Japan Academy Prize to:

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for "Control of Homeostasis by Newly Discovered Klotho Gene Family"



Outline of the work:

Dr. Yo-ichi Nabeshima's research is based on molecular genetics and is classified into two main theses; firstly, the genetic program which regulates animal development, consisting of (1) the discovery of the alternative splicing system which allows the generation of multiple proteins from a single gene, (2) the genetic programs of muscle growth, differentiation and regeneration, (3) the genetic programs regulating central nervous system development and (4) the molecular mechanisms regulating stem cells; and second, research into the mechanisms underlying the maintenance of homeostasis in individuals, which is based on his discovery of the Klotho gene family and the analysis of its physiological and molecular functions. Both themes have thrown light on some of the fundamental questions in life science, and his findings have been highