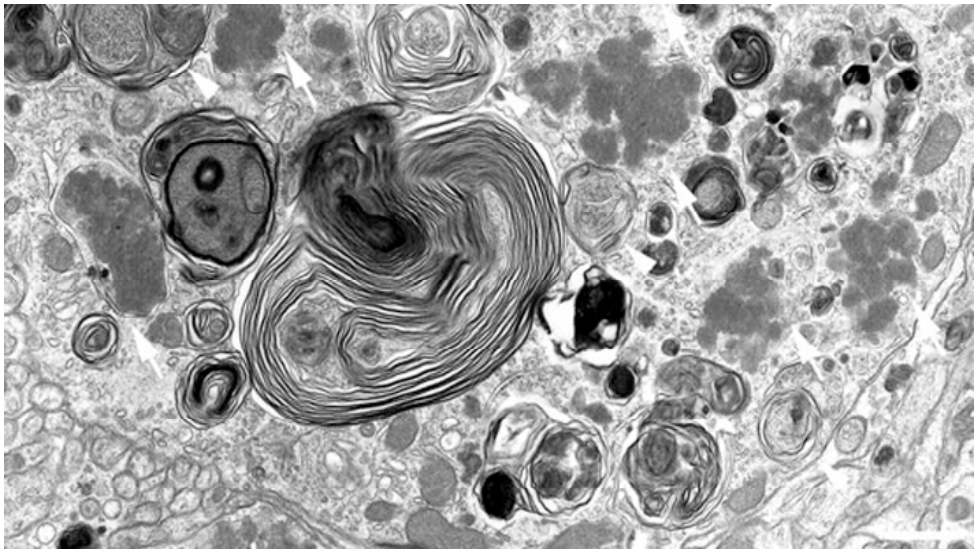
Kyudai Oral Bioscience 2017 (KOB2017)

Physiological and Pathological Roles of Lysosomal Proteolytic System

February 11th (Sat) 2017

Collaboration Station I, 2F Audiovisual Hall
Kyushu University

PROGRAM & ABSTRACTS



The School of Dentistry Kyushu University will celebrate its 50th Anniversary in 2017.

〇会期：平成 29 年 2 月 11 日（土） 13:00～18:40

〇会場：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

〇主催：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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PROGRAM

Kyudai Oral Bioscience 2017 (*KOB2017*)

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

Opening Remarks
Masato Hirata (*Dean, Faculty of Dental Science, Kyushu University*)

【Special Lectures】
Physiological and Pathological Roles of Lysosomal Proteolytic System

Chair person Masato Hirata (*Laboratory of Molecular and Cellular Biochemistry*)

13:10～14:10 *Special Lecture 1*
Veronika Stoka
Lysosomal cysteine cathepsins and their protein inhibitors, cystatins, in health and disease
Department of Biochemistry and Molecular and Structural Biology, J. Stefan Institute, Ljubljana, Slovenia

Chair persons Seiji Nakamura (*Section of Oral and Maxillofacial Oncology*)
Hiroshi Nakanishi (*Department of Aging Science and Pharmacology*)

14:20～15:00 *Special Lecture 2*
Masato Koike
The role of autophagy and lysosomal proteolysis for the maintenance of the normal environment of central nervous system
Department of Cell Biology and Neuroscience, Juntendo University Graduate School of Medicine, Tokyo, Japan

15:00～15:40 *Special Lecture 3*
Yoichi Ezura
Paradoxical osteoclast functions in congenital osteolytic disorders
Skeletal Molecular Pharmacology, Division of Advanced Molecular Medicine,
Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan

15:40～16:20　*Special Lecture 4*

Xian-Wu Cheng

Role of cysteinyl protease cathepsins in atherosclerosis-based cardiovascular disease: Focus on novel biology and mechanisms

Institute of Innovation for Future Society, Nagoya University, and the Department of Community Health & Geriatrics, Nagoya University Graduate School of Medicine, Nagoya Japan

