Interface Oral Health Science 2009

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Interface Oral Health Science 2009

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Preface

Since 2002, the Tohoku University Graduate School of Dentistry has proposed "Interface Oral Health Science" as a major theme for next-generation dental research. That theme is based on the following new concept: healthy oral function is maintained by biological and biomechanical harmony among three systems: (1) oral tissues (host); (2) parasitic microorganisms of the oral cavity (parasites); and (3) biomaterials. The concept implies that oral diseases such as dental caries, periodontal disease, and temporomandibular disorders should be interpreted as "interface disorders" that result from disruption of the intact interface among these systems. The uniqueness of this concept rests on the fact that it not only encompasses the field of dentistry and dental medicine, but also expands the common ground shared with other fields, including medicine, agriculture, material science, engineering, and pharmacology. We aim to promote advances in dental research and to activate collaboration with related fields by putting interface oral health science into practice. On this basis, we have already organized the 1st and 2nd International Symposiums for Interface Oral Health Science, which included inspiring special lectures, symposiums, poster presentations, and other discussions. The contents of the two symposiums were published as monographs entitled Interface Oral Health Science in 2005 and 2007.

The 3rd International Symposium was held in January 2009 as part of this project. With prominent researchers invited from Japan and other countries, the symposium included a keynote lecture by Professor Joji Ando of the Graduate School of Medicine of The University of Tokyo. In addition, there were three symposiums: "Novel Bioengineering," "Mechanobiology," and "Biomaterial Surface." In the poster session, more than 100 poster presentations (the largest number ever) were listed from a wide variety of fields related to interface oral health science, including "Social Interface" as a new section. In addition, the Poster Award for Young Researchers was newly announced, and the winners made a presentation at the Tohoku–Forsyth Symposium (the second part of the Sendai Symposium), held in March in Boston, U.S.A.

This book, containing the presentations at the symposium, is being published in 2010 as a serial entitled Interface Oral Health Science. We hope that our project, including the symposium and the book, will accelerate the progress of dental science and point the way for dental research for future generations.

In closing, I would like to extend our best wishes for the health and success of those who participated in this symposium and who presented such outstanding papers.

Takashi Sasano
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January 2009

Commentary to The 1st Tohoku-Forsyth Symposium

On March 10th and 11th, 2009, the Tohoku–Forsyth Symposium was held at The Forsyth Institute in Boston. The Forsyth Institute was founded in 1910 as a free dental clinic for the children of Boston through a generous gift from the Forsyth family. Between 1914 and the 1950s, more than 500,000 children received care at Forsyth. In the 1950s, realizing that dental diseases could not simply be "treated away," Forsyth's mission evolved to one of research into the causes and pathogenic mechanisms that underlie these conditions, and to the application of this knowledge to the development of better modes of disease prevention and treatment. Today Forsyth is recognized as a world leader in oral and craniofacial research, focusing on the disciplines of microbiology, immunology, bone and mineralized tissue biology, developmental biology, and clinical research.

Tohoku University, located in the city of Sendai, Miyagi Prefecture, is one of the foremost academic research institutions in Japan. Because the Tohoku Faculty of Dentistry, much like Forsyth, fosters an interdisciplinary approach to dental biomedicine, a sister relationship was established between the two institutions in 2005.

The Tohoku Faculty of Dentistry is to be commended for its long history of scientific scholarly activity and for contributing to our mutual efforts to understand basic biological processes, to elucidate host–pathogen interactions at the molecular level, and to develop cutting-edge technology and biomaterials for diagnostic and therapeutic applications. During the course of our two-day symposium, we heard many outstanding presentations of pioneering research activities by both the Tohoku and Forsyth scientists. It is in the spirit of our shared purpose that this book entitled Interface Oral Health Science 2009 has been prepared as a compendium that attests to the Forsyth–Tohoku collaboration. Its publication will be a milestone, leading us into the next century of dental and craniofacial sciences and service to the public.

