○会期：平成 28 年 2 月 28 日（日）10:00～18:00

○会場：福岡リーセントホテル　レインボーホール　：下図参照

住所：福岡市東区箱崎 2-52-1
電話：092-641-7741

○主催：九州大学大学院　歯学研究院

日本学術振興会　頭脳循環を加速する戦略的国際研究ネットワーク推進プログラム

口腔から健康長寿を支えるプロジェクト推進に向けた

研究拠点構築プログラム

連絡先：　西村　英紀
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PROGRAM

頭脳循環第二回シンポジウム

2nd Symposium:
Program for

2016年2月28日

■ 10:00～10:10　Opening remarks　Masato Hirata

■ 10:10～11:10　Special Lecture 1　　Chair :　Hidefumi Maeda

Dr. Junichi Iwata：
Role of cholesterol in craniofacial development and diseases
The University of Texas Health Science Center at Houston (UTHealth) School of Dentistry Department of Diagnostic and Biomedical Sciences

■ 11:20～12:20　Special Lecture 2　　Chair :　Seiji Nakamura

Dr. Ophir Klein：
Sinking our teeth into development, evolution and regeneration
The Larry L. Hillblom Distinguished Professor in Craniofacial Anomalies, Professor of Orofacial Sciences and Pediatrics, and Chair of the Divisions of Craniofacial Anomalies and Orthodontics at the University of California, San Francisco (UCSF). Medical Director of the UCSF Craniofacial Center and Director, UCSF Program in Craniofacial Biology.

■ 13:30～14:30　Plenary Lecture　　Chair :　Masato Hirata

Dr. Yuji Mishina：
Enhanced BMP signaling in neural crest cells leads to Craniosynostosis
University of Michigan, School of Dentistry, Department of Biologic and Materials Sciences

■ 14:50～15:30 Special Lecture 3 Chair : Naohisa Wada

Dr. Keigo Yoshizaki :
Comprehensive analysis for the functions of genes during epithelial-mesenchymal interactions in tissue morphogenesis
Section of Orthodontics and Dentofacial Orthopedics, Faculty of Dental Science, Kyushu University, Japan

■ 15:30～16:10 Reports after circulation Chair : Fusaniri Nishimura

Dr. Takashi Maehara :
Lesional CD4+ IFN-γ ++ cytotoxic T lymphocytes in IgG4-related dacryoadenitis and sialoadenitis
Section of Oral and Maxillofacial Oncology
Division of Maxillofacial Diagnostic and Surgical Sciences
Faculty of Dental Science,Kyushu University
Ragon Institute of MGH, MIT and Harvard
Harvard Medical School

■ 16:30～17:30 Young researchers for next circulation Chair : Takayoshi Yamaza

Takao Fukuda : Molecular basis of amelogenin-induced periodontal tissue regeneration

Sachiko Furukawa : IL-33 secreted by M2 macrophages promotes the pathogenesis of IgG4-related disease

Shusuke Iwata : Stimulus repetition enhances responses of the chorda tympani nerve to sweeteners via endocannabinoid system

Hideki Sugii : Activin A reversely works between human pre-osteoblastic cells and periodontal ligament cells on their osteoblastic differentiation

■ 17:30 Closing remarks Prof. Fusanori Nishimura

Photo-taking

【MEMO】

Plenary Lecture

Enhanced BMP signaling in neural crest cells leads to Craniosynostosis

Yuji Mishina

University of Michigan, School of Dentistry, Department of Biologic and Materials Sciences

Craniosynostosis is caused by premature cranial suture fusion resulting in abnormal facial features. Approximately 20% of human craniosynostoses are thought to result from gene mutations altering growth factor signaling; however, the molecular mechanisms by which these mutations cause craniosynostosis are incompletely characterized, and the causative genes for diverse types of syndromic craniosynostosis have yet to be identified.

Bone morphogenetic protein (BMP) signaling plays broad roles in developmental patterning, including skull morphogenesis. We have previously reported enhanced BMP signaling through the BMP type IA receptor (BMPR1A) in cranial neural crest cells causes craniosynostosis. We have noticed approximately 55% of the mutants show neonatal lethality characterized by distended gastrointestinal tract. TUNEL assay demonstrated that an increase of apoptosis in condensing mesenchymal tissues and chondrocytes in the nasal region of the mutants. Furthermore, immunohistochemical staining for p53 revealed an increase of p53 in mutant nasal cartilage. To rescue the phenotype, pifithrin-a, a chemical inhibitor of p53, was injected to the pregnant female. The treatment resulted in prevention of apoptosis in mesenchymal cells and neonatal lethality. Furthermore, the survived mutant pups showed suture patency strongly suggesting that enhanced BMP signaling induces p53-mediated apoptosis leading to depletion of stem cell/progenitor population within suture mesenchyme resulting in premature suture fusion.

To dissect the molecular mechanism of how enhanced BMP signaling leads to p53-mediated apoptosis in our mutants, we examined the expression level of p53 and its regulatory network for apoptosis. We found that expressions of Bax and Caspase3 were increased in the mutants; however, p53 expression level was not changed. It is previously reported that Mdm2 ubiquitinates p53 to prompt its degradation and SMAD1 prevents from the MDM2-mediated p53 ubiquitination. We compared ubiquitinated p53 level between controls and the mutants by western blotting and immunoprecipitation. We found that ubiquitination of p53 was decreased in the mutants. These results indicate that BMP signaling induces p53-mediated apoptosis by preventing p53 ubiquitination in early stage of chondrogenesis and thus appropriate level of BMP signaling is required for proper craniofacial morphogenesis.

CURRICULUM VITAE

Bibliography

Education and Advanced Training:

1983-1986	Ph.D. University of Tokyo, Tokyo, Japan (Molecular Biology)
1981-1983	M.S. University of Tokyo, Tokyo, Japan (Molecular Biology)
1977-1981	B.S. University of Tokyo, Tokyo, Japan (Biology)

Academic Appointment:

1992-1998	Postdoctoral Fellow with Dr. Richard Behringer, The University of Texas, M.D. Anderson Cancer Center, Texas
1998-2008	Group Head, Molecular Developmental Biology Group, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC
2008-2014	Associate Professor, University of Michigan, School of Dentistry, Department of Biologic and Materials Sciences, Ann Arbor, MI
2014-Present	Professor, University of Michigan, School of Dentistry, Department of Biologic and Materials Sciences, Ann Arbor, MI

【MEMO】

Special Lecture 1

Role of cholesterol in craniofacial development and diseases

Junichi Iwata

The University of Texas Health Science Center at Houston (UTHealth) School of Dentistry Department of Diagnostic and Biomedical Sciences

Abstract

Cholesterol is 1) important as a structural lipid in cellular membranes to maintain membrane structural integrity and fluidity; 2) abundant in specialized microdomains called lipid rafts, which are organizing centers for the assembly of signaling molecules; 3) an obligatory biogenic precursor for steroid hormones, oxysterols, vitamin D, and bile acids. In addition, recent studies indicate that cholesterol can control a cell signaling activity. Therefore, the proper amount of cellular cholesterol is crucial for a wide variety of biological processes, including bone formation and salivary glands homeostasis. This talk will cover two topics related to cholesterol metabolic disorders; craniofacial bone formation and saliva production.

