New developments in **heart disease research**

Dr. Takahiro Yoshizawa

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Dr. Takahiro Yoshizawa is interested in unveiling the role of homeostasis in health. Here he discusses the mechanisms of homeostasis regulation and his work on the role of a small protein, Adrenomedullin, in heart health.

As a starting point, can you discuss how your research background led to your interest in healthcare and more specifically in Adrenomedullin's role in regulating homeostasis?

I have always been interested in human health and disease treatment and prevention. In a healthy organism, all organs and cells work in harmony to sustain the life. In an unhealthy one, the organism is attempting to return to homeostasis.

Consequently, maintaining homeostasis is essential for health, with Adrenomedullin (AM) playing a key role. AM is a peptide involved in anti-hypertrophy, anti-oxidant stress, anti-inflammation and organ protection. Our work focuses on RAMP2, a regulating factor of the AM-receptor system in the heart. Understanding the AM-receptor system may shed light on cardiac homeostasis regulation.

Your research interests are highly focused on exploring the role and functioning of the 52-amino acid Adrenomedullin. Why this specific protein and why now?

We are generally interested in the AM-receptor system. AM is secreted by many organs and cells where it participates in autocrine and/or paracrine signalling by receptor binding. Receptor-activity-modifying protein (RAMP) is one example of AM receptor-related molecule, and three subtypes of RAMPs were identified. Our work suggests that RAMP2 (whose genetic deletion was embryonic lethal with abnormality of cardiovascular development in knockout mice) is essential for AM function.

Has your work revealed any significant findings to date, and if so, what are the potential implications?

Prior reports indicate that AM or RAMP2 homozygote knockout mice are dead in utero, which hinders analysis of AM and RAMP2's cardiac function. To address that, we engineered drug-induced cardiac-specific RAMP2 knockout mice (C-RAMP2 KO). Prior work suggested AM-dependent regulation of NADPH oxidase was the cause of AM's organ protective effect. Our work showed instead that AM-RAMP2 system regulates a range of mitochondrial functions, thereby maintaining cardiac homeostasis. This finding may help understand the mechanisms of mitochondrial regulation in the heart (a common dysfunction in heart failure patients). This could in turn help the development of new treatments for heart failure.

Much of your work uses transgenic mice as a model organism. What is the degree of genetic and functional similarity between Adrenomedullin in humans and mice? In other words, how likely is your research to help understand and possibly treat human disease?

AM is produced in many mammals including humans and mice. While AM shows many structural differences across species, on the functional level there are many similarities. In humans, AM blood levels increase in hypertension, heart failure, kidney diseases and myocardial infection, which suggests involvement in organ damage. Similar to the effect observed in humans, transgenic mice overexpressing AM show lower blood pressure and resistance to organ damage. However, AM knockout mice can show embryonic lethality with abnormal cardiovascular development (homozygote KO) or higher blood pressure and cardiac hypertrophy (heterozygote KO).

A RAMP2 homozygotic knockout is lethal in utero and is phenotypically similar to an AM knockout, suggesting that the AM-receptor system is essential for AM functions and homeostasis. In humans, naturally-occurring variations of RAMP2 gene may induce hypertension. However, almost of the functions of RAMPs are still unclear in humans.

This study focused on understanding the AM-RAMP2 system may help develop a therapeutic or diagnostic method for cardiac disease based on the AM-receptor system.

Given Adrenomedullin's role in a range of illnesses, do you envision any type of Adrenomedullin-based treatments and how they might be implemented?

Since AM levels in the blood are increased in hypertension, heart failure and myocardial infarction in humans, AM is likely involved in cardiovascular disease. AM may be useful as a biochemical marker and even as a therapeutic agent. In this latter role it has been tested with heart failure patients by Dr. Kitayama (Miyazaki University).

AM binds two types of receptors (CLR-RAMP2 and CLR-RAMP3), which may explain AM's versatility. The drawback is that non-specific effects may cause unwanted side effects. Investigation of the AM-receptor system is important to provide safe and effective cure for a range of diseases.

What implications might this study have on future work and potentially on healthcare, society and policy?

