

**Kyudai Oral Bioscience  
OBT Research Center  
&  
DDR Research Center  
8<sup>th</sup> Joint International Symposium 2024**

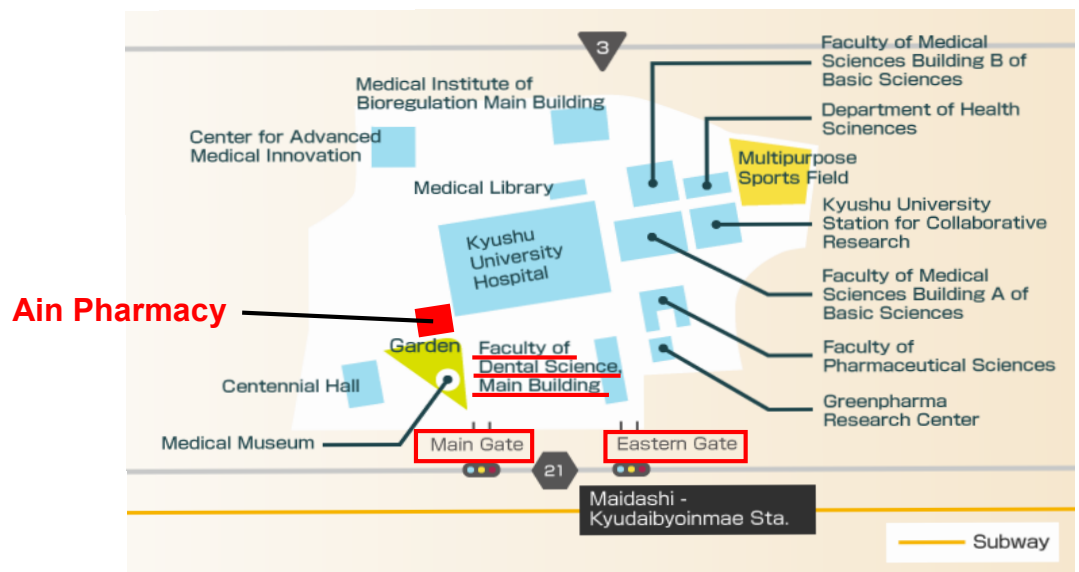
**PROGRAM & ABSTRACTS**

■ **Date**

February 8–9, 2025

■ **Location**

Ain Pharmacy Kyushu Minami Store, 2nd floor conference room  
Kyushu University Hospital Campus



■ **Organization**

Organizers

Kyudai Oral Bioscience (KOB)

Oral Health • Brain Health • Total Health (OBT) Research Center

Dento-Craniofacial Development & Regeneration (DDR) Research Center

Co-organizers

Kyusyu-daigaku Shigaku-kai

Iwadare Scholarship Foundation

■ **Organizer**

Chair: Fusanori Nishimura

Co-Chair: Takashi Kanematsu

- Zhou Wu
- Tomomi Sano
- Ayano Ogura

## ■ Contact

Takashi Kanematsu

Department of Cell Biology, Aging Science, and Pharmacology,

Division of Oral Biological Sciences, Faculty of Dental Science, Kyushu University,

Fukuoka 812-8582, Japan

Tel: +81 92-642-6412

E-mail: [taka-kanematsu@dent.kyushu-u.ac](mailto:taka-kanematsu@dent.kyushu-u.ac)

## ■ Conference Dinner

Venue: BISTRO au bascou

Date: Saturday, February 8, 2025

Time: 18:15–

## Program

February 8

Time	Title	Presenter
13:00–13:10	Opening Remark	Prof. Fusanori Nishimura
<b>Session 1</b>	<b>DDR Session</b>	Chair: Prof. Satoshi Fukumoto
13:10–13:40	Molecular Mechanism of Head & Neck Cancer Progression	Prof. Yasusei Kudo
13:40–14:10	The oral tumor-stromal interaction in the induction of jaw bone destruction	Dr. Shinsuke Fujii
14:10–14:20	Break	
<b>Session 2</b>	<b>Graduate Student Session 1</b>	Chair: Dania Alkhatib, Miyu Shida
14:20–14:30	Contribution of T cell differentiation and autophagy in neonatal thymus to the onset of autoimmunity	Shigefumi Matsuzawa
14:30–14:40	rM180Amelogenin prolongs the survival of skin allograft in mice	Miyu Shida
14:40–14:50	Elucidation of the mechanism to promote tumorigenesis of adenoid cystic carcinoma: A cancer-neuro interaction	Tatsufumi Fujimoto
14:50–15:00	Poly (L-lactide-co-ε-caprolactone)/carbonate apatite composite sponge scaffold for bone regeneration: fabrication and biological evaluation	Zhanrui Lou
15:00–15:10	Identification of a novel bone resorption regulator downstream of c-Src/p130Cas axis	Aonan Li
15:10–15:30	Break	
<b>Session 3</b>	<b>KOB Special Lecture</b>	Chair: Prof. Takashi Kanematsu
15:30–16:30	RNA Modifications and Disease Onset	Prof. Kazuhito Tomizawa
16:30–16:40	Break	
<b>Session 4</b>	<b>OBT Special Lecture</b>	Chair: Prof. Ejiro Jimi
16:40–17:40	The emotional side of autoimmune inflammatory disorders - a new venue for treatment?	Prof. Fulvio D'Acquisto