It has been largely unknown how disturbances in cholesterol production lead to bone abnormalities. In this study, we investigated the effects of cholesterol metabolism on craniofacial bone formation using mouse genetic approaches. So far, we discovered that mice with cholesterol metabolic aberrations altered: 1) calvaria bone formation; 2) osteoblast differentiation; 3) the formation of primary cilia (a microtubule-based organelle that functions in sensory and signaling pathways); 5) cell signaling cascades in osteoblasts.

It has long been appreciated that individuals with metabolic syndromes and abnormal diets (high cholesterol diets, poor nutrition, etc.) are at risk of chronic dry mouth (a.k.a. xerostomia). Hypofunction of the salivary glands results in severe periodontitis, dental caries, and oral ulcers, as well as difficulty in swallowing, tasting, chewing and speaking and substantially decreased quality of life. Despite such important physiological functions, we know very little about the regulatory mechanism of saliva exocytosis because of the lack of animal models for xerostomia and of tools for tracking exocytosis. In this study, we investigated the association between high cholesterol and xerostomia. The mice with a high cholesterol level displayed xerostomia with the accumulation of secretory vesicles in acinar cells of the salivary glands. We found that autophagic machinery (non-autophagic roles of autophagy-related molecules) was compromised in the salivary glands.

Thus, the role of cholesterol is diverse in craniofacial development and diseases. Taking advantage of our animal models, we are trying to determine new roles of cholesterol and its related molecules. Our findings will provide new insights into the role of cholesterol metabolism and lead to innovations in the prevention, diagnosis, and treatment of cholesterol-related disorders.

CURRICULUM VITAE

Biography

Dr. Iwata graduated from Kyushu University School of Dentistry in 2000, and received his Ph.D. at Kyushu University Graduate School of Dentistry in 2004. He carried out his Ph.D. training under Dr. Kenji Yamamoto, a well-established investigator in the fields of protease and molecular biology. He analyzed the properties of cathepsin E using knockout and transgenic mice as well as optical techniques, and found that cathepsin E could induce anti-angiogenic factors. He then worked as an Assistant Professor at Juntendo University Medical School through 2004 to 2007. He worked with a number of collaborators in the field of autophagy which is a major protein degradation system. They investigated a variety of genetic mouse models from 2004 to 2007. Their characterization of developmental defects in autophagy knockout mice inspired his interest in developmental regulation. To expand this interest, he joined the laboratory of Dr. Yang Chai, a well-established investigator in the fields of transforming growth factor beta (TGF β) signaling and craniofacial development, at University of Southern California School of Dentistry from 2007 to 2013. They discovered ectopic noncanonical TGF β signaling in the absence of TGF β receptor type II and identified downstream target molecules of this altered TGF β signaling. In his laboratory at The University of Texas Health Center at Houston (UTHealth) School of Dentistry from August 2013, his group focuses on understanding molecular and cellular mechanisms that cause developmental defects, and work closely with researchers from the Medical as well as the Dental School. As a DDS-PhD scientist, Dr. Iwata is very pleased to contribute to the craniofacial and dental research community. He is named on more than 30 peer-reviewed publications.

Selected Publications

1. Iwata J, Ezaki J, Komatsu M, Yokota S, Ueno T, Tanida I, Chiba T, Tanaka K, and Kominami E. (2006) Excess peroxisomes are degraded by autophagic machinery in mammals. J. Biol. Chem., 281, 4035-41.
2. Sou Y*, Waguri S*, Iwata J*, Ueno T, Fujimura T, Hara T, Sawada N, Yamada A, Mizushima N, Uchiyama Y, Kominami E, Tanaka K, Komatsu M. (2008). The Atg8 conjugation system is indispensable for proper development of autophagic isolation membranes in mice. Mol. Biol. Cell, 19(11):4762-75. (*These authors contributed equally to this work.)
3. Iwata J, Hosokawa R, Sanchez-Lara PA, Urata M, Slavkin H, and Chai Y. (2010) Transforming growth factor-beta regulates basal transcriptional regulatory machinery to control cell proliferation and differentiation in cranial neural crest–derived osteoprogenitor cells. J. Biol. Chem., 285(7):4975-82.
4. Iwata J, Tung L, Urata M, Hacia JG, Suzuki A, Ramenzoni L, Chaudhry O, Parada C, Sanchez-Lara PA, and Chai Y. (2012) FGF9–PITX2 pathway mediates TGF β signaling to regulate cell proliferation in palatal mesenchyme during mouse palatogenesis. J. Biol. Chem., 287(4):2353-63.
5. Iwata J, Hacia JG, Suzuki A, Sanchez-Lara PA, Urata M, and Chai Y. (2012) Modulation of non-canonical TGF- β signaling prevents cleft palate in Tgfbr2 mutant mice. J. Clin. Invest., 122(3):873-85.
6. Pelikan RC*, Iwata J*, Suzuki A, Chai Y, and Hacia JG. (2013) Identification of candidate downstream

targets of TGF β signaling during palate development by genome-wide transcript profiling. J. Cell Biochem., 114:796-807. (*These authors contributed equally to this work.)
7. Iwata J, Suzuki A, Pelikan RC, Ho TV, Sanchez-Lara PA, Urata M, Dixon MJ, and Chai Y. (2013) Smad4–Irf6 genetic interaction and TGF β –mediated IRF6 signaling cascade are crucial for palatal fusion in mice. Development, 140(6):1220-30.
8. Song ZC*, Liu C*, Iwata J*, Gu SP, Suzuki A, Sun C, He W, Shu R, Li L, Chai Y, and Chen YP. (2013) Mice with Tak1-deficiency in neural crest lineage exhibit cleft palate associated with abnormal tongue development. J. Biol. Chem., 288(15):10440-50. (*These authors contributed equally to this work.)
9. Iwata J, Suzuki A, Pelikan RC, Ho TV, and Chai Y. (2013) Cranial neural crest cells regulate tongue muscle formation via TGF β –mediated BMP and FGF signaling. J. Biol. Chem. 288:29760-70.
10. Iwata J, Suzuki A, Pelikan RC, Ho TV, Sanchez-Lara PA, and Chai Y. (2014) Modulation of lipid metabolic defects rescues cleft palate in Tgfbr2 mutant mice. Hum. Mol. Genetics, 23(1):182-93.
11. Iwata J, Suzuki A, Yokota T, Ho TV, Pelikan RC, Urata M, Sanchez-Lara P, and Chai Y. (2014) TGF β regulates epithelial–mesenchymal interactions through WNT signaling activity to control muscle development in the soft palate. Development, 14 (4):909-17.
12. Ho TV*, Iwata J*, Ho HA, Grimes WC, Park S, Sanchez-Lara PA, and Chai Y. (2015) Integration of comprehensive 3D microCT and signaling analysis reveals differential regulatory mechanisms of craniofacial bone development. Dev. Biol. 400(2):180-90. (*These authors contributed equally to this work.)
13. Suzuki A, Pelikan RC, and Iwata J. (2015) WNT/ β -catenin signaling regulates multiple steps of myogenesis by regulating step-specific targets. Mol. Cell. Biol. 35(10):1763-76.