To elucidate the mechanisms of receptor specificity is essential to discovering new drugs. RAMPs regulate AM receptor activity and specificity. Our studies on the newly-discovered function of RAMP2 in the heart show that the AM-RAMP2 system is essential for cardiac metabolism and homeostasis. This makes the AM-RAMP2 system a promising therapeutic target of heart failure.

New Cures for the Aching Heart?

Heart disease is the single deadliest illness of our times, responsible for nearly a quarter of human losses worldwide. Research in the biomedical field by Dr. Takahiro Yoshizawa may help develop new treatments for heart failure.

21ST CENTURY MEDICINE

Improved standards of living and advances in medicine have led to an unprecedented increase in life expectancy from about 30 years just a century ago to a 2010 world average of 67 years. We are no longer dying from the epidemics of early 20th century, but from old-age diseases such as cancer and heart disease.

Heart disease alone is responsible for 17 million deaths worldwide. Healthy diets, exercise, and reducing smoking and alcohol are well-publicised preventative strategies. Blood clot prevention, medicines that lower cholesterol levels, and various surgical procedures are but a few of the treatments available. In addition, biomedical researchers make great efforts to shed more light on the causes of the illness, in the hopes of new and possibly improved cures.

This finding may help understand the mechanisms of mitochondrial regulation in the heart (a common dysfunction in heart failure patients). This could in turn help the development of new treatments for heart failure.

HEART DISEASE AND THE ADRENOMEDULLIN SYSTEM

A long standing interest in health and disease treatment and prevention is the motivation of Dr. Takahiro Yoshizawa's work to understand the Adrenomedullin system. An Assistant Professor at Shinshu University and recipient of several Japanese and international Young Investigator Awards and a Research Fellowship for Young Scientists, Dr. Yoshizawa is specifically interested in how a healthy organism maintains homeostasis and what goes wrong when that balance is upset. Adrenomedullin (AM) is a 52-aminoacid peptide which plays a key role in the maintenance of homeostasis and Yoshizawa's research focuses on the RAMP2 protein, a component of the AM-receptor system in the heart.

According to Dr. Yoshizawa, understanding the AM-receptor system may shed light on cardiac homeostasis regulation. His hypothesis is based on recent studies showing that AM plays an important role in vasodilation, depression, anti-hypertrophy, antioxidant stress, anti-inflammation, and in organ protection, suggesting that AM may be secreted to compensate organ damage. Dr. Yoshizawa collaborated extensively in this work with Professor Takayuki Shindo of Shinshu University Graduate School of Medicine.

A STORY OF MICE AND MEN

Although coming from a country known for its healthy diet and consequent low rate of heart disease, Dr. Yoshizawa is interested in better understand the AM-RAMP2 system as that knowledge may help develop a therapeutic or diagnostic method for cardiac disease based on the AM-receptor system. His work shows that the AM-RAMP2 system maintains cardiac homeostasis by regulating a range of mitochondrial functions. A dysfunctional mitochondrial regulatory system is often encountered in heart failure patients; therefore, this finding may help develop new treatments for heart failure.

AM is produced in many mammals including humans and mice, and as much of the biomedical research, this work is done in mice.

While AM shows many structural differences across species, on the functional level there are many similarities. AM's involvement in organ damage is shared between humans and mice. Genetically-engineered mice that do not express AM can show embryonic lethality with abnormal cardiovascular development (homozygote KO) or higher blood pressure and cardiac hypertrophy (heterozygote KO).

The AM-receptor system exists in many organs and cells where it participates in autocrine and/or paracrine signalling. Receptor-activity-modifying protein (RAMP) is one example of AM receptor-related molecule, and RAMP2 (whose genetic deletion was embryonic lethal with abnormality of cardiovascular development in knockout mice) is essential for AM function. A RAMP2 homozygotic knockout is lethal in utero and is phenotypically similar to an AM knockout, suggesting that the AM-receptor system is essential for AM functions and homeostasis. In humans, naturally-occurring variations of RAMP2 gene may induce hypertension. However, almost of the functions of RAMPs remain unclear in humans. Dr. Yoshizawa set out to address important questions about the role of RAMP2 in heart function, using modern genetic engineering and the latest techniques in the heart described below.