## Program

February 9

Time	Title	Presenter
9:00–9:10	Award Presentation	Prof. Fusanori Nishimura
<b>Session 5</b>	<b>Award Lecture</b>	Chair: Prof. Takashi Kanematsu
9:15– 9:30	Translocation of oral bacteria to the gut in community-dwelling adults	Dr. Shinya Kageyama
9:30– 9:45	Elucidating Dynamic Allostery in the Transmembrane Domain of TAS1R3 a Sweet Taste Receptor Subunit	Dr. Keisuke Sanematsu
9:45–10:00	Oligoclonally-expanded cytotoxic T cells and activated MKI67+B cells are potential drivers of IgG4 -related disease	Dr. Risako Koga
10:00–10:10	Break	
<b>Session 6</b>	<b>Study Abroad Reports from Undergraduate Students</b>	Chair: Dr. Zhou Wu
10:10–10:20	Participating in the International Exchange Program at Pusan National University	Yui Iihoshi
10:20–10:30	Participating in Kyushu University's English Language Training Program in Canada (University of Victoria)	Kazuha Sueyoshi
10:30–10:40	Participating in Stovit Community Outreach program 2024 held in AIRLANGGA University, Indonesia	Natuki Eguchi Teppeï Kouno
10:40–10:50	Break	
<b>Session 7</b>	<b>Graduate Student Session 2</b>	Chair: Malaz Elsheikh, Shigefumi Matsuzawa
10:50–11:00	Ligature-induced periodontitis in mice has the potential to enhance senescent CD4+ T cells	Jinfeng Li
11:00–11:10	Multistep activation of p63 and the MEK/ERK induces ARL4C expression to promote oral squamous cell carcinoma cell proliferation	Dania Alkhatib
11:10–11:20	MYBPC1 and FCHSD2 as potential autoantigens in IgG4-related dacryoadenitis and sialadenitis	Tomoki Sueyoshi
11:20–11:30	Experimental periodontitis may contribute to the progression of tubular pathology in a diabetic nephropathy model of KK-Ay mice	Al-Kafee Ahmed
11:30–11:40	Break	
<b>Session 8</b>	<b>KOB Session</b>	Chair: Dr. Akiko Mizokami
11:40–12:10	The Dynamic Role of Cathepsin B in the Brain: Physiological Functions, Pathological Alterations, and Mechanisms of Change	Dr. Junjun Ni
12:10–12:40	Association of Reduced Occlusal Support with the Development of Alzheimer's disease: Life Study	Prof. Yasunori Ayukawa
12:40–12:45	Closing Remarks	Prof. Takashi Kanematsu

# **DDR Session**

Chair: Prof. Satoshi Fukumoto

# Molecular Mechanism of Head & Neck Cancer Progression

**Yasusei Kudo**

*Department of Oral Bioscience, Tokushima University Graduate School of Biomedical Sciences, Japan*

Head and neck squamous cell carcinoma (HNSCC) is a globally prevalent cancer with a rising incidence rate. While chemotherapy, radiation therapy, and targeted drugs such as cetuximab and nivolumab have been approved for treatment, the 5-year survival rate for HPV-negative HNSCC has not improved significantly. Genetic mutation analysis in HNSCC cases has consistently shown frequent mutations in *TP53*, *FAT1*, and *CDKN2A* genes in HPV-negative cases. To better understand their roles, we generated knock-in mice with these commonly observed genetic mutations in HNSCC patients, aiming to simulate HNSCC development. Among these mice, Fat1 knock-in mice exhibited embryonic lethal, revealing mandibular and tongue defects associated with morphological abnormalities in the first pharyngeal arch.

Furthermore, we conducted subset classification of HNSCC cases to enhance our understanding of the disease. The reanalysis of publicly available single-cell RNA sequencing data resulted in the classification of HNSCC into three subsets. One of these subsets demonstrated a strong correlation with partial epithelial-mesenchymal transition (partial-EMT), which represents an intermediate state in EMT induction and plays a significant role in cancer progression. Notably, we found that partial-EMT-related genes were associated with poor prognosis in HNSCC.

Additionally, we observed that *Fusobacterium nucleatum*, commonly associated with periodontal disease, converts oral cancer cells with epithelial properties into cells with partial-EMT properties. In this talk, we would like to introduce our recent findings regarding the involvement of FAT1 in maxillofacial development and the roles of partial-EMT in the mechanism of HNSCC progression.

# **The oral tumor-stromal interaction in the induction of jaw bone destruction**

**Shinsuke Fujii<sup>1,2</sup>, Tamotsu Kiyoshima<sup>1</sup>**

*<sup>1</sup>Laboratory of Oral Pathology, Division of Maxillofacial Diagnostic and Surgical Sciences,*

*<sup>2</sup>Dento-craniofacial Development and Regeneration Research Center, Faculty of Dental Science, Kyushu University, Japan.*

Oral tumors affect more than 10,000 people each year. Although survival rates have increased with the development of surgical treatment, the current situation is that tumor formation causes bone resorption and destruction, which can lead to an increase in the extent of surgical operation and seriously impair quality of life. Therefore, it is required to develop a treatment that suppresses jaw bone destruction caused by oral tumors. However, current therapies that target osteoclasts, which are directly involved in jaw bone destruction, affect normal systemic bone metabolism. Then, it is needed to elucidate the specific mechanism of jaw bone destruction caused by oral tumors and establish a new treatment strategy based on that.

We previously reported that the elevated expression of ADP-ribosylation factor (ARF)-like 4c (ARL4C), a member of small GTP-binding superfamily, is involved in the tumorigenesis of both ameloblastoma and oral squamous cell carcinoma, which are oral tumors that develop destructing the jaw bone. However, it is unclear whether ARL4C is involved in jaw bone destruction. We recently found that the characteristics of stromal cells, existing between oral tumors and the jaw bone, is transitioned by secreted factors released from oral tumors in an ARL4C-dependent manner, and that this transition activates osteoclasts. In this presentation, we would like to discuss the detailed molecular basis of stromal cell transition by oral tumors.

# **Graduate Student Session 1**

Chair: Dania Alkhatib, Miyu Shida

# **Contribution of T cell differentiation and autophagy in neonatal thymus to the onset of autoimmunity**

**Shigefumi Matsuzawa<sup>1,2</sup>, Aya Ushio<sup>2,3</sup>, Ruka Nagao<sup>2</sup>, Kunihiro Otsuka<sup>2</sup>, Takaaki Tsunematsu<sup>2</sup>, Masafumi Moriyama<sup>1</sup>, Naozumi Ishimaru<sup>2,3</sup>**

*<sup>1</sup> Section of Oral and Maxillofacial Surgery, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan*

*<sup>2</sup> Department of Oral Molecular Pathology, Institute of Biomedical Sciences, Tokushima University, Tokushima, Japan*

*<sup>3</sup> Department of Oral Pathology, Graduate School of Medical and Dental Sciences, Institute of Science Tokyo, Tokyo, Japan*

## **Background**

Sjögren syndrome (SS) is the autoimmune disease which affects exocrine glands, such as lacrimal and salivary glands. We previously reported that the T cell property including Treg component were altered in the SS model generated with neonatal thymectomy. In this study, we investigated the impact of T cell development in the neonatal thymus on the development of autoimmune diseases. In addition, it was recently reported that starvation-independent autophagic activities contribute to production of autoreactive T cells during T cell differentiation in the thymus. Therefore, we also examined the relationship between T cell selection related with the autophagy in the neonatal thymus and the onset of autoimmune disease.

