Kyudai Oral Bioscience
&
OBT Research Center・DDR Research Center

7th Joint International Symposium 2023

PROGRAM & ABSTRACTS

February 18, 2024

Lecture Room AB / Zoom meeting

Faculty of Dental Science,

Kyushu University

◆ Date:

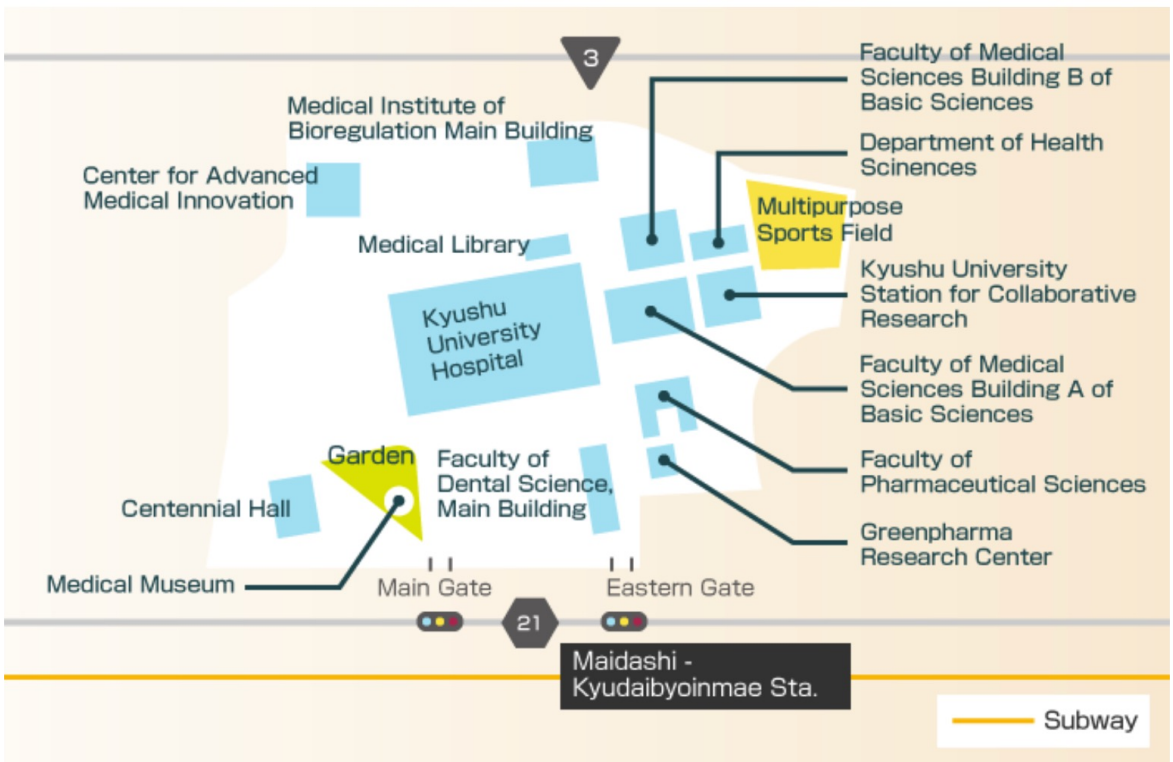
February 18, 2024

◆ Hybrid:

Zoom meeting / Lecture room AB

◆ Organization

- Kyudai Oral Bioscience
- Dento-Craniofacial Development and Regeneration Research Center
- Oral Health・Brain・Health Total Health Research Center



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7th KOB & OBT & DDRJoint International Symposium 2023