16:20～16:40　*Group Photograph*

【PhD Student Session (PSS)】 Chair person：Yuka Harada

16:40～17:00　*Peports of Short-term Exchange Program (JASSO)*

Sanako Nakaya, Wakako Nakayama,Tihiro Furumi, Masato Yamamoto

Report after attending Gadjah Mada University dental summer course

School of Dental Science, Kyushu University

17:00～17:10　*PSS-1*

Erika Tomoda

Epigenetic regulation of PITX2 causes dysfunction of stem cells from apical papilla in dentin dysplasia type I

Departments of Molecular Cell Biology and Oral Anatomy, Faculty of Dental Science, Kyushu University

17:10～17:20　*PSS-2*

Yoshikazu Hayashi

Uncarboxylated osteocalcin involves antitumor immunity in cancer growth

Laboratory of Molecular and Cellular Biochemistry, Faculty of Dental Science, Kyushu University

17:20～17:30　*PSS-3*

Yicong Liu

Infection of microglia with *Porphyromonas gingivalis* promotes cell migration and an inflammatory response through the gingipain-mediated activation of protease-activated receptor-2

Department of Aging Science and Pharmacology, Faculty of Dental Science, Kyushu University

17:30～17:40　*PSS-4*

Shinya Kageyama

Exploration of IgA-binding bacteria in salivary microbiome

Section of Preventive and Public Health Dentistry, Faculty of Dental Science, Kyushu University

17:40～17:50　*Coffee Break*

17:50～18:00　*PSS-5*

Masahiko Morioka

Exosomes from oral squamous carcinoma cells define the tropism of invasiveness and lymphatic dissemination

Section of Oral and Maxillofacial Oncology, Faculty of Dental Science, Kyushu University

18:00～18:10　*PSS-6*

Mitsudai Tsuruta

The effect of innate micro-inflammation on pancreatic islet cells

Department of Periodontology, Faculty of Dental Science, Kyushu University

18:10～18:20　*PSS-7*

Hiromi Mitarai

Transgelin mediates the proliferation of human periodontal ligament cells induced by TGF-β1

Department of Endodontology and Operative Dentistry, Faculty of Dental Science, Kyushu University

18:20～18:30　*PSS-8*

Kota Tsutsumi

Characterisation of sucrose-independent supragingival plaque produced in an *in vitro* biofilm model

Section of Oral Health Promotion and Technology, Graduate School of Dental Science, Kyushu University

Closing Remarks

18:30～18:40

Fusanori Nishimura　(*Department of Periodontology*)

ABSTRACTS

Special Lecutures

Physiological and Pathological Roles of Lysosomal Proteolytic System

Special Lecture 1

Lysosomal cysteine cathepsins and their protein inhibitors, cystatins, in health and disease

Veronika Stoka

Department of Biochemistry and Molecular and Structural Biology, J. Stefan Institute, Ljubljana, Slovenia

Lysosomes and endosomes contain proteases, primarily cathepsins, namely, the aspartic cathepsins D and E (pepsin family) and 11 human cysteine cathepsins, B, C, F, H, K, L, O, S, V, W and X (papain family). These enzymes differ in their localization and tissue distribution, expression profiles, biochemical properties, structures and the regulation of their proteolytic activities **[1,2]**. Cysteine cathepsins are synthesized as inactive precursors and activated at acidic pH into mature active enzymes. They are all monomeric proteins except the tetrameric cathepsin C. Their extracellular localization is often bound to an increased expression and activity such as elastinolytic and collagenolytic activity i.e. cathepsins S, K and V responsible for the remodelling of the extracellular matrix (ECM) **[2]**. The structures of distinct cathepsins explain their broad substrate specificities. Cathepsin activities can be controlled by various mechanisms such as pH, zymogen activation, and endogenous protein and exogenous low Mr inhibitors **[1]**.