## **Materials and Methods**

Male and female C57BL/6 (B6) mice at 1, 3, 5 and 7 days after birth were used for each experiment. Thymus tissues were digested with liberase in the presence of 0.01% DNase I recombinant to obtain thymic epithelial cell (TEC). Surface or intracellular molecules of TECs and thymocytes were analyzed by flow cytometer. Microtubule-associated protein light chain 3 (LC3) expression-related to the autophagy was examined by western blotting using extracted proteins from the thymus tissue.

## **Results**

The number of TECs was significantly increased from day 3 to day 7 after birth, and the number of thymocytes was dramatically increased at neonatal stage consistent with TECs expansion. LC3 expression associated with autophagy was elevated in the day 3 thymus, and autophagy in TECs was enhanced at day 3 after birth by autophagy detection.

## **Conclusion**

Autophagic activity during the early stage in the neonatal thymus may affect the dramatic T cell differentiation associated in B6 mice. Now, we are trying to find the relationship between T cell differentiation and autophagy in neonatal thymus of SS model mice by comprehensive gene expression analysis using neonatal thymus samples.

## **rM180Amelogenin prolongs the survival of skin allograft in mice.**

**Miyu Shida<sup>1</sup>, Terukazu Sanui<sup>1</sup>, Li Jinfeng<sup>1</sup>, Karen Yotsumoto<sup>1</sup>, Yuki Nishimura<sup>1</sup>, Chikako Hayashi<sup>1</sup>, Takao Fukuda<sup>1</sup>, Fusanori Nishimura<sup>1</sup>**

<sup>1</sup>*Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University.*

### *Background and objective:*

It is empirically known that enamel matrix proteins appear to exhibit minimal post-surgical inflammation and favorable healing outcomes. Previous studies reported that amelogenin inhibits the expression of major histocompatibility complex class II (MHC II) through histone H3 euchromatin suppression with reduced antigen presentation. However, the physiological significance of lowered MHC II expression remains unclear. This study aimed to investigate the influence of amelogenin on skin graft rejection among the mice with different haplotype antigens.

### *Methods and Results:*

Recombinant amelogenin was applied to the skin graft from C57BL/6J mice, followed by the transplantation onto recipient sites of BALB/c mice. The median survival period of the grafts was extended by up to almost 6 days compared to a PBS control group, accompanied by a reduction in necrotic area. Amelogenin applied group showed lower splenic size, weight and number of the cells than control. Histological analysis of skin tissues at day 7 after transplantation showed significantly decreased inflammatory cell infiltration and MHC II+ cells in test group, while large thickening and massive immune cell infiltration was observed in the control group. Furthermore, a decrease in CD4+ and CD19+ cells was observed with significant reduction in IFN- $\gamma$ +CD4+ cells. However, CD25+Fox3+CD4+ cells increased controversially. Serum IFN- $\gamma$ , IL-2 and IL-17 levels in amelogenin applied group were significantly lower than control group.

### *Conclusion:*

The results may provide insights into the mechanisms by which amelogenin accelerates post-surgical wound healing during flap surgery. This study demonstrates that the application of amelogenin significantly extended graft survival time by six days, facilitated enhanced tissue regeneration, and promoted systemic immunosuppression. The findings elucidate the underlying mechanisms through which amelogenin exerts its beneficial effects in the context of periodontal surgical procedures. Moreover, these results suggest the potential of amelogenin as a promising immunosuppressive agent, with potential therapeutic applications in the treatment of autoimmune diseases and allergic conditions.

## **Elucidation of the mechanism to promote tumorigenesis of adenoid cystic carcinoma: A cancer-neuro interaction**

**Tatsufumi Fujimoto<sup>1,2</sup>, Shinsuke Fujii<sup>1,3</sup>, Tamotsu Kiyoshima<sup>1</sup>**

<sup>1</sup>*Laboratory of Oral Pathology, and* <sup>2</sup>*Section of Oral and Maxillofacial Surgery, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan*

<sup>3</sup>*DDR center, Faculty of Dental Science, Kyushu University, Fukuoka, Japan*

Adenoid cystic carcinoma (AdCC) is a rare malignant tumor that arises in the salivary glands and has the tendency for perineural invasion. The current first-line treatment for AdCC is surgical removal. Although the 5-year survival rate for AdCC is high, the long-term prognosis is still relatively poor. Therefore, the development of new cancer treatment based on the molecular basis of AdCC formation is needed to improve the long-term survival rate.

Based on the idea that organ development and tumor formation have a common molecular mechanism, we focused on a certain axonal guidance factor and hypothesize that the axonal guidance factor functions as a common molecule associated with salivary gland development and AdCC tumorigenesis. Then, we have revealed that the axonal guidance factor can play an important role in salivary gland development and AdCC tumorigenesis (Fujii, Fujimoto *et al.* Pathol Res Pract 2022).

The autonomic nervous system controls the physiological functions of salivary glands. Recently, it has also been reported that the autonomic nervous system controls the tumor formation derived from exocrine glands (Claire Magnon *et al.* Science 2013, Michelle Monje *et al.* Cell 2020, Steven W Cole *et al.* Nat Rev Cancer 2015). Considering these reports, organ development and tumor formation in exocrine glands are regulated by nerves. However, the interaction between AdCC development and nerve tissue remains unclear. Therefore, the aim of this study is to clarify the mechanism of cancer-nerve interaction in AdCC formation.

We performed immunohistochemical analysis in human AdCC and oral squamous cell carcinoma (OSCC) specimens. We found that the number of nerve bundles (S100-positive) per specimen was higher in AdCC than in OSCC. Furthermore, in AdCC specimens, the short diameter and area of nerve bundles in the perineural invasion area were larger than those in the non-perineural invasion area. In addition, the Ki-67 (proliferative cell marker) positive rate of tumor cells in the area-- of perineural invasion was significantly higher than that in the non-perineural invasion area in the AdCC specimens. These results suggest that the interaction of AdCC and nerve tissue enhances AdCC proliferation.

