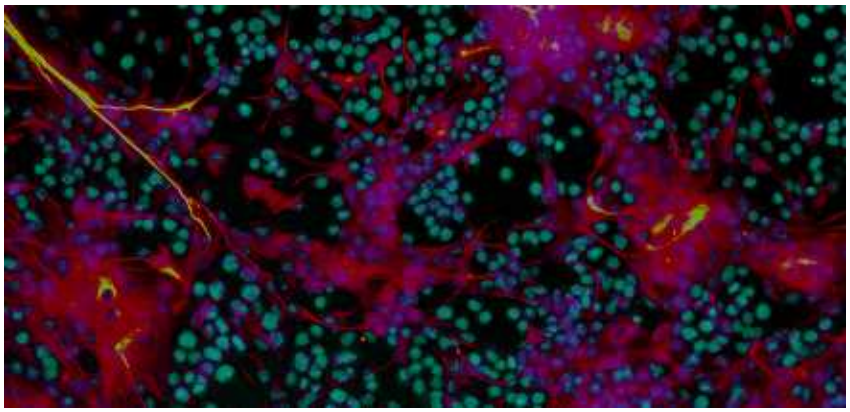
The 5th International Symposium on “Dental and Craniofacial Morphogenesis and Tissue Regeneration: A View from Stem Cell Research”

第5回国際シンポジウム「口腔組織の再生・再建医療研究」
(文部科学省　特別教育研究経費「口腔から QOL の向上を目指す連携研究」)



February 5, 2010
Centennial Hall, Kyushu University School of Medicine, Fukuoka, Japan

PROGRAM & ABSTRACTS

The 5th International Symposium on “Dental and Craniofacial Morphogenesis and Tissue Regeneration: A View from Stem Cell Research”

■ 日時：平成２２年２月５日（金）9:30~18:30

■ 場所：九州大学医学部　百年講堂

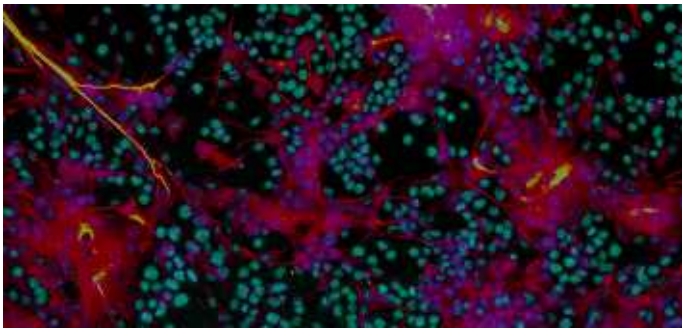
福岡市東区馬出 3-1-1（TEL: 092-642-6030）

■ 主催：九州大学大学院　歯学研究院

「口腔組織の再生・再建医療研究」プロジェクト

共催：文部科学省 特別教育研究経費「口腔から QOL の向上を目指す連携研究」

九州大学リサーチコア「口腔組織の再生医療の総合的開発」



■ プログラム

・プロジェクト成果報告会（9:40~11:40）

・ランチョンセミナー（12:00~13:00）共催：日機装株式会社

Dr. Hideo Iwasaka（Oita University, Japan）

・特別講演（13:20~18:10）

Dr. Yang Chai（University of Southern California, USA）

Dr. Richard O. Oreffo（University of Southampton, UK）

Dr. Songtao Shi (University of Southern California, USA)

Dr. Hidefumi Maeda (Kyushu University, Japan)

Dr. Takayoshi Yamaza (Kyushu University, Japan)

・交流懇親会（19:00~20:30）

■参加費無料

Centennial Hall
Kyushu University
School of Medicine

九州大学医学部
百年講堂

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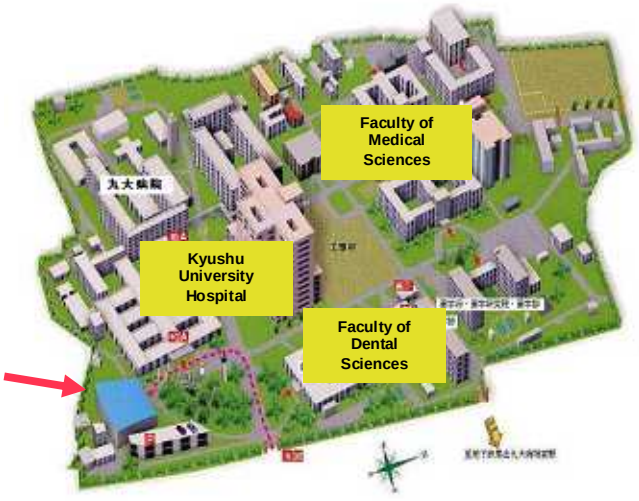

PROGRAM

■ **Opening remarks**

9:25 – 9:30 **Hiroshi Nakanishi**, ***Project leader***

9:30 – 9:40 **Kazunori Yoshiura**, ***Dean of Kyushu University Faculty of Dental Science***