The endogenous protein inhibitors of the cystatin superfamily have been classified into three types: the stefins (Family 1), the cystatins (Family 2), and the kininogens (Family 3) **[3]**. Cystatin inhibitory activity is essential for the delicate regulation of normal physiological processes by limiting the potentially harmful activity of their target proteases. Failures in biological mechanisms controlling protease activities result in many diseases such as neurodegeneration **[4,5]**, cardiovascular diseases, inflammatory diseases, such as rheumatoid arthritis, and cancer, among others **[1]**.

There are a few genetic disorders involving mutations in cathepsin genes. Genetic studies revealed that loss-of-function mutations in the cathepsin C (DPPI) gene results in early-onset

periodontitis and palmoplantar keratosis, characteristics of Haim-Munk and Papillon-Lefevre syndromes **[6]**. In the cathepsin K gene, at least fifteen mutations in humans leading to pycnodysostosis, are known **[7]**. Studies on cysteine cathepsins biology contribute to development of new therapies in various diseases with high probability of being incorporated into clinical trials.

References

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Special Lecture 2

The role of autophagy and lysosomal proteolysis for the maintenance of the normal environment of central nervous system

Masato Koike

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We have been analyzing brain tissues from cathepsin D (CD) deficient mice, a model for the most severe type of neuronal ceroid lipofuscinoses (NCLs). NCLs are a group of inherited, neurodegenerative, lysosomal storage disorders characterized by progressive intellectual and motor deterioration, seizures, and early death. The group of human neuronal NCLs currently comprises 14 genetically distinct disorders (CLN1-14).

Most types of NCLs are pathologically characterized by storage of autofluorescent material containing subunit c of mitochondrial ATP synthase (SCMAS) within lysosomes. Mitochondria are sequestrated by autophagy/mitophagy resulting in the degradation by lysosomal proteinases, such as tripeptidyl peptidase 1 (TPP1)/CLN2 and CD/CLN10. NCLs are classified as rare diseases. However, taking into account that in many cases of early-onset familial Parkinson's disease (PD), genes essential for mitophagy are mutated, NCL and PD may share etiological features. Indeed, mutations in ATP13A2 are a known cause of Kufor-Rakeb syndrome (KRS) with both PD phenotypes and NCL pathology. Thus ATP13a2 is called both Park9 and CLN13.

In this symposium I will review our previous studies on the several genetic mouse models for elucidation of autophagy-lysosomal systems in neurons under physiologic and pathologic conditions and further introduce about the recent studies on the comparative analyses of CD and ATP13a2 deficient mice and Purkinje cells-selective CD and Atg7-deficient mice.

Special Lecture 3

Paradoxical osteoclast functions in congenital osteolytic disorders

Yoichi Ezura

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1-5-45 Yushima Bunkyo-ku, Tokyo, 113-8510 Japan

The osteolysis occurs in various clinical situations such as loosening orthopaedic prosthesis, infectious dental implants, and metastatic malignant tumors. In addition, various genetic disorders are known to be associated with osteolysis. In most situations, activated osteoclast function and inflammation would explain the symptoms. However, in some situations, the findings are paradoxical. For example, an osteopetrotic disorder named Pycnodysostosis by defective homozygous mutation in Cathepsin K is known to be associated with paradoxical fingertip bone erosion, named as "acro-osteolysis". Similarly, carpal bone osteolysis could be somehow paradoxically caused by homozygous *MMP2* deficiency or by *MMP14* deficiency in the patients of “multicentric osteolysis nodulosis arthropathy (MONA)”. Indeed in our experiment, *Mmp2* deficient mice had paradoxical osteopenia in the long bone metaphysis in contrast to the thickened cortical and calvarial bones, consistent with the symptoms found in human patients. Although the pathophysiological mechanisms of osteolysis are still need to be understood, we are trying to unveil the relationships between the osteoclast functions and location specific osteolysis in several mutant mouse lines. In this talk, I would like to talk about the osteolytic and osteopenic phenotypes of the mutant mice that we had analyzed in the past few years. The relationships between the findings on Dok-deficient mice, Cnot3-hemizygous mice, conditional Profilin-deficient mice and genetic disorders would be discussed.