PROGRAM

Time	Title	Presenter
9:00–9:10	**Opening Remark**	**Prof. Fusanori Nishimura**
Session 1	**Yonsei University Session**	**Chair: Dr. Shingo Takai**
9:10–9:25	Construction of 3D anatomical data using ultrasound and motion capture technology.	Hyungkyu Bae
9:25–9:40	Salt diet affect salt preference in Drosophila melanogaster.	LINNI JIN
9:40–9:55	Tuning circadian rhythm of cancer-associated fibroblasts against cancer progression.	Shujin Li
9:55–10:10	**Break**	
Session 2	**DDR Session**	**Chair: Prof. Satoshi Fukumoto**
10:10–10:50	Dual nature of K8 expressing differentiated cells drives epithelial regeneration.	Han-Sung Jung
10:50–11:10	Human fibrotic autoimmune disease: latest immunological approaches into the pathogenesis	Takashi Maehara
11:10–11:30	Temperature-controlled ex vivo culture for organ development and drug-induced side effect mitigation: insights from mouse tooth germ model	Tian Tian
11:30–11:45	**Break**	
Session 3	**Undergaduate Student`s Session**	**Chair: Dr. Kenichi Ogata**
11:45–12:00	Short term summer study abroad program at Universitas Airlangga (Indonesia)	Kanto Nakamura
12:00–12:15	Study Abroad at The University of Waikato, Waikato College (New Zealand)	Miku Tokuhisa
12:15–13:15	**Lunch Break**	
13:15–13:25	**Award Presentation (IF and FWCI Award)**	**Prof. Fusanori Nishimura**
Session 4	**Award Lectures (IF Award winners)**	**Chair: Prof. Takashi Kanematsu**
13:25–13:45	p130Cas is required for androgen-dependent postnatal development regulation of submandibular glands.	Jing Gao
13:45–14:05	Distinct disease-specific Tfh cell populations in two different fibrotic diseases: IgG4-related disease and Kimura's disease.	Ryusuke Munemura
14:05–14:25	GATA3 mediates nonclassical β-catenin signaling in skeletal cell fate determination and ectopic chondrogenesis.	Daigaku Hasegawa
14:25–14:45	Long-term administration of bisphosphonates enhances aversive behavioral responses to HCl.	Asami Oike
14:45–15:00	**Break**	
Session 5	**Graduate Student`s Session**	**Chair: Dr. Takao Fukuda Dr. Takashi Maehara**
15:00–15:15	The function of membrane-permeable itaconate in cancer cell growth.	Ayaka Saeki
15:15–15:30	Arl4c is involved in tooth germ development through osteoblastic differentiation.	Truong Thi Kim Thinh
15:30–15:45	Involvement of B cells in tumor immunity of oral squamous cell carcinoma.	Junsei Sameshima
15:45–16:00	Theme of presentation: Fabrication and histological evaluation of ant-nest type porous carbonate apatite artificial bone.	Janice Tan Lay Tin
16:00–16:15	Cytoplasmic signaling molecule CySM2 in LTi cells plays critical role on Payer's patch formation.	Naoko Kiya
16:15–16:30	Fabrication of granular apatite cement using partially set α-tricalcium phosphate granules.	Abdulrahman Diabi
16:30–16:45	Establishment of a new peripheral nerve regeneration therapy using artificial nerves with extracellular vesicles derived from dental pulp stem cells.	Aiko Yano
16:45–16:55	**Closing Remarks**	**Prof. Satoshi Fukumoto**

Session1

Yonsei University Session

Chaird by Dr. Shingo Takai

Construction of 3D anatomical data using ultrasound and motion capture technology.

Hyungkyu Bae[1], Kang-Woo Lee[1], Soo-Bin Kim[1], Kyung-Seok Hu[1], Hee-Jin Kim[1,2]

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Ultrasound (US) imaging is widely used in both treatment and diagnosis because it can visualize the internal structure in real time, is simple to utilize, and less harmful to patients. Recently, various three-dimensional (3D) methods including 3D scanning, computed tomography (CT), magnetic resonance imaging (MRI) have been used in the field, but US has been able to be analyzed 3D only in a limited area due to its characteristics. The aim of the study was to construct and verify 3D anatomic data by specifying the location of the US transducer using motion capture technology.

A total of 57 healthy Korean volunteers (average age 43.2 years, 57 females) were included in this study. Real-time two-dimensional B-mode ultrasound images of the intraoral structures were acquired using a high-frequency (11.5 MHz) intraoral transducer. Image reconstruction was additionally performed to identify structures in intraoral US images. A motion capture camera was used to track using an intraoral transducer attached with a marker, and the structure was scanned at the reference points. Simultaneously, the marker attached to the subject's forehead was tracked and used as the Reference Coordinate System. The face of the volunteer was scanned with the mouth open 2.7cm using an Artec Space Spider scanner, which is a high-resolution 3D scanner based on blue light technology. The US image position of the tracked intraoral transducer and the scanned face were visualized with software that is part of the SlicerIGT extension. For the verification, MRI images were taken from one volunteer using a 3.0T scanner. The visualized image and MRI of the volunteer were superimposed on the nose and ear and used for image analysis.

The superimposed model of the MRI and 3D scanned face with tracked intraoral US image was obtained. The location of the Internal anatomic structures of intraoral area were confirmed in the model and verified through comparison with MRI image. The association with motion capture technology can extend the ultrasonographic visualization from two dimensions to the three dimensions. This method can provide 3D model for the internal structure in real time, and it is expected to be utilized as a powerful diagnostic tool for clinicians.

Salt diet affect salt preference in *Drosophila melanogaster.*

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Taste plays a pivotal role in evaluating the nutritional value and potential toxicity of food. Typically, sweet and umami are perceived as appealing, while bitter and sour are considered aversive, and salt elicits a concentration-dependent preference. Salt taste is essential for maintaining electrolyte homeostasis and facilitating nutrient absorption; however, prolonged high salt intake can be detrimental. In mammals, elevated salt consumption is linked to an increased risk of cardiovascular and chronic kidney diseases. Despite considerable knowledge about the molecular and cellular underpinnings of salt taste in mammals and insects, the impact of daily dietary salt on salt perception remains unclear. In this study, utilizing the *Drosophila* model system, we investigated the effects of daily salt intake on salt perception. Our findings reveal that daily salt intake suppresses low salt sensitivity so that reduces the intake of salt-containing food in flies. Exposure to a sodium-rich diet leads to the abolition of low salt electrophysiological responses in low salt-sensing gustatory neurons through receptor-mediated endocytosis, reshaping both low salt perception and feeding behavior. While the desensitization mechanism exhibits sexual dimorphism, both male and female flies counteract salt desensitization by inhibiting clathrin-mediated endocytosis following the salt diet. Notably, the macropinocytosis pathway also modulates low salt perception exclusively in female flies after the salt diet. Collectively, our study provides insights into how salt diet-induced alterations in salt perception are regulated in *Drosophila*, shedding light on the intricate interplay between dietary factors and sensory mechanisms.