■ **Annual Research Reports (9:40 – 11:40)**

<Chair person: Tamotsu Kiyoshima>

(A-1) 9:40 – 9:55

"Angiotensin II mediates the loading signal in human periodontal ligament cells"

S. Monnouchi[1], H. Maeda[2], S. Fujii[2], A. Tomokiyo[2], K. Hori[1], and A. Akamine[1,2]

[1]Division of Oral Rehabilitation, Department of Endodontology and Operative Dentistry, Faculty of Dental Science, Kyushu University, and [2]Department of Endodontology, Kyushu University Hospital, Fukuoka 812-8582, Japan

(A-2) 9:55 – 10:10

"Neural stem/progenitor cells in the leptomeningeal niche differentiate into astrocytes during systemic inflammation"

Zhou Wu, Li Sun, Yoshinori Hayashi, Hiroshi Nakanishi

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

(A-3) 10:10 – 10:25

"Fibroblast growth factor 10 and Meckel's cartilage formation in the early mandibular morphogenesis"

Fumie Terao[1], Ichiro Takahashi[1,2], Hidetoshi Mitani[1], Naoto Haruyama[1,3], Yasuyuki Sasano[1], Osamu Suzuki[1], Teruko Takano-Yamamoto[1]

[1]Division of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry, Tohoku University. [2]Department of orthodontics, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, [3]Tokyo Medical and Dental University, Tokyo113-8549, Japan

(A-4) 10:25-10:40

"Interleukin-22-induced STAT3 signal transduction in oral squamous cell carcinoma"

Lutfun Naher[1,2], Tamotsu Kiyoshima[1], Seiji Nakamura[2], Hidetaka Sakai[1]

[1]Laboratory of Oral Pathology and Medicine, and [2]Department of Oral and Maxillofacial Oncology, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, Japan

Break 10:40 – 10:55

<Chair Person: Kanji Tsuru>
(A-5) 10:55 – 11:10
"Analysis of the molecular mechanisms of periodontal regeneration by EMD"
Takao Fukuda, Terukazu Sanui, Takaharu Taketomi, Takafumi Hamachi, Katsumasa Maeda
Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, Japan

(A-6) 11:10 – 11:25
"Studies on characterization of the gene structure and transcriptional regulation of a novel inositol 1,4,5-trisphosphate binding protein, possibly involved in bone metabolism"
Ayako Murakami[1,2], Miho Matsuda[2], Koushirou Tsutsumi[2], Masato Hirata[2], and Ichiro Takahashi[1]
[1]Department of orthodontics, Faculty of Dental Science, Kyushu University
[2]Laboratory of Molecular and Cellular Biochemistry, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, Japan

(A-7) 11:25 – 11:40
"Preparation of porous apatite for bone tissue regeneration"
Giichiro Kawachi, Kanji Tsuru and Kunio Ishikawa
Department of Biomaterials, Faculty of dental science, Kyushu University, Fukuoka 812-8582, Japan

Break 11:40 – 12:00

■ Nikkiso Co. Inc. presenting Lunchon Seminor
<Chair person: Takeshi Yokoyama>
12:00 – 13:00
"Eating for better health-Perioperative nutritional management-"
Hideo Iwasaka
Department of Anesthesiology and Intensive Care Medicine, Oita University Faculty of Medicine, Yufu City, Oita, Japan

Break 13:00 – 13:20
■ Special Lecture (SL-1)
<Chair persons: Haruyoshi Yamaza / Kazuaki Nonaka>
SL-1. 13:20 – 14:20
"Stem Cell Property of Post-migratory Cranial Neural Crest Cells and Their Utility in Tissue Regeneration."
Yang Chai
Center for Craniofacial Molecular Biology, School of Dentistry, University of Southern California, USA

Break 14:20 – 14:30

■ **Special Lectures (SL-2 & SL-3)**
<Chair person: Toshio Kukita>
SL-2. 14:30 – 15:10
"What are the Characteristics of PDL Stem Cells ?"
Hidefumi Maeda
Department of Endodontology, Faculty of Dental Science, Kyushu University, Fukuoka 812-8582, Japan

SL-2. 15:10 – 16:10
Richard O. Oreffo
"Skeletal Stem Cells – Bridging the Gap in Bone Regeneration from Cell Biology to Clinical Translation"
University of Southampton DOHA, Bone & Joint Research Group M887, General Hospital Hampshire, S016 6YD, UK

Break 16:10 – 16:30

■ **Special Lectures (SL-4 and SL-5)**
<Chair person: Ichiro Takahashi>
SL-4. 16:30 – 17:10
"Immunomodulatory Properties of Stem Cells from Human Exfoliated Deciduous Teeth"
Takayoshi Yamaza
Department of Oral Anatomy and Cell Biology, Kyushu University Graduate School of Dental Science, Fukuoka 812-8285, Japan

SL-5. 17:10 – 18:10
"Mesenchymal Stem Cells: From Clinics to Bench-top and Back"
Songtao Shi
Center for Craniofacial Molecular Biology, School of Dentistry, University of Southern California, USA