Special Lecture 2

Sinking our teeth into development, evolution and regeneration

Ophir David Klein, MD, PhD

The Larry L. Hillblom Distinguished Professor in Craniofacial Anomalies, Professor of Orofacial Sciences and Pediatrics, and Chair of the Divisions of Craniofacial Anomalies and Orthodontics at the University of California, San Francisco (UCSF). Medical Director of the UCSF Craniofacial Center and Director, UCSF Program in Craniofacial Biology.

An important challenge facing medicine today is the development of strategies for organ regeneration and repair. The tooth provides a model that allows us to integrate investigations into organogenesis, stem cells and evolution with studies into therapeutic approaches. I will present data from our recent work focusing on development and renewal of the rodent incisor, which unlike human teeth grows throughout the life of the animal. The growth of this organ, like that of the skin, gastrointestinal tract, hematopoietic system, and others, is dependent on the continuous generation of progeny from stem cells that have the capacity to self-renew as well as to give rise to the required differentiated cell types. In addition, I will discuss new mechanisms of tooth formation as well as how evolutionarily informative transitions can be experimentally reproduced to provide development-based expectations for evolutionary studies. I will conclude with some connections between the evolutionary studies and therapies for human disease.

CURRICULUM VITAE

Name: Ophir David Klein, MD, PhD

Position: Professor
Orofacial Sciences & Pediatrics Schools of Dentistry & Medicine
Larry L. Hillblom Distinguished Professor in Craniofacial Anomalies Chair, Division of Craniofacial Anomalies
Chair, Division of Orthodontics
Director, Program in Craniofacial Biology

EDUCATION

1989 - 1993	University of California, Berkeley	B.A.	Cum Laude, Spanish	(Research Advisor: Daniel Koshland, Ph.D.)
1993 - 2000	Yale University School of Medicine	M.D.		
1995 - 1999	Yale University School of Medicine	Ph.D.	Genetics	(Thesis Advisor: Daniel DiMaio, M.D., Ph.D.)
2000 - 2003	Yale University School of Medicine	Resident	Pediatrics	
2003 - 2007	University of California, San Francisco	Resident	Medical Genetics	(Research Advisor: Gail Martin, Ph.D.)

HONORS AND AWARDS

1989	Chancellor's Scholarship	University of California, Berkeley
1993	Medical Scientist Training Program	National Institutes of Health
1999	Dean's Distinguished Thesis Commendation	Yale University Graduate School
2004	Fellow	American Academy of Pediatrics
2004	Fellow	Pediatric Scientist Development Program
2005	David W. Smith Research Award	Western Society for Pediatric Research
2005	Fellow Research Award	Society for Pediatric Research
2005	Young Investigator Research Grant	American Academy of Pediatrics Section on Genetics and Birth Defects
2008	Young Investigator	United States Bone and Joint Decade
2008	Charles E. Culpeper Scholar	Goldman Philanthropic Partnerships
2008	New Faculty Award	California Institute of Regenerative Medicine

2008	Elected Member	Society for Pediatric Research
2009	Basil O'Connor Award	March of Dimes
2009	Elected Member	Western Society for Pediatric Research
2009	Harold M. Frost Young Investigator Award	American Society for Bone and Mineral Research
2010	New Innovator Award	National Institutes of Health
2013	Physician Scientist Award	California Institute of Regenerative Medicine
2013	Elected Member	American Society for Clinical Investigation
2013	Outstanding Faculty Mentorship Award	UCSF Graduate Students' Association
2014	Larry L. Hillblom Distinguished Professor in Craniofacial Anomalies	
2014	Fellow	American Association for the Advancement of Science (AAAS)
2015	E. Mead Johnson Award	Society for Pediatric Research

KEYWORDS/AREAS OF INTEREST

Developmental biology, stem cell biology, clinical genetics, receptor tyrosine kinase signaling, craniofacial, tooth, intestinal stem cells

SERVICE TO PROFESSIONAL PUBLICATIONS

2007 - present Ad hoc referree for American Journal of Medical Genetics, Archives of Oral Biology, Birth Defects Research Part A, BMC Developmental Biology, BMC Musculoskeletal Disorders, Cancer Research, Cell Proliferation, Cell Reports, Cell Stem Cell, Cells Tissues Organs, Developmental Biology, Developmental Cell, Developmental Dynamics, Development, e Cells and Materials, European Journal of Oral Sciences, Frontiers in Physiology, Human Molecular Genetics, International Journal of Developmental Biology, Journal of Cellular and Molecular Medicine, Journal of Dental Research, Journal of Experimental Medicine, Journal of Experimental Zoology B, Mammalian Genome, Mechanisms of Development, Odontology, Oral Diseases, Nature Biotechnology, Nature, Nature Cell Biology, Nature Communications, Nucleic Acids Research, Odontology, Oral Diseases, PLoSONE, PNAS, Stem Cells Development, Stem Cell Research.

2010 - present Editorial Board, Frontiers in Craniofacial Biology 2011 - present Section Editor, Dentistry 3000

2012 - present Editorial Board, Molecular Genetics and Genomic Medicine 2013 - 2013 Guest Editor, PNAS

2013 - present Editorial Board, International Journal of Oral Science

PEER REVIEWED PUBLICATIONS

1. Lee ME, Dyer DH, Klein OD, Bolduc JM, Stoddard BL, Koshland DE. Mutational analysis of the catalytic residues lysine 230 and tyrosine 160 in the NADP(+)-dependent isocitrate dehydrogenase from Escherichia coli. Biochemistry. 1995 Jan 10; 34(1):378-84. PMID: 7819221

2. Klein O, Polack GW, Surti T, Kegler-Ebo D, Smith SO, DiMaio D. Role of glutamine 17 of the bovine papillomavirus E5 protein in platelet-derived growth factor beta receptor activation and cell transformation. J Virol. 1998 Nov; 72(11):8921-32. PMID: 9765437. PMCID: PMC110309

3. Surti T, Klein O, Aschheim K, DiMaio D, Smith SO. Structural models of the bovine papillomavirus E5 protein. Proteins. 1998 Dec 1; 33(4):601-12. PMID: 9849943

4. Klein O, Kegler-Ebo D, Su J, Smith S, DiMaio D. The bovine papillomavirus E5 protein requires a juxtamembrane negative charge for activation of the platelet-derived growth factor beta receptor and transformation of C127 cells. J Virol. 1999 Apr; 73(4):3264-72. PMID: 10074180. PMCID: PMC104090

5. Raga F, Bonilla-Musoles F, Casañ EM, Klein O, Bonilla F. Assessment of endometrial volume by three-dimensional ultrasound prior to embryo transfer: clues to endometrial receptivity. Hum Reprod. 1999 Nov; 14(11):2851-4. PMID: 10548635

6. Klein OD, Cotter PD, Albertson DG, Pinkel D, Tidyman WE, Moore MW, Rauen KA. Prader-Willi syndrome resulting from an unbalanced translocation: characterization by array comparative genomic hybridization. Clin Genet. 2004 Jun; 65(6):477-82. PMID: 15151506

7. Klein OD, Backstrand K, Cotter PD, Marco E, Sherr E, Slavotinek A. Case report: Y;6 translocation with deletion of 6p. Clin Dysmorphol. 2005 Apr; 14(2):93-6. PMID: 15770132