Tuning circadian rhythm of cancer-associated fibroblasts against cancer progression

Shujin Li[1], Yan Cui[1], Senthil Kumar Baskaran[1], Ji-Sun Kim[1], Anish Ashok Adpaikar[1], Hyun-Yi Kim[1], Han-Sung Jung*

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Cancer-associated fibroblasts (CAFs) are essential stromal cell populations of the tumor microenvironment (TME) with diverse functions, including matrix deposition and remodeling, extensive reciprocal signaling interactions with cancer cells and crosstalk with infiltrating leukocytes. However, owing to the limitation of understanding the origin and biological function of CAFs, many challenges are present in ongoing attempts to modulate CAFs for therapeutic benefit.

The circadian clock coordinates the daily rhythmicity of metabolic, physiologic, and behavioral processes with a 24-hour periodicity in humans. Intriguingly, dysfunction of the circadian clock is intimately related to tumorigenesis and facilitates the circadian rhythm disruption of the adjacent tissues.

In the present study, we are utilizing ameloblastoma, an odontogenic tumor arising in the jawbone, as a model system, to investigate the circadian rhythm of CAFs and the subsequential biological effect on tumor progression. We established the stromal-rich ameloblastoma tumoroid that recapitulated the histological and molecule characteristics of ameloblastoma. The CAFs that isolated from ameloblastoma patients, showed accelerated growth and metabolic rhythm and disrupted circadian clock expression pattern compared to the control. Time-series RNA sequencing results confirmed that circadian clock genes were upregulated, the rhythmically expressed genes were decreased, and gene ontology terms related to extracellular matrix remodeling, secretory process, and metabolism process were enriched in ameloblastoma tumoroid co-cultured with the CAFs group. Interestingly, CRISPR/Cas9 based-BMAL1 knockout in CAFs results in the reduces of the growth factors and inflammatory cytokines secretion, matrix contraction in transcription levels. Furthermore, administration of the BMAL1 inhibitor (GSK4112) in CAFs increase the anti-tumor efficacy of PLX4023 on ameloblastoma tumoroid. In conclusion, we demonstrated that targeting CAFs by tuning circadian rhythm regulate the secretion and ECM remodeling properties that beneficial for the anti-cancer treatment on ameloblastoma.

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Session2

DDR Session

Chaird by Prof. Satoshi Fukumoto

Dual nature of K8 expressing differentiated cells drives epithelial regeneration

Han-Sung Jung, Anish Adpaikar, Jong-Min Lee

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Taste receptor cells are epithelial cells dependent upon the innervating nerve for continuous renewal and maintained by resident tissue stem/progenitor cells. Transection of the innervating nerve causes degeneration of taste buds and taste receptor cells. Genetic lineage tracing has identified that Lgr5+ cells present at the trench of the circumvallate papilla give rise to all taste and non-taste epithelial cells during homeostasis and regeneration. However, whether Lgr5 expressing cells are the only source of stem cells in circumvallate papillae (CVP) remains to be investigated. This study revealed that a subset of the taste receptor cells is maintained without nerve contact after glossopharyngeal nerve transection in the CVP of adult mice.

Furthermore, the injury caused by nerve transection triggers these surviving differentiated K8-positive taste receptor cells to dedifferentiate and acquire a transient progenitor cell-like state. Dedifferentiated taste receptor cells proliferate, express progenitor cell markers (K14, Sox2, PCNA), and form organoids *in vitro.* In another model, using Lgr5-DTR mice, ablation of Lgr5+ expressing cells led to the depletion of half the CVP organ by disrupting the basal cells and basement membrane after 24 hours. Interestingly, the entire CVP with taste buds was regenerated within 2 weeks. This data indicates that another population of cells acts as reserve stem cells in the CVP that activate upon Lgr5 stem/progenitor cell depletion.

This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korea Government (MSIP) (2022R1A2B5B03001627 and NRF-2021R1A2C1005506)

Human fibrotic autoimmune disease: latest immunological approaches into the pathogenesis