■ **Concluding Remarks**
18:10 – 18:30 Drs. Richard Oreffo, Yang Chai, Songtao Shi

■ **Getting Together Party**
19:00 – 20:30

■ Nikkiso Co. Inc. presenting Lunchon Seminor

Eating for Better Health-Perioperative Nutritional Management-

Hideo Iwasaka
Department of Anesthesiology and Intensive Care Medicine, Oita University Faculty of Medicine, Yufu City, Oita, Japan

食べて健康になるー周術期の栄養管理ー

食べるという文字は人を良くすると書きます。健康であるためには食べることが必須条件になります。近年は過食によるメタボリックシンドロームが注目されていますが、周術期において患者さんは絶食による食事摂取不良が問題となり、周術期の合併症とも関連することが考えられます。低栄養により細胞へのエネルギー供給が低下するとオートファジーと呼ばれる自食作用が発現します。ユビキチンープロテオソーム系が選択的蛋白質分解を行うことに対して、オートファジーは非選択的に蛋白質や細胞内小器官を分解しエネルギーを得る方法です。基礎的レベルのオートファジーは変性蛋白質の処理機構として必須の機構です。しかし、侵襲とエネルギー供給の低下はオートファジーを過剰に活性化し細胞死をもたらす可能性のあることが指摘されています。周術期の合併症の発現にオートファジーが活性化されることが関連している可能性があります。術前の経口摂取不能に伴うエネルギー不足を補うため私どもの施設では炭水化物含有飲料水を術前２時間まで術前補水食として手術患者さんに提供しています。

手術侵襲はストレス誘導性高血糖をもたらします。高血糖による糖毒性は死亡率の増加と関連することが指摘されています。したがって術中、術後は低栄養をきたさない程度のエネルギー供給と高血糖をもたらさない血糖管理の両面をみたす管理が大切になってきます。至適なエネルギー投与と高血糖を防止するため人工膵臓による管理が最も安全、効果的管理法と考えられます。私どもの施設での管理方法について説明します。

■ Special lecture-1

Yang CHAI

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EDUCATION & CAREER

1984	MB, Peking University School of Stomatology, Beijing, China
1984-1986	Teaching Faculty and Surgical Resident, Peking University, School of Stomatology, Department of Oral-Maxilofacial Surgery
1987-1991	Research Associate, School of Dentistry, University of Southern California
1991-1994	Postdoctoral fellow, Center for Craniofacial Molecular Biology, University of Southern California
1996-2002	Assistant Professor, School of Dentistry, University of Southern California
2002-2006	Associatet Professor, School of Dentistry, University of Southern California
2004-2009	Chairman, Division of Craniofacial Sciences and Therapeutics, School of Dentistry, University of Southern California
2006-Present	Professor, University of Southern California School of Dentistry
2007-present	Director, Center for Craiofacial Molecular Biology, School of Dentistry, University of Southern California
2009-present	Associate Dean of Research, School of Dentistry, University of Southern California

Stem Cell Property of Post-Migratory Cranial Neural Crest Cells and Their Utility in Tissue Regeneration

Yang Chai

Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry Los Angeles, CA 90033, USA

The vertebrate neural crest is a multipotent cell population that gives rise to a variety of different cell types. We have discovered that post-migratory cranial neural crest cells (CNCCs) maintain mesenchymal stem cell (MSC) characteristics and show potential utility for the regeneration of craniofacial structures. We are able to induce the osteogenic differentiation of post-migratory CNCCs, and this differentiation is regulated by bone morphogenetic protein (BMP) and transforming growth factor- b (TGF-b) signaling pathways. After transplantation into a host animal, post-migratory CNCCs form bone matrix. CNCC-formed bones are distinct from bones regenerated by bone marrow mesenchymal stem cells (BMMSCs). In addition, CNCCs support tooth germ survival via BMP signaling in our CNCC-tooth germ co-transplantation system. Thus, we conclude that post-migratory CNCCs preserve stem cell features, contribute to craniofacial bone formation, and play a fundamental role in supporting tooth organ development. These findings reveal a novel function for post-migratory CNCCs in organ development, and demonstrate the utility of these CNCCs in regenerating craniofacial structures.

■ Special lecture-2

Hidefumi MAEDA

AFFILIATION Kyushu University Hospital, Department of Endodontology and Operative Dentistry

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EDUCATION & CAREER

1984-1990	Kyushu University, School of Dentistry
1990-1994	Kyushu University Graduate School of Dentistry Ph.D. program in Oral Science
1994-1995	Research Associate, Kyushu University, Faculty of Dentistry, Department of Pharmacology
1995-1999	Assistant Professor, Kyushu University, Faculty of Dentistry, Department of Endodontology and Operative Dentistry
1999-2001	Research Associate, University of Texas Health Science Center at San Antonio, Department of Medicine, Division of Hematology, TX, USA
2001-2003	Assistant Professor, Kyushu University Hospital, Department of Endodontology and Operative Dentistry
2004-present	Lecturer, Kyushu University Hospital, Department of Endodontology and Operative Dentistry

What are the Characteristics of PDL Stem Cells?

