Kyudai Oral Bioscience 2018 (KOB2018)

Health Longevity from Oral Brain Science: From Basic to Clinical Research

February 11th (Sun) - 12th (Mon) 2018

Collaboration Station I, 2F Audiovisual Hall
Kyushu University

PROGRAM & ABSTRACTS

Kyushu University Faculty of Dental Science

○ Photograph on the cover : A monument commemorating the 50th anniversary of the School of Dentistry Kyushu University

５０周年記念モニュメント　「口腔の健康が世界を救う」

○ 会期：平成 29 年 2 月 11 日（日）　13:00 ～ 17:10
2 月 12 日（月）　9:30 ～ 12:35

○ 会場：九州大学コラボステーションⅠ・視聴覚ホール（2 階）
住所：福岡市東区馬出 3－1－1

○ 主催：九州大学大学院 歯学研究院

九州大学コラボ・ステーションⅠ
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Information for Speakers

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・For the speakers in Special Lecture, please bring your own personal computer to the Computer Operating Desk at 12:40.

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・Your cooperation in finishing your presentation within the allotted time is appreciated.

・After your presentation, please reclaim your computer from the Computer Operating Desk.

PROGRAM

Kyudai Oral Bioscience 2018 (*KOB2018*)

Feb 11 (Sun) 13:00～17:10

13:00～13:10
Hiroshi Nakanishi (*Representative Organizer of KOB, Vice Dean*)

Opening Remarks
Kiyoshi Koyano (*Dean, Faculty of Dental Science, Kyushu University*)

【Symposium】
Healthy Longevity from Oral Brain Science: From Basic to Clinical Research

Session 1　Oral Health and Cognitive Function

Chair person　Noriatsu Shigemura (*Kyushu University*)

13:10～13:40　*Special Lecture 1*
Tetsuya Goto (*Kagoshima University*)
Neurodegeneration by Tooth Loss Causes Cognitive Impairment

13:40～14:10　*Special Lecture 2*
Makoto Inoue (*Niigata University*)
Physiological Study on Swallowing Performance

14:10～14:20　*Coffee Break*

Session 2　Periodontitis and Alzheimer's Disease

Chair person　Hiroshi Nakanishi (*Kyushu University*)

14:20～15:10　*Special Lecture 3*
Angela R Kamer (*New York University*)
Peripheral Inflammatory Conditions Contribute to Alzheimer's Disease: *the Periodontal disease model*

15:10～15:40　*Special Lecture 4*

Zhou Wu　(*Kyushu University*)

Mechanisms Linking Periodontal Disease to Alzheimer's Disease: ***Key functions of cathepsins in microglia activation and chronic systemic inflammation***

15:40～15:50　*Coffee Break*

Session 3　Treatments and Biomarkers for and Alzheimer’s Disease

Chair person　Haruhiko Kashiwazaki　(*Kyushu University*)

15:50～16:20　*Special Lecture 5*

Hachiro Sugimoto　(*Doshisha University*)

Development of Drug (GT863) for Alzheimer’s Disease

16:20～16:50　*Special Lecture 6*

Kazuhiko Uchida　(*Tsukuba University*)

Sequester Proteins Involved in Amyloid-β Clearance as Blood-based Biomarkers for Alzheimer’s Disease

16:50～17:00

Group Photograph

17:00～17:10

Closing Remarks

Fusanori Nishimura　(*Vice Dean, Faculty of Dental Science, Kyushu University*)

Feb 12 (Mon) 9:30～12:35

9:30～9:40
Opening Remarks
Ichiro Takahashi (*Vice Dean, Faculty of Dental Science, Kyushu University*)

【Educational Lectures】

Chair person Ichiro Takahashi (*Kyushu University*)

9:40～10:10 *Educational Lecture 1*
Haruhiko Kashiwazaki (*Kyushu University*)
Oral Supportive Care for Cancer Therapy

Chair person Kasuaki Nonaka

10:10～11:00 *Educational Lecture 2*
Janice A Townsend (*Louisiana State University*)
Innovation in Education and Research at LSU School of Dentistry

11:00～11:10 *Coffee Break*

【PhD Student Session (PSS)】

Chair persons：Yicong Liu & Yuka Harada

11:10～11:25 *PSS-1*
Eddy (*Departments of Biometerials*)
Fabrication of self-setting β-tricalcium phosphate granular cement through bridging with calcium sulfate hemihydrate

11:25～11:40 *PSS-2*
Yuka Harada (*Department of Aging Science and Pharmacology*)
Cathepsin E-dependent elastase secretion in neutrophils derives experimental autoimmune encephalomyelitis-induced neuropathic pain

11:40～11:55　*PSS-3*

Misaki Abe (*Laboratory of Oral Pathology of Dental &Section of Oral and Maxillofacial Surgery*)

Expression and function of Piezo1 and Piezo2 in tooth germ development

11:55～12:10　*PSS-4*

Mai Arima　(*Department of Endodontology and Operative Dentistry*)

R-spondin2 enhances osteogenesis of immature human periodontal ligament cells through the canonical Wnt signaling pathway

12:10～12:25　*PSS-5*

Keita Funada　(*Section of Orthodontics and Dentofacial Orthopedics*)