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We examined tissue infiltrating T cells in human fibrotic atutoimmune disease. IgG4-related disease (IgG4-RD) is an immune-mediated fibrotic disorder characterized by the dysregulated resolution of severe inflammation and wound healing and represents an example of a Type I atutoimmune disease. We performed single-cell RNA-sequencing and T-cell receptor (TCR) sequencing to obtain a comprehensive, unbiased view of tissue infiltrating T-cells. Almost all clonally expanded T-cells in these lesions were either GZMK expressing $CD4^+$ cytotoxic T-cells (CTLs) or $GZMK^+$ $CD8^+$ CTLs. These Granzyme K^+ cytotoxic T cells also express amphiregulin and TGFb, but did not express immune-checkpoints, and the tissue infiltrating $CD8^+$ T cells are phenotypically heterogeneous. Other abundant infiltrating T-cells in IgG4-RD lesions included two T helper cell populations, $IL10^+LAG3^+Foxp3^-$ Tfh cells and $CXCL13^+TIGIT^+PD1^+$Tfh cells. Infiltrating regulatory T cells with an altered dysregulated transcriptome similar to that seen in tumors and severe COVID-19 were also observed in this inflammatory disease. We also conducted quantitative analyses of the T cell subsets in 68 IgG4-RD and 30 Sjögren's syndrome (SjS) patients. Our findings, validated using orthogonal approaches, support a central role for cytotoxic $CD4^+$ and $CD8^+$T-cells expressing Granzyme K, amphiregulin and TGFb in the pathogenesis of inflammatory fibrotic disorders. Therapies should be focused on targeting these cells and studies on pathogenesis should examine the antigenic sources and mechanisms of their generation.

Temperature-controlled ex vivo culture for organ development and drug-induced side effect mitigation: insights from mouse tooth germ model

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In recent years, with the advancement of regenerative technologies, the prospect of organ regeneration using pluripotent stem cells has become increasingly realistic. However, for the clinical application of these achievements in regenerative medicine, the timely provision of regenerated organs to patients is crucial. This study aimed to establish a temperature screening method for controlling the development of organs by applying an ex vivo organ culture model using mouse tooth germs. The development of cultured tooth germs was delayed by low-temperature stimulation and could be resumed by subsequent cultivation at 37°C. Furthermore, the optimal temperature for the long-term preservation of organs was found to be 25°C, maintaining the expression of stem cell markers under these conditions. Comprehensive analysis revealed that low-temperature stimulation induces the expression of cold shock proteins (CSP). These results suggest that maintaining stem cell viability and CSP expression may play an important role in the long-term preservation of organs under the low-temperature culture conditions at 25°C.

Additionally, with a focus on clinical applications, we investigated the potential of avoiding the side effects of anticancer drugs that affect developing organs. By applying low-temperature stimulation, it was possible to circumvent the inhibition of tooth formation caused by cyclophosphamide (CPA), an anticancer drug used in the treatment of childhood leukemia. Furthermore, low-temperature stimulation inhibited Rb phosphorylation and induced cell cycle arrest at the G1 phase during CPA treatment. These findings suggest the potential for preventing cell damage associated with DNA replication caused by CPA through low-temperature stimulation.

This study indicates the potential utility of a screening method for organ culture using tooth germs in screening for organ regeneration technology and medical techniques.

Session3

Undergraduate Student Session

Chaird by Dr. Kenichi Ogata

Session4

Award Lectures

Chaird by Prof. Takashi Kanematsu

p130Cas is required for androgen-dependent postnatal development regulation of submandibular glands

Jing Gao[1], Aonan Li[1], Shinsuke Fujii[2,3], Fei Huang[1], Tamotsu Kiyoshima[2], Eijiro Jimi[1,4]

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Salivary glands develop through epithelial-mesenchymal interactions and are formed through repeated branching. The Crk-associated substrate protein (p130Cas) serves as an adapter that forms a complex with various proteins via integrin and growth factor signaling, with important regulatory roles in several essential cellular processes. We found that p130Cas is expressed in ductal epithelial cells of the submandibular gland (SMG). We generated epithelial tissue-specific p130Cas-deficient (*p130Cas*$^{\Delta epi-}$) mice and aimed to investigate the physiological role of p130Cas in the postnatal development of salivary glands.

Submandibular gland were extracted from *p130Cas*$^{flox/flox}$ (control) and *p130Cas*$^{\Delta epi-}$ mice and measured for weight. Mice were anesthetized and stimulated with pilocarpine to collect saliva for secretion measurement and components analysis. For histological analysis, submandibular gland were processed for H/E staining, immunohistochemistry and transmission electron microscopy (TEM), respectively. Quantitative RT-PCR was performed to examine mRNA expression levels in submandibular gland.

Histological analysis showed immature development of granular convoluted tubules (GCT) of the SMG in male *p130Cas*$^{\Delta epi-}$ mice. Immunofluorescence staining showed that nuclear-localized androgen receptors (AR) were specifically decreased in GCT cells in *p130Cas*$^{\Delta epi-}$ mice. Furthermore, epidermal growth factor (EGF)-positive secretory granules contained in GCT cells were significantly reduced in *p130Cas*$^{\Delta epi-}$ mice with downregulated AR signaling. GCTs lacking p130Cas showed reduced numbers and size of secretory granules, disrupted subcellular localization of the cis-Golgi matrix protein GM130, and sparse endoplasmic reticulum membranes in GCT cells. These results suggest that p130Cas plays a crucial role in androgen-dependent GCT development accompanied with ER-Golgi network formation in SMG by regulating the AR signaling.