Special Lecture 4

Role of cysteinyl protease cathepsins in atherosclerosis-based cardiovascular disease: Focus on novel biology and mechanisms

Xian-Wu Cheng

Institute of Innovation for Future Society, Nagoya University, and the Department of Community Health & Geriatrics, Nagoya University Graduate School of Medicine, Nagoya Japan

Until recently, the role of lysosomal cysteine protease cathepsins in intracellular protein degradation was believed to be mainly restricted to scavenging. However, recent studies have revealed nontraditional roles for cysteine protease cathepsins in the extracellular space during the development and progression of cardiovascular disease. Although the precise mechanisms are unknown, data from animal studies suggest that members of the cathepsin family, like other extracellular proteases, contribute to extracellular matrix protein remodeling and interstitial matrix degradation, as well as to cell signaling and cell apoptosis in heart disease. Serum levels of cathepsins L, S, and K and their endogenous inhibitor cystatin C may be useful predictive biomarkers in patients with coronary artery disease and cardiac disease. Furthermore, *in vivo* pharmacological intervention with a synthetic cathepsin inhibitor and cardiovascular drugs has the potential for pharmacologic targeting of cathepsins in cardiovascular disease. This review focuses on cathepsin biology and the involvement of cysteinyl cathepsins in the pathogenesis of several heart and vessel diseases, especially with respect to their potential application as diagnostic and prognostic markers and drug targets to prevent inappropriate proteolysis in cardiovascular disease.

PhD Student Session

Peports of Short-term Exchange Program (JASSO)

Report after attending Gadjah Mada University dental summer course

Sanako Nakaya, Wakako Nakayama,Tihiro Furumi, Masato Yamamoto

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We visited Gadjah Mada University in Yogyakarta, Indonesia. It takes about 1 hour by air plane from the capital city, Jakarta. There are many islands in Indonesia and Yogyakarta is located in Java island. The city has many temples like Kyoto. Gadjah Mada University is a national university built in 1949. It has 18 faculties, for example, law, medicine, engineering and so on. We participated in UGM dental summer course hosted by the Faculty of Dentistry. We studied with the students from Yonsei University in Korea and Malaya University in Malaysia. From Japan, there were 5 attendants from Kyushu and Tokushima University. The course contained visiting regional hospital, clinical skill training, language class (English, Javanese), wearing traditional clothes, sightseeing, and so on. Here, we talk about what we experienced, felt, and learned about the dentistry and culture in Indonesia, and would like to give a message to the junior students coming after us.

PSS-1

Epigenetic regulation of PITX2 causes dysfunction of stem cells from apical papilla in dentin dysplasia type I

Erika Tomoda[1,2], Haruyoshi Yamaza[2], Soichiro Sonoda[1], Yosuke Tanaka[1], Norihisa Uehara[1], Yukari N. Kyumoto[1], Toshio Kukita[1], Sontao Shi[3], Kazuaki Nonaka[2], Takayoshi Yamaza[1]