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Contents

Plenary Lecture

Shear-stress-sensing and response mechanisms in vascular endothelial cells

Joji Ando and Kimiko Yamamoto

Abstract. Vascular endothelial cells (ECs) change their morphology, function, and gene expression in response to shear stress, a fluid mechanical force generated by flowing blood. This fact suggests that ECs recognize shear stress and transmit signals to the interior of the cell. Shear-stress-sensing and response mechanisms, however, have not been fully understood. We have demonstrated that ECs are capable of converting information regarding shear stress intensity into changes in intracellular Ca^{2+} concentration. The Ca^{2+} signaling is based on cell-surface ATP synthase-mediated ATP release and subsequent activation of an ATP-operated cation channel P2X4, which leads to a Ca^{2+} influx. Our studies using *P2X4*-deficient mice revealed that P2X4-mediated Ca^{2+} signaling of shear stress plays a crucial role in the homeostasis of the circulatory system, including the control of blood pressure, blood-flow-dependent vasodilation, and vascular remodeling, through endothelial nitric oxide production.

Key words. shear stress, endothelial cell, P2X purinoceptor, ATP, Ca^{2+}, ATP synthase

1 Introduction

Endothelial cells (ECs) lining blood vessels are constantly exposed to shear stress, a fluid mechanical force generated by flowing blood. A number of recent studies have revealed that ECs recognize changes in shear stress and transmit signals to the interior of the cell, which leads to cell responses that involve changes in cell

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morphology and cell functions, including the production of a potent vasodilator, nitric oxide (NO), and an antithrombotic protein, thrombomodulin [1–3]. It has also become clear that shear stress regulates endothelial gene expression through transcription and/or mRNA stabilization [4–6]. Our DNA microarray analysis showed that approximately 3% of all genes examined in ECs showed some kind of response to shear stress, indicating that more than 600 genes are shear-stress-responsive [7]. These EC responses to shear stress are thought to play important roles in blood-flow-dependent phenomena, such as vascular tone control, angiogenesis, vascular remodeling, and atherogenesis. However, the precise mechanisms of the shear-stress-sensing are not yet completely understood. Here, we demonstrate the existence of ATP receptor-mediated Ca^{2+} signaling that occurs in ECs in response to shear stress and its physiological role in the vascular system.

2 Ca^{2+} Signaling of Shear Stress

Our previous studies demonstrated that Ca^{2+} signaling plays an important role in shear-stress-sensing and signal transduction [8, 9]. Human pulmonary artery ECs (HPAECs) that had been labeled with a fluorescent Ca^{2+} indicator, Indo-1, were exposed to controlled levels of shear stress in a parallel-plate-type flow chamber, and changes in the intracellular Ca^{2+} concentration were monitored. The intracellular Ca^{2+} concentration increased in a shear-stress-dependent manner (Fig. 1a). There is a good, almost linear, correlation between shear stress and Ca^{2+} concentration. This means that ECs can accurately convert information regarding shear stress intensity into changes in Ca^{2+} concentration. When extracellular Ca^{2+} was removed with EGTA, the shear-induced Ca^{2+} response completely disappeared, indicating that the response was due to an influx of extracellular Ca^{2+} across the cell membrane.

3 P2X4 Channels Mediate Ca^{2+} Influx in Response to Shear Stress

We found that P2X4, a subtype of ATP-operated cation channels known as P2X purinoceptors, plays a crucial role in the shear-stress-dependent Ca^{2+} influx [10, 11]. HPAECs were treated with antisense-oligonucleotides (AS-oligos) targeted to the P2X4 receptor or control scramble-oligos (S-oligos), and their Ca^{2+} responses were determined. A shear-stress-dependent Ca^{2+} response was seen in the cells treated with control S-oligos but not in those treated with AS-oligos (Fig. 1b). To further examine the role of P2X4 in flow-related Ca^{2+} signaling, we transfected P2X4 cDNA into human embryonic kidney (HEK) cells, which are basically insensitive to flow, and established cell lines that stably express P2X4 receptors. The