Distinct disease-specific Tfh cell populations in two different fibrotic diseases: IgG4-related disease and Kimura's disease

Ryusuke Munemura[1], Takashi Maehara[1,10], Yuka Murakami[1], Risako Koga[1], Ryuichi Aoyagi[1], Naoki Kaneko[1], Atsushi Doi[2], Cory A. Perugino[3,4], Emanuel Della-Torre[5],Takako Saeki[6], Yasuharu Sato[7], Hidetaka Yamamoto[8], Tamotsu Kiyoshima[9], John H. Stone[3], Shiv Pillai[4], and Seiji Nakamura[1].

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How T follicular (Tfh) cells contribute to many different B-cell class-switching events during T-cell–dependent immune responses has been unclear. Diseases with polarized isotype switching offer a unique opportunity for the exploration of Tfh subsets. Secondary and tertiary lymphoid organs in patients with elevated tissue expression levels of IgE (Kimura disease, KD) and those of IgG4 (IgG4-related disease, IgG4-RD) can provide important insights regarding cytokine expression by Tfh cells. We sought to identify disease-specific Tfh cell subsets in secondary and tertiary lymphoid organs expressing IL-10 or IL-13 and thus identify different cellular drivers of class switching in 2 distinct types of fibrotic disorders: allergic fibrosis (driven by type 2 immune cells) and inflammatory fibrosis (driven by cytotoxic T lymphocytes).

Peripheral blood and affected organs of 11 patients with KD and 25 patients with IgG4-RD were used. Single-cell RNA sequencing, in situ sequencing, and multicolor immunofluorescence analysis were used to investigate B cells, Tfh cells, and infiltrating type 2 cells in lesion tissues from patients with KD or IgG4-RD.

Infiltrating Tfh cells in tertiary lymphoid organs from IgG4-RD were divided into 6 main clusters. We encountered abundant infiltrating IL-10–expressing LAG3$^+$Tfh cells in patients with IgG4-RD. Furthermore, we found that infiltrating AICDA$^+$CD19$^+$ B cells expressing IL-4, IL-10, and IL-21 receptors correlated with IgG4 expression. In contrast, we found that infiltrating IL-13–expressing Tfh cells were abundant in affected tissues from patients with KD. Moreover, we observed few infiltrating IL-13 expressing Tfh cells in tissues from patients with IgG4-RD, despite high serum levels of IgE (but low IgE in the disease lesions). Cytotoxic T cells were abundant in IgG4-RD;
in contrast, type 2 immune cells were abundant in KD.

Our analysis revealed a novel subset of IL-10$^+$LAG3$^+$Tfh cells infiltrating the affected organs of IgG4-RD patients. In contrast, IL-13$^+$Tfh cells and type 2 immune cells infiltrated those of KD patients. IL-10$^+$LAG3$^+$Tfh cells might contribute to IgG4 isotype switching in SLOs and TLOs of patients with IgG4-RD. In contrast, IL13$^+$Tfh cells might contribute to high-affinity IgE secretion by B cells in KD.

GATA3 mediates nonclassical β-catenin signaling in skeletal cell fate determination and ectopic chondrogenesis

Takamitsu Maruyama[1,2] †, Daigaku Hasegawa[1,2] †, Tomas Valenta[3], Jody Haigh[4], Maxime Bouchard[5], Konrad Basler[3], Wei Hsu[1,2,6,7,8*]

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Skeletal precursors are mesenchymal in origin and can give rise to distinct sublineages. Their lineage commitment is modulated by various signaling pathways. The importance of Wnt signaling in skeletal lineage commitment has been implicated by the study of β-catenin–deficient mouse models. Ectopic chondrogenesis caused by the loss of β-catenin leads to a long-standing belief in canonical Wnt signaling that determines skeletal cell fate. As β-catenin has other functions, it remains unclear whether skeletogenic lineage commitment is solely orchestrated by canonical Wnt signaling. The study of the Wnt secretion regulator Gpr177/Wntless also raises concerns about current knowledge.

Therefore, we have created several mouse models to examine details of the skeletal cell fate decision mediated by β-catenin. Our findings provide evidence supporting that an alternative mechanism mediated by β-catenin independent of the transcriptional output of canonical Wnt signaling is necessary for inhibiting ectopic chondrogenesis. The whole genomics study further examines downstream effectors, leading to the identification of GATA3 as the key modulator associated with this alternative signaling effects of β-catenin. Functional analyses further demonstrate that the GATA3 transcription factor is sufficient to promote the commitment of skeletogenic mesenchyme to chondrocyte lineage. In this study, the single pioneer factor GATA3 can program skeletal cells to become chondrocytes that are responsible for cartilage formation, demonstrating its essential role in mediating nonclassical β-catenin signaling in skeletogenic lineage specification.

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

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

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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.

Session5

Graduate Student`s Session

Chaird by Dr. Takao Fukuda

Dr. Takashi Maehara

The function of membrane-permeable itaconate in cancer cell growth

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Introduction: Itaconate (IA) is synthesized in the citric acid cycle *via* cis-aconitate decarboxylase (ACOD1). Previous reports demonstrated that IA is an anti-inflammatory metabolite in macrophage-mediated acute inflammation, making it possible to link with cellular and molecular events involved in various pathophysiological conditions such as cancer. In this study, we thus focused on the effect of IA on cancer progression by using 4-occtyl itaconate (OI) which is a membrane-permeable IA.