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Tooth root formation is associated with epithelial-mesenchymal interaction between epithelial sheath of Hertwig and odonotoblasts. Stem Cells from Apical Papilla (SCAP) are responsible mesenchymal stem cells for forming tooth root dentin. Dentin dysplasia type I (DDI) is an autosomal-dominant irritant clinically associated with tooth root aplasia and/or hypoplasia. However, the critical gene and mechanism of DDI have not been elucidated. Recently, we clinically met with a patient of non-autosomal-dominant DDI non-associated with ectodermal deficiency, suggesting that the tooth root abnormality might be occurred by dysfunction of SCAP. Cells were isolated from the apical papilla-like tissue of the patient teeth and characterized as SCAP-like cells, DDI-SCAP. We then evaluated that DDI-SCAP impaired odontogenic differentiation capacity associated with dentin sialophosphoprotein and enhanced Runx2 expression when compared with healthy-donor derived control SCAP (control SCAP). In addition, DDI-SCAP damaged the cell cycle, especially G1 phase associated with cyclin dependent kinase 6 (CDK6), CDK2 and cyclin D1. Molecular and biochemical analyses revealed that PITX2 gene and protein was markedly reduced in DDI-SCAP in comparison with control SCAP. However, the genetic deletion and mutation of PITX2 were not observed in DDI-SCAP. Furthermore, we evaluated that PITX2 siRNA-knockdown suppressed odontogenic differentiation and G1 phase of cell cycle in control SCAP, suggesting that epigenetically regulated PITX2 gene cause the dysfunction of cell differentiation and cell cycle of SCAP in DDI.

Key words: Dentin Dysplasia Type I (DDI), Stem Cells from Apical Papilla (SCAP), PITX2, Cell Cycle

PSS-2

Uncarboxylated osteocalcin involves antitumor immunity in cancer growth

Yoshikazu Hayashi[1,2], Tomoyo Kawakubo-Yasukochi[1,4], Akiko Mizokami[1,3], Seiji Nakamura[2], Masato Hirata[1]

[1]Laboratory of Molecular and Cellular Biochemistry, [2]Section of Oral and Maxillofacial Oncology, [3]OBT Research Center, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, Japan, [4]Department of Immunological and Molecular Pharmacology, Faculty of Pharmaceutical Science, Fukuoka University, Fukuoka 814-0180, Japan

Osteocalcin (OC), a noncollagenous bone matrix protein secreted by osteoblasts, in serum has been correlated with bone remodeling under pathological status including cancer bone metastasis, as well as normal skeletal turnover. OC in serum exits as two types, γ-carboxylated (GlaOC) or lower- (or un-)γ-carboxylated (GluOC). Recent studies demonstrated that high circulating OC levels constitute a marker for bone metastasis in prostate cancer patient. We previously reported that GluOC potentially suppresses human prostate cancer cell growth by inhibiting receptor tyrosine kinases (RTKs) activities. However, the mechanisms *in vivo* have not been elucidated.

In this study, we found that GluOC suppressed tumor growth of B16 mouse melanoma transplants in C57Bl/6N wild-type mice, but GluOC did not exhibit antitumor activity in human prostate cancer xenografts using athymic nude mice. Moreover, stimulation of primary mouse splenocytes with concanavalin A, a polyclonal T-cell mitogen, in the presence of GluOC promoted T cell proliferation and their interferon-γ (IFN-γ) production. Besides, GluOC directly suppressed B16 cell growth through downregulating phosphorylation levels of RTKs *in vitro*.

These results indicate that GluOC exerts antitumor effects not only *in vitro*, but also *in vivo* via cellular immunostimulatory effects in B16 mouse melanoma cells.

Key words: GluOC, antitumor effects, immunostimulatory effects, IFN-γ

PSS-3

Infection of microglia with *Porphyromonas gingivalis* promotes cell migration and an inflammatory response through the gingipain-mediated activation of protease-activated receptor-2

Yicong Liu[1], Zhou Wu [1,2], Junjun Ni[1], Yoshinori Hayashi[1], Fumiko Takayama[1], Yurika Nakanishi[1], Yamin Zhou[3], Tomoko Kadawaki[4], Hiroshi Nakanishi[1]

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Despite a clear cause and effect relationship having been demonstrated between periodontitis and cognitive decline in Alzheimer's disease, the precise mechanism underlying the relationship remains unclear. The periodontal pathogen *Porphyromonas gingivalis* produces a unique class of cysteine proteinases termed gingipains that comprises Arg-gingipain (Rgp) and Lys-gingipain (Kgp). Rgp and Kgp are important in the bacterium-mediated host cell responses and the subsequent intracellular signaling in infected cells. In the present study, we attempted to crarify the potential effects of Rgp and Kgp on the cellular activation of brain-resident microglia. We provided the first evidence that Rgp and Kgp cooperatively contribute to the *P. gingivalis*-induced cell migration and expression of proinflammatory mediators through the activation of protease-activated protease 2 and phosphatidylinositol 3-kinase/Akt pathway in microglia.