The roles of miR875-5p as a specific marker during early tooth development

Closing Remarks

12:25～12:35

Seiji Nakamura　(*Associate Director of Kyushu University Hospital*)

ABSTRACTS

Symposium

Health Longevity from Oral Brain Science: From Basic to Clinical Research

Session 1

Oral Health and Cognitive Function

Special Lecture 1

Neurodegeneration by Tooth Loss Causes Cognitive Impairment

Tetsuya Goto

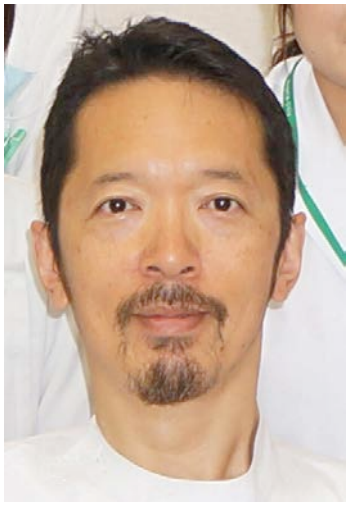
Division of Oral Anatomy and Cell Biology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8544, Japan

Brief curriculum vitae: Dr. Goto graduated from Kyushu University in 1988, completed Doctoral Course of Kyushu University and received a PhD degree in Dental Science in 1992. Served as a post-doctoral fellow at University of British Columbia and University of Toronto in Canada until 1996. Worked as Assistant Professor at Kyushu University, until 2001, and as Associate Professor at Kyushu Dental University until 2014. Currently, working as a Professor at Faculty of Dentistry, Kagoshima University.

Abstract: Alzheimer's disease (AD) is a major cause of dementia in elderly people. The main neuropathological changes of AD are amyloid beta (Aβ) deposition, phosphorylation of tau (pTau), and neural cell loss in the brain. Poor oral health condition such as periodontitis or tooth loss has been thought to be involved in the progression of AD. However, it was unclear how neurodegeneration by tooth loss affects the progress of AD. At first, we confirmed which trigeminal neurons was induced the neurodegeneration of AD in 3xTg AD model mice using immunohistochemistry. Then, we found the strong Aβ deposition and pTau in the neuron at the trigeminal mesencephalic nucleus (Vmes) among trigeminal nuclei. Next, we extracted bilateral maxillary molars of 12-month-old 3×Tg AD model mice and examined the pathological changes. At one-month after tooth extraction the number of Aβ- and pTau-immunoreactive neurons at the caudal side of Vmes was intensively reduced. Iba-1 immunoreactive microglia distributed around the neural loss site of Vmes. Close to the caudal site of Vmes with microglia, there is locus coeruleus (LC) that is well know the place where the initial lesion of AD appears and its cell death causes a reduction in nerve cells in the hippocampus. We also found the neural loss of LC neuron with active microglia close to the place where neural loss of Vmes was occurred after tooth

extraction. Therefore, we propose the following cascade model based on these results. After the extraction of the molars in AD model mice, neurons on the caudal side of Vmes cause cell death. Dead neurons are phagocytosed by microglia, coinstantaneously Aβ is released and activates microglia. Activated microglia also phagocytose the neighboring neurons of LC. Deposition of Aβ on neurons is known to begin at the early age, indicating that tooth loss is a risk factor of AD.

Memo



Special Lecture 2

Physiological study on swallowing performance

Makoto Inoue

Division of Dysphagia Rehabilitation, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Brief curriculum vitae: Dr. Inoue graduated from Niigata University in 1994, completed Doctoral Course of Niigata University and received a PhD degree in Dental Science in 1998. Worked as Assistant Professor at 1998, until 2004. Worked as Lecturer at 2004, until 2006. Worked as Associate Professor at 2006, until 2008. Currently, working as a Professor at 2008, Niigata University.

Abstract: Japanese population is aging, and more than 27% to total population is now over 65 years old. One of the most common cause of death in Japanese elderly people is aspiration pneumonia caused by dysphagia. Dysphagia is a symptom of difficulty of swallowing due to diseases such as stroke, neurological diseases, head and neck cancer and so on. Major pathological problems of dysphagia is decline of swallowing initiation. In my presentation, I will introduce our basic study to investigate which type of receptors in the pharyngeal/laryngeal cavity is involved in swallowing initiation. To initiate swallow, thermal, chemical or electrical stimulation is available. Thermal and chemical stimulation may be expected to activate some types of channels, i.g. TRPs, ASICs etc. On the other hand, electrical stimulation is used to widely activate the peripheral nerve or nerve endings. Peripheral stimulation can activate swallow-related neural network not only in the brain stem but also in the higher centers such as the cerebral cortex. We will also introduce our human study to investigate how we evaluate the swallow-related neural activity.

Memo

Session 2

Periodontitis and Alzheimer's disease

Special Lecture3

Peripheral Inflammatory Conditions Contribute to Alzheimer's Disease: *the Periodontal disease model*

Angela Kamer

Department of Periodontology and Implant Dentistry,

New York University (NYU) College of Dentistry, New York, USA.