Materials and Methods: OI, IA (membrane-impermeable IA), and 2-ethylhexanoic acid (EHA) were applied to *in vitro* (murine (B16) and human (COLO679 and G361) melanoma cell lines) and *in vivo* (C57BL/6N mice) assays. Cell proliferation potential was evaluated by using the WST-8 and the BrdU incorporation assays. RNA sequencing was performed with the MGI DNBSEQ G400 FAST. LCMS-8050 was used for HPLC-MS/MS assays. Glutathione, ROS, and γH2AX quantifications were measured by GSSG/GSH quantification kit, ROS assay kit, and DNA damage detection kit-γH2AX, from Dojindo, respectively. Lipid hydroperoxide (LPO) and ROS were quantified by FACSAria Fusion and FlowJo software from BD Biosciences with Liperfluo (Dojindo). Succinate dehydrogenase (SDH) activity was measured by Complex II assay kit from Cayman Chemical. Mitochondrial respiratory capacity was assessed using a Seahorse XF Extracellular Flux analyzer.

Results: Cell proliferation of melanoma cells treated with OI, but not IA and EHA, were significantly suppressed in its concentration-dependent manner. Comprehensive transcriptome analysis in B16 cells revealed that OI treatment induced a marked change in the expression of genes related to glutathione metabolism. Subsequent analysis showed OI treatment induced a decreased glutathione levels followed by increased levels of ROS, LPO, and γH2AX. Furthermore, OI treatment suppressed the activity of mitochondrial complex II and induced a marked decrease in mitochondrial respiratory capacity. In addition, OI markedly suppressed tumor growth *in vivo*, partly caused by abnormal glutathione metabolism, with the minimum side effect.

Conclusion: This study demonstrated the direct antiproliferative effects of OI on melanoma cells, mediated by increased ROS accumulation, resulted from impaired glutathione metabolism and dysfunction of mitochondrial respiratory capacity. These intracellular events consequently led the melanoma cells to senescence.

Arl4c is involved in tooth germ development through osteoblastic differentiation.

Truong Thi Kim Thinh, Shinsuke Fujii, Tamotsu Kiyoshima

Laboratory of Oral Pathology

Murine tooth germ development proceeds in continuous and sequential steps with reciprocal interactions between the odontogenic epithelium and the adjacent mesenchyme, and several growth factor signaling pathways and their activation are required for tooth germ development. The expression of ADP-ribosylation factor (Arf)-like 4c (Arl4c) has been shown to induce cell proliferation, and is thereby involved in epithelial morphogenesis and tumorigenesis. In contrast, the other functions of Arl4c (in addition to cellular growth) are largely unknown. Although our laboratory members recently demonstrated the involvement of the upregulated expression of Arl4c in the proliferation of ameloblastoma (J Pathol., 2022), which has the same origin as odontogenic epithelium, its effect on tooth germ development remains unclear.

In this study, single-cell RNA sequencing (scRNA-seq) analysis revealed that the expression of *Arl4c*, among 17 members of the Arf-family, was specifically detected in odontogenic epithelial cells, such as those of the stratum intermedium, stellate reticulum and outer enamel epithelium, of postnatal day 1 (P1) mouse molars. scRNA-seq analysis also demonstrated the higher expression of *Arl4c* in non-ameloblast and inner enamel epithelium, which include immature cells, of P7 mouse incisors. In the mouse tooth germ rudiments culture, treatment with SecinH3 (an inhibitor of the ARNO/Arf6 pathway; a possible downstream of Arl4c signaling) reduced the size, width and cusp height of the tooth germ and thickness of the eosinophilic layer, which would involve the synthesis of dentin and enamel matrix organization. In addition, loss-of-function experiments using siRNAs and shRNA revealed that Arl4c expression was involved in cell proliferation and osteoblastic cytodifferentiation in odontogenic epithelial cells. Finally, RNA-seq analysis with gene set enrichment analysis (GSEA) and Gene Ontology (GO) analysis showed that osteoblastic differentiation-related gene sets and/or GO terms were downregulated in shArl4c-expressing odontogenic epithelial cells.

These results suggest that the Arl4c-ARNO/Arf6 pathway axis contributes to tooth germ development through osteoblastic differentiation.

Involvement of B cells in tumor immunity of oral squamous cell carcinoma

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(Background)
Understanding the significance of lymphocyte infiltration in the tumor lesion is pivotal in cancer immunotherapy. Traditionally, cytotoxic T cells have been shown to play a significant role in eliminating tumor cells. Recent research has also exhibited that tumor-infiltrating B cells strikingly influence the prognosis and efficacy of cancer immunotherapy. While B cells primarily produce humoral immunity through antibody production, their role in tumor immunity remains unclear. This study thus aimed to clarify the role of B cells infiltrating locally and antibodies in oral squamous cell carcinoma (OSCC).