Hidefumi Maeda[1], Shinsuke Fujii[1], Atsushi Tomokiyo[1], Naohisa Wada[2], Satoshi Monnouchi[3], Kiyomi Hori[3], Katsuaki Koori[3], Naohide Yamamoto[3], Akifumi Akamine[3]

[1] Kyushu University Hospital, Department of Endodontology and Operative Dentistry,

[2] University of Adelaide, Colgate Australian Clinical Dental ResearchCentre,

[3]Kyushu University, Department of Endodontology and Operative Dentistry, Fukuoka 812-8582, Japan

Once the periodontal ligament (PDL) tissue is severely damaged by the periodontal diseases and lost, its reconstruction is well-known to be difficult. Therefore it is recognized that the regeneration of PDL tissue is the concern for conserving teeth. To address this issue, researchers have been to try to identify the stem cells, scaffolds, and the signaling factors responsible for the PDL regeneration. So far we have been focusing on the stem cells present in PDL tissue. However, as such PDL stem cells are very few to analyze their characteristics, we developed the immortalized PDL cells derived from human PDL tissue, and cloned these cells. Then we have succeeded in establishing two clones, 1-11 and 1-17, with multi-differential potentials, capable of characterizing the PDL stem cells. Line 1-11 contained the potentials to differentiate into osteoblastic cells and adipocytic cells, while line 1-17 differentiated into osteoblastic cells, adipocytic cells, chondrocytic cells, and neurocytic cells. Furthermore, both cell lines expressed CD13, CD29, CD44, CD71, CD90, CD105, and CD166 at higher ratios as expressed by mesenchymal stem cells. Therefore using these cell lines we have been evaluating the characteristics of PDL stem cells, analyzing the interaction of these cell lines with the scaffold components or the signaling elements.

■ Special lecture-3

Richard OC Oreffo

AFFILIATION	Bone and Joint Research Group, Center for Human Development, Stem Cell and Regeneration, Instutute of Developmental Sciences, University of Southampton
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EDUCATION & CAREER

1980-1983	BSc Hons II I, Biochemistry, University of Liverpool, UK
1983-1986	D.Phil. University of Oxford. (Thesis: “Vitamin A and Bone”)
1987-1989	Research Fellow, Division of Endocrinology, University of Texas, USA
1990-1993	Reswarch Fellow and Principal Research Scientist, Zeneca Pharmaceuticals, Cheshire, UK
1993-1999	MRC Research Fellow, University of Oxford, Oxford, UK
1999-2002	Non-Clinical Lecturer, University of Southampton, Southampton, UK
2002-2004	Senior lecturer and Reader, University of Southampton, Southampton, UK
Present	Professor of Musculoskeletal Science Associate Dean Innovation and Enterprise - University of Southampton, UK

Skeletal Stem Cells – Bridging the Gap in Bone Regeneration from Cell Biology to Clinical Translation

Richard O. Oreffo

University of Southampton DOHA, Bone & Joint Research Group M887, General Hospital Hampshire, S016 6YD, United Kingdom

Tissue engineering strategies have sought to repair skeletal defects resulting from trauma and disease with the application of cells, typically isolated from the patients themselves, in combination with porous biomaterials or scaffolds. Human bone marrow stromal stem cells or skeletal stem cells are multipotent progenitor cells with the ability to generate stromal lineages including cartilage, bone, fat and connective tissue. These primitive progenitors exist postnatally and exhibit stem cell characteristics, namely low incidence and extensive renewal potential. Advances in our understanding of skeletal stem cells and their role in bone development and repair, offer the potential to open new frontiers in bone regeneration.

Our work is centered on isolation, expansion and translational studies of skeletal populations, including enriched skeletal stem cell populations for skeletal repair using biomimetic scaffolds together with judicious selection of osteotropic growth factors to generate appropriate skeletal tissue constructs. A number of areas of work will be reviewed using data from the group including: i) innovative isolation strategies for of fetal and adult skeletal populations, ii) derivation of niche environments through combination of progenitor cells with tailored biomimetic scaffolds in an attempt to modulate the osteogenic and angiogenic repair process and iii) translational studies to examine the efficacy of skeletal populations using impaction bone grafting as an exemplar. The development of protocols, tools and multidisciplinary approaches that integrate materials, cell, molecular and clinical techniques for skeletal tissue regeneration for de novo tissue formation using skeletal progenitor and stem cell populations offers exciting opportunities to improve the quality of life of many in an increasing ageing population.