Brief curriculum vitae: Dr. Kamer is associate professor in the Department of Periodontology and Implant Dentistry, NYU College of Dentistry, New York, USA. Her research activities involved periodontal disease, implant dentistry, diabetes, cancer, bone research and currently Alzheimer's disease. After completing her studies in Stomatology at the University "Iuliu Hatieganu" in Cluj Napoca, Romania, and practicing dentistry for a few years, Angela Kamer went on to receive her MS in Oral Sciences, Ph.D. in Pathology and training in Periodontics from SUNY at Buffalo and later in Implant Dentistry from NYU, USA. Subsequently, she started working at NYU College of Dentistry. As a trained periodontist at the NYU College of Dentistry and a research scientist at NYU Center for Brain Health, Angela is in a unique position to bridge the medical-dental research gap by developing new hypotheses and strengthening collaborations across disciplines. Her research focuses on the role of peripheral inflammatory and dysbiotic conditions in the pathogenesis of Alzheimer's disease using periodontal disease as a model. Periodontal disease is a peripheral chronic polymicrobial inflammatory condition affecting about 50% of people over 55 years of age in USA. It is characterized by high Gram-negative pathogenic bacterial load and often increased systemic inflammation. Studies coming from Angela's group will present data showing that periodontal disease may contribute to AD-specific pathology and cognitive performance. She published in well-regarded peer-reviewed journals, she wrote book chapters, and presented to several international conferences. She also co-edited a book entitled "A Clinician's Guide to Systemic Effects of Periodontal Diseases" and a special journal issue.

Abstract: Worldwide, more than 31 million persons suffer from dementia, with 50-60% having AD and these numbers are estimated to double by 2030 and double again by 2050. Alzheimer's disease is a continuum condition characterized by specific pathology such as amyloid β plaques (Aβ), neurofibrillary tangles, inflammation and neurodegeneration. In the preclinical phase, there is

AD pathology but no symptomatology suggesting that the AD pathology did not cause irreversible brain damage. Therefore, identifying potentially reversible risk factors before or during the preclinical phase would provide an opportunity for prevention and intervention.

It is accepted that Inflammation is involved in the pathogenesis of AD, but it remains unknown to what extent peripheral inflammation and infections and dysbiotic conditions play a role. Studies have shown that peripheral cytokines, infections and their products including bacterially derived lipopolysaccharide (LPS) can promote AD-specific pathology. Periodontal disease a localized peripheral chronic inflammatory condition affecting about 50% of people over 55 years of age is characterized by dysbiosis with high Gram-negative pathogenic bacterial load and increased systemic inflammation.

It is our general hypothesis that peripheral sources of inflammation, with its inflammatory/bacterial burden, contribute to Alzheimer's disease (AD) pathogenesis by altering AD-specific pathology. In support of this hypothesis we find that in cognitively normal individuals clinical measures of periodontal disease associate with brain amyloid load (assessed by ^{11}C-PIB PET imaging) in AD amyloid-vulnerable regions and enhances tau pathology thus, supporting a role of peripheral inflammations/infections in AD pathogenesis.

Memo

Special Lecture 4

Mechanisms Linking Periodontal Disease to Alzheimer's Disease: *Key functions of cathepsins in microglia activation and chronic systemic inflammation*

Zhou Wu

Department of Aging Science and Pharmacology, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, Japan

Brief curriculum vitae: Dr. Wu graduated from Bethune Medical College of Ji Lin University in China, completed Doctoral Course of Kyushu University and received a PhD degree in Dental Science. Served as a research fellow of Japan Society for the Promotion of Science at Kyushu University from 2002 to 2004. Worked as Assistant Professor, Lecturer and Associate professor at Kyushu University Faculty of Dental Sciences from 2005 until now. She is a visiting Professor at Ji Lin University and China Medical University, China. She is also a Diamond Jubilee International Visiting Fellow in University of Southampton, UK. Her research interest is functions of cathepsins in innate and adaptive immunity that regulate functions of mononuclear phagocytes including dendritic cells and microglia during chronic systemic inflammation.

Abstract: Many lines of evidence show that chronic neuroinflmmation play an essential role in onset and progression of Alzheimer's disease (AD). Furthermore, there is increasing evidence that chronic systemic inflammation enhances neuroinflammation through activation of microglia. During the last decade, numerous clinical studies have found that as a chronic oral infectious disease, periodontal disease, is correlated with AD and is involved in persisting systemic inflammation. We have previously found that cathepsin B, a lysosomal cysteine protease, is involved in microglia-mediated neuroinflammation, which induces memory impairment. Therefore, we have proposed "microglia-aging" hypothesis, which microglia-mediated neuroinflammation promotes the brain aging [1]. Lipopolysaccharide derived from *Porphyomonas gingivalis* (Pg-LPS), a major causative agent of periodontal disease, induces microglia-mediated neuroinflammation through activating leptomeningeal cells, which cover the entire cortex [2]. Furthermore, the chronic systemic exposure of Pg-LPS in low concentration causes the cathepsin B-dependent AD-like pathologies, including microglia-mediated neuroinflammation, the amyloid β accumulation in

neurons and memory impairment in middle-aged mice [3]. More recently, we have shown that chronic systemic exposure of Pg-LPS amplifies cathepsin S-dependent systemic inflammation by promoting the differentiation of Th17 cells through activation of dendritic cells in middle-aged mice [4].