(Materials and Methods)
Quantitative analysis of infiltrating antibody-secreting cells (ASCs) and the binding of IgG antibodies to tumor cells were conducted using multiplex immunofluorescence. Additionally, we performed gene expression analysis and B cell receptor (BCR) repertoire analysis through single-cell RNA-sequencing (scRNA-seq) on CD45-positive cells extracted from three OSCC resected tissues. Moreover, we generated recombinant antibodies with binding sites identical to the BCRs that showed substantial expansion among B cells infiltrating OSCC. Subsequently, we investigated the binding of these antibodies to tongue SCC cell lines using multiplex immunofluorescence.

(Results)
Multiplex immunofluorescence revealed significant infiltration of ASCs in the lesion of OSCC and more frequent IgG binding to tumor cells compared with that to adjacent oral mucosal epithelial cells. The scRNA-seq with BCR repertoire analysis demonstrated the infiltration of B cells at various differentiation stages in OSCC, particularly showing clonal expansion of the ASCs cluster with high IgG expression. The binding of recombinant antibodies referring to BCR sequences of clonally expanded ASCs to the tongue SCC cell lines was also observed. The investigation into the impact of IgG antibody binding to tumor cells on prognosis revealed no significant correlation with clinical findings.
(Conclusion)
This study suggests that B cells, especially ASCs, may play an essential role in tumor immunity by producing tumor-specific IgG antibodies in the lesion of OSCC. However, no significant correlation was found between IgG antibody binding to tumor cells and clinical outcomes, including prognosis. Further investigation is required to elucidate the antibodies produced locally and their functions, with identifying these antigens being particularly imperative.

Fabrication and histological evaluation of ant-nest type porous carbonate apatite artificial bone

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Biomaterials

Background

Carbonate apatite (CO_3Ap) artificial bone has been used as bone substitute due to its superior osteoconductivity. Besides composition, morphology of the artificial bone is an crucial factor for bone regeneration. Generally, the porosity and mechanical strength of artificial bone are trade-offs. Previously, the polyurethane foam have been used as replica template to fabricate interconnected porous CO_3Ap with a low compressive strength (25kPa) which is not suitable to be used in load bearing area. In contrast, polyurethane foam used as porogen able to provide CO_3Ap an ant-nest type porous (ANP) structure without creating an excessive amount of pores. This study aimed to evaluate the effectiveness of using polyurethane foam as a porogen in fabricating CO_3Ap artificial bone and its potential in bone regeneration.

Materials and methods

The porogen employed in this investigation was polyurethane foam with strut thickness of approximately 150 μm. Calcium carbonate ($CaCO_3$) slurry was incorporated into the polyurethane foam's pores, followed by the burned out of the foam to produce an interconnected porous $CaCO_3$ precursor block. $CaCO_3$ block were then transformed into CO_3Ap block through a dissolution-precipitation reaction. The resulting CO_3Ap blocks were characterized in terms of composition and morphology. Additionally, the ANP-structured CO_3Ap artificial bone's mechanical and biological performances were investigated.

Results & Discussion

The polyurethane foam was completely burned out after the heat treatment. As a result, the samples were converted to interconnected porous CO_3Ap blocks which revealed an ANP structure with a compressive strength comparable to that of human trabecular bone (around 15MPa). Moreover, in vivo result showed that the migration of cells and tissues towards the interior of the artificial bone through the ANP structure. The results indicated that the the usage of polyurethane foam as a porogen gave rise to an ANP-structured CO_3Ap block which possessed adequate mechanical strength and promoted bone regeneration.

Conclusion

The fabrication of an ANP-structured CO_3Ap artificial bone with adequate mechanical strength was successful. The interconnected porous architecture aided cell and tissue migration into the CO_3Ap artificial bone, thereby replacing CO_3Ap with new bone. This approach were found to be beneficial in creating artificial bone for bone regeneration.

Cytoplasmic signaling molecule CySM2 in LTi cells plays critical role on Payer's patch formation.

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＜Background＞
The intestinal tract is the largest immune organ continuously exposed to antigens from ingested food, symbiotic bacteria and pathogens. Intestinal mucosal immunity develops gut-associated lymphoid tissue (GALT) which achieves both tolerance to harmless symbionts and defense against pathobionts. Failure of such fine-tuned intestinal immune system is supposed to cause inflammatory bowel diseases. Peyer's patches (PPs) and ILFs are major components of GALT formed via such common cellular mechanism as mutual activation of Lymphoid Tissue inducer (LTi) cells and mesenchymal fibroblastic cell called Lymphoid Tissue organizer (LTo) cells. Recently, we coincidentally found that ubiquitously expressed cytoplasmic signaling molecule family, hereafter CySM, was involved in the development of PPs and ILFs in mouse. From these results, we hypothesized that CySM molecules contribute to PPs and ILFs via previously unappreciated mechanisms.
＜Methods＞
In mouse, CySM is composed of two subfamily molecules CySM 1 and CySM 2. First, to determine which CySM molecule plays predominant roles on GALT development, we generated Cysm 1/2-DKO, Cysm 1-KO, and Cysm 2-KO mice. Second, to determine cell population which plays critical role on CySM-dependent GALT formation, we generated lymphocyte-specific Cysm2-deficient Cd127Cre; Cysm2 $^{flox/flox}$ mice and two mesenchymal-specific Cysm2-deficient mouse lines such as Cxcl13Cre; Cysm2 $^{flox/flox}$ and Twist2Cre; Cysm2 $^{flox/flox}$. In these Cysm deficient and the control mice, formations of PPs and ILFs were assessed with microscopy.
＜Results and Discussion＞
In Cysm 1/2-DKO mice, PP formation was totally impaired. As the size and number of PP in Cysm2 KO mice were significantly reduced compared to WT or Cysm1 KO mice, we concluded that CySM 2 plays predominant role on PP formation. While mesenchymal-specific Cysm2-deficient mice showed no significant loss of PP in size and number, lymphocyte-specific CySM 2-deficient mice showed severely impaired PP formation. From these results, we speculate that CySM 2 in LTi cells plays critical role on PP formation. However, precise role of CySM2 in LTi cells during PP formation remains to be elucidated.