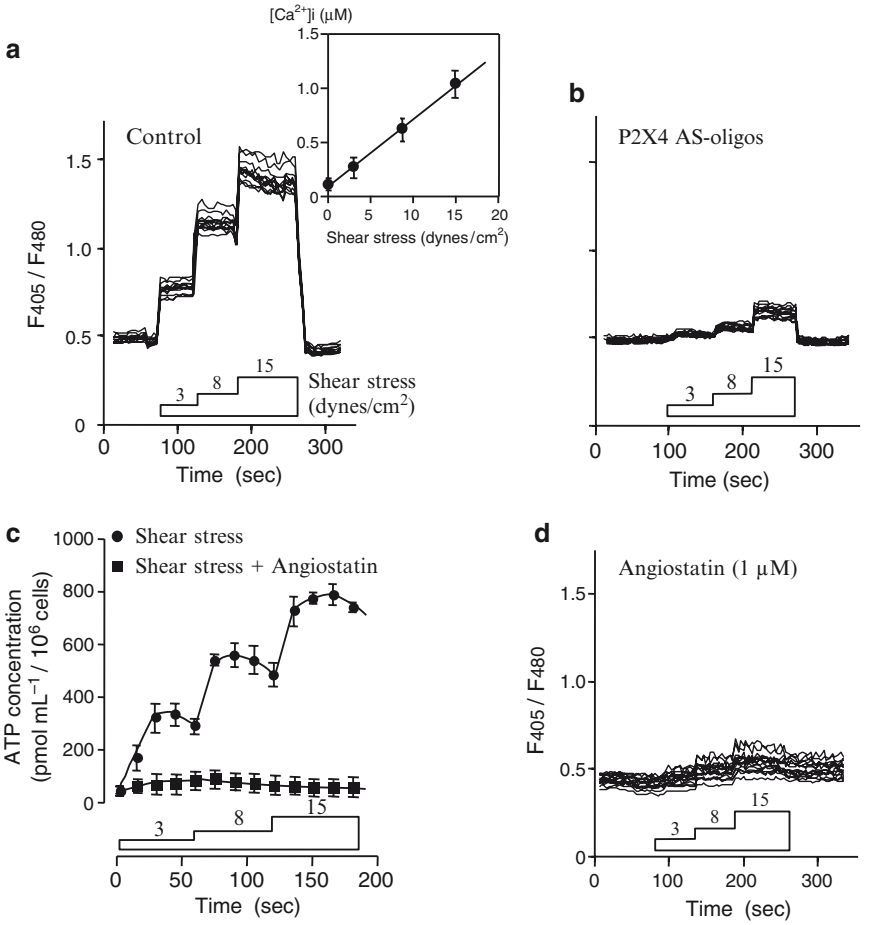

Fig. 1. Shear-stress-dependent Ca^{2+} influx via P2X4 channels. (**a**) Shear-stress-induced Ca^{2+} response. Intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$) increased in a stepwise manner when cultured human pulmonary artery ECs (HPAECs) were exposed to stepwise increases in shear stress, and a linear relationship was found between the Ca^{2+} concentration and shear stress, indicating that ECs are capable of accurately converting information on shear stress into changes in Ca^{2+} concentration. The Ca^{2+} response was attributable to an influx of extracellular Ca^{2+} because it did not occur in the absence of extracellular Ca^{2+}. The ratio of the emitted light of the fluorescent Ca^{2+} indicator Indo-1/AM at 405 nm (F405) and 480 nm (F480) reflects $[Ca^{2+}]i$. (**b**) Involvement of P2X4 in the Ca^{2+}influx. Antisense-oligonucleotides (AS-oligos) targeted against P2X4 that knockout P2X4 expression in HPAECs markedly suppressed the shear-stress-dependent Ca^{2+} responses. (**c**) ATP release in response to shear stress. HPAECs released ATP in a shear-stress-dependent manner, and the ATP-releasing response was completely blocked by angiostatin, a membrane-impermeable ATP synthase inhibitor, suggesting the involvement of cell-surface ATP synthase in the shear-stress-induced ATP release. (**d**) Involvement of ATP release in shear-stress-dependent Ca^{2+} influx. A membrane-impermeable ATP synthase inhibitor, anigostatin, almost completely blocked the Ca^{2+} response to shear stress, suggesting that ECs have shear stress mechanotransduction mechanisms in which shear stress stimulates ECs to release ATP via cell-surface ATP synthase, which leads to P2X4 activation followed by a Ca^{2+} influx

control HEK cells showed no Ca^{2+} response when exposed to flow, whereas the HEK cells that stably expressed P2X4 exhibited a stepwise increase in Ca^{2+} concentrations in response to graded increments in shear stress. These findings suggest that P2X4 receptors have a 'shear-transducer' property through which shear stress signals are transmitted into the cell interior via the Ca^{2+} influx.