8. Ahn CP, Lachman RS, Cox VA, Blumberg B, Klein OD. Brachydactylic multiple delta phalanges plus syndrome. Am J Med Genet A. 2005 Sep 15; 138(1):41-4. PMID: 16092122

9. Klein OD, Cotter PD, Schmidt AM, Bick DP, Tidyman WE, Albertson DG, Pinkel D, Rauen KA. Interstitial deletion of chromosome 12q: genotype-phenotype correlation of two patients utilizing array comparative genomic hybridization. Am J Med Genet A. 2005 Nov 1; 138(4):349- 54. PMID: 16200635

10. Klein OD, Minowada G, Peterkova R, Kangas A, Yu BD, Lesot H, Peterka M, Jernvall J, Martin GR. Sprouty genes control diastema tooth development via bidirectional antagonism of epithelial-mesenchymal FGF signaling. Dev Cell. 2006 Aug; 11(2):181-90. PMID: 16890158. PMCID: PMC2847684

11. Klein OD, Cotter PD, Moore MW, Zanko A, Gilats M, Epstein CJ, Conte F, Rauen KA. Interstitial

deletions of chromosome 6q: genotype-phenotype correlation utilizing array CGH. Clin Genet. 2007 Mar; 71(3):260-6. PMID: 17309649

12.

13. Klein OD, Lyons DB, Balooch G, Marshall GW, Basson MA, Peterka M, Boran T, Peterkova R, Martin GR. An FGF signaling loop sustains the generation of differentiated progeny from stem cells in mouse incisors. Development. 2008 Jan; 135(2):377-85. PMID: 18077585. PMCID: PMC2555979

14. Metzger RJ, Klein OD, Martin GR, Krasnow MA. The branching programme of mouse lung development. Nature. 2008 Jun 5; 453(7196):745-50. PMID: 18463632. PMCID: PMC2892995

15. Klein OD*, Kostiner DR, Weisiger K, Moffatt E, Lindeman N, Goodman S, Tuchman M, Packman S. Acute fatal presentation of ornithine transcarbamylase deficiency in a previously healthy male. Hepatol Int. 2008 Sep; 2(3):390-4. PMID: 19669271. PMCID: PMC2716892

16. Kalfa N, Liu B, Klein O, Audran F, Wang MH, Mei C, Sultan C, Baskin LS. Mutations of CXorf6 are associated with a range of severities of hypospadias. Eur J Endocrinol. 2008 Oct; 159(4):453-8. PMID: 18635673

17. Kalfa N, Liu B, Klein O, Wang MH, Cao M, Baskin LS. Genomic variants of ATF3 in patients with hypospadias. J Urol. 2008 Nov; 180(5):2183-8; discussion 2188. PMID: 18804813

18. Catón J, Luder HU, Zoupa M, Bradman M, Bluteau G, Tucker AS, Klein O, Mitsiadis TA. Enamel-free teeth: Tbx1 deletion affects amelogenesis in rodent incisors. Dev Biol. 2009 Apr 15; 328(2):493-505. PMID: 19233155. PMCID: PMC2880856

19. Peterkova R*, Churava S, Lesot H, Rothova M, Prochazka J, Peterka M, Klein OD*. Revitalization of a diastemal tooth primordium in Spry2 null mice results from increased proliferation and decreased apoptosis. J Exp Zool B Mol Dev Evol. 2009 Jun 15; 312B(4):292- 308. PMID: 19127536. PMCID: PMC2880865

20. Boran T, Peterkova R, Lesot H, Lyons DB, Peterka M, Klein OD. Temporal analysis of ectopic enamel production in incisors from sprouty mutant mice. J Exp Zool B Mol Dev Evol. 2009 Jul 15; 312B(5):473-85. PMID: 19101957. PMCID: PMC2837846

21. Charles C, Lazzari V, Tafforeau P, Schimmang T, Tekin M, Klein O*, Viriot L.* Modulation of Fgf3 dosage in mouse and men mirrors evolution of mammalian dentition. Proc Natl Acad Sci U S A. 2009 Dec 29; 106(52):22364-8. PMID: 20018768. PMCID: PMC2799759

22. Kettunen P, Kivimäe S, Keshari P, Klein OD, Cheyette BN, Luukko K. Dact1-3 mRNAs exhibit distinct expression domains during tooth development. Gene Expr Patterns. 2010 Feb- Mar; 10(2-3):140-3. PMID: 20170752. PMCID: PMC2849867

23. B § Prochazka J §, Pantalacci S, Churava S, Rothova M, Lambert A, Lesot H, Klein O, Peterka M, Laudet V, Peterkova R. Patterning by heritage in mouse molar row development. Proc Natl Acad Sci

U S A. 2010 Aug 31; 107(35):15497-502. PMID: 20709958. PMCID: PMC2932592

24. Ahn Y, Sanderson BW, Klein OD, Krumlauf R. Inhibition of Wnt signaling by Wise (Sostdc1) and negative feedback from Shh controls tooth number and patterning. Development. 2010 Oct; 137(19):3221-31. PMID: 20724449

25. Jelin A, Perry H, Hogue J, Oberoi S, Cotter PD, Klein OD. Clefting in trisomy 9p patients: genotype-phenotype correlation using microarray comparative genomic hybridization. J Craniofac Surg. 2010 Sep; 21(5):1376-9. PMID: 20856024

26. Parsa S, Kuremoto K, Seidel K, Tabatabai R, Mackenzie B, Yamaza T, Akiyama K, Branch J, Koh CJ, Al Alam D, Klein OD*, Bellusci S*. Signaling by FGFR2b controls the regenerative capacity of adult mouse incisors. Development. 2010 Nov; 137(22):3743-52. PMID: 20978072. PMCID: PMC3049274

27. Seidel K, Ahn CP, Lyons D, Nee A, Ting K, Brownell I, Cao T, Carano RA, Curran T, Schober M, Fuchs E, Joyner A, Martin GR, de Sauvage FJ*, Klein OD*. Hedgehog signaling regulates the generation of ameloblast progenitors in the continuously growing mouse incisor. Development. 2010 Nov; 137(22):3753-61. PMID: 20978073. PMCID: PMC3049275

28. Milunsky JM, Maher TM, Zhao G, Wang Z, Mulliken JB, Chitayat D, Clemens M, Stalker HJ, Bauer M, Burch M, Chénier S, Cunningham ML, Drack AV, Janssens S, Karlea A, Klatt R, Kini U, Klein O, Lachmeijer AM, Megarbane A, Mendelsohn NJ, Meschino WS, Mortier GR, Parkash S, Ray CR, Roberts A, Roberts A, Reardon W, Schnur RE, Smith R, Splitt M, Tezcan K, Whiteford ML, Wong DA, Zori R, Lin AE. Genotype-phenotype analysis of the branchio-oculo-facial syndrome. Am J Med Genet A. 2011 Jan; 155A(1):22-32. PMID: 21204207

29. Jheon AH, Mostowfi P, Snead ML, Ihrie RA, Sone E, Pramparo T, Attardi LD, Klein OD. PERP regulates enamel formation via effects on cell-cell adhesion and gene expression. J Cell Sci. 2011 Mar 1; 124(Pt 5):745-54. PMID: 21285247. PMCID: PMC3039019