■ Special lecture-4

Takayoshi YAMAZA

AFFILIATION Department of Oral Anatomy and Cell Biology, Kyushu University Graduate School of Dental Science

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EDUCATION & CAREER

1988-1994	Kyushu University, School of Dentistry
1994-1998	Kyushu University Graduate School of Dentistry Ph.D. program in Oral Science
1998- 2001	Research Associate, Department of Endodontlogy and Operative Dentistry, Kyushu University Graduate School of Dental Science,
2001-2003	Research Associate, Department of Oral Anatomy and Cell Biology, Kyushu University Graduate School of Dental Science
2005-2006	Special Volunteer, Dental Unit, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA, Dr. Songtao Shi's Laboratory
2006-2007	Postdoctoral Research Associate, Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry, Los Angels, CA, USA, Dr. Songtao Shi's Laboratory
2008-2009	Research Associate, Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry, Los Angels, CA, USA, Dr. Songtao Shi's Laboratory
2003-present	Assistant Professor, Department of Oral Anatomy and Cell Biology, Kyushu University Graduate School of Dental Science

Immunomodulatory Properties of Stem Cells from Human Exfoliated Deciduous Teeth

Takayoshi Yamaza

Department of Oral Anatomy and Cell Biology, Kyushu University Graduate School of Dental Science, Fukuoka 812-8582, Japan.

Stem cells from human exfoliated deciduous teeth (SHED) have been identified as a population of postnatal stem cells capable of differentiating into osteogenic and odontogenic cells, adipogenic cells, and neural cells. Herein we have characterized mesenchymal stem cell properties of SHED in comparison to human bone marrow mesenchymal stem cells (BMMSCs). **Methods**: We used *in vitro* stem cell analysis approaches, including cytometric flow, inductive differentiation, telomerase activity, and Western blot analysis to assess multipotent differentiation of SHED and *in vivo* implantation to assess tissue regeneration of SHED. In addition, we utilized systemic SHED transplantation to treat systemic lupus erythematosus (SLE)-like MRL/*lpr* mice. We found that SHED are capable of differentiating into osteogenic and adipogenic cells, expressing mesenchymal surface molecules (STRO-1, CD146, SSEA4, CD73, CD105, and CD166), and activating multiple signaling pathways, including TGFβ, ERK, Akt, Wnt, and PDGF. Recently, BMMSCs were shown to possess an immunomodulatory function that leads to successful therapies for immune diseases. We examined the immunomodulatory properties of SHED in comparison to BMMSCs and found that SHED had significant effects on inhibiting T helper 17 (Th17) cells and promoting regulatory T cells (Tregs) *in vitro*. Moreover, we found that SHED transplantation is capable of effectively reversing SLE-associated disorders in MRL/*lpr* mice. At the cellular level, SHED transplantation elevated the ratio of Tregs via Th17 cells. These data suggest that SHED are an accessible and feasible mesenchymal stem cell source for treating immune disorders like SLE.

■ Special lecture-5

Songtao SHI

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EDUCATION & CAREER

1986	MS, Peking University School of Stomatology, Beijing, China
1986-1989	Assistant professor, Peking University, School of Stomatology, Department of Pediatric Dentistry
1989-1994	Research Associate, University of Southern California, Doheny Eye Institute Dr. Isaac Bekhor's Laboratory
1994-1997	Postdoctoral fellow, University of California at San Francisco. Dr. Arnold J. Kahn's Laboratory
1997-1998	Contractor and IRTA fellow, Craniofacial and Skeletal Diseases Branch, NIDCR/NIH Skeletal Biology Section
1998-1999	Dentist, S&S Best Dental Center, Los Angeles, CA, Clinical Fellow Craniofacial and Skeletal Diseases Branch, NIDCR/NIH
2002-2003	Clinical Fellow (Supervisor), Craniofacial and Skeletal Diseases Branch, NIDCR/NIH
2003-2006	Principal Investigator, Craniofacial and Skeletal Diseases Branch, NIDCR/NIH. Dental Biology Section
2006-2008	Assistant Professor, University of Southern California School of Dentistry
2008-present	Tenured Associate Professor, University of Southern California School of Dentistry,

Mesenchymal Stem Cells: From Clinics to Bench-top and Back

Songtao Shi

Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry Los Angeles, CA 90033, USA

Mesenchymal stem cells (MSCs) are a population of hierarchical postnatal stem cells with the potential to differentiate into mesodermal lineage-derived cells including osteoblasts, chondrocytes, adipocytes, cardiomyocytes, myoblasts and non mesodermal lineage-derived cells such as neural cells. The orofacial region contains multiple lineage mesenchymal stem cells, including bone marrow derived MSCs, dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), Stem cells from Human Exfoliated Deciduous teeth (SHED), and Stem Cells from root Apical Papilla (SCAP). First, we found that MSCs contribute to diseases such as osteoporosis and benign tumors. Next, we used autologous PDLSCs and periodontal progenitors to cure periodontitis with regeneration of cementum and Sharpey's fibers in swine and humans. More interestingly, we used PDLSCs and SCAP to regenerate root/periodontal complexes that are capable of supporting artificial porcelain crowns resulting in normal tooth function restoration in swine. Finally, we generated jaw osteonecrosis model in mouse and employed autologous bone marrow MSC transplantation to cure the necrosis, suggesting a novel therapeutic approach to recover jaw necrosis by bone marrow MSC-generated new bone and bone marrow. Our data indicate a great potential of using MSCs to cure a variety of disorders and to regenerate functional tissues.