These findings demonstrate that cathepsins B and S are key enzymes for controlling microglia-mediated neuroinflmmation and the systemic inflammatory signaling from periodontal disease to the brain. Because AD is a disease that progresses with a long span of more than 25 years and fundamental therapeutic agents have not yet been developed, it is important to prevent or delay the onset and progression of AD. As a treatable disease, oral care for periodontal disease will reduce the risk for AD. In addition to the dental treatment, specific inhibitors of cathepsin B or S can be used in the prevention and treatment of AD.

References

1. Nakanishi H, Wu Z. Microglia-aging: roles of microglial lysosome- and mitochondria- derived reactive oxygen species in brain aging. *Behav Brain Res.* 201(1), 1-7, 2009.
2. Liu Y, Wu Z, Zhang X, Ni J, Yu W, Zhou Y, Nakanishi H. Leptomeningeal cells transduce peripheral macrophages inflammatory signal to microglia in response to Porphyromonas gingivalis LPS. *Mediators Inflamm* 2013:407562, 2013.
3. Wu Z, Ni J, Liu Y, Teeling JL, Takayama F, Collcutt A, Ibbett P, Nakanishi H. Cathepsin B plays a critical role in inducing Alzheimer's disease-like phenotypes following chronic systemic exposure to lipopolysaccharide from Porphyromonas gingivalis in mice. *Behav Brain Immun* 65: 350-361 2017.
4. Dekita M, Wu Z, Ni J, Zhang X, Liu Y, Yan X, Nakanishi H, Takahashi I. Cathepsin S is involved in Th17 differentiation through the upregulation of IL-6 by activating PAR-2 after systemic exposure to lipopolysaccharide from *Porphyromonas gingivalis*. *Front Pharmacol* 8:470, 2017.

Memo

Session 3

Treatments and Biomarkers for Alzheimer's disease

Special Lecture 5

Development of Drug (GT863) for Alzheimer's Disease

Hachiro Sugimoto

Doshisha University Faculty of Life and Medical Science, Kyoto, Japan.

Brief curriculum vitae: Dr. Sugimoto joined Eisai Co., Ltd. in 1961. While working for Eisai, he graduated from Chuo University in 1969 and obtained his doctorate in pharmacology at Hiroshima University in 2002. In 2003, he resigned from Eisai, and became Professor in the Graduate School of Pharmaceutical Sciences, Kyoto University. Currently, working as a Professor at Doshisha University Faculty of Life and Medical Science.

His research on E2020 (now called *Donepezil*), an acetylcholinesterase inhibitor, first began at Eisai's Tsukuba Research Laboratories in 1983, because his mother suffered from dementia. His research group was finally able to successfully create Donepezil with a promising enough profile for the compound to become a drug candidate. For his achievement, he received the Award of Science from Eisai in October 1993, the Award of Engineering from the Pharmaceutical Society of Japan in March 1998, the Special Galien Prize in the UK in April 1998, the Chemistry-Bio Tsukuba Award in May 1998, and the Imperial Award for Invention in June 2002.

Abstract:

Outline of disease

Alzheimer's disease (AD) starts with an early memory impairment, gradually decreases comprehension ability and judgmental ability, interferes with social life, and then nursing care is required in 5 to 6 years. Eventually it is also called "a disease that loses personality" and it is one of the most cruel diseases among all ones. In Japan, about 4 million people suffer from dementia, and 4 million people are said　mild cognitive impairment (MCI) and the number of patients is rapidly increasing (Ministry of Health、Labor and Welfare in Japan). About 2/3 of dementia patients are Alzheimer's disease, the incidence of disease has increased nearly threefold since 1990 and it is expected to increase to 4 times from now in 2050.

Therapeutic drug development

The biggest challenge for AD is the lack of effective radical therapies. Until now, the development of a large number of therapeutic drugs has been promoted by pharmaceutical companies, universities, laboratories and venture companies, but everything has failed at the clinical trials. GT 863 is a candidate drug for AD with new profiles developed by Green Tech Co., Ltd., and is promoting commercialization ahead of the rest of the world.

Current status of development of therapeutic drug for AD

Development based on amyloid hypothesis: Target the inhibition of Aβ cascade. Amyloid β (Aβ) has been considered the most noteworthy as a therapeutic target. Aβ is elevated in familial AD patients, and APP as a precursor is also elevated in Down syndrome patients who become young AD, so it has been noted as a potent inducer of AD. Furthermore, aggregates of Aβ actually have neurotoxicity (inducing nerve cell death).

Development of therapeutic drugs

1) The development of enzyme inhibitors of β / γ secretase responsible for the production of Aβ from APP was advanced. However, the development has been halted because of strong side effects, no efficacy, or symptomatic worsening.
2) Development of drugs targeting inhibition of the formation of aggregates of Aβ has been advanced, and the development of small molecules and antibodies, but everything has failed in phase 3 clinical trials.