Key words: *Porphyromonas gingivalis*, gingipains, microglia, cell migration, neuroinflammation

PSS-4

Exploration of IgA-binding bacteria in salivary microbiome

Shinya Kageyama, Toru Takeshita, Mikari Asakawa, Yukie Shibata, Rie Matusmi, Michiko Furuta, Kenji Takeuchi, Yoshihisa Yamashita

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Numerous bacteria inhabit and construct a complex but stable ecosystem in the oral cavity, and are involved in the progression of dental caries and periodontitis, as well as systemic disorder such as pneumonia. However, the regulation of their homeostasis remains unclear. In this study, we focused on immunoglobulin A (IgA) secreted into saliva, and investigated the relationship between IgA and oral microbiome. Stimulated saliva was collected from 8 healthy individuals (26-61 years old). Their salivary bacteria were stained with APC-labelled anti-human IgA, and sorted into IgA-negative bacteria and IgA-positive bacteria by cell sorter. The bacterial DNA was extracted from each pre- and post sorting sample, and their bacterial composition was determined by 16S rRNA gene sequencing analysis using the next generation sequencer, Ion PGM. Of 342 species-level operational taxonomic unit (OTUs), 22 OTUs corresponding to bacteria such as *Prevotella melaninogenica*, *Neisseria flavescens*, and *Streptococcus salivarius* exceeded 1% in pre-sorting samples and constitute 82.0 ± 5.7% of each microbiome. Most of the salivary bacteria (84.3 ± 2.9%) were bound by IgA, whereas 3 OTUs corresponding to *Fusobacterium periodonticum*, *Lautropia mirabillis*, and *Veillonella atypica* were characteristically uncoated with IgA in saliva of the most individuals, regardless of the concentration of IgA, age, and sex. These results suggested that some specific oral bacteria might evade mucosal immunity-recognition.

Key words: salivary microbiome, IgA, 16S rRNA gene, cell sorter

PSS-5

Exosomes from oral squamous carcinoma cells define the tropism of invasiveness and lymphatic dissemination

Masahiko Morioka[1,2], Tomoyo Kawakubo-Yasukochi[2], Seiji Nakamura[1], and Manabu Nakashima[2]

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Emerging evidence indicates that cancer-derived exosomes increase the tumorigenic potential of tumor cells by reprogramming the cells associated with the tumor microenvironment.

Our study aimed to examine the effect of cancer cell-derived exosomes on invasive and metastatic process, using two oral squamous carcinoma cell (OSCC) clones, SQUU-A and SQUU-B, from the same patient. Our data demonstrated that exosomes derived from highly metastatic SQUU-B cells conferred invasive ability to nonmetastatic SQUU-A cells and subsequently reduced mRNA expression of cytokeratin 13, which is strongly linked to malignant transformation of OSCCs.

We further examined the effect of the OSCC-derived exosomes on angiogenesis and lymphangiogenesis, closely related with hematogenous and lymphatic metastasis, respectively, using HUVECs (human umbilical vein endothelial cells) and HDLECs (human dermal lymphatic endothelial cells). As a result of expression assays, HUVECs exposed to the exosomes had little change in the expression levels of VEGFs (vascular endothelial growth factors) and VEGFRs (VEGF receptors), which are closely related with tube formation. In contrast, the expression levels of VEGFR1, VEGFR2 and VEGFR3 in HDLECs were significantly increased by the exosomes, and those of VEGF-A, VEGF-C and VEGF-D were increased only by the exosomes from SQUU-B. Additionally, these results were reflected to structure-forming ability in tube formation assay.

