yang Y, Ni Z, Dong Y, Cai G, Foncelle A, Ma S, Sang K, Tang S, Li Y, Shen Y, Berry H, Wu S, Hu H*. ***Nature,* 2018.**
7. Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. Yang Y[#], **Cui Y[#]**, Sang K[#], Dong Y[#], Ni Z, Ma S, Hu H*. ***Nature,* 2018.**

[#] equal contribution, * corresponding author

Targeting hypothalamus-habenula synapses – a novel circuitry mechanism for prevention strategy of chronic stress induced depression

Yihui CUI

School of medicine, Zhejiang University

Depression is a leading cause of disability worldwide and a major contributor to the overall global burden of diseases. However, numerous studies have focused on the "cure", but less on the "prevention". Among the complicated etiologies of depression, chronic stress is a major risk factor. Stress dynamically leads to synaptic dysfunction, which can be a potential therapeutic target for stress-related mood disorders. Given the essential role of lateral habenula (LHb) in depression, here, we attempted to clarify how LHb-centric neural circuitry integrates stress-related information. We identified lateral hypothalamus (LH) as the most physiologically relevant input to LHb under stress. LH neurons fire with a unique pattern that efficiently drives postsynaptic potential summation and a closely followed LHb bursting (EPSP-burst pairing) in response to various stressors. We found that LH-LHb synaptic potentiation is determinant in stress-induced depression. Mimicking this repeated EPSP-burst pairings at LH-LHb synapses by photostimulation, we artificially induced an ''emotional status'' merely by potentiating this pathway in mice. In contrast, by de-potentiating LH-LHb synaptic transmission during chronic stress, we successfully prevented depression onset. Notably, this de-potentiation is activity-pattern dependent, timing-sensitive and relies on the activation of eCB systems. Together, we delineated the spatiotemporal dynamics of chronic stress processing from forebrain onto LHb in a pathway-, cell-type-, and pattern-specific manner, and discovered a novel neuromodulation strategy on early interventions before depression onset.

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JICA Session

Chaired by Dr. Tomoyo Yasukochi

Some memorandums about International Activities of Kyushu University Faculty of Dentistry

Minoru Nakata, PhD, Professor Emeritus

A couple of years later since I was promoted to be a professor of Kyushu University in 1979, I was appointed as the chairman of the international exchange program for entire Kyushu University. This position gave me a very good opportunity to be acquainted with personals who were working in regard with the international activities in governmental levels or easy access to the officers who were dealing with the international projects supported by the Government. In early 1980s, the chief officer of JICA Kyushu Branch visited me and asked whether any specialty could contribute to JICA Projects. So, I asked whether any dental project was being conducted and I could know that no dental project existed. Then I tried to propose that our dental faculty may be possible to manage the dental training course.

Then I realized that setting up of this course was no so easy as I thought. Firstly, some faculty members did no not show any positive interests or did not like to pay extra efforts on such activities that may not be so much academic. In next, I was surprised when the governmental officer of the Department of Foreign Affairs that control the JICA's activities, told me that they had never received the requests for assistance in dental fields and said if the number of applications were less than ten, this course may not be realized.

Then I mailed this information with application papers to a lot of friends in the developing countries whom I could be acquainted through my activities in FDI (World Dental Congress) or visits to give lectures in those countries. I was very glad to know that most of participants in the year of 1988 when this course started, were promoted to the higher position in each area such as academic or public health, since what they learned at Kyushu University was evaluated.

In 1999, I planned the International Symposium on the Global Cooperation on Oral Health at our Faculty of Dental Kyushu University. JICA supported financially this project, then we invited several former participants and the Heads of WHO, FDI, and IADR as special advisors. This was very successful to make our Faculty very impressive and active in the field of International collaboration for better oral health promotion for whole nations. I am sure this event gave a big momentum for Kyushu University Faculty of Dentistry to be known, as the one which has been very eager to work with the global partners in the field of Oral Health and its science. As far as I

know, 242 dentists participated to this course from 52 countries during 1988-2009. I hope the participation to this course may be benefitted to not only to the participant himself or herself also to the people of their countries.

In addition, I would like to mention my activity in concerned with OBT Research Center projects. I am the first one who proposed that recent studies have shown that mastication is of great importance, not only for the intake of food but also for the systemic, mental and physical functions of the body.

At the FDI general assembly in the year of 2003, as a delegate from Japan Dental Association to FDI, I proposed that we have a lot of evidences indicating the chewing function gives good effects on general health and proposed that the FDI should approve this and transfer to all dental associations about these facts. My proposal was that FDI should adopt that Effect of Masticatory ability on general health, as the official statement of FDI for all over the world. This proposal caused a lot of debates and the decision was postponed until the last day assembly of FDI Annual Meeting, where the decision was undertaken by votes by representatives of dental associations from each country and finally this proposal was adopted. You can see this in the home page of FDI official site.