Development based on the Tau hypothesis : Target the inhibition of tau cascade factor Tau. Factors that have recently drawn attention as therapeutic targets are neurofibrillary tangles accumulated in neurons, an aggregate of phosphorylated tau protein. It has been confirmed that this aggregate possesses neuro cytotoxicity and has attracted attention as a therapeutic target for AD. However, the development of AD therapeutic drugs targeting tau has only just begun, there are few examples that have been developed yet. Currently, all clinical trials, including the first compound MB, have failed.

AD Patient: Onset mechanism of disease

Presently, hundreds of AD therapeutic drugs are probably developed in the world and clinical

trials are being conducted, but all of them have failed to develop. Most of these developments target Aβ or Tau, but unlike these developments, when developing new AD therapeutic drugs in the future, it is necessary to construct an accurate development strategy based on an accurate understanding of AD pathology.

Accurate understanding of AD pathology

AD is caused by "neuronal cell death"

AD is a disease leading to a significant loss of reduced cognitive brain function by cell death in the brain. Neurons decrease due to cell death and the brain atrophies. → Detected as remarkable brain atrophy image of MRI.

Neuronal cell death is induced by "①Aβ" and "②Tau"

Characteristic pathological image of AD patients

①Amyloid β (Aβ) aggregates accumulate outside the brain cells (senile plaques)

② Aggregates of phosphorylated tau proteins appear in cells

Since neurofibrillary tangles directly induce nerve cell death, it is also a potential therapeutic target. Aβ and Tau are both promoted simultaneously in the majority of AD patient tissues. Both are expressed simultaneously in the same patient.

Development strategy for AD therapeutic drug (GT863)

Compound synthesis was conducted with the aim of the concept of "compounds that simultaneously inhibit both Aβ aggregation and Tau aggregation", which is considered to be an essential requirement for AD therapeutic drug development. Curcumin has an inhibitory effect on aggregation of Aβ · Tau at the *in vitro* / *in vivo* experimental level. However, it did not show reasonable efficacy in clinical trials for AD. The reason for this is that curcumin itself is still low aggregation inhibitory activity, bad pharmacokinetics (Low absorptivity, easy metabolism, low brain migration). We have synthesized approximately 1,000 compounds using this curcumin as a seed compound and searched for compounds with high inhibitory activity on aggregation of Aβ · Tau and high pharmacokinetics. As a result, GT863 was obtained as the most suitable compound.

Memo

Special Lecture 6

Sequester Proteins Involved in Amyloid-β Clearance as Blood-based Biomarkers for Alzheimer's Disease

Kazuhiko Uchida

Department of Molecular Biology and Oncology,

Faculty of Medicine, University of Tsukuba, Tsukuba , Japan

Brief curriculum vitae: Dr. Uchida graduated from Nara Medical University in 1983, completed Doctoral Course of Nara Medical University and received a M.D., Ph.D. degree in Medical Sciences in 1988. Served as a Research Resident at National Cancer Center Research Institute from 1985 to 1989. Worked as Research Staff at National Cancer Center Research Institute in 1989. Worked as Assistant Professor at University of Tsukuba, until 1995. Currently, working as an Associate Professor and Research Staff at Center for Tsukuba Advanced Research Alliance, at University of Tsukuba, and CEO/CTO at MCBI, Inc., a spin-out company from University of Tsukuba.

Abstract: Currently, the number of people who have been diagnosed with dementia worldwide is 35.6 million, and it is predicted that this rate will increase to more than 100 million people in 2050. Diagnosis and intervention at early stages of dementia may greatly reduce the number of individuals suffering from this debilitating disease. Mild cognitive impairment (MCI) is a syndrome defined as cognitive decline that is greater than expected for an individual's age and is regarded as a risk group for dementia. MCI due to Alzheimer's Disease (AD) is considered as a pre-stage of AD, and without clinical intervention, 40% of patients with MCI may convert to AD within four years of diagnosis.

Biomarker discovery for neurological disorders is challenging but rewarding because it facilitates early diagnosis, monitors disease progression, and assesses response to medical treatment. Development of blood-based biomarkers for early detection of cognitive impairment is important to potentially reduce the number of patients with dementia.

AD is distinguished from other neurological diseases, including other types of dementia and normal aging, by abundant deposits of fibrillar amyloid β (Aβ). Aβ-derived diffusible ligands cause

abnormal spine morphology and a significant decrease in spine density, and trigger synaptic dysfunction, which may lead to memory loss in AD. In healthy individuals, synapse damage by amyloid-β (Aβ) is prevented by clearance mechanisms via blood-brain-barrier. Apolipoprotein and transthyretin are involved in sequestration of Aβ from brain to blood. Aβ may also be removed by clearance mechanisms such as phagocytosis by microglia. Complement proteins sequestrate Aβ via an innate immune response of microglia in the brain.

We analyzed serum levels of apolipoprotein A1 (apoA1), complement protein C3 (C3), and TTR in development of blood-based biomarkers for MCI and AD, and revealed the clinical potential of these proteins involved in sequestration of Aβ in the assessment of cognitive decline by longitudinal and cross-sectional studies using 3 independent cohorts[1]. The least absolute shrinkage and selection operator (LASSO) was used to evaluate the combination of multiple biomarkers in MCI vs. NDC. A combination of apoA1, C3, and TTR achieved an area under the curve of 0.89 in MCI vs. healthy controls and also discriminated individuals with mild cognitive decline from non-demented age-matched control (NDC).