4 Shear-Stress-Induced ATP Release Via Cell-Surface ATP Synthase

Our recent study revealed that shear-stress-induced activation of P2X4 requires ATP, which is supplied in the form of endogenous ATP released by ECs [12]. We determined the amount of ATP released into the perfusate using a sensitive luciferase luminometric assay. HPAECs released ATP in response to shear stress, and the ATP release was dose-dependent (Fig1c). A membrane-impermeable ATP synthase inhibitor, angiostatin, and an antibody against ATP synthase markedly suppressed the shear-stress-dependent ATP release, which resulted in significant inhibition of the shear-stress-dependent Ca^{2+} response (Fig. 1d). This means that endogenously released ATP plays an important role in the shear-stress-induced activation of P2X4 receptors. These findings also indicated that ATP synthase is involved in the shear-stress-induced ATP release.

We found that HPAECs express ATP synthase on their cell surface [13]. The cell-surface ATP synthase is distributed in caveolae/lipid rafts and colocalized with caveolin-1, a marker protein of caveolae. Depletion of plasma membrane cholesterol with methyl-β cyclodextrin disrupted the lipid rafts and abolished the colocalization of ATP synthase with caveolin-1, which resulted in a marked reduction in shear-stress-induced ATP release. To further examine the role of caveolin-1 in the flow-induced ATP release, we used siRNA to specifically knock down expression of caveolin-1. Transfection of ECs with caveolin-1 siRNA resulted in a significant reduction in caveolin-1 protein expression and markedly inhibited the flow-induced ATP release. These results suggest that the localization and targeting of ATP synthase to caveolae/lipid rafts is critical for shear stress-induced ATP release. However, it remains unknown how shear stress activates cell-surface ATP synthase.

5 Vascular Physiological Roles of Ca^{2+} Signaling of Shear Stress

To gain insight into the roles of this shear-stress-sensing mechanism via P2X4 in vascular homeostasis, we generated a *P2X4*-knockout (KO) mouse [14]. The absence of P2X4 impaired the EC response to flow stimulation. When the pulmonary microvascular ECs of wild-type (WT) mice were exposed to flow, intracellular Ca^{2+} concentration increased stepwise in tandem with the increase in shear stress,