# **Poly (L-lactide-co-ε-caprolactone)/carbonate apatite composite sponge scaffold for bone regeneration: fabrication and biological evaluation**

**Zhanrui Lou<sup>1</sup>, Ryo Kishida<sup>1</sup>, Koichiro Hayashi<sup>1</sup>, Kunio Ishikawa<sup>1</sup>**

*<sup>1</sup>Department of Biomaterials, Faculty of Dental Science, Kyushu University, Japan*

Shape-conforming bone graft materials offer versatile solutions for bone regeneration. Commonly used granular bone grafts can accidentally dislodge from the graft site, and block grafts inevitably create gaps with the host bone. Combining ceramic powders with mechanically flexible polymers can create both shape-conforming and osteoconductive bone grafts. Notably, the bone mineral phase, carbonate apatite (CAp), known for its superior osteoconductivity, is considered a promising candidate for enhancing the osteogenic capability compared to conventional bone graft compositions.

This study presents a novel PLCL/CAp composite sponge for bone regeneration. The composite was prepared by freeze-drying a PLCL/CaCO<sub>3</sub> suspension, followed by immersion in sodium phosphate solution to convert the CaCO<sub>3</sub> into CAp. Pure PLCL sponges were also prepared as controls. To examine the impact of macroporosity on bone regeneration, sponges with 500 μm macropores (PLCL/CAp<sub>500</sub>) were fabricated using NaCl as a porogen. The biological responses of the sponges were assessed using MC3T3-E1 cell cultures and rabbit femoral defect models.

X-ray diffraction (XRD) confirmed complete conversion of CaCO<sub>3</sub> to CAp in both the PLCL/CAp and PLCL/CAp<sub>500</sub> groups. Scanning electron microscopy (SEM) revealed that the phosphatization treatment exposed CAp particles on the PLCL surface, with micropores (~10 μm) and macropores (~500 μm) in the PLCL/CAp<sub>500</sub> group.

Biological evaluation using MC3T3-E1 cells showed that the PLCL/CAp and PLCL/CAp<sub>500</sub> composites enhanced cell attachment and osteogenic differentiation compared to pure PLCL sponges. At 4 weeks post-implantation, histological analysis revealed significant new bone formation in the PLCL/CAp and PLCL/CAp<sub>500</sub> groups, with bone area percentages of 8.4% and 17.7%, respectively, compared to just 2.3% in the pure PLCL group. These results suggest that PLCL/CAp sponges, particularly those with macropores, offer superior osteoconductivity and hold promise for effective bone repair applications.

## **Identification of a novel bone resorption regulator downstream of c-Src/p130Cas axis**

**Aonan Li<sup>1</sup>, Jing Gao<sup>1</sup>, Eijiro Jimi<sup>1,2</sup>**

<sup>1</sup>*Department of Molecular and Cellular Biochemistry, Faculty of Dental Science, Kyushu University, Japan*

<sup>2</sup>*Department of Oral, Brain and Total Health Science, Faculty of Dental Science, Kyushu University, Japan*

Podosome formation in osteoclasts is a critical initial step in osteoclastic bone resorption. Mice deficient in either c-Src (c-SrcKO) or its downstream effector, p130Cas (Crk-associated substrate) exhibit osteopetrosis due to impaired podosome formation in osteoclasts, highlighting the significance of the c-Src/p130Cas axis in osteoclastic bone resorption. We have previously demonstrated that c-Src and p130Cas form a complex with Pyk2, which is crucial for bone resorption. In this study, our objective was to identify new downstream molecules of the c-Src/p130Cas axis that are essential for osteoclastic bone resorption and to further elucidate the underlying molecular mechanisms. We performed immunoprecipitation with an anti-Pyk2 antibody using cell lysate extracted from osteoclasts of wild-type (WT), c-SrcKO, and osteoclast-specific p130Cas-deficient (p130Cas<sup>ΔOCL-/-</sup>) mice, followed by mass spectrometry analysis of the Pyk2-immunocomplex. Our aim was to identify proteins that could form a complex with Pyk2, c-Src and p130Cas. Using this screening strategy we identified 34 proteins. Among these 34 proteins, Y-box binding protein 3 (Ybx3), which has been reported to control cytoskeleton organization and is distributed in a wide variety of tissues including osteoclasts, was investigated for its role in osteoclast differentiation and activation. When Ybx3 was knocked down using siRNA in an osteoclast precursor cell line (Raw 264.7) or bone marrow cells, the number of osteoclasts with actin rings and the number of large multinucleated osteoclasts were significantly reduced. Immunofluorescence staining of primary osteoclast derived from WT mice revealed that YBX3 and p130Cas colocalize on the actin ring in osteoclasts. Furthermore, we overexpressed long and short isoforms of YBX3 in HEK cells and performed immunoprecipitation. Interestingly, we found that only the long isoform of YBX3 could be co-immunoprecipitated with Pyk2, c-Src and p130Cas. These results indicate that Ybx3 is important for the formation of actin rings and the multinucleation process during osteoclast differentiation, probably acting as a downstream effector of c-Src/p130Cas.

# **KOB Special Lecture**

Chair: Prof. Takashi Kanematsu

# RNA Modifications and Disease Onset

**Kazuhito Tomizawa**

*Department of Molecular Physiology, Faculty of Life Sciences, Kumamoto University,  
Japan*

Accurate and efficient protein translation is one of the most fundamental biological processes in all living organisms. The basic molecular mechanism of translation whereby transfer RNA (tRNA) binds to codon nucleotides of messenger RNA (mRNA) on the ribosome to decode genetic information and transfer amino acids to form peptide chains according to the mRNA's instructions, is universally accepted as a core principle of the central dogma of life sciences. One remarkable feature of tRNA is the diverse array of post-transcriptional modifications it undergoes. More than 100 types of modifications are present in the nucleotide bases of tRNA in both prokaryotes and eukaryotes, making base editing in tRNA one of the most evolutionarily conserved cellular functions. Our research has previously elucidated molecular mechanisms through which the loss or abnormalities in tRNA modifications contribute to the onset of various diseases, including diabetes, mitochondrial disorders, myopathies, and type 1 allergies.