We further developed conventional immuno-assays for these sequester proteins to investigate their clinical validity as blood-based test for MCI. In addition, we developed novel C3 assay which differentially measured a native form (nC3) and activated (fragmented) forms (aC3) of C3 indicating status of activation of C3. The levels of theses serum biomarkers were compared with MMSE score, voxel-based specific regional analysis system for Alzheimer Disease (VSRAD) score in MRI and reginal cerebral blood flow (rCBF) in SPECT from 257 participants.

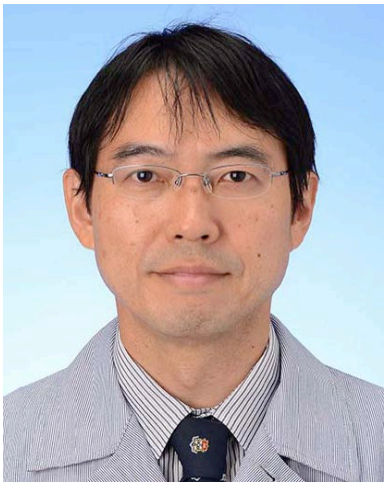
A combination apoA1, TTR and aC3/nC3 achieved an area under the curve of 0.89 in MCI vs. NDC. Atrophy in medial temporal were associated with decreased levels of apoA1 and HDL. Significantly lower levels of aC3, apoA1, HDL, and total cholesterol were observed in participants with severe reductions in rCBF. Furthermore, lower nC3 and higher aC3 levels were observed in AD hippocampus tissues by immunohistochemical analysis.

The current results are consistent with the hypothesis that individuals with decreased levels or impaired function of sequester proteins resulting in impaired Aβ clearance are susceptible to cognitive impairment. Dysfunction of sequestration and/or low levels of sequester proteins may promote pathological progression of AD. Thus, apoA1, TTR and complement protein profiles could be blood-based biomarkers for assessment of early stages of cognitive decline including of preclinical AD.

1. Uchida, K., *et al*. Amyloid-β sequester proteins as blood-based biomarkers of cognitive decline. Alzheimers & Dement (*Amst*), 1, 270-280 (2015).

Memo

Educational Lectures



Educational Lecture 1

Oral Supportive Care for Cancer Therapy

Haruhiko Kashiwazaki

Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, Japan

Brief curriculum vitae: Dr. Kashiwazai graduated from Hokkaido University in 1992, completed Doctoral Course of Hokkaido University and received a PhD degree in Dental Science in 1997. Served as a post-doctoral fellow at Hokkaido University until 2001. Worked as Assistant Professor until 2013, as Lecturer until 2015 at Hokkaido University. Currently, working as a Professor at Section of Geriatric Dentistry and Perioperative Medicine in Dentistry, Kyushu University.

Abstract:

Oral supportive care is required as part of the cancer therapy pretreatment assessment to manage conditions that may impact the cancer therapy and affect the ability of the person to complete the planned therapy. Programs to prevent acute and chronic complications of the cancer treatment are initiated.

Oral complications of cancer therapy can be the most distressing of side effects and affect quality of life. The acute complications include oral infection, oral mucositis, altered saliva, change in taste, and coincidental dental infection, all of which can result in pain, and affect all aspects of oral function.

Prevention and management of chronic complications and observance for recurrent or second primary cancers is mandatory following cancer therapy. The most distressing chronic complication is dry mouth, which leads to increased risk of dental cavities, gum disease, mucosal infection, reduced taste, dysphagia, poor denture function and affect nutrition.

Oral supportive care providers, acting as part of the medical team, can facilitate the delivery of cancer treatment, and affect the quality of life during and following cancer therapy.

Memo

Educational Lecture 2

Innovation in Education and Research at LSU School of Dentistry

Janice A. Townsend

Department of Pediatric Dentistry at LSU School of Dentistry in New Orleans, Louisiana, USA.

Brief curriculum vitae: Dr. Townsend is Associate Professor and Chair of the Department of Pediatric Dentistry at LSU School of Dentistry in New Orleans, Louisiana. She received a bachelor of arts in English with a minor in chemistry in 2001 from the University of North Carolina (UNC) in Chapel Hill, received her doctorate of dental surgery from Marquette University School of Dentistry in Milwaukee, Wisconsin, in 2005 and then completed her certificate in pediatric dentistry and her master of dental science at the Ohio State University in Columbus, Ohio, in 2007. In 2008, she received her board certification from the American Board of Pediatric Dentistry and in 2009, awarded the Art Nowak Award for scoring the highest on the board's oral clinical examination. She has served on numerous committees for the university, including the leadership committee for the LSUHSC accreditation process for Southern Association of Schools and Colleges. She also has served on Children's Hospital of New Orleans Medical Executive Committee. Her current areas of research and publication are pain control in children, behavior guidance dental education, and biomaterials.