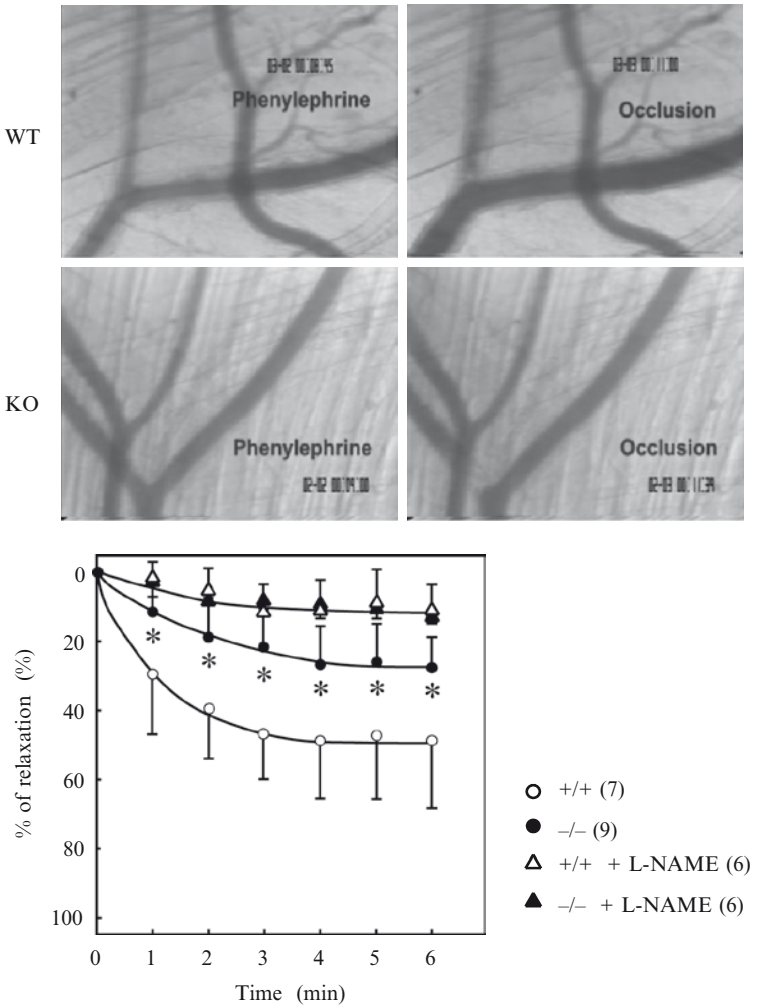

Fig. 2. Impaired flow-induced vasodilation in P2X4 knockout (KO) mice. *Top panel*, intravital microscopic images of vasodilator responses of cremaster muscle arterioles to an increase in blood flow. Arterioles were pre-constricted with phenylephrine. *Bottom panel*, results of a quantitative analysis of flow-induced vasodilation. The increase in blood flow caused marked vasodilation in the wild-type (WT) mice and much less prominent vasodilation in the KO mice. Blockade of NO synthesis with L-NAME markedly reduced the flow-induced vasodilation in both groups of mice, indicating that P2X4 plays an important role in the blood-flow-mediated vasodilation through endothelial NO production. Sample numbers are indicated in parentheses. * $p<0.01$ WT mice vs KO mice

whereas no flow-induced Ca^{2+} response occurred in the ECs of KO mice. Since increases in intracellular Ca^{2+} concentrations directly lead to the production of a potent vasodilator, NO, the ECs were examined for changes in NO production with a fluorescence indicator, diaminofluorescein (DAF-2). NO production by the ECs of

WT mice increased in response to flow, and the response was shear-stress-dependent. The ECs of KO mice, however, did not show flow-induced NO production, indicating that P2X4 channels are involved in endothelial NO production.

We examined the effects of *P2X4* deficiency on the endothelium-dependent vasodilator response in the murine cremaster muscle. Occlusion of one of the branches of an arteriole with a glass micropipette increases blood flow through the other branch, and the increase in blood flow caused marked vasodilation in the WT mice but much less prominent vasodilation in the KO mice (Fig. 2). Blockade of NO synthesis by N^G-nitro-L-arginine methyl ester (L-NAME) markedly reduced the flow-induced dilation in both types of mice. These findings suggest that the blood-flow-sensitive vasomotor mechanisms that regulate vascular tone are impaired in *P2X4* KO mice. A marked difference was observed in mean blood pressure determined by an intra-arterial catheter measurements, with significantly higher values recorded in the KO mice than in the WT mice (125 ± 8 mmHg vs 104 ± 10 mmHg).

Chronic changes in blood flow through large arteries induce structural remodeling of the vascular wall. Increases in blood flow cause enlargement of vessel diameter while decreases in blood flow have the opposite effect. To examine the role of the P2X4 receptor in flow-dependent vascular remodeling, the left external carotid artery of mice was ligated for 2 weeks. The ligation reduced blood flow in the left common carotid artery (LC), and we compared the diameter of the LC and the right common carotid artery (RC) histologically at the end of the 2-week period. Ligation of the left external carotid artery resulted in a significant reduction in lumen diameter in the LC in the WT mice but not in the KO mice. The absence of the blood-flow-induced change in diameter in the *P2X4* KO mice resembled the structural changes that occurred in eNOS-deficient mice [15], suggesting that P2X4 plays a critical role through endothelial NO production in controlling vascular structural adaptation to chronic changes in blood flow.

These results indicate that Ca^{2+} signaling of shear stress via endothelial P2X4 channels play an important role in the control of blood pressure, blood-flow-dependent vasodilation, and vascular remodeling, through endothelial NO production.