Dr. Yoshizawa and Professor Shindo's research team solved the function of RAMP2 by generating drug inducible cardiac myocyte-specific RAMP2 knockout mice (C-RAMP2-/-) using the *α*MHC-MerCreMer-lox system. The Cre-lox system is a sophisticated tool for conditional knockouts, which uses the Cre recombinase, an enzyme that catalyses recombination between two lox sites. The alpha myosin heavy chain (*α*MHC) promoter regulates the place of gene expression (cardiac motet-specific) on the downstream side. In C-RAMP2-/-, cardiac motet-specific deletion of RAMP2 can be caused in embryonic stage to senility stage.

C-RAMP2-/- mice showed dilated cardiomyopathy-like heart failure with cardiac dilatation and disarray of myofibrils after induction of the gene-deletion. Abnormality of mitochondrial structure was also found by electron microscopy. Using real-time PCR, the mitochondrial regulatory factor PGC-1 was also found to be down-regulated. Primary-cultured cardiac myositis showed reduced mitochondrial-membrane potential and increased mitochondrial reactive oxygen species (ROS) production in RAMP2-deletion dependent manner. C-RAMP2-/- showed the down-regulated activation of CREB, one of the regulator of PGC-1. Finally, forskolin treatment recovered the cardiac remodelling, CREB activity, and the expression of mitochondria-related genes.

The complex set of experiments, accessible only to specialists in the field, demonstrated that the AM-RAMP2 system is essential for cardiac metabolism and homeostasis.

HEALTHCARE APPLICATIONS

Commenting on his results in a 2013 article published in the reputable medical journal Hypertension, Dr. Yoshizawa concludes that the AM-RAMP2 system is a promising therapeutic target of heart failure. AM's involvement in hypertension, heart failure and myocardial infarction in humans makes the peptide a major player in cardiovascular disease. Many researchers propose to use AM as a biochemical marker for early detection of heart health problems and even as a therapeutic agent. Ongoing work with heart failure patients by Dr. Kitayama at Miyazaki University already explores that possibility.

Dr. Yoshizawa nonetheless warns that the clinical applicability of AM, like that of other bioactive endogenous peptides, has two major limitations: AM has a very short half-life in the blood, and the cost of the recombinant protein is very high, which together make the use of AM impractical for treatment of chronic diseases. However, his findings show that the cardiac activity of AM can be affected by modulating RAMP2. Medicines targeting the AM-RAMP2 system, if developed, would directly promote both energy production and free radical suppression in cardiac myocytes. Since elucidating the mechanisms of receptor specificity is essential to drug discovery, Prof. Shindo's research team recommend further investigation of the AM-receptor system to provide safe and effective cure for a range of diseases.

Genetic studies and in vivo studies such as Dr. Yoshizawa's aimed at understanding the causes of heart disease add to the repertoire of biomedical research undertaken at world's medical and academic centres. Additional concerted efforts are, by way of examples, ongoing R&D by pharmaceutical companies who develop new medicines targeting cholesterol levels, heart inflammation, etc. or new developments in imaging techniques such as computed tomography, positron emission tomography, and magnetic resonance imaging. It would appear that while the cure for the aching heart is just about as complex as the problem itself, we are getting closer to finding it by the day.

Researcher Profile

Takahiro Yoshizawa
Assistant Professor

Dr Takahiro Yoshizawa obtained his Ph.D. in medical sciences at Shinshu University where he is now an Assistant Professor and recipient of several Japanese and international Young Investigator Awards and a Research Fellowship for Young Scientists. His research focuses on homeostasis maintenance in healthy organisms and what goes wrong when that balance is upset. A major component of his work unveiled that the AM-RAMP2 system maintains cardiac homeostasis by regulating a range of mitochondrial functions, thereby proving that the AM-RAMP2 system is essential for cardiac metabolism and homeostasis. This finding can form the basis for developing new treatments for heart failure.

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