Our data indicate that the cancer cell-derived exosome undertakes crosstalk with different malignant cell clones in an identical tumor microenvironment and luminal cells closely related to cancer dissemination, and which may define clinical prognosis.

Key words: oral squamous carcinoma, exosome, invasion, matastasis

PSS-6

The effect of innate micro-inflammation on pancreatic islet cells

Mitsudai Tsuruta, Misaki Iwashita, Shinjo Takanori, Akiko Yamashita, Hiroaki Matsunaga, Fusanori Nishimura

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【Background】In subjects with Type 2 diabetes, infiltration of inflammatory cells such as macrophages is reportedly observed in islet in addition to the dysfunction of islet cells such as alpha and beta cells associated with disease progression. However, the role of infiltrated inflammatory cells on islet function remains unclear. We previously reported that the interaction between tissue infiltrated macrophages and adipocytes or glomerular mesangial cells contributed to the development of insulin resistance or renal dysfunction through inflammation.

【Research objectives】The aim of this study is to investigate the effect of interaction between infiltrated inflammatory cells and pancreatic alpha or beta cells on islet function.【Methods】Mouse adenoma-derived pancreatic α cell line αTC1 or insulinoma-derived pancreatic β cell line βTC6 were co-cultured with mouse macrophage-derived cell line RAW 264.7 using a transwell system. Changes in gene expression in the co-cultured αTC1 or βTC6 stimulated with *E.coli* lipopolysaccharide (LPS) were comprehensively analyzed by DNA microarray method. Then mRNA and protein expression in these cells were determined by real-time PCR and western blotting. In addition, LPS-stimulated interferon (IFN) β secretion from macrophages was measured by ELISA.

【Results】Expression of the genes belonging to Type 1 IFN gene cluster was shown to be significantly elevated in both co-cultured αTC1 and βTC6 by LPS stimulation. Among these genes, mRNA expression of X-linked inhibitor of apoptosis protein associated factor 1 (Xaf1), involved in the induction of apoptosis, was found to be markedly up-regulated particularly in βTC6. Xaf1 protein expression in βTC6 was also significantly elevated by stimulation with IFNβ or addition of culture supernatant of LPS-stimulated RAW 264.7. Furthermore, RAW 264.7 was found to secrete IFNβ by LPS stimulation, indicating that Xaf1 expression in the co-cultured βTC6 was induced by IFNβ secreted from macrophages in response to toll-like receptor (TLR) 4 ligand.

【Conclusions】These results suggest that IFNβ produced from macrophages activated by micro-inflammation induces Xaf1 expression in pancreatic beta cells, thereby promoting apoptotic pathway in β cells initially caused by cellular stress such as hyperglycemia and dyslipidemia, which potentially promotes the progression of diabetes.

Key word: Micro inflammation, Pancreatic beta cell, Xaf1

PSS-7

Transgelin mediates the proliferation of human periodontal ligament cells induced by TGF-β1

Hiromi Mitarai[1], Naohisa Wada[2], Daigaku Hasegawa[3], Shinichiro Yoshida[3], Mai Sonoda[1], Atsushi Tomokiyo[3], Sayuri Hamano[1,4], Suguru Serita[1] , Hiroyuki Mizumachi[1] , Hidefumi Maeda[1,3]

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【Background】Human periodontal ligament (PDL) cells express transforming growth factor β1 (TGF-β1). As TGF-β1 signaling pathway promotes many physiological processes, including cell growth, differentiation, proliferation, and collagen production in PDL cells, TGF-β1 is thought to play key roles in homeostasis of PDL tissue. Transgelin, known as a cytoskeleton-associated protein, has a Smad-binding element in gene promoter, suggesting that transgelin could be a target gene of TGF-β1 signaling. In this study, we examined the localization and the potential function of transgelin in PDL tissue and cells.