Fabrication of granular apatite cement using partially set α-tricalcium phosphate granules

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Background

Apatite cements are synthetic bone grafts with the ability to set and harden in bone defects. These are prepared by mixing a powder, typically α-tricalcium phosphate (α-TCP), with a liquid to form a moldable paste. This paste sets by transforming into apatite products such as hydroxyapatite (HAp). However, traditional apatite cements form dense blocks with limited bone regeneration inside. To address this, introducing pores into the cement is crucial. Using larger particles, or granules, instead of fine powder, helps create porous structures by leaving gaps between the granules. The challenge, however, lies in maintaining a high surface area with larger particles, as this is crucial for the setting reaction. Our study introduces a novel method for producing α-TCP granules with a high specific surface area, aiming to develop a granular apatite cement that is both porous and sets effectively.

Materials and methods

We created α-TCP granules by freeze-drying partially set apatite cement. The process involved mixing α-TCP powder with a sodium phosphate solution, incubating it at 37°C for partial setting, followed by freeze-drying and crushing into granules of 50–100 μm diameter. For comparison, we also produced α-TCP granules using a traditional sintering method, where β-tricalcium phosphate was compacted and sintered at 1500°C.

Results and discussion

The partial setting of α-TCP created a unique microporous, raspberry-like structure in the granules, maintaining gaps between particles and resulting in a large specific surface area (5.9 m^2/g), significantly higher than the sintered granules (0.2 m^2/g). Importantly, these freeze-dried granules preserved the original α-TCP composition. When mixed with a weakly acidic sodium phosphate solution, the granules set within 25 minutes, which is clinically acceptable, unlike the slower setting sintered granules (91 minutes). The freeze-dried granules rapidly transformed into HAp, while the sintered ones predominantly remained as α-TCP even after a week. The final set blocks featured macropores around 20–40 μm. These findings indicate that our method is promising for creating apatite cement with enhanced properties suitable for clinical applications.

Establishment of a new peripheral nerve regeneration therapy using artificial nerves with extracellular vesicles derived from dental pulp stem cells.

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Backgrounds;
Recently, extracellular vesicles (EVs) released by stem cells have also attracted attention, as they are transported to target cells and provide various biological functions such as regulating immune functions, suppressing inflammatory reactions, and even repairing tissues. Previous reports have shown that EVs from dental pulp stem cells (DPSCs) have higher anti-inflammatory and tissue regeneration properties than those from other stem cells.
In this study, we compared EVs from DPSCs (DPSC-EVs) with known artificial nerves *in vivo* to confirm whether the addition of DPSCs-EVs to artificial nerves, which are already commercially available, improves nerve regeneration.
Methods;
EVs were collected from the conditioned media of human DPSCs using ultracentrifugation; EVs were evaluated by Western blotting using CD9 and CD81, and their presence was confirmed by cryo-EM. *In vivo* study, a 15-mm transected sciatic nerve model of 8-week-old male SD rats was created, and five groups were established. At 1 and 2 weeks after the nerve defect, the footprints were measured and the sciatic functional index (SFI) was calculated. Two weeks later, the artificial nerve was harvested, and electrophysiological evaluation was performed using an oscilloscope. Finally, the gastrocnemius muscle innervated by the sciatic nerve was harvested and its weight measurement and Masson's trichrome staining were used to compare the degree of fibrosis of the muscle.
Results;
DPSC-EVs were CD9-positive and CD81-positive by Western blot, and were spherical in size, around 200 nm, and surrounded by a bilayer membrane. *In vivo* study, electrophysiological evaluation showed that the EVs group recovered to values closer to the amplitude of normal nerves than the PBS group. In functional evaluation, SFI values in the EVs group were improved over those in the PBS group; histological findings showed infantile nerve regeneration with angiogenesis in the EVs group compared to the PBS group. In addition, gastrocnemius muscle was collected and weighed, which was heavier in the EVs group than in the PBS group.
Conclusion;
In the EVs group, it was found that nerve regeneration was triggered earlier by stimulating angiogenesis from the surrounding area.