Abstract: LSU Department of Pediatric Dentistry is a comprehensive unit that provides dental education to pediatric dental residents, dental students, and dental hygiene student, conducts research, and provides service to the community in New Orleans Louisiana. The department is enhancing the educational experience of pediatric dental residents and dental students through utilization of a pioneering "flipped classroom" education design and objective structured clinical examination (OSCE) assessment. This talk will address how both of these pedagogical innovations can be implemented into any department.

LSU Department of Pediatric Dentistry is also a leader in the area of biomaterials research, especially in the area of sealants. Recent department research is addressing fundamental gaps in knowledge pertaining to sealant placement techniques as well as partnering with well-known basic science researchers to revolutionize the sealant composition to better inhibit caries.

Memo

PhD Student Session

PSS-1

Fabrication of self-setting β-tricalcium phosphate granular cement through bridging with calcium sulfate hemihydrate

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Introduction: Previously, we have proposed beta tricalcium phosphate granular cement (β-TCPGC) that is useful to prevent flowing out of the β-TCP granules from the bone defect. When the β-TCP granules were mixed with acidic calcium phosphate solution, it set to form interconnected porous structure. Although it seems promising, β-TCP granules need to be mixed with the acidic calcium phosphate solution. Calcium sulfate hemihydrate (CSH) has self-setting ability by converting to calcium sulfate dihydrate (CSD) when exposes to water. Furthermore, CSD is the component of Osteoset®, which is a commercially available as a bone substitute. In this study, we fabricated CSH coated β-TCP granules through dissolution-precipitation method. This setting ability could inhibit the flowing out of the β-TCP granules from the bone defect.

Experiment method: β-TCP granules were immersed in $NaHSO_4$ solution for 1, 3, 5 and 7 days at 70°C. The samples were then heated at 120°C for 4 hours. β-TCPGC was prepared by mixing the granules with saline solution at a L/P ratio of 0.3 and identified by XRD and SEM. The mechanical strength of the β-TCPGC was measured as a DTS by universal testing machine. β-TCPGC implanted in rabbit femur for 4 weeks and the percentage of newly formed bone was calculated from histological analysis.

Results: β-TCP granules immersed in $NaHSO_4$ solution were coated by CSD and CSD became CSH after heating at 120ºC for 4 hours. CSH coated β-TCP granules mixed with saline solution were set and DTS value of β-TCPGC with 75 wt% of CSH was 0.8±0.1 MPa. The percentage of newly formed bone of β-TCPGC with 75 wt% of CSH was 28.7±0.5 %, meanwhile β-TCP granules without coating was 19.9±1.1 %.

Conclusion: CSH coated β-TCP were successfully fabricated and formed interconnected porous structure with good mechanical strength after mixed with saline solution.

Key words: beta tricalcium phosphate, interconnected porous structure, calcium sulfate

PSS-2

Cathepsin E-dependent elastase secretion in neutrophils derives experimental autoimmune encephalomyelitis-induced neuropathic pain

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Multiple sclerosis (MS) is a chronic disease characterized by the destruction of central nervous system (CNS). Infiltration of immune cells into CNS is a hallmark of MS, leading to neuronal dysfunction such as motor disorder and paresthesia. It has been estimated that 55 % of MS patients experienced pain symptom that cause sleep disturbance, leading to fatigue and depression, and seriously affect the patient's daily activities and quality of life. Although pain is a common problem in MS patients, no effective therapeutics are commercially available. In the present study, we try to find molecular mechanism of neuropathic pain in MS using by experimental autoimmune encephalomyelitis (EAE), a mouse model of MS.

Neuropathic pain in C57BL/6 mice was observed 3 days after myelin oligo-glycoprotein (MOG) immunization before EAE onset (days 9-12 post immunization). In contrast, cathepsin E (CatE)-knockout mice were highly resistant to neuropathic pain caused by MOG-immunization. We observed that neutrophils infiltrated into the dorsal root ganglion (DRG) after MOG immunization. Isolated neutrophils from bone marrow increased both protein and mRNA levels of CatE following MOG stimulation. Adoptive transfer of WT neutrophils that stimulated with MOG induced pain behaviors in recipient mice, whereas that of *CatE*$^{-/-}$ neutrophils did not. Further study revealed that sivelestat, a neutrophil elastase specific inhibitor, significantly suppressed neuropathic pain caused by adoptive transfer of MOG-stimulated neutrophils. In addition, neutrophils increase elastase activity in response to MOG stimulation, which was a CatE-dependent manner. Elastase derived from neutrophils activated protease-activated receptor-2 (PAR2) in the DRG neurons, which triggered neuropathic pain in EAE. We finally identified that CatE induction in neutrophils by MOG was mediated by TLR4.

Together, CatE in neutrophils thus become a promising therapeutic target for neuropathic pain in MS patient.

Key words: multiple sclerosis, EAE, neutrophils, Cathepsin E, neutrophil elastase

PSS-3

Expression and function of Piezo1 and Piezo2 in tooth germ development

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Mechanotransduction, the conversion of mechanical force into biological signals, has a crucial role in physiology. In mammals, embryonic development, touch, pain, adjustment of vascular tone and blood flow, lung growth, and bone and muscle homeostasis are all regulated by mechanotransduction (Coste B et al., Science, 2010). The epithelial-mesenchymal interaction in the tooth germ development dynamically produces to figure complicated tooth forms, however it is unknown whether Piezo family is involved as mechanosensitive receptor in the developing tooth germ. In this study, we examined to clarify expression patterns and functions of Piezo1 and Piezo2 in tooth germ development.