【Materials and Methods】Microarray analysis of human PDL cell lines (2-14, 2-23, and 2-52) was performed. Transgelin expression in primary human PDL cells (HPDLCs) was examined by quantitative RT-PCR, immunofluorescence staining and western blotting analysis. The effects of rhTGF-β1 and TGF-β1 activin receptor-like kinases (ALK5) inhibitor, SB431542, on HPDLCs were examined by western blotting analysis. The effects of transgelin knockdown by siRNA on the proliferation of HPDLCs were assessed by WST-1 assay.

【Results】 In microarray and quantitative RT-PCR analyses, transgelin expression level in 2-14 and 2-23 cells, which highly express PDL markers, such as periostin, alkaline phosphatase, α-smooth muscle actin, and type 1 collagen, was significantly higher than that in 2-52 cells. Immunohistochemical and immunofluorescence staining revealed the expression of transgelin in rat PDL tissue and HPDLCs. In HPDLCs, rhTGF-β1 upregulated transgelin expression while the inhibition of ALK5 by SB431542 suppressed the TGF-β1-induced transgelin expression. Furthermore, transgelin siRNA transfection did not promote the proliferation of HPDLCs treated with TGF-β1. The expression levels of cyclinA2 and cyclinE1, which regulate DNA synthesis and mitosis through the cell cycle, were also not upregulated in HPDLCs transfected with transgelin siRNA.

【Conclusion】Transgelin is expressed in PDL tissue and might be essential for the proliferation of human PDL cells induced by TGF-β1.

Key words: transgelin; TGF-β1; periodontal ligament

PSS-8

Characterisation of sucrose-independent supragingival plaque produced in an *in vitro* biofilm model

Kota Tsutsumi, Uchiyama A, Ken-ichiro Shibasaki

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A dental plaque is a complex biofilm comprising a consortium of microorganisms. The accumulation of supragingival plaque causes dental caries and periodontal diseases. The amount of sugar consumption in Japan has been decreasing, suggesting that the rate of sucrose-independent plaque formation is increasing in the current Japanese population. We therefore developed an *in vitro* model of a sucrose-independent supragingival plaque to examine the effect of new anti-cariogenic agents on a sucrose-independent plaque formation. In this study, we characterised a sucrose-independent biofilm model in comparison with a 1% sucrose-dependent biofilm model in terms of their products' properties physical strength, type of extracellular polymeric substance (EPS) and acidogenic potential.

For testing the physical strength, the biofilm models were treated with two different washing methods: (i) manual cleaning with different washing frequencies and (ii) mechanical cleaning with different shaking strengths. The type of EPS was evaluated using confocal laser scanning microscopy, after fluorescence labelling of either dextran or lectin (concanavalin A: Con A, wheat germ agglutinin: WGA). The biofilm model was also incubated with a glucose solution ranging from 0 to 10% for 3 h and then the pH of the solution was evaluated.

The sucrose-independent biofilm model retained more than approximately 70% of its original level after ‘performing manual cleaning two times’ and ‘750-rpm mechanical cleaning’, although the physical strength of the sucrose-independent biofilm model was slightly lower than that of the sucrose-dependent biofilm model. Glucan, an EPS produced by the synthesis from sucrose, was observed only in the sucrose-dependent biofilm model, whereas other EPSs were detected by Con A and WGA in both biofilm models. On the other hand, the decrease in pH was similar level in both biofilm models.

These results suggest the sucrose-independent *in vitro* biofilm model may produce biofilm without glucan EPSs, but with physical strength and an acidogenic potential at the same level as that in produced in the sucrose-dependent biofilm model.

Key words: supragingival plaque, physical strength, EPS, acidogenic potential

Memo


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