In this symposium, I will focus on the molecular mechanisms of Asian-type type 2 diabetes. *Cdk5 regulator subunit associated protein 1-like 1 (CDKAL1)* is one of the most reproducible genes associated with type 2 diabetes. While the physiological role of CDKAL1 was previously unclear, our research identified it as an enzyme responsible for thiomethylating adenosine at position 37 near the anticodon of lysine-specific tRNA. A deficiency in CDKAL1 leads to mistranslation during lysine incorporation, resulting in abnormalities in insulin processing and reduced insulin secretion. Furthermore, we developed a drug screening system to suppress the mistranslation caused by CDKAL1 deficiency during lysine translation. Using this system, we identified compounds capable of inhibiting mistranslation. Clinical studies on these compounds confirmed their efficacy. This presentation will detail these findings and provide insights into the molecular mechanisms of Asian-type type 2 diabetes, offering new perspectives on potential therapeutic approaches.

Furthermore, we have revealed that modified nucleosides, which are metabolites of modified RNAs, are actively excreted in urine. I will also introduce that urinary modified nucleosides serve as sensitive markers for bacterial and viral infections.

# **OBT Special Lecture**

Chair: Prof. Eijiro Jimi

## **The emotional side of autoimmune inflammatory disorders - a new venue for treatment?**

**Fulvio D'Acquisto**

*School of Life and Health Science, University of Roehampton,  
London, United Kingdom; William Harvey Research Institute, School of Medicine and  
Dentistry, Queen Mary University of London, London, United Kingdom*

Emotions and feelings are the building blocks of our social lives, yet we often overlook their significant impact on our physical well-being. A growing body of research has demonstrated that both imbalanced and improved emotional states can greatly influence our immune system's response. This talk presents a summary of our recent findings, which focus on the effects of various emotional states on the immune system, and vice versa. Our hope is that this research will encourage scientists and clinicians to consider the therapeutic value and potential of emotions and feelings in immune-related diseases, shedding light on the scientific truth behind the well-known proverb "a sound mind in a sound body."

# **Award Lecture**

Chair: Prof. Takashi Kanematsu

## Translocation of oral bacteria to the gut in community-dwelling adults

**Shinya Kageyama<sup>1</sup>, Satoko Sakata<sup>2,3</sup>, Jiale Ma<sup>1</sup>, Mikari Asakawa<sup>1</sup>,  
Toru Takeshita<sup>1</sup>, Michiko Furuta<sup>1</sup>, Toshiharu Ninomiya<sup>2,3</sup>, Yoshihisa  
Yamashita<sup>1</sup>**

<sup>1</sup>*Section of Preventive and Public Health Dentistry, Faculty of Dental Science, Kyushu University, Japan*

<sup>2</sup>*Center for Cohort Studies, Graduate School of Medical Sciences, Kyushu University*

<sup>3</sup>*Department of Epidemiology and Public Health, Graduate School of Medical Sciences, Kyushu University, Japan*

Ectopic enrichment of oral microbes in the gut is a notable alteration in gut microbial balance. These microbes are likely delivered from the oral cavity with saliva and food; however, evidence of oral-gut microbial transmission is insufficient and needs further investigation. In this observational study, we examined 144 pairs of saliva and stool samples collected from community-dwelling adults to verify the oral-gut microbial link and identify the relevant influencing factors on the increased abundance of oral microbes within the gut. The bacterial composition of each sample was determined using PacBio single-molecule long-read sequencing of the full-length 16S rRNA gene and amplicon sequence variant (ASV) analysis. Although the bacterial compositions of salivary and gut microbiota were distinctly different, at least one ASV was shared between salivary and gut microbiota in 72.9% of subjects. Shared ASVs accounted for 0.0 to 63.1% (median 0.14%) of the gut microbiota in each subject and frequently included abundant *Streptococcus salivarius* and *Streptococcus parasanguinis*. Their total relative abundance in the gut was significantly higher in older subjects or those with dental plaque accumulation. The gut microbiota with  $\geq 5\%$  of shared ASVs displayed a higher abundance of *Streptococcus*, *Lactobacillus*, and *Klebsiella* and a lower abundance of *Faecalibacterium*, *Blautia*, *Megamonas*, and *Parabacteroides*. Our study presents evidence for the translocation of oral bacteria to the gut in community-dwelling adults and suggests that aging and dental plaque accumulation contribute to an increased abundance of oral microbes in the gut, which might be relevant to the compositional shift in the gut commensals.

## Elucidating Dynamic Allostery in the Transmembrane Domain of TAS1R3, a Sweet Taste Receptor Subunit

**Keisuke Sanematsu<sup>1,2,3</sup>, Masato Yamamoto<sup>1,4</sup>, Yuki Nagasato<sup>1,5</sup>, Yuko Kawabata<sup>1</sup>, Yu Watanabe<sup>1</sup>, Shusuke Iwata<sup>1,3</sup>, Shingo Takai<sup>1</sup>, Kiyoshi Toko<sup>3,6</sup>, Toshiro Matsui<sup>3,5</sup>, Naohisa Wada<sup>4</sup>, and Noriatsu Shigemura<sup>1,3</sup>**

<sup>1</sup>*Oral Neuroscience, Graduate School of Dental Science, Kyushu University, Japan*

<sup>2</sup>*Oral Health/Brain Health/Total Health Research Center, Graduate School of Dental Science, Kyushu University, Japan*

<sup>3</sup>*Research and Development Center for Five-Sense Devices, Kyushu University, Japan*

<sup>4</sup>*Department of General Dentistry, Division of Interdisciplinary Dentistry, Faculty of Dental Science, Kyushu University, Japan*

<sup>5</sup>*Department of Bioresources and Biosciences, Faculty of Agriculture, Graduate School of Kyushu University, Japan*

<sup>6</sup>*Institute for Advanced Study, Kyushu University, Japan.*

Sweet taste receptor functions as an energy sensor by detecting carbohydrates, a primary source of calories. Understanding the mechanisms of sweet taste receptor activation could enable the development of taste modifiers beneficial for individuals with diabetes mellitus or obesity, where calorie management is essential. While previous studies have primarily focused on the static binding interactions between the sweet taste receptor and their ligands, the dynamic mechanisms governing receptor activation remain poorly understood. In this study, we explored the interactions between the transmembrane domain of TAS1R3, a G protein-coupled sweet receptor subunit, and various allosteric modulators using molecular dynamics (MD) simulations. Our simulations successfully reproduced species-specific ligand sensitivities, offering novel insights into receptor behavior. One key finding was that cyclamate, a human-specific sweetener, interacts with the mouse receptor as a negative allosteric modulator, demonstrating how ligand-receptor interactions can vary across species. Furthermore, agonist-induced allostery during receptor activation was observed to destabilize the intracellular portion of the receptor, potentially interfacing with the G $\alpha$  subunit, through the opening of an ionic lock. This destabilization represents a critical step in the activation mechanism. We also investigated a common human TAS1R3 variant, R757C, which showed a reduced response to sweet taste stimuli, consistent with our predictions. Additionally, histidine residues within the binding site were found to act as pH-sensitive microswitches, modulating receptor sensitivity to saccharin. These findings enhance our understanding of the dynamic activation mechanisms of TAS1R3 and provide a framework for predicting similar mechanisms in other G protein-coupled receptors.