FLOW OF LIFE WITH AND WITHOUT KYUSHU UNIVERSITY

Comparison Of The Effect Of Proanthocyanidin Surface Treatments On Shear Bond Strength Of Different Cements

A. Nehir Özden[1], Şifa Atabek[2]

Department of Prosthodontics, Faculty of Dentistry, Eastern Mediterranean University, Cyprus[1].
Department of Prosthodontics, Faculty of Dentistry, Final International University, Cyprus[2].

Participation to the course of JICA in Kyushu University enriched not only my academic but social life also. While we were learning Japanese culture and science and technology in dental school of Kyushu University, because of the multicultural structure of course participants I experienced different behaviours also which I remember with a smiling face.

I have worked many different subjects of dentistry and one of my latest study was a PhD thesis '' Comparison Of The Effect Of Proanthocyanidin Surface Treatments On Shear Bond Strength Of Different Cements''.

Cements for prosthodontic restorations advanced and application techniques developed. Minimally invasive approach within dentistry created a new demand in the use of restorative properties at the micro- and macro-level, and this perspective also had an effect on bioactive cements. A bioactive material is defined as a substance that results in the formation of an apatite-like material as a surface layer in the presence of a simulated body fluid.

This study aimed to compare the effect of proanthocyanidin-rich grape seed extract (Pa-rich GSE) in two different concentrations (6.5% &12.5%) on the bond strength to dentin tissue for four different cement groups (resin cement (P), resin modified glass ionomer cement (K), calcium aluminate glass ionomer cement (C), glass ionomer cement (G).

As a result; Resin cement showed higher bond strength to dentin than other cements (P12.5: 15.08) . The application of the extract containing 6.5% proanthocyanidin to dentin surfaces increased the bonding of conventional glass ionomer cement to dentin. While there was no difference in dentin–cement bonding between resin and calcium aluminate glass ionomer cement by applying 12.5% Pa-rich GSE to dentin surfaces, resin modified, and conventional glass ionomer cement weakened dentin bonding. Calcium aluminate glass ionomer cement showed similar shear bond strength values with other cements except resin cement.

According to these results, as an alternative to resin modified glass ionomer cement and glass ionomer cement, calcium aluminate glass ionomer cement can be tried in clinical studies, and more studies can be done with glass ionomer cements and 6.5% Pa-rich GSE.

Keywords: Grape seed extract; Dental cements; Shear strength; Bioactive material; Proanthocyanidin

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OBT Special Lecture (2)

Chaired by Prof. Eijiro Jimi

Osteoblast Lineage-Specific Cell-Surface Antigen Regulating Osteoclastogenesis and Calcification: A Possible New Player in Bone Remodeling

Tamer Badawy[1,2], Yukari Kyumoto-Nakamura [3], Norihisa Uehara [3], Jingqi Zhang [3], Takayoshi Yamaza[3], Akiko Kukita[4] and Toshio Kukita[3]

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2. Department of Oral Biology, Faculty of Denistry, Galala University, Egypt.
3. Department of Molecular Cell Biology and Oral Anatomy, Faculty of Dental Science, Kyushu University, Japan.
4. Department of Microbiology, Faculty of Medicine, Saga University, Nabeshima, Saga, Japan.