At the bud and cap stages in the mouse lower first molar, Piezo1 mRNA expression was prominently observed in the enamel organ, while Piezo2 mRNA expression was seen in the enamel organ and the surrounding mesenchymal cells. At the bell stage, Piezo1 and Piezo2 mRNA expression was detected in both the ameloblasts and odontoblasts. At the tooth root formation stage, Piezo1 and Piezo2 mRNAs were noted in the odontoblasts of the tooth root. The expression patterns of the Piezo1 and Piezo2 proteins were similar to those of the Piezo1 and Piezo2 mRNAs, respectively. In addition, to investigate the effects of knockdown of Piezo1 on the tooth germ morphogenesis, organ culture was performed in cultured embryonic day 15.0 (E15.0) for 8 days. Tooth germs treated with Piezo1 siRNA showed irregular arrangements of the ameloblasts and odontoblasts. Immunochemical staining for Ki-67 was carried out to examine the cell proliferative activity in the tooth germs since the development of the E15.0 tooth germs was disturbed by treatment with Piezo1 siRNA. The number of Ki-67 positive cells increased in the irregular-arrangemented ameloblasts in the tooth germs treated with Piezo1 siRNA.

These results suggested that Piezo1 and Piezo2 might play functional roles in tooth germ development.

Key words: Piezo1, Piezo2, mechanosensitive channels

PSS-4

R-spondin2 enhances osteogenesis of immature human periodontal ligament cells through the canonical Wnt signaling pathway

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R-spondin family proteins (RSPOs), secreted glycoproteins, are known to play an important role in embryonic development and tissue homeostasis through activation of the canonical Wnt/β-catenin signaling pathway. R-spondin2 (RSPO2), a member of RSPOs, has been reported to enhance osteogenesis in mice. In this study, we examined the localization of RSPO2 in periodontal ligament (PDL) tissue and the effect of RSPO2 on osteoblastic differentiation of PDL cells.

Immunohistochemical staining revealed that RSPO2 was expressed in rat PDL tissue. Semi-quantitative RT-PCR and immunofluorescence staining demonstrated that the immature human PDL cell line (2-14 cells) predominantly expressed the receptor of R-spondin, LGR4, whereas we found little expression of LGR5 and LGR6. Alizarin red S staining and von Kossa staining revealed that RSPO2 stimulation significantly enhanced osteoblastic differentiation of 2-14 cells cultured in osteogenic induction medium. In addition, DKK1, an inhibitor of the canonical Wnt/β-catenin signaling pathway, suppressed the RSPO2-induced osteoblastic differentiation of 2-14 cells.

These results suggest that RSPO2 enhanced osteoblastic differentiation of immature human PDL cells through the canonical Wnt signaling pathway. Therefore, RSPO2 may be a therapeutic molecule in PDL healing or regeneration.

Key words: canonical Wnt/β-catenin signaling, immature human periodontal ligament cell line, osteoblastic differentiation, R-spondin2

PSS-5

The roles of miR875-5p as a specific marker during early tooth development

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Epithelial–mesenchymal interaction plays critical roles for the development of organs such as tooth, lung, salivary gland, kidney and hair. During this process, epithelial thickening and budding into mesenchyme are common phenomenon, while the decision of cell fate is made by the specific transcriptional controls of individual organs. We explored specific transcriptional start sites (TSS) of each organ by CAGE (Cap Analysis of Gene Expression) analysis using embryonic day 11 (E11), E12, E13 and E14 ICR mouse. We identified a tooth specific TSS, which has been detected on chromosome 15qD1 region. This TSS potentially codes both microRNA 599 (*miR599*) and *miR875*. To identify which microRNA is coded from the TSS during tooth development, we performed RT-qPCR to determine the expression level of *miR599* and *miR875* at early developmental stage of tooth. According to this assay, we found that *miR875-5p* is specifically expressed in E14 tooth germ. We also tested the expression pattern of *miR875-5p* in tooth development from E11 to postnatal day 7 (P7). We found that *miR875-5p* was highly expressed in E14 tooth germ and the expression decreased gradually after that. In addition, we found that *miR875-5p* was expressed in mesenchyme of E14 tooth germ by RT-qPCR and in situ hybridization. To assess the role of miR875-5p in dental mesenchyme during tooth development, we transfected mimic of *miR875-5p* into mouse dental pulpal (mDP) cells, which do not express *miR875-5p* originally. We identified that the expression of odontoblast marker, *Dspp* (dentin sialophosphoprotein) was significantly induced by miR875-5p in mDP cells. In this study, we identified tooth specific miRNA at early developmental stage. miR875 may regulate odontoblast differentiation and can be used as a specific marker for early stages of dental mesenchymal cells.

Key words: Tooth, dental mesenchymal cell, CAGE analysis, miRNA

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