30. Hovorakova M, Prochazka J, Lesot H, Smrckova L, Churava S, Boran T, Kozmik Z, Klein O, Peterkova R, Peterka M. Shh expression in a rudimentary tooth offers new insights into development of the mouse incisor. J Exp Zool B Mol Dev Evol. 2011 Jul 15; 316(5):347-58. PMID: 21455944

31. Hochheiser H, Aronow BJ, Artinger K, Beaty TH, Brinkley JF, Chai Y, Clouthier D, Cunningham ML, Dixon M, Donahue LR, Fraser SE, Hallgrimsson B, Iwata J, Klein O, Marazita ML, Murray JC, Murray S, de Villena FP, Postlethwait J, Potter S, Shapiro L, Spritz R, Visel A, Weinberg SM, Trainor PA. The FaceBase Consortium: a comprehensive program to facilitate craniofacial research. Dev Biol. 2011 Jul 15; 355(2):175-82. PMID: 21458441. PMCID: PMC3440302

32. Petersen CI, Jheon AH, Mostowfi P, Charles C, Ching S, Thirumangalathu S, Barlow LA, Klein OD. FGF signaling regulates the number of posterior taste papillae by controlling progenitor field size. PLoS Genet. 2011 Jun; 7(6):e1002098. PMID: 21655085. PMCID: PMC3107195

33. Charles C, Hovorakova M, Ahn Y, Lyons DB, Marangoni P, Churava S, Biehs B, Jheon A, Lesot H, Balooch G, Krumlauf R, Viriot L, Peterkova R, Klein OD. Regulation of tooth number by fine-tuning levels of receptor-tyrosine kinase signaling. Development. 2011 Sep; 138(18):4063- 73. PMID: 21862563. PMCID: PMC3160100

34. Jheon AH, Li CY, Wen T, Michon F, Klein OD. Expression of microRNAs in the stem cell niche of the adult mouse incisor. PLoS One. 2011; 6(9):e24536. PMID: 21931743. PMCID: PMC3169592

35. Tian H, Biehs B, Warming S, Leong KG, Rangell L, Klein OD*, de Sauvage FJ*. A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature. 2011 Oct 13; 478(7368):255-9. PMID: 21927002. PMCID: PMC4251967

36. Iraqi FA, … Broman KW, Hallgrimsson B, Klein O, Weinstock G, Warren WC, Yang YV, Schwartz D. The genome architecture of the Collaborative Cross mouse genetic reference population. Genetics. 2012 Feb; 190(2):389-401.

37. Purcell P*, Jheon A, Vivero MP, Rahimi H, Joo A, Klein OD*. Spry1 and spry2 are essential for development of the temporomandibular joint. J Dent Res. 2012 Apr; 91(4):387-93. PMID: 22328578. PMCID: PMC3310757

38. Kyrylkova K, Kyryachenko S, Biehs B, Klein O, Kioussi C, Leid M. BCL11B regulates epithelial proliferation and asymmetric development of the mouse mandibular incisor. PLoS One. 2012; 7(5):e37670. PMID: 22629441. PMCID: PMC3358280

39. Li CY, Cha W, Luder HU, Charles RP, McMahon M, Mitsiadis TA, Klein OD. E-cadherin regulates the behavior and fate of epithelial stem cells and their progeny in the mouse incisor. Dev Biol. 2012 Jun 15; 366(2):357-66. PMID: 22537490

40. Juuri E, Saito K, Ahtiainen L, Seidel K, Tummers M, Hochedlinger K, Klein OD, Thesleff I, Michon F. Sox2+ stem cells contribute to all epithelial lineages of the tooth via Sfrp5+ progenitors. Dev Cell. 2012 Aug 14; 23(2):317-28. PMID: 22819339. PMCID: PMC3690347

41. Justice CM, Yagnik G, Kim Y, Peter I, Jabs EW, Erazo M, Ye X, Ainehsazan E, Shi L, Cunningham ML, Kimonis V, Roscioli T, Wall SA, Wilkie AO, Stoler J, Richtsmeier JT, Heuzé Y, Sanchez-Lara PA, Buckley MF, Druschel CM, Mills JL, Caggana M, Romitti PA, Kay DM, Senders C, Taub PJ, Klein OD, Boggan J, Zwienenberg-Lee M, Naydenov C, Kim J, Wilson AF, Boyadjiev SA. A genome-wide association study identifies susceptibility loci for nonsyndromic sagittal craniosynostosis near BMP2 and within BBS9. Nat Genet. 2012 Nov 18; 44(12):1360-4.

42. Chavez MG, Yu W, Biehs B, Harada H, Snead ML, Lee JS, Desai TA, Klein OD. Characterization of dental epithelial stem cells from the mouse incisor with two-dimensional and three-dimensional platforms. Tissue Eng Part C Methods. 2013 Jan; 19(1):15-24. PMID: 22742471. PMCID: PMC3522131

43. Wan M, Gao B, Sun F, Tang Y, Ye L, Fan Y, Klein OD, Zhou X, Zheng L. microRNA miR- 34a

regulates cytodifferentiation and targets multi-signaling pathways in human dental papilla cells. PLoS One. 2012; 7(11):e50090. PMID: 23226240. PMCID: PMC3511455

44. Goodwin AF, Oberoi S, Landan M, Charles C, Groth J, Martinez A, Fairley C, Weiss LA, Tidyman WE, Klein OD, Rauen KA. Craniofacial and dental development in cardio-facio- cutaneous syndrome: the importance of Ras signaling homeostasis. Clin Genet. 2013 Jun; 83(6):539-44. PMID: 22946697. PMCID: PMC4115672

45. Griffin JN, Compagnucci C, Hu D, Fish J, Klein O, Marcucio R, Depew MJ. Fgf8 dosage determines midfacial integration and polarity within the nasal and optic capsules. Dev Biol. 2013 Feb 1; 374(1):185-97. PMID: 23201021

46. Felfly H, Klein OD. Sprouty genes regulate proliferation and survival of human embryonic stem cells. Sci Rep. 2013; 3:2277. PMID: 23880645. PMCID: PMC3721083

47. Plaks V, Brenot A, Lawson DA, Linnemann JR, Van Kappel EC, Wong KC, de Sauvage F, Klein OD, Werb Z. Lgr5-expressing cells are sufficient and necessary for postnatal mammary gland organogenesis. Cell Rep. 2013 Jan 31; 3(1):70-8. PMID: 23352663. PMCID: PMC3563842

48. Yamada Y, Nakamura S, Ito K, Umemura E, Hara K, Nagasaka T, Abe A, Baba S, Furuichi Y, Izumi Y, Klein OD, Wakabayashi T. Injectable bone tissue engineering using expanded mesenchymal stem cells. Stem Cells. 2013 Mar; 31(3):572-80. PMID: 23225744. PMCID: PMC4043220

49. Juuri E, Jussila M, Seidel K, Holmes S, Wu P, Richman J, Heikinheimo K, Chuong CM, Arnold K, Hochedlinger K, Klein O, Michon F, Thesleff I. Sox2 marks epithelial competence to generate teeth in mammals and reptiles. Development. 2013 Apr; 140(7):1424-32. PMID: 23462476. PMCID: PMC3596986