■ Annual Research Reports

(A-1) Angiotensin II Mediates the Loading Signal in Human Periodontal Ligament Cells

S. Monnouchi[1], H. Maeda[2], S. Fujii[2], A. Tomokiyo[2], K. Hori[1], and A. Akamine[1,2]

[1]Division of Oral Rehabilitation, Department of Endodontology and Operative Dentistry, Faculty of Dental Science, Kyushu University, and [2]Department of Endodontology, Kyushu University Hospital, Fukuoka 812-8582, Japan

The loading caused by occlusion and mastication plays an important role in maintaining periodontal ligament (PDL) tissues. We hypothesized that a loading magnitude would be involved in the production of biological factors that function in the maintenance of PDL tissues. Here, we identified up-regulated gene expressions of transforming growth factor-β1 (TGF-β1), alkaline phosphatase (ALP) and angiotensinogen (AGT) in human PDL fibroblastic cells (HPLF) that were exposed to 8% stretch loading. Immuno-localization of angiotensin I/II (Ang I/II), which were converted from AGT, were detected in rat PDL tissues. HPLFs that were stimulated by Ang II also increased their gene expressions of TGF-β1 and ALP. Furthermore, the antagonist for Ang II type 2 receptor, rather than for type 1, significantly inhibited gene expressions induced by the stretch loading. These data suggest that Ang II mediates the loading signal in stretched HPLFs to induce expressions of TGF-β1 and ALP.

(A-2) Neural Stem/Progenitor Cells in the Leptomeningeal Niche Differentiate into Astrocytes during Systemic Inflammation

Zhou Wu, Li Sun, Yoshinori Hayashi, Hiroshi Nakanishi

Department of Aging Science and Pharmacology, Faculty of Dental Science, Kyushu University, Fukuoka 812-8581, Japan

Neural stem/progenitor cells (NSCs) are recognized by expression of nestin, an intermediate filament of neuroepithelial derivation. In the adult rat brain, nestin-positive NSCs are known to exclusively reside in two neurogenic regions, subventricular zone and dentate gyrus of the hippocampus. NSCs can differentiate into both neurons and glial cells including astrocytes in tight contact with the local environment. Recently, however, we have found that nestin-positive NSCs also reside in the leptomenigeal niche which covers the convolusin. By flow cytometrical analyses, nestin-positive NSCs in cultured leptomenigeal cells from embryonic day 19 rats showed both neuronal and glial spheres. On the other hand, the nestin-positive astrocytes identified by glial fibrillary acidic protein (GFAP) were significantly increased after treatment with lipopolysaccharide (LPS). By immunohistochemal analyses, the immunoreactivity for nestin localized in the leptomeniges of the cerebral cortex and choroid plexus in the young adult rats. Furthermore, the nestin-positive astrocytes in the proximity of leptomeniges significantly increased during systemic inflammation caused by adjuvant injection. Moreover, the cultured astrocytes expressed interkeukin (IL)-10 after treatment with LPS. In the cerebral cortex of the adjuvant injected rats, astrocytes in the proximity of the leptomeniges also expressed IL-10.

Our observations may aid in understanding the important roles of leptomeninges on the brain functions during systemic inflammation. We have previously demonstrated that the leptomeninges modulate neuronal and glial functions by secretion of diffusible molecules during systemic inflammation. Now we have provided first evidence that nestin-positive NSCs in the leptomeningeal niche increasingly differentiate into astrocytes during systemic inflammation. These differentiated astrocytes from leptomeningeal NSCs can in turn regulate neuroinflammation by secreting anti-inflammatory mediators including IL-10.

(A-3) Fibroblast Growth Factor 10 and Meckel's Cartilage Formation in the Early Mandibular Morphogenesis

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During organogenesis, fibroblast growth factor (FGF) signaling plays an important role in early pattern formation and tissue differentiation. In craniofacial development, differentiation of various tissues and organs involves FGF signaling, such as craniofacial skeletogenesis and mandibular development. However, the role of FGF10 in mandibular morphogenesis is still unclear. In the present study, we evaluated the role of FGF10 in mandibular chondrogenesis and morphogenesis by using rat mandibular cell micromass culture and mandibular organ culture in combination with either recombinant FGF10 treatment or electroporation of an FGF10 expression vector, respectively. In mandibular cell micromass culture, FGF10 treatment enhanced chondrogenic differentiation and endogeneous ERK (extracellular signal-regulated kinase) phosphorylation in the cells derived from lateral area of mandible. In organ culture system, not only the Meckel's cartilage was spirally deformed by ectopic expression of FGF10, but also the cartilage characteristic *Col2a1* and *Sox9* gene expression were up-regulated. The Meckel's cartilage was deformed and the size increased when FGF10 was overexpressed in the lateral area, but not in the medial region of the mandible. In conclusion, FGF10 regulates the shape and size of Meckel's cartilage during rat early mandibular morphogenesis through the differentiation of lateral cells of mandible.