References

1. Korenaga R, Ando J, Tsuboi H et al (1994) Laminar flow stimulates ATP- and shear stress-dependent nitric oxide production in cultured bovine endothelial cells. Biochem Biophys Res Commun 198:213–219
2. Takada Y, Shinkai F, Kondo S et al (1994) Fluid shear stress increases the expression of thrombomodulin by cultured human endothelial cells. Biochem Biophys Res Commun 205:1345–1352
3. Davies PF (1995) Flow-mediated endothelial mechanotransduction. Physiol Rev 75:519–560
4. Korenaga R, Ando J, Kosaki K et al (1997) Negative transcriptional regulation of the VCAM-1 gene by fluid shear stress in murine endothelial cells. Am J Physiol 273:C1506–C1515

5. Kosaki K, Ando J, Korenaga R et al (1998) Fluid shear stress increases the production of granulocyte-macrophage colony-stimulating factor by endothelial cells via mRNA stabilization. Circ Res 82:794–802
6. Ando J, Korenaga R, Kamiya A (1999) Flow-induced endothelial gene regulation. In: Lelkes PI (ed) Mechanical Forces and the Endothelium. Harwood Academic Publishers, London, pp 111–126
7. Ohura N, Yamamoto K, Ichioka S et al (2003) Global analysis of shear stress-responsive genes in vascular endothelial cells. J Atheroscler Thromb 10:304–313
8. Ando J, Komatsuta T, Kamiya A (1988) Cytoplasmic calcium response to fluid shear stress in cultured vascular endothelial cells. In Vitro Cell Dev Biol 24:871–877
9. Ando J, Ohtsuka A, Korenaga R et al (1993) Wall shear stress rather than shear rate regulates cytoplasmic Ca^{2+} responses to flow in vascular endothelial cells. Biochem Biophys Res Commun 190:716–723
10. Yamamoto K, Korenaga R, Kamiya A et al (2000) P2X4 receptors mediate ATP-induced calcium influx in human vascular endothelial cells. Am J Physiol Heart Circ Physiol 279:H285–H292
11. Yamamoto K, Korenaga R, Kamiya A et al (2000) Fluid shear stress activates Ca^{2+} influx into human endothelial cells via P2X4 purinoceptors. Circ Res 87:385–391
12. Yamamoto K, Sokabe T, Ohura N et al (2003) Endogenously released ATP mediates shear stress-induced Ca^{2+} influx into pulmonary artery endothelial cells. Am J Physiol Heart Circ Physiol 285:H793–H803
13. Yamamoto K, Shimizu N, Obi S et al (2007) Involvement of cell surface ATP synthase in flow-induced ATP release by vascular endothelial cells. Am J Physiol Heart Circ Physiol 293:H1646–H1653
14. Yamamoto K, Sokabe T, Matsumoto T et al (2006) Impaired flow-dependent control of vascular tone and remodeling in P2X4-deficient mice. Nat Med 12:133–137

Symposium I
Novel Bioengineering

Cleft formation and branching morphogenesis of salivary gland: exploration of new functional genes

Takayoshi Sakai, Tomohiro Onodera, and Kenneth M. Yamada

Abstract. Epithelial branching morphogenesis is important to form many organs. Embryonic salivary glands provide an excellent model for clarifying the mechanisms of this phenomenon. As clefts form, epithelial cell–cell adhesions are converted to cell–matrix adhesions. Nevertheless, the mechanism of cleft formation is not well understood. Here, we describe a set of approaches being used to identify and characterize molecules necessary for branching morphogenesis. A combination of laser microdissection with T7-SAGE has been established as a gene discovery method for identifying candidate molecules that may be essential for early organ morphogenesis. Progress in understanding the mechanisms of salivary branching morphogenesis will provide novel approaches to future tissue engineering or regeneration of damaged salivary glands.

Key words. salivary gland, branching morphogenesis, cleft formation, molecular analysis, T7-SAGE

1 Introduction

Branching morphogenesis is an important developmental process that is required for the formation of a number of organs, including kidney, lung, pancreas, prostate, and salivary gland. Salivary epithelium undergoes repetitive cycles of branching

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