50. Lagronova-Churava S, Spoutil F, Vojtechova S, Lesot H, Peterka M, Klein OD, Peterkova R. The dynamics of supernumerary tooth development are differentially regulated by Sprouty genes. J Exp Zool B Mol Dev Evol. 2013 Jul; 320(5):307-20. PMID: 23606267

51. Jones KB, Goodwin AF, Landan M, Seidel K, Tran DK, Hogue J, Chavez M, Fete M, Yu W, Hussein T, Johnson R, Huttner K, Jheon AH, Klein OD. Characterization of X-linked hypohidrotic ectodermal dysplasia (XL-HED) hair and sweat gland phenotypes using phototrichogram analysis and live confocal imaging. Am J Med Genet A. 2013 Jul; 161A(7):1585-93. PMID: 23687000. PMCID: PMC4414120

52. Biehs B, Hu JK, Strauli NB, Sangiorgi E, Jung H, Heber RP, Ho S, Goodwin AF, Dasen JS, Capecchi MR, Klein OD. BMI1 represses Ink4a/Arf and Hox genes to regulate stem cells in the rodent incisor. Nat Cell Biol. 2013 Jul; 15(7):846-52. PMID: 23728424. PMCID: PMC3735916

53. Cao H, Jheon A, Li X, Sun Z, Wang J, Florez S, Zhang Z, McManus MT, Klein OD, Amendt BA. The Pitx2:miR-200c/141:noggin pathway regulates Bmp signaling and ameloblast differentiation. Development. 2013 Aug; 140(16):3348-59. PMID: 23863486. PMCID: PMC3737717

54. Oishi PE, Klein OD, Keller RL. Developing physician-scientists in the fields of neonatology and pediatric critical care medicine: an effort to formulate a departmental policy. J Pediatr. 2013 Sep; 163(3):616-7.e1. PMID: 23973233

55. Zheng L, Warotayanont R, Stahl J, Kunimatsu R, Klein O, Denbesten PK, Zhang Y. Inductive ability of human developing and differentiated dental mesenchyme. Cells Tissues Organs. 2013; 198(2):99-110. PMID: 24192998

56. Goodwin AF, Tidyman WE, Jheon AH, Sharir A, Zheng X, Charles C, Fagin JA, McMahon M, Diekwisch TG, Ganss B, Rauen KA, Klein OD. Abnormal Ras signaling in Costello syndrome (CS) negatively regulates enamel formation. Hum Mol Genet. 2014 Feb 1; 23(3):682-92. PMID: 24057668

57. Sun F, Wan M, Xu X, Gao B, Zhou Y, Sun J, Cheng L, Klein OD, Zhou X, Zheng L. Crosstalk between miR-34a and Notch Signaling Promotes Differentiation in Apical Papilla Stem Cells (SCAPs). J Dent Res. 2014 Apr 7; 93(6):589-595. PMID: 24710391

58. Mues G, Bonds J, Xiang L, Vieira AR, Seymen F, Klein O, D'Souza RN. The WNT10A gene in ectodermal dysplasias and selective tooth agenesis. Am J Med Genet A. 2014 Oct; 164(10):2455-60. PMID: 24700731. PMCID: PMC4167166

59. Chavez MG, Hu J, Seidel K, Li C, Jheon A, Naveau A, Horst O, Klein OD. Isolation and culture of dental epithelial stem cells from the adult mouse incisor. J Vis Exp. 2014; (87). PMID: 24834972

60. Ching ST, Cunha GR, Baskin LS, Basson MA, Klein OD. Coordinated activity of Spry1 and Spry2 is required for normal development of the external genitalia. Dev Biol. 2014 Feb 1; 386(1):1-11. PMID: 24361260

61. Zhao H, Feng J, Seidel K, Shi S, Klein O, Sharpe P, Chai Y. Secretion of shh by a neurovascular bundle niche supports mesenchymal stem cell homeostasis in the adult mouse incisor. Cell Stem Cell. 2014 Feb 6; 14(2):160-73. PMID: 24506883

62. Mendelsohn BA, Jeng LL, Oberoi S, Klein OD. Dental findings in 14q terminal deletion syndrome. Clin Dysmorphol. 2014 Apr; 23(2):60-2. PMID: 24535103

63. Goodwin AF, Oberoi S, Landan M, Charles C, Massie JC, Fairley C, Rauen KA, Klein OD. Craniofacial and dental development in Costello syndrome. Am J Med Genet A. 2014 Jun; 164(6):1425-30. PMID: 24668879

64. Michailovici I, Harrington HA, Azogui HH, Yahalom-Ronen Y, Plotnikov A, Ching S, Stumpf MP, Klein OD, Seger R, Tzahor E. Nuclear to cytoplasmic shuttling of ERK promotes differentiation of muscle stem/progenitor cells. Development. 2014 Jul; 141(13):2611-20. PMID: 24924195. PMCID: PMC4067960

65. Castillo D, Seidel K, Salcedo E, Ahn C, de Sauvage FJ, Klein OD*, Barlow LA*. Induction of ectopic taste buds by SHH reveals the competency and plasticity of adult lingual epithelium. Development. 2014 Aug; 141(15):2993-3002. PMID: 24993944. PMCID: PMC4197660

66. Harjunmaa E, Seidel K, Häkkinen T, Renvoisé E, Corfe IJ, Kallonen A, Zhang ZQ, Evans AR, Mikkola ML, Salazar-Ciudad I, Klein OD*, Jernvall J*. Replaying evolutionary transitions from the dental fossil record. Nature. 2014 Aug 7; 512(7512):44-8. PMID: 25079326. PMCID: PMC4252015

67. Goodwin AF, Larson JR, Jones KB, Liberton DK, Landan M, Wang Z, Boekelheide A, Langham M, Mushegyan V, Oberoi S, Brao R, Wen T, Johnson R, Huttner K, Grange DK, Spritz RA, Hallgrímsson B*, Jheon AH, Klein OD*. Craniofacial morphometric analysis of individuals with X-linked hypohidrotic ectodermal dysplasia. Mol Genet Genomic Med. 2014 Sep; 2(5):422- 9. PMID: 25333067. PMCID: PMC4190877

68. Jheon AH, Prochazkova M, Sherman M, Manoli DS, Shah NM, Carbone L, Klein O. Spontaneous emergence of overgrown molar teeth in a colony of Prairie voles (Microtus ochrogaster). Int J Oral Sci. 2015 Mar; 7(1):23-6. PMID: 25634121.

69. Dye BR, Hill DR, Ferguson MA, Tsai YH, Nagy MS, Dyal R, Wells JM, Mayhew CN, Nattiv R, Klein OD, White ES, Deutsch GH, Spence JR. In vitro generation of human pluripotent stem cell derived lung organoids. Elife. 2015 March 24; 4. PMID: 25803487. PMCID: PMC4370217

70. Tian H, Biehs B, Chiu C, Siebel CW, Wu Y, Costa M, de Sauvage FJ*, Klein OD*. Opposing activities of notch and wnt signaling regulate intestinal stem cells and gut homeostasis. Cell Rep. 2015 Apr 7; 11(1):33-42. PMID: 25818302. PMCID: PMC4394041

71. Thys A, Vandenberghe P, Hague P, Klein OD, Erneux C, Vanderwinden JM. Hyperplasia of interstitial cells of cajal in sprouty homolog 4 deficient mice. PLoS One. 2015 Apr 29; 10(4):e0124861. PMID: 25923139. PMCID: PMC4414615

72. Zarate YA, Perry H, Ben-Omran T, Sellars EA, Stein Q, Almureikhi M, Simmons K, Klein O, Fish J, Feingold M, Douglas J, Kruer MC, Si Y, Mao R, McKnight D, Gibellini F, Retterer K, Slavotinek A. Further supporting evidence for the SATB2-associated syndrome found through whole exome sequencing. Am J Med Genet A. 2015 May; 167(5):1026-32. PMID: 25885067.