(A-4) Interleukin-22-induced STAT3 signal transduction in oral squamous cell carcinoma

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Cytokines or cytokine-related mediators influence the cellular behavior of malignant tumors. Interleukin (IL)-22 is a member of the IL-10 family. Its main targets are epithelial cells, but not immune cells. The objectives of this study were to examine IL-22 signal transduction in oral squamous cell carcinoma (OSCC) cells. RT-PCR showed that human OSCC cells, MISK81-5 and HSC-3 cells, expressed IL-22 receptor chains. Immunoblotting showed that IL-22 induced a transient tyrosine phosphorylation of STAT3 (pY705-STAT3) in MISK81-5 cells after IL-22 stimulation. The change in the serine phosphorylation of STAT3 was subtle during the examination periods. Meanwhile pY705-STAT3 activation in HSC-3 cells was undetectable after IL-22 stimulation. The immunocytochemistry demonstrated that IL-22 induced translocation of phosphorylated STAT3 into the nucleus of MISK81-5 cells. IL-22 temporarily up-regulated the expression of anti-apoptotic and mitogenic genes such as Bcl-x, survivin and c-Myc as well as SOCS3. IL-22 transiently activated ERK1/2 and induced a delayed phosphorylation of p38 MAP kinase. IL-22 negligibly involved activation of NF-κB as detected by using MISK81-5 cells stably transfected with pGL4.26 [*luc2*/minP/Hygro] encompassed four tandem copies of the NF-κB consensus sequence. MISK81-5 cells treated with IL-22 showed mild cellular proliferation and down-regulation of the keratinocyte differentiation-related genes in comparison to unstimulated cells. IL-22 also increased MMP-2 protein in MISK81-5 cells, but not MMP-9. These results indicated that IL-22 differentially activated the STAT3 signaling system depending on the type of OSCC. IL-22 may play roles in the tumor growth, cell differentiation and progression through the activation of the STAT3 pathway. Modifying the function of IL-22 and/or blockade of JAK/STAT signals will lead to new perspectives and therapies with relatively few side effects in order to improve the patient management in OSCC.

(A-5) Analysis of the Molecular Mechanisms of Periodontal Regeneration by EMD

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I. Objective: Emdogain® (enamel matrix derivative, EMD) is well recognized in periodontology, where it is used as a local adjunct to periodontal surgery to stimulate regeneration of periodontal tissues lost to periodontal disease. However, the exact molecular mechanisms of periodontal regeneration remain largely unknown. In this study, we investigated the proteins bound to amelogenin, the major (>95%) component of EMD, to elucidate the molecules involved in the tissue regeneration during periodontal treatment.

II. Materials & Methods: We made GST (glutathione S-transferase) fusion amerogenin using the fetal mouse cDNA, to analyze the proteins bound to amelogenin by GST pull down assay. Purified GST fusion amelogenin immobilized on glutathione was incubated with the lysates of periodontal tissue calls. SaOS-2 and Sa-3, an osteosarcoma cell line widely used as a model system for osteoblastic cells and human gingival squamous carcinoma cell line for epithelial cell were mainly used in this study. The cell lysates were isolated as soluble proteins and membrane proteins respectively. After extensive washing, bound proteins ware resolved by SDS-PAGE, and analyzed by silver staining or Western blotting. Thio-His fusion protein was also made to confirm the direct interation with amelogenin by GST pull down assay.

III. Results: Silver stining analysis showed several proteins bound to amelogenin. Some proteins bound to amelogenin ware ubiquitous, others ware cell specific. YB-1, human Y-box protein, was identified as one of the amelogenin binding proteins by the Western blot analysis.

IV. Conclusion: We identified new amelogenin binding protein, YB-1 (human Y-box binding protein-1). YB-1 has multiple functions but was initially identified as a transcription factor. YB-1 protein is abundant and expressed ubiquitously in human cells, functioning in cell proliferation. Our data suggest that YB-1 is involved in regeneration of periodontal tissues by binding to amelogenin.

(A-6) Studies on Characterization of the Gene Structure and Transcriptional Regulation of a Novel Inositol 1,4,5-Trisphosphate Binding Protein, Possibly Involved in Bone Metabolism

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Phospholipase C-related but catalytically inactive protein (comprising PRIP-1 and -2) has been isolated as a novel inositol 1,4,5-trisphosphate binding protein with a domain organization similar to phospholipase C-delta but lacking the emzyme activity. We have recently reported that PRIP-1 and –2 double knockout (DKO) mice showed upregulation of gonadotropin secretion, but lower levels of serum sex steroid hormones were observed. Therefore we analyzed the bone condition of PRIP-DKO mice and the results indicated the involvement of PRIP in the regulation of bone metabolism. The PRIP-1 gene is expressed predominantly in the brain, while PRIP-2 exhibits a relatively ubiquitous expression. We speculated that the expression of PRIP-1 in brain is important for the regulation of hormone balance because central nervous system is involved in the regulation of many of hormones secretions.