## Oligoclonally-expanded cytotoxic T cells and activated MKI67<sup>+</sup>B cells are potential drivers of IgG4 -related disease

**Risako Koga<sup>1</sup>, Takashi Maehara<sup>1,2</sup>, Ryuichi Aoyagi<sup>1</sup>, Ryusuke Munemura<sup>1</sup>, Yuka Murakami<sup>1</sup>, Atsushi Doi<sup>3</sup>, Michihito Kono<sup>4</sup>, Hidetaka Yamamoto<sup>5</sup>, Hiroaki Niiro<sup>6</sup>, Tamotsu Kiyoshima<sup>7</sup>, Mika Tanabe<sup>8</sup>, Toshiaki Nakano<sup>9</sup>, Yuta Matsukuma<sup>9</sup>, Mitsuhiro Kawano<sup>10</sup>, John H. Stone<sup>11</sup>, Shiv Pillai<sup>12</sup>, Shintaro Kawano<sup>1</sup>, Seiji Nakamura<sup>1</sup>**

<sup>1</sup> Section of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan; <sup>2</sup> Dento-craniofacial Development and Regeneration Research Center, Faculty of Dental Science, Kyushu University; <sup>3</sup> Cell Innovator, Inc., Fukuoka, Japan; <sup>4</sup> Department of Rheumatology, Endocrinology and Nephrology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan; <sup>5</sup> Graduate School of Medicine, Dentistry & Pharmaceutical Science, Okayama University, Okayama, Japan; <sup>6</sup> Department of Medical Education, Faculty of Medical Sciences, Kyushu University, Fukuoka, Japan; <sup>7</sup> Laboratory of Oral Pathology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan; <sup>8</sup> Department of Ophthalmology, Graduate School of Medicine Sciences, Kyushu University, Fukuoka, Japan; <sup>9</sup> Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; <sup>10</sup> Division of Rheumatology, Department of Internal Medicine, Kanazawa University Hospital, Kanazawa, Japan; <sup>11</sup> Division of Rheumatology, Allergy, and Immunology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; <sup>12</sup> Ragon Institute of MGH, MIT, and Harvard, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

IgG4-related disease (IgG4-RD) is an immune-mediated fibrotic disorder characterized by dysregulated resolution of severe inflammation and wound healing. However, truly dominant or pathognomonic autoantibodies related to IgG4-RD are not identified. We sought to perform single-cell RNA sequencing and T-cell receptor and B-cell receptor sequencing to obtain a comprehensive, unbiased view of tissue-infiltrating T and B cells.

We performed unbiased single-cell RNA-sequencing analysis for the transcriptome and T-cell receptor sequencing and B-cell receptor sequencing on sorted CD3<sup>+</sup> T or CD19<sup>+</sup> B cells from affected tissues of patients with IgG4-RD. We also conducted quantitative analyses of CD3<sup>+</sup> T-cell and CD19<sup>+</sup> B-cell subsets in 68 patients with IgG4-RD and 30 patients with Sjögren's syndrome.

Almost all clonally expanded T cells in these lesions were either Granzyme K (GZMK)-expressing CD4<sup>+</sup> cytotoxic T cells or GZMK<sup>+</sup>CD8<sup>+</sup> T cells. These GZMK-expressing cytotoxic T cells also expressed amphiregulin and TGF- $\beta$  but did not express immune checkpoints, and the tissue-infiltrating CD8<sup>+</sup> T cells were phenotypically heterogeneous. MKI67<sup>+</sup> B cells and IgD<sup>-</sup>CD27<sup>-</sup>CD11c<sup>-</sup>CXCR5<sup>-</sup> double-negative 3 B cells were clonally expanded and infiltrated affected tissue lesions. GZMK<sup>+</sup>CD4<sup>+</sup> cytotoxic T cells colocalized with MKI67<sup>+</sup> B cells in the extrafollicular area from affected tissue sites.

The above-mentioned cells likely participate in T-B collaborative events, suggesting possible avenues for targeted therapies. Our findings were validated using orthogonal approaches, including multicolor immunofluorescence and the use of comparator disease groups, to support the central role of cytotoxic CD41 and CD81 T cells expressing GZMK, amphiregulin, and TGF- $\beta$  in the pathogenesis of inflammatory fibrotic disorders.

# **Study Abroad Reports from Undergraduate Students**

Chair: Dr. Zhou Wu

## **List of Presenters of KOB/OBT International Symposium 2024**

1.

Participating in the International Exchange Program at Pusan National University  
Yui Iihoshi, 4th year student, School of Dentistry

2.

Participating in Kyushu University's English Language Training Program in Canada  
(University of Victoria)  
Kazuha Sueyoshi, 3rd year student, School of Dentistry

3.

Participating in Stovit Community Outreach program 2024 held in AIRLANGGA  
University, Indonesia  
Natsuki Eguchi, 3rd year student, School of Dentistry  
Teppei Kono 3rd year student, School of Dentistry

# **Graduate Student Session 2**

Chair: Malaz Elsheikh, Shigefumi Matsuzawa

## **Ligature-induced periodontitis in mice has the potential to enhance senescent CD4+ T cells**

**Jinfeng Li<sup>1</sup>, Terukazu Sanui<sup>1</sup>, Miyu Shida<sup>1</sup>, Mwannes Ahmad<sup>1</sup>,  
Chikako Hayashi<sup>1</sup>, Takao Fukuda<sup>1</sup>, Fusanori Nishimura<sup>1</sup>**

*<sup>1</sup>Department of periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Japan*

Chronic periodontitis typically develops in individuals aged 40–50 and is rare in those under 30. Aging weakens immune function while enhancing chronic inflammation and autoimmune responses, contributing to diseases like Type 2 diabetes, rheumatoid arthritis, and certain cancers. The age-related changes in the immune system are known as "immunosenescence" and can be broadly divided into three symptoms: a decline in adaptive immune response ability, an overproduction of inflammatory cytokines, and an increased risk of autoimmunity. Thymic involution after puberty reduces naive T cells, leading to compensatory proliferation of senescent T cells. Among these, senescent CD4+ T cells, characterized by PD-1 and CD153 expression, have impaired proliferation, shortened telomeres, and high secretion of inflammatory cytokines, forming the senescence-associated secretory phenotype (SASP).

This study aims to clarify the molecular mechanism by which chronic periodontitis exacerbates systemic diseases using ligature-induced periodontitis (LIP) as an experimental model from the perspective of senescent CD4+ T cells.

5, 10, 18, 26, 34, and 42 weeks old Balb/c mice were subjected to induction of periodontitis through silk ligatures tied around both sides of the maxillary second molar (LIP group). Periodontitis was induced via silk ligatures around maxillary second molars, and splenic CD4+ T cells were analyzed. Initial findings revealed no significant differences in PD-1/CD153 double-positive cells between the LIP and control groups at any age. However, when cells were stimulated *in vitro* with IL-2, anti-TCR $\beta$ , and anti-CD28 for three days to mimic "homeostatic proliferation," the LIP group showed a significantly higher proportion of PD-1/CD153 double-positive cells, peaking at 18 weeks of age. This stimulation also increased SASP cytokine secretion, such as osteopontin and IL-6, in the LIP group's culture supernatant. SA  $\beta$ -gal staining confirmed that LIP accelerated CD4+ T cell senescence through homeostatic proliferation.

These results suggest that LIP promotes CD4+ T cell senescence, potentially contributing to systemic inflammation. Future experiments will involve transferring splenic CD4+ T cells from LIP and control groups into T cell-deficient mice to evaluate homeostatic proliferation *in vivo* and its impact on experimental rheumatoid arthritis. This approach will further elucidate the link between periodontitis-induced senescence and systemic diseases.

## **Multistep activation of p63 and the MEK/ERK induces ARL4C expression to promote oral squamous cell carcinoma cell proliferation**

**Dania Alkhatib<sup>1</sup>, Shinsuke Fujii<sup>1,2</sup>, Tamotsu Kiyoshima<sup>1</sup>**

*<sup>1</sup>Laboratory of Oral Pathology, Division of Diagnostic and Surgical Sciences,  
Faculty of Dental Science, Kyushu University, Japan*

*<sup>2</sup>DDR center, Faculty of Dental Science, Kyushu University, Fukuoka, Japan*

Carcinogenesis is a process consisting of ordered steps accompanied by genetic changes to drive the transition from non-tumorous epithelia through a preneoplastic lesion/benign tumor to cancer. Oral squamous cell carcinoma (OSCC) progresses in sequential steps beginning with epithelial hyperplasia, followed by dysplasia, carcinoma in situ, and invasive carcinoma. This leads to the hypothesis that OSCC development may involve a series of genetic alterations driving each step of progression. Nevertheless, the detailed molecular mechanisms are unknown. In this study, the comprehensive gene expression patterns were examined using DNA microarray data from a pathological specimen of OSCC (including a non-tumor region, carcinoma in situ lesion, and invasive carcinoma lesion) and enrichment analyses were performed. Then, the expression of several genes and signal activation were found to be changed in the process of OSCC development. Of these, the p63 expression was increased and the MEK/ERK-MAPK pathway was activated in carcinoma in situ lesion and in invasive carcinoma lesion, respectively. Reportedly, ADP-ribosylation factor (ARF)-like 4c (ARL4C) has been shown to promote tumorigenesis in several types of cancer. Immunohistochemical analyses revealed that p63 was initially upregulated in carcinoma in situ followed by ERK activation in invasive carcinoma lesions in OSCC specimens and that ARL4C was more frequently expressed in tumor lesions, especially in invasive carcinoma lesions, compared to its expression in carcinoma in situ lesions. Moreover, ARL4C and phosphorylated ERK were frequently colocalized in invasive carcinoma lesions. Loss-of-function experiments using inhibitors and siRNAs revealed that p63 and MEK/ERK-MAPK cooperatively induce ARL4C expression and cell growth in OSCC cells. These results suggest that the stepwise activation of p63 and the MEK/ERK-MAPK pathway is involved in OSCC tumor cell proliferation by regulating ARL4C expression.

## MYBPC1 and FCHSD2 as potential autoantigens in IgG4-related dacryoadenitis and sialadenitis

Tomoki Sueyoshi<sup>1</sup>, Naoki Kaneko<sup>1,2</sup>, Junsei Sameshima<sup>1</sup>, Hu Chen<sup>1</sup>, Shiho Yokomizo<sup>1</sup>, Haruki Nagano<sup>1</sup>, Yan Lijin<sup>1</sup>, Masafumi Moriyama<sup>3</sup>, Shintaro Kawano<sup>1</sup>

<sup>1</sup> *Section of Oral and Maxillofacial Oncology Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan*

<sup>2</sup> *OBT Research Center, Faculty of Dental Science, Kyushu University, Fukuoka, Japan*

<sup>3</sup> *Section of Oral and Maxillofacial Surgery Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka, Japan*

**Background:** IgG4-related disease (IgG4-RD) is a chronic inflammatory condition characterized by elevated serum IgG4 levels and tissue infiltration of IgG4-positive plasma cells, affecting various organs with storiform fibrosis. Particularly, the disease targets lacrimal and salivary glands, leading to IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS). Despite presumed autoimmune mechanisms, specific autoantibodies and target antigens remain unidentified. Studies using peripheral blood have reported oligoclonal expansion of unique B cells producing autoantibodies against autoantigens like galectin-3, laminin-511, and prohibitin. However, their expression varies among patients and a unified view on autoantigens has not been obtained. Since affected organs directly reflect disease pathogenesis, this study aimed to identify autoantigens using both peripheral blood and affected tissues.

**Materials and Methods:** We conducted protein microarray analyses on serum from IgG4-DS. Single-cell RNA-sequencing (scRNA-seq) was employed to perform gene expression and B cell receptor (BCR) repertoire analysis on CD45-positive cells from five resected submandibular glands of IgG4-DS. Recombinant antibodies based on BCR sequences were generated. Protein microarray analyses was conducted and the results were compared with published scRNA-seq of total cells from IgG4-DS submandibular glands, identifying candidate molecules for autoantigens. Furthermore, immunofluorescence staining was used to evaluate their expression in tissues.