(Purpose) Bone remodeling is a dynamic lifelong process involving interactions between various bone cells in a precisely coordinated manner at specific multi-cellular units. Osteoclasts play a pivotal role in bone remodeling. Although RANKL/RANK axis is considered the major factor determining the total number of osteoclasts in bone tissues, detailed molecular events regulating the efficiency of bone remodeling are still obscure. We postulated that osteoblast-specific cell-surface molecules could have regulatory roles in bone remodeling. Therefore, we carried out an extensive immunological survey searching for such molecules. In the course of our survey, we identified a unique antigen (A7 antigen) highly specific to cells in the osteoblast-lineage. We described on the detailed expression pattern of A7 antigen *in vitro* and *in vivo* and its possible role in modulating bone remodeling.
(Materials and Methods) BALB/c mice were immunized with a clone of the rat osteoblastic cell line ROS17/2.8. Hybridomas were formed by fusing splenocytes with murine myeloma cell line (P3X63-AG8-U1). Antibodies of each hybridoma were initially screened by staining of rat bone marrow, osteoblast cell line, mesenchymal stem cells. The osteoblast-specific hybridoma was selected and extensively cloned to obtain hybridoma secreting anti-A7 monoclonal antibody (A7 MAb). Immunoreactivity of A7 MAb was examined using cultures of osteoblasts and bone marrow cultures and by use of bone tissue sections. Bioactivity of this MAb was assessed using *in vitro* calcification system of primary calvarial-derived osteoblasts and bone marrow cultures for forming osteoclasts.
(Results and conclusions) *In vitro*, A7 antigen showed typical cell-surface expression pattern in osteoblast cell line and bone marrow stromal cells. *In vivo*, the antigen showed membrane expression in a subset of bone surface osteoblasts and in osteocytes. Tissue array studies demonstrated preferential expression of A7 antigen on cell-surface of osteocytes located just close to the bone surface. Immunoblotting revealed that A7 antigen was lineage-specific of approximate molecular weight of 45 KDa. Immunoprecipitation of A7 antigen from biotinylated osteoblast cell-surface proteins also resulted in a single band of 45 KDa protein. Cross-linking of A7 antigen with A7 MAb completely abrogated calcification in cultures of primary calvarial-derived osteoblasts and slightly stimulated osteoclasogenesis in bone marrow cultures. A7 antigen could be an important regulator of osteoclast recruitment and osteoblast differentiation and calcification and could be useful in the development of novel therapeutic approaches for treatment of bone diseases.

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KOB Session

Chaired by Prof. Eijiro Jimi

CURRICULUM VITAE

Kaan Orhan, DDS MSc MHM PhD, BBAc is the Dean and a Professor of DentoMaxilloFacial Radiology at the Ankara University, Faculty of Dentistry, where he serves as a faculty in Dentomaxillofacial Radiology Department, Ankara University, Ankara, Turkey, and visiting Professor at the Medical University of Lublin, Poland and was also visiting Professor between 2018-2020 at the OMFS-IMPATH Research Group, Department of Imaging and Pathology, University of Leuven, Belgium. He is currently also a visiting Professor at the Lublin University Faculty of Medicine in Poland.

Dr. Orhan received his dental degree in 1998 and completed his PhD and Maxillofacial radiology residency studies in 2002 at the Osaka University Faculty of Dentistry in Osaka, Japan and Ankara University, Faculty of Dentistry. In 2004, he started his academic career in Ankara University as a consultant at the Faculty of Dentistry. Between 2004-2006, he worked as Maxillofacial consultant and lecturer in the same University. He became an associate professor in 2006 and a full-time professor in 2012. 2007-2010, he was the founder and the chairman of Dentomaxillofacial Radiology Department, Near East University, Cyprus.

He has over 350 SCI international publications on peer-reviewed journals, and received over 5000 citations from his studies with an h index 40. He particularly made significant contributions in the Maxillofacial Radiology. He has been invited to give many lectures in national and international scientific meetings. He served as the chairman of Research and Scientific Committee, European Academy of DentoMaxillofacial Radiology (EADMFR) between 2008-2012 and he was elected for the Vice president position (2012-2014) and then as the President of EADMFR.

He is also still serving in the Research and Scientific com in IADMFR. He is a fellow of American Academy of Oral and Maxillofacial Radiology (AAOMFR), Japanese Board of DentoMaxillofacial Radiology, European Head and Neck Radiology Society (ESHNR), European society of Magnetic Resonance in Medicine and Biology (ESRMB), Turkish Magnetic Resonance Society,

He was a Board member of specialization committee in Ministry of Health and served as the recognition of Dentomaxillofacial Radiology specialty in Turkey. He is editor of several journals and also reviewer more than 50 different medical journals. He is co-author and contributor of more than 10 books both in English and Turkish.

Prof. Dr. Kaan Orhan was included in the "World's Most Influential Scientist" list, which was published by Stanford University in 2021, at the rate of 2% according to academic and scientific performance among 7 million researchers all over the world. In 2020, he received the "Innovative Dentist of the Year" award with his work titled Artificial Intelligence Application in Dentistry. Besides, The Lincoln R. Manson-Hing Honorable Mention Award given by the Oral Maxillofacial Radiology Association of America in 2018 and 2019, and the Ankara University Science Award in 2018, the Turkish Dental Society Oral and Dental Health 2nd Prize in 2015, and the "52nd Prize in Dental Health" in 2011. Japanese Congress of DentoMaxillofacial Radiology". He received the European Congress of DentoMaxillofacial Radiology", the best study award in 2008. "Yoshida Manufacturing Award" at the "7th Asia Oral and Maxillofacial Radiology" congress in 2008.

Artifical Intelligence and Blockchain in Dentistry and Radiology: What does the future hold?