In this study, we investigated PRIP-1 gene structure and the possible mechanisms involved in the expression. The tissue distribution pattern of PRIP gene expression in humans was similar to that in rodents. 5'RACE (rapid amplification of cDNA ends) analysis using PRIP-1 gene specific primers with human brain mRNA revealed the presence of three new exons, indicating that the PRIP-1 gene is organized into 8 exons intervened by 7 introns. Although three transcripts resulting from the alternative splicing of exon 2 and/or 3 were detected, a transcript lacking exons 2 and 3 was predominantly expressed in humans, suggesting that the translation start codon of human PRIP-1 exists in exon 1. To characterize the human PRIP-1 promoter, transient luciferase assay was carried out. The results indicated that the positive regulatory region is located –237 to –108 bp upstream from the transcription start site. Gel shift assay revealed the specific binding of some nuclear proteins to this region, suggesting that the existence of transcription factors contributes to the positive regulation of PRIP-1 gene expression. Mutation analyses revealed that the binding of a transcription factor, MAZ to the regulatory site leads to the promoter activity, indicating that MAZ is involved in the expression regulation of the human PRIP-1 gene.

(A-7) Preparation of Porous Apatite for Bone Tissue Regeneration

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Introduction

For bone tissue regeneration, the materials should have osteoconductivity and biodegradablity. Autograft is the standard choice for bone tissue regeneration. Because autograft or bone tissue is absorbed by osteoclasts and then replaced by new bone. Hydroxyapatite (HAp; $Ca_{10}(PO_4)_6(OH)_2$) has been commonly known to have high biocompatibility and bioactivity [1]. So, conventional bone substitute (HAp) is wildly used in orthopedic and dental fields. HAp shows osteoconductivity but it never shows biodegradablity. Because HAp with stoichiometric composition (Ca/P atomic ratio = 1.67 can not be absorbed by osteoclasts. So, it could not be replaced with new bone. One of differences between bone tissue and hydroxyapatite is chemical compositions. Biological apatite in bone tissue has a lot of carbonate ion non-stoichiometric composition (calcium deficient composition). We think this is the key for bone substitute. We already prepared a lot of porous apatite with unique structures by hydrothermal technique [2, 3, 4, 5].

In this study, we will report the reparation of porous apatite; carbonate apatite and calcium deficient apatite and discuss its application for bone tissue regeneration.

Experimental method

As starting material, tricalcium phosphate (TCP; $Ca_3(PO_4)_2$) and calcium sulfate were used. These powders were shaped as cylindrical shape of about 8 mmϕ × 2 mmL by molding under 100 MPa. The formed samples were set in a 100 cm^3 autoclave with 20 cm^3 of NH_4Cl / NH_3 buffer solution or ammonium carbonate solution. The pH of solutions was adjusted from 7.3 to 9.5 at room temperature. Then, samples were treated hydrothermally in liquid phase and vapor phase at 120 or 160 ºC for time up to total 24 h under saturated vapor pressure. Hydrothermal soaking method means that samples were soaked into liquid and treated hydrothermally in liquid phase. These buffer solutions were kept same pH volume after hydrothermal treatment.

Results

In case of TCP and NH_4Cl / NH_3 buffer solution, rod-shaped apatite crystals with about 20 μm in length were obtained. Due to crystal growth under the hydrothermal conditions, shape of obtained crystal became rod-shaped.

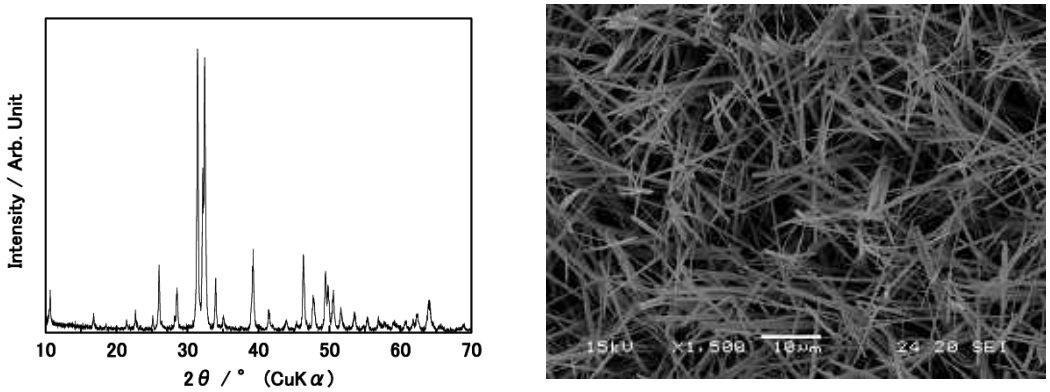

Fig. 1. XRD pattern and SEM image of apatite crystals prepared hydrotherally in NH_4Cl / NH_3 buffer solution (pH7.3) at 160 ℃ for 24 h.

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