**Results:** Protein microarray analyses using serum revealed variations in known autoantibodies across patients, some of which were also found in healthy controls. scRNA-seq with BCR repertoire analysis highlighted B cell infiltration of various differentiation stages, including clonal expansion of antibody-secreting cells. Recombinant antibodies were generated from BCR sequences. The results from protein microarray analyses using serum and recombinant antibodies differed, with the latter revealing several potential autoantigens. Further analysis with published scRNA-seq data identified MYBPC1 and FCHSD2 as candidate autoantigens. Immunofluorescence staining showed that these candidate molecules are expressed in the epithelial cells of submandibular gland.

**Conclusion:** This study highlights the significant B cell infiltration in submandibular glands of IgG4-DS and emphasizes the potential for antibody-secreting cells to produce autoantibodies. MYBPC1 and FCHSD2 were identified as potential autoantigens, expressed in the epithelial cells, suggesting that these autoantibodies may contribute to the pathogenesis of IgG4-DS. Understanding these phenomena opens avenues for developing targeted diagnostics and therapeutic strategies.

## **Experimental periodontitis may contribute to the progression of tubular pathology in a diabetic nephropathy model of KK-A<sup>y</sup> mice**

**Al-Kafee Ahmed<sup>1</sup>, Takanori Shinjo<sup>1</sup>, Kohei Sato<sup>1</sup>, Zeze Tatsuro<sup>1</sup>,  
Honoka Otsuka<sup>1</sup>, Mio Imagawa<sup>1</sup>, Yuki Nishimura<sup>1</sup>,  
Gulinigeer Dilimulati<sup>1</sup>, Naoaki Ryo<sup>1</sup>, Akiko Yamashita<sup>1</sup>,  
Misaki Iwashita<sup>1</sup>, Fusanori Nishimura<sup>1</sup>**

*<sup>1</sup> Department of Periodontology Division of Oral Rehabilitation Faculty of Dental Science*

**Background:** Previous studies suggested an association between diabetic nephropathy (DN) and periodontal disease (PD), although the precise underlying mechanism is still unclear. Here, we focused on the tubular pathology which might be potentially influenced by PD. **Method:** Thirteen-week-old male C57BL/6 and KK-Ay mice were divided into groups with or without experimental periodontitis induced by 6-0 silk ligation around the maxillary second teeth for 3 weeks. Urinary tubular injury markers were assessed, while tubular fibrosis and inflammatory cell infiltration were evaluated using Masson's Trichrome and CD68 immunofluorescent staining. Real-time PCR measured inflammatory and fibrotic gene expressions in tubular fractions, and RNA sequencing explored PD-associated factors in the tubules of DN mice. **Results:** The urinary lipocalin2-creatinine ratio in KK-Ay mice was significantly elevated by experimental periodontitis. Fibrotic areas and CD68-positive cells in the tubules were also significantly increased in KK-Ay mice with ligation compared to those without. Tubular gene expressions related to fibrosis and inflammation, such as Fibronectin1, Tnf $\alpha$ , IL-1 $\beta$ , and Mcp-1, were significantly upregulated in KK-Ay mice with ligation. RNA sequencing revealed significant alterations in ion transport-related pathways, and western blot analysis showed increased tubular expression of parvalbumin in KK-Ay mice with ligation. Further studies are needed to clarify the role of these proteins in DN progression. **Conclusion:** Experimental periodontitis may contribute to the progression of tubular pathology in DN.

# **KOB Session**

Chair: Dr. Akiko Mizokami

# **The Dynamic Role of Cathepsin B in the Brain: Physiological Functions, Pathological Alterations, and Mechanisms of Change**

**Junjun Ni**

*Department of Biology, School of Life Science, Beijing Institute of Technology, China*

Cathepsin B (CatB), a lysosomal cysteine peptidase, is implicated in various brain pathologies, including hypoxic brain injury, infection-induced neuroinflammation, and brain aging. However, its physiological functions and the mechanisms underlying its transition between beneficial and detrimental roles in the brain remain unclear.

Our recent findings show that CatB is highly expressed in the cerebral cortex of mice from embryonic day 12.5 (E12.5) to E16.5 and plays a key role in cortical development by regulating neuronal migration and differentiation. Mice lacking CatB exhibit normal cognitive function but display depressive behaviors. Additionally, we observed that CatB regulates young microglia to clear amyloid plaques in the brain, suggesting its critical role in both brain development and homeostasis.

Despite these beneficial effects, CatB also exhibits harmful functions in certain conditions. Using aging and inflammation cell models, we found that CatB translocate from the lysosome to the cytosol and other organelles, where a shift in pH may alter its substrate preference. Notably, pharmacological inhibition of CatB in the lysosome had no impact on microglial senescence, while either genetic deletion or inhibiting cytosolic CatB significantly alleviated microglial senescence. These results indicate that the subcellular localization of CatB—whether within the lysosome or extralysosomal compartments—determines its functional outcomes in the brain.

Our findings suggest the potential for a targeted, cellular location- or pH-dependent inhibitor strategy to modulate CatB activity and intervene in associated brain disorders.

# **Association of Reduced Occlusal Support with the Development of Alzheimer's disease: Life Study**

**Yasunori Ayukawa**

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

A link between poor oral health and cognitive decline has been reported. Most of these studies have used the number of teeth as a reference, and only a few have analyzed the relationship between occlusal status and Alzheimer's disease (AD). We focused on the Eichner classification to clarify whether posterior occlusal contacts are associated with AD in a Japanese population aged 65 years or older.

In this study, we used monthly claims data from the Japanese National Health Insurance from April 2017 to March 2020. The outcome was newly diagnosed Alzheimer's disease, defined according to ICD-10 code G30. The number of teeth was estimated from dental code data, and occlusal contacts were classified into three categories (A, B, and C) according to the Eichner classification. We used a multivariate Cox proportional hazards model to analyze the association between new diagnosis of AD and the Eichner classification.

The hazard ratios for Eichner classification B and C were 1.34 and 1.54, respectively, after adjusting for covariates. In conclusion, advanced age, reduced posterior occlusal contact, and tooth loss are associated with AD. It can be emphasized the importance of paying attention to occlusal support to reduce the risk of AD.