73. Tapaltsyan V, Eronen JT, Lawing AM, Sharir A, Janis C, Jernvall J*, Klein OD*. Continuously Growing Rodent Molars Result from a Predictable Quantitative Evolutionary Change over 50 Million Years. Cell Rep. 2015 May 5; 11(5):673-80. PMID: 25921530. PMCID: PMC4426059

74. Wu D, Mandal S, Choi A, Anderson A, Prochazkova M, Perry H, Gil-Da-Silva-Lopes VL, Lao R, Wan E, Tang PL, Kwok PY, Klein O, Zhuan B, Slavotinek AM. DLX4 is associated with orofacial clefting, and abnormal jaw development. Hum Mol Genet. 2015 May 7. pii: ddv167. [Epub ahead of print] PMID: 25954033

75. Finkbeiner SR, Hill DR, Altheim CH, Dedhia PH, Taylor MJ, Tsai YH, Chin AM, Mahe MM, Watson CL, Freeman JJ, Nattiv R, Thomson M, Klein OD, Shroyer NF, Helmrath MA, Teitelbaum DH, Dempsey PJ, Spence JR. Transcriptome-wide Analysis Reveals Hallmarks of Human Intestine Development and Maturation In Vitro and In Vivo Stem Cell Reports. 2015 Jun 3; pii: S2213-6711(15)00122-8. doi: 10.1016/j.stemcr.2015.04.010. [Epub ahead of print] PMID: 26050928. PMCID: PMC4471827

76. Tapaltsyan V, Charles C, Hu J, Mindell D, Ahituv N, Wilson GM, Black BL, Viriot L, Klein OD. Identification of novel Fgf enhancers and their role in dental evolution. Evol Dev. 2015 Jun 18. doi: 10.1111/ede.12132. [Epub ahead of print]

76. Marangoni P, Charles C, Tafforeau P, Laugel-Haushalter V, Joo A, Bloch-Zupan A, Klein OD, Viriot L. Phenotypic and evolutionary implications of modulating the ERK-MAPK cascade using the dentition as a model. Sci Rep. 2015 June 30; 5:11658. PMID: 26123406. PMCID: PMC4485067

77. Jheon AH*, Prochazkova M, Meng B, Wen T, Lim YJ, Naveau A, Espinoza R, Cox TC, Sone ED, Ganss B, Siebel CW, Klein OD*. Inhibition of Notch Signaling During Mouse Incisor Renewal Leads to Enamel Defects. J Bone Miner Res. 2015 Jul 14. [Epub ahead of print] PMID: 26179131

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Special Lecture 3

Comprehensive analysis for the functions of genes during epithelial-mesenchymal interactions in tissue morphogenesis

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Epithelial-mesenchymal (E-M) interactions play pivotal roles in the morphogenesis of various organs, such as tooth, lung, kidney, submandibular grand (SMG) and hair. In previous study, we found that the transcriptional coactivator, Mediator 1 (Med1), conditional deficient mice showed an ectopic hair generation in incisor (Yoshizaki et al., PLoS One 2014). This result indicated that the cell fate of tooth and hair could be switched by alteration of specific transcriptional programs. In E-M interactions, thickening of epithelial layer forming lamina, as the first event, is followed by bud formation. We hypothesized that the critical genes for E-M interactions are expressed in every organs during these early developmental stages. To identify the specific genes for E-M interactions, we performed Cap Analysis of Gene Expression (CAGE) for comprehensive analysis, using the early developmental stages of tooth, lung, kidney, SMG and hair, and performed bioinformatics analysis. CAGE is genome-wide transcription starting detection technology, which is capable of determining the transcriptional start sites (TSS) by sequencing 5' end of single-strand cDNA. We finally identified 42 tooth specific TSS and 19 TSS commonly peaked during various developing organs as the candidate genes critical for E-M interactions. In this presentation, I would like to discus about the candidate genes regulating E-M interaction and the possibility of tooth regeneration.

CURRICULUM VITAE

EDUCATION

1998-2004 DDS, Kyushu University School of Dentistry, Fukuoka, Japan

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【MEMO】

Reports after circulation

Lesional $CD4^+$ IFN- γ^+ + cytotoxic T lymphocytes in IgG4-related dacryoadenitis and sialoadenitis

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Background: IgG4-related disease (IgG4-RD) is a chronic, systemic, inflammatory condition of unknown etiology characterized by storiform fibrosis and tumefactive lesions. We have recently described clonally expanded circulating $CD4^+$ cytotoxic T lymphocytes (CTLs) in IgG4-RD that secrete IL-1b and TGFb1 in affected tissues. However, the contributions of $CD4^+$CTLs to IgG4-RD pathogenesis remain to be fully defined.

Objective: To examine the pathogeneis of IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS) and to determine whether $CD4^+$ CTLs in IgG4-RD lesions secrete IFN- γ.

Methods: Gene expression was analyzed by DNA microarray in submandibular glands (SMGs) from five patients with IgG4-DS, three with chronic sialoadenitis (CS) and three healthy controls. Infiltrating $CD4^+$ CTLs were examined by quantitative multi-color imaging in tissue samples from eleven patients with IgG4-DS, ten with Sjögren's syndrome (SS), five with CS, and five healthy controls.

Results: In IgG4-DS tissues, nine genes associated with $CD4^+$ CTLs were overexpressed. The expression of granzyme A mRNA was significantly higher in samples from patients with IgG4-RD compared to corresponding tissues from SS and healthy controls. Quantitative imaging showed that infiltrating $CD4^+$GranzymeA^+ CTLs were more abundant in IgG4-DS patients than in the other groups. The ratio of $CD4^+$GranzymeA^+ CTLs in submandibular salivary glands (SMGs) from IgG4-DS patients correlated with serum IgG4 concentrations and the number of affected organs. The percentage of $CD4^+$GranzymeA^+IFN- γ^+ CTLs in SMGs was significantly higher in IgG4-DS than in SS and CS patients.

Conclusions: The pathogenesis of IgG4-DS is associated with tissue infiltration by $CD4^+$GranzymeA^+ CTLs that secrete IFN- γ.

Young researchers for next circulation

Molecular basis of amelogenin-induced periodontal tissue regeneration

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Amelogenin, the major component of enamel matrix derivative (Straumann® Emdogain), is well recognized in periodontology. It is used in periodontal surgery to regenerate cementum, periodontal ligament, and alveolar bone. However, the precise molecular mechanisms underlying periodontal regeneration are still unclear. Although the involvement of other enamel matrix proteins in the regeneration process cannot be excluded, recent studies have demonstrated that recombinant amelogenin induced the in vivo regeneration of all tooth-supporting tissues. In order to gain further insight into how amelogenin induces periodontal tissue regeneration, we conducted a protein interaction screen using recombinant full-length amelogenin (rM180) as bait. Using this proteomic screen we identified glucose-regulated protein 78 (Grp78) as an amelogenin-binding protein, present in both the cytosolic and membrane-enriched fractions of osteoblastic cells.