Prof. Dr. Kaan ORHAN

Ankara University Faculty of Dentistry Department of Dentomaxillofacial Radiology, Ankara, TURKEY

Abstract

Artificial intelligence (AI) in healthcare is the use of algorithms and software to approximate human cognition in the analysis of complex medical data. Specifically, AI is the ability for computer algorithms to approximate conclusions without direct human input. What distinguishes AI technology from traditional technologies in health care is the ability to gain information, process it and give a well-defined output to the end-user. AI does this through machine learning algorithms, which can recognize patterns in behavior and create its own logic. This lecture explains the basic principles of deep learning and its application as well blockchain technology will be discussed technical requirements, and presents examples of successful application of deep learning techniques in dentistry and radiology based on our clinical studies.

Keywords: Artifical Intelligence, Block chain, health, NFT, IoT

Prevalence and predictors of bruxism patients with dental implants: a symptom-based diagnosis and prevention strategy

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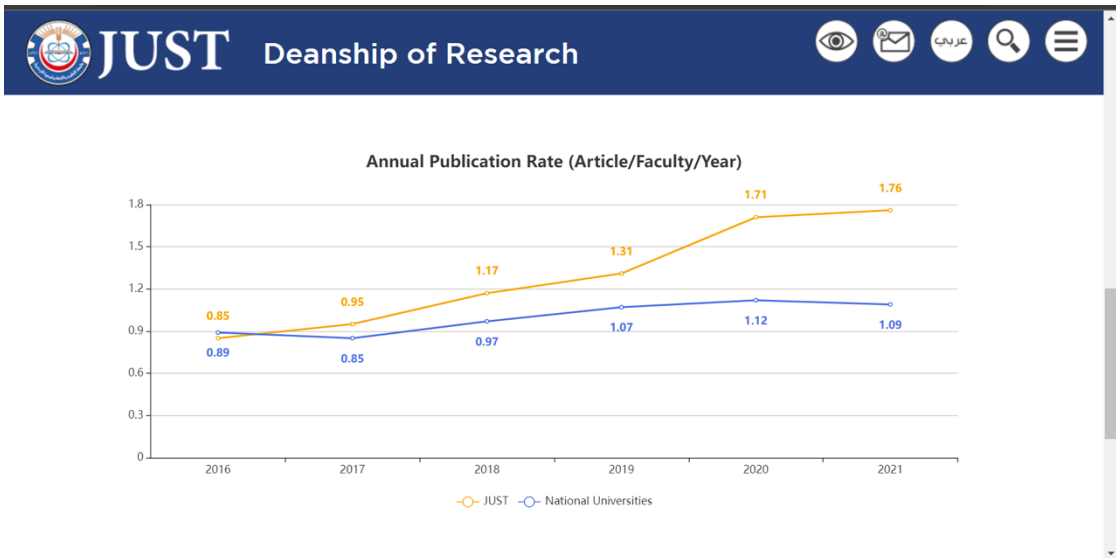
Involuntary clenching and/or bracing of the teeth -bruxism- can yield detrimental effects on the stomatognathic system. Despite the growing number of patients carrying dental implants the frequency, indicators and the consequences of bruxism is not yet fully understood. In to determine the prevalence of bruxism a survey including clinical and patient-reported findings was conducted and 1688 patients with a total of 1856 prostheses supported by 4702 implants were analyzed. 54.04% of the prostheses experienced complications consisting of 1003 technical and 353 mechanical complications. 333 patients (19.72%) identified as a probable bruxer. Besides self-reported bruxism ($p < 0.022$), antidepressant use ($p < 0.002$), frequent headaches ($p < 0.014$), and observation of linea alba ($p < 0.028$) were the predictors for probable bruxism. Technical and mechanical complications were frequent in the probable bruxers ($p < 0.05$). In this lecture diagnostic predictors of bruxism will be discussed with relation to potential prevention strategies in patients with dental implants.

Jordan University of Science and Technology (JUST) is an honored academic and research center that is committed to giving students a stimulating learning environment and a welcoming community. Among Jordanian universities, JUST is the jewel. As a part of a community of researchers, who are working to improve the education, and industry in Jordan and the region through research, worldwide scientific associations as well as local community involvement , JUST has given its major priority to academic research. The Deanship of Research started its activities at the early stages of the University establishment during the academic year 1986/1987. It works in passion to recognize, encourage, and reward success and quality in research and development by creating a research environment that encourages both basic and applied research.

The annual reports through years showed a noticeable gradual improvement in the field of research in JUST . A distinguished research publication rate was recorded in the last few years in comparison with the national universities recorded rates. The following diagram showed that proudly.



Regarding dentistry field, JUST supports dentistry research financially and scientifically. And the faculty itself provides the researchers with all the facilities needed either equipment, machines and materials. In addition, an appreciated role of faculty staff members in research can be highlighted in all dentistry specialties. The scientific environment that is provided by the university and the faculty encourages the staff members to keep up the good work in research field with passion.

In addition, JUST provides MClinDent degrees in six specialties. These programs are: Fixed and Removable Prosthodontics, Restorative Dentistry, Periodontics, Endodontics , Orthodontics and Pediatric Dentistry. To achieve the full certificate in the specialty, residents are obligated to go through a full formal research experience (master's thesis) including article submission after thesis defense under the supervision of staff members.

ABSTRACT

In Vitro immunomodulation of Human Periodontal Ligament Stem Cells on Dendritic Cells Phenotype and Function in Type 1 Diabetes

Rola Alhabashneh

Jordan University of Science and Technology (JUST), Jordan

Background: Human periodontal ligament stem cells (PDLSCs) have recently been found a viable source of mescenchymal stem cells with properties similar to those seen in bone marrow stromal stem cells (BMSSCs). Their ability to modulate dendritic cell DCs phenotype and function along with with its immunosuppressive effects on other cells of the immune system makes an investigation of PDLSCs effect in type 1 diabetes context worth pursuing Aim: The aim of this study is to understand the immunological function of human PDLSCs in DC-mediated T-cell immune responses and if they are able to modulate the islet β-cell destruction. This is done by evaluating whether periodontal ligament stem cells PDLSCs have an effect on the phenotype, differentiation and maturation of monocyte-derived DCs isolated from T1DM patients. Furthermore, this work aims to investigate the effect on gene profile for selected immunoregulatory and immunostimulatory cytokines. Material and methods: Mature dendritic cells from type 1 diabetic patients are derived from monocytes, characterized by flow cytometry at different stages (monocyte, immature DCs, and mature DCs) from four donors. mDCs where then co-culured with PDLSCs for two days and changes in level of maturation and costimulatory molecules measured by flow cytometry. qPCR was then done to analyse the gene transcription profile of co-cultured mDCs in comparison to mDCs in terms of their upregulation or downregulation of IL-6, IL-10, TGF-b, IL-1b, TNF-a Results: PDLSCs exerted an immunosuppressive effect on fully mature dendritic cells by significantly reducing expression of all maturation markers. A significant upregulation of the immunoregulatory cytokine IL6 and a significant downregulation of immunostimulatory cytokine TNF-a was also noted.

Conclusion: this in vitro study provides strong evidence of the immunomodulatory capacities of PDLSCs in T1DM context

The Association between Anti-Asthma Medications and Periodontal Health

Objective: The present study aims to assess clinical and inflammatory parameters as indicators for periodontal disease in patients with bronchial asthma who are on a combination of inhaled corticosteroids and long acting beta 2 agonists, and those on regular short acting beta 2 agonist.
Methods: 116 patients visiting the outpatient pulmonary clinics were divided into four groups according to the used medications. BA was diagnosed by a pulmonary consultant and being on inhaled asthma medication for at least 12 months. Participants were examined for clinical periodontal parameters and samples of saliva were taken and analyzed for the acidity of saliva, un-stimulated whole salivary flow rate and for the levels of 4 different inflammatory cytokines.
Results: Compared with controls, asthma groups had significant higher mean gingival index (GI) ($p = 0.001$). Also, the occurrence of periodontitis was significantly higher among asthma patients compared to non-asthma participants ($p=0.006$). Asthma patients who were treated by inhaled corticosteroids had significantly lower salivary flow rate compared to asthma patient who were used short acting beta 2 agonists (SABA) who had also lower salivary flow rate compared to control participants (0.5, 0.7, 0.9 ml/ min, $p < 0.001$). The levels of IL-1β and MCP-1 were significantly higher in the asthma group compared to the control group ($p<0.05$). The level of hsCRP was significantly higher in asthma groups who treated by inhaled corticosteroids compared to the other groups ($p<0.001$). Obese patients had significantly higher concentration of hsCRP compared to non-obese patients ($p<0.001$). There was no significant difference in the level of MMP-8 between groups.
Conclusions: Patients with bronchial asthma especially those on inhaled corticosteroids are at increased risk of having periodontitis. This association was supported by the increased level of local pro-inflammatory cytokines which might have local and systemic inflammatory burden.

Award Lectures

Chaired by Prof. Hidefumi Maeda
and Prof. Tamotsu Kiyoshima

RAF1–MEK/ERK pathway-dependent ARL4C expression promotes ameloblastoma cell proliferation and osteoclast formation

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Faculty of Dental Science, Kyushu University, Japan.

Ameloblastoma is an odontogenic neoplasm characterized by intraosseous slow growth with progressive bone resorption of the jaw. Recent reports have demonstrated that ameloblastoma harbors an oncogenic *BRAF* V600E mutation with MAPK pathway activation. Recently, we have examined the expression and function of ADP-ribosylation factor (ARF)-like 4c (ARL4C), a member of small GTP-binding superfamily, in the oral fields, especially in tumorigenesis and morphogenesis. ARL4C expression is reportedly induced by EGF/Ras signaling, and ARL4C overexpression, due to its signaling alterations, is involved in tumorigenesis. However, ARL4C expression and function in ameloblastoma remain unclear. Here, we conducted to investigate the expression of ARL4C in human ameloblastoma tissue specimens and elucidate its function in ameloblastoma cell line (AM-1). Immunohistochemical analyses demonstrated that ARL4C was strongly expressed in tumor lesions at high frequencies alongside the expression of both BRAF V600E and RAF1 (also known as C-RAF). The experiments using siRNA and inhibitors revealed that ARL4C expression depended on RAF1-MAPK, but not *BRAF* V600E mutation in ameloblastoma cell line. ARL4C-depleded AM-1 cells using CRISPR/Cas9 system showed a decrease of cellular growth and osteoclast formation when AM-1 was co-cultured with mouse bone marrow cells and primary osteoblasts. These results suggest that RAF1-MEK/ERK-mediated ARL4C expression promotes ameloblastoma cell proliferation and osteoclast formation.

These results lead to the conclusion that the RAF1-MEK/ERK-ARL4C axis, which may function in cooperation with the BRAF V600E-MEK/ERK pathway, promotes ameloblastoma development. It is therefore intriguing to speculate that cooperative activation of BRAF V600E and RAF1 would be the etiology of ameloblastoma cell proliferation and that such cooperative activation might induce the clinical aggressiveness of ameloblastoma beyond the category with benign neoplasia.

Regeneration of glomerular metabolism and function by podocyte pyruvate kinase M2 in diabetic nephropathy

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Diabetic Nephropathy (DN) is the major cause of end-stage kidney disease (ESKD) in people with diabetes, affecting 30% of patients with diabetes. DN arises from systemic and local changes in glucose metabolism and hemodynamics. In people with type 1 diabetes, hyperglycemia is the major risk factor for DN since glycemic control can prevent and delay its progression. Podocyte loss in DN is related to the duration and severity of hyperglycemia, and it correlates with the progression of DN. It has reported that many glycolytic and mitochondrial enzymes, such as pyruvate kinase M2 (PKM2), were elevated in renal glomeruli of DN-protected patients with type 1 and 2 diabetes (Gordin et al., *Diabetes Care*, 2019, Qi et al., *Nat Med*, 2017).

Here, mice with PKM2 overexpression specifically in podocytes (PPKM2Tg) were generated to uncover the renal protective function of PPKM2Tg as a potential therapeutic target that prevented elevated albumin/creatinine ratio (ACR), mesangial expansion, basement membrane thickness, and podocyte foot process effacement after 7 months of streptozotocin-induced (STZ-induced) diabetes. Furthermore, diabetes-induced impairments of glycolytic rate and mitochondrial function were normalized in diabetic PPKM2Tg glomeruli, in concordance with elevated Ppargc1a and Vegf expressions. Restored VEGF expression improved glomerular maximal mitochondrial function in diabetic PPKM2Tg and WT mice. Mechanistically, the preservations of mitochondrial function and VEGF expression were dependent on tetrameric structure and enzymatic activities of PKM2 in podocytes.

In this presentation, we report that PKM2 structure and enzymatic activation in podocytes can preserve the entire glomerular mitochondrial function against toxicity of hyperglycemia via paracrine factors such as VEGF and prevent DN progression.

Targeted inhibition of EPAS1–driven IL-31 production by a small-molecule compound

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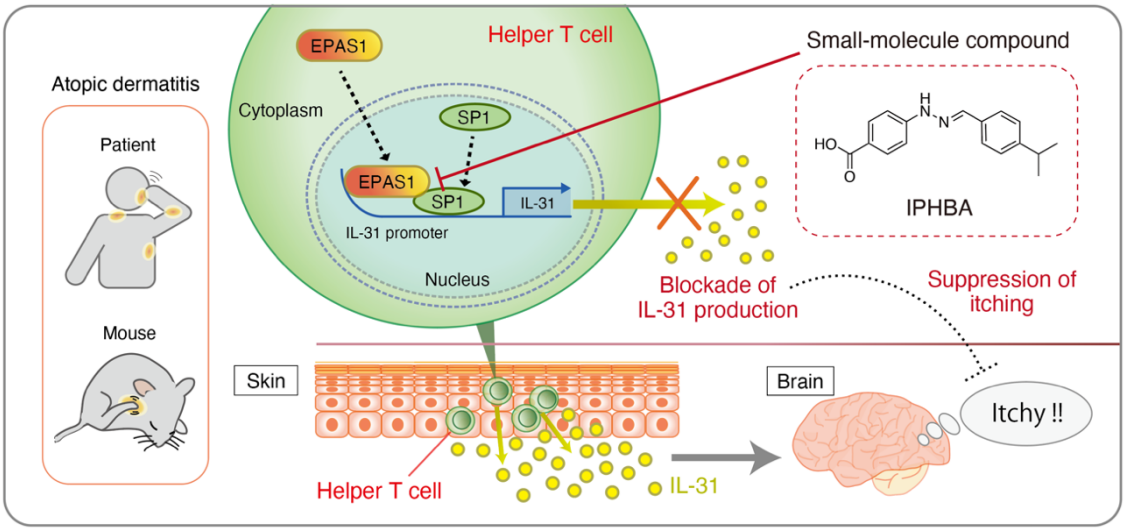

Graphical Abstract; An overview of mechanisms of IPHBA identified as a small-molecule inhibitor targeting EPAS1-driven IL-31 production.

Highlights

- IPHBA was identified as a small-molecule inhibitor of IL-31 (interleukin-31) production.
- IPHBA inhibits IL-31 induction in murine and human helper T cells without affecting other cytokine productions and hypoxic responses.
- IPHBA inhibits the association between EPAS1 and SP1 and impairs recruitment of both transcription factors to the specific sites of *IL31* promoter.

【Introduction】 Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by recurrent eczematous legions and intense itch. Although itch can be induced by various chemical mediators, IL-31 is a major pruritogen associated with AD. A specific antibody for IL-31 receptor has been shown to alleviate pruritus in patients with AD, however, therapeutic approaches to inhibit IL-31 production remain unexploited. IL-31 production by helper T cells critically depends on the transcription factor EPAS1. Therefore, EPAS1–mediated *IL-31* promotor activation might be a drug target for treatment of AD-associated itch. In this study, we aimed at developing small-molecule inhibitors that selectively block IL-31 production.

【Methods】 We generated the reporter cell line that inducibly expressed EPAS1 in the presence of doxycycline to mediate *Il31* promoter activation, and screened 9,600 chemical compounds. The selected compounds were further examined using helper T cells from a spontaneous mouse model of AD and those from patients with AD.

【**Results**】We have identified (E)-4-(2-(4-isopropylbenzylidene)hydrazinyl)benzoic acid (IPHBA) as an inhibitor of *Il31* induction. Although IPHBA did not affect non-specific T cell proliferation, IPHBA inhibited antigen–induced *Il31* induction in helper T cells from *Dock8*$^{-/-}$ AND Tg mice without affecting *Il2* and *Il4* induction. Similar results were obtained when helper T cells from AD patients were treated with IPHBA. Mechanistically, IPHBA inhibited recruitment of EPAS1 to the specific site of *Il31* promoter region.

【**Discussion**】We have shown that IPHBA selectively inhibits IL-31 induction without affecting other cytokine productions and hypoxic responses. IPHBA could be a lead drug to inhibit EPAS1-driven IL-31 production and treat AD–associated itch.

CD163^{+} M2 macrophages promote fibrosis in IgG4-related disease via TLR7/IRAK4/NF-κB signaling.

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ABSTRACT

Backgrounds: IgG4-related disease (IgG4-RD) is characterized by elevated serum IgG4 and marked fibro-inflammatory conditions that can affect multiple organs. We previously reported that human Toll-like receptor 7-transgenic C57BL/6 (huTLR7 Tg) mice showed elevated serum IgG1 (equaled to human IgG4) levels and inflammation with fibrosis in the salivary glands (SGs), lungs, and pancreas. Moreover, we observed extensive TLR7-positive CD163-positive M2 macrophage infiltration in SGs from IgG4-RD patients. However, fibrotic mechanism via the TLR-7 pathway remains unclear. In this study, we thus examined the downstream of TLR-7 and the mechanism of fibrosis in IgG4-RD.

Methods: Gene expression in SGs from huTLR7 Tg mice and IgG4-RD patients was analyzed using DNA microarrays. Common up-regulated TLR-7-related molecules in SGs from huTLR7 Tg mice and IgG4-RD patients were validated by real-time PCR and immunohistochemical staining in SGs from patient with IgG4-RD (n=15), Sjögren's syndrome (SS) (n=15), chronic sialadenitis (CS) (n=15), and controls (n=15). Next, CD163-positive M2 macrophages isolated by peripheral blood mononuclear cells from healthy controls were stimulated by TLR7 agonist (loxoribine), and then measured the cytokine concentration of culture supernatant by ELISA. Finally, we investigated the interaction between CD163-positive M2 macrophages and fibroblast. before and after stimulation with loxoribine.

Results: In both huTLR7 Tg mice and IgG4-RD patients, interleukin-1 receptor-associated kinase 3 (IRAK3) and IRAK4 were significantly overexpressed by DNA microarray. Real-time PCR validated the up-regulation of only IRAK4 in IgG4-RD patients compared with the other groups (P<0.05). IRAK4 was strongly detected in and around germinal centers in SGs from patients with IgG4-RD alone. Double immunofluorescence staining showed that IRAK4-positive cells were mainly colocalized with CD163-positive M2 macrophages in SGs (P<0.05). After stimulation with loxoribine, CD163-positive M2 macrophages exhibited significantly enhanced expression of IRAK4 and NF-κB and increased supernatant concentrations of fibrotic cytokines (IL-1β and TGF-β). Finally, we confirmed that the number of fibroblasts was increased by culture with the supernatant of CD163-positive M2 macrophages following stimulation with loxoribine (P<0.05).

Conclusion: CD163-positive M2 macrophages promote fibrosis in IgG4-RD by increasing the production of fibrotic cytokines via TLR-7/IRAK4/NF-κB signaling.

6th KOB & OBT Joint International Symposium 2022

DDR Session

Chaired by Prof. Takayoshi Yamaza

Regulation of tooth development by Epiprofin

Takashi Nakamura[1], Minoru Wakamori[1],

and Satoshi Fukumoto,[2,3]

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The balanced synchronization of cell proliferation, cell differentiation, and cell migration of dental epithelial and mesenchymal cells is required for the tooth morphogenesis with the functional shapes and optimum sizes. This synchronization is precisely operated by the reciprocal interaction between the dental epithelium and dental mesenchyme in tooth development. Epiprofin (Epfn), a transcription factor belonging to the Sp family, regulates the number of teeth as well as dental epithelial cell proliferation and differentiation into ameloblasts. Epfn deficiency results in the lack of enamel and the development of an excess number of teeth and Epfn overexpression driven by epithelial specific promoter induces ectopic enamel formation and reduction of missing teeth. The results of the analysis of abnormal dental phenotypes in these mutant mice suggest that Epfn play important roles in tooth development by regulating the synchronization of cell proliferation, cell differentiation, and cell migration of dental epithelial.

In this presentation, we discuss about the mechanism of abnormal tooth formation in Epfn mutant mice based on recent findings on the molecular regulation of signaling pathways interacting with Epfn in epithelial cells.

Strategies for functional enamel formation using hiPSCs

Han-Sung Jung

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

Tooth enamel is the highly mineralized tissue of the body, but it is well-known not to regenerate. An ideal solution for enamel regeneration is using patients' cells. Human induced pluripotent stem cells (hiPSCs) have emerged as a promising bioengineering source for organ replacement, with great potential for treating incurable diseases. Recently, many studies have kept investigating efforts to regenerate functional tooth enamel using hiPSC, which is still not established yet. Also, yet, hiPSC-derived dental organoids have not been reported. This study confirmed the optimal 2D and 3D culture protocol to induce differentiation for dental epithelial cells (hDECs) and dental epithelial organoids (hDEOs) from hiPSCs without the animal-derived factors and cell feeders for future clinical application. For hDECs differentiation with enamel deposition, we attempted to increase the efficiency and proliferation of DECs by BMP-Noggin modulation. To fabricate mineralized hard tissues, including bone and tooth, we considered extracellular matrix microenvironments, such as GelMA, collagen, and agar matrix. Recombination of hiPSC-derived dental epithelial cells and dental mesenchymal cells encapsulated in ECM led to various mineralized tissue lineage.

Furthermore, to establish a 3D dental organoid culture system, the ectodermal cells from EBs were collected, dissociated into single cells, and seeded in Matrigel with a differentiation medium. hDEOs derived from hiPSCs had the characterization of dental epithelial cells as well as ameloblasts. In conclusion, our findings provide a suitable hiPSC-derived dental epithelial cell source using both 2D and 3D cultures for tooth regeneration, especially enamel regeneration. These results will be a new insight that can be used in multiple regenerative medicine fields.