Mesenchymal stem cell-derived periodontal ligament stem cells (PDLSCs) play pivotal roles in successful periodontal regeneration. Thus, we evaluated the biological interaction between amelogenin and Grp78, and its effect on cellular responses in human PDLSCs by using the cell line 1-17. Confocal co-localization experiments revealed the internalization of recombinant amelogenin (rM180) via binding to cell surface Grp78. Microarray analysis indicated that rM180 and Grp78 regulate the expression profiles of cell migration-associated genes in 1-17 cells. Our study revealed that Grp78 is essential for enhancing amelogenin-induced cell migration in 1-17 cells. Furthermore, we demonstrated that Rac1 activation and lamellipodia formation are critical steps in amelogenin-induced cell migration.

In conclusion, our study demonstrates that the biological interaction of Grp78 with amelogenin enhances amelogenin-induced cell migration in PDLSCs. Although the effect of the biological interaction of amelogenin with Grp78 on osteogenic differentiation must be further explored, our findings provide a better understanding of the molecular basis of amelogenin-induced periodontal regeneration.

IL-33 secreted by M2 macrophages promotes the pathogenesis of IgG4-related disease

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Objectives.
IgG4-related disease (IgG4-RD) is characterized by elevated serum IgG4 and marked infiltration of IgG4-positive cells in multiple organs. Regarding immunological aspects, it has been suggested that helper T type 2 (Th2) immune responses play a major role in IgG4 production of IgG4-RD. Interleukin-33 (IL-33) is a recently described cytokine that is secreted by damaged epithelial cells, macrophages (M Φ s) and dendritic cells (DCs) and potently activates Th2 immune responses. Here, we assessed the expression of IL-33 and the producing cells in the submandibular glands (SMGs) from IgG4-RD patients compared with those from Sjögren's syndrome and controls.
Patients and methods.
SGs from patients with IgG4-RD (n=7), Sjögren's syndrome (n=10), and healthy subjects (n=10) were screened for 1) expression of IL-33, ST2, Th1 cytokine (IFN- γ), Th2 cytokines (IL-4 and IL-13), IL-33 producing cells including M Φ detected by CD68 (M1+M2 M Φ) and CD163 (M2 M Φ) and DCs detected by CD11c (myeloid DC) and CD123 (plasmacytoid DC) by immunohistochemical staining; 2) the relationship between mRNA expression of IL-33 and cytokines; 3) the co-localization of IL-33 and IL-33-producing cells by double immunofluorescence staining; 4) the population of IL-33-producing cell by flow cytometry.
Results.
mRNA expression of IL-33, ST2, IL-4, and IL-13 in IgG4-RD was significantly higher than that in the other groups. Moreover, mRNA expression of IL-33 was positively correlated with that of Th2 cytokines only in IgG4-RD and didn't show positive or negative correlation with IFN- γ . IL-33 was detected in/around epithelial cells in all the groups, while was strongly detected in infiltrating lymphocytes around ectopic germinal centers only in IgG4-RD. Both of CD68 and CD163-positive cells almost merged with IL-33. In addition, flow cytometric analysis revealed that IL-33+ cells were mainly contituted by CD68+CD163+ M2 M Φ s in IgG4-RD patients.
Conclusion. These results suggest that IL-33 produced by M2 M Φ s might contribute to the pathogenesis of IgG4-RD via aberrant activation of Th2 immune responses.

Stimulus repetition enhances responses of the chorda tympani nerve to sweeteners via endocannabinoid system

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2-Arachidonoylglycerol (2-AG), one of endocannabinoids (eCBs), enhances responses of sweet-sensitive taste cells via activation of the cannabinoid receptor 1 (CB1). We recently found that diacylglycerol lipase (DAGL), which synthesizes 2-AG through hydrolyses of diacylglycerol by in the GPCR-phospholipase cascade, is expressed in T1R3-positive taste cells. This suggests the possibility that the activation of sweet taste receptor T1R2/T1R3 by sweeteners may lead to generation of 2-AG and subsequent enhancement of sweet taste responses. In order to assess this possibility, here, we investigated potential effects of stimulus repetition with a single taste compound on responses of the mouse chorda tympani (CT) nerve. The results indicated that CT nerve responses to sweeteners but not to other tastants were gradually increased by stimulus repetition. Such effect was not observed in T1R3 knockout mice and was inhibited by CB1 blocker AM251. Intriguingly, intraperitoneal injection with Angiotensin II (AngII), which is a major mediater of body fluid and sodium homeostasis, enhanced such increase of sweet taste response by stimulus repetition. CV11974, AngII type1 receptor antagonist, inhibited the AngII's effects. Taken together, our results suggest that the repeated T1R2/T1R3 activation leads to generation of 2-AG, which enhances sweet taste responses by acting on CB1 expressed in sweet sensitive taste cells. AngII may cooperatively act on this eCB sweet-enhancing effect.

Activin A reversely works between human pre-osteoblastic cells and periodontal ligament cells on their osteoblastic differentiation

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Objectives : Activin A (ActA) that is a member of the TGF- β superfamily and is composed of a dimer of inhibin β a. Our recent report suggested that ActA exerted an inhibitory effect on osteoblastic differentiation of human periodontal ligament cells (HPDLCs) and could be applied as a therapeutic factor that promotes healing of periodontal ligament (PDL) tissue. In this study, we have examined the effect on osteoblastic differentiation of human pre-osteoblastic cells (Saos2) and compared the signal pathway between HPDLCs and Saos2 treated with ActA.

Materials and Methods : We purchased Saos2 from RIKEN (Saitama, Japan) and used HPDLCs that were isolated from a healthy third molar from a 23-year-old male. These cells were cultured with or without ActA in the presence or absence of CaCl2 that was used for inducing osteoblastic differentiation. The cells were then subjected to Alizarin red S staining and von Kossa staining. Gene expressions were evaluated by semi-quantitative or quantitative RT-PCR, and protein expressions were investigated by western blotting and immunocytochemical analyses.

Results : Immunocytochemical analysis with an anti-ActA antibody revealed positive staining in Saos2. Expression of Activin receptors (ALK4, IIa and IIb) was detected in Saos2. ActA promoted mineralization and expression of bone-related genes in Saos2. The effect of ActA on osteoblastic differentiation of Saos2 was inhibited by an anti-TGF- β 1 neutralizing antibody, but the effect of TGF- β 1 was not affected by an anti-ActA neutralizing antibody. Western blotting data showed that ActA up-regulated the phosphorylation of Smad1/5/8 in Saos2, whereas promoted the phosphorylation of Smad2/3 in HPDLCs.

Discussion and Conclusion : Our results showed that ActA and its receptors were expressed in Saos2 and that promoted osteoblastic differentiation of Saos2. It was suggested that ActA promoted osteoblastic differentiation of Saos2 through phosphorylation of Smad1/5/8 pathway, while inhibited osteoblastic differentiation of HPDLCs through phosphorylation of Smad2/3 pathway. Thus, ActA may reversely work between these cells through activation of different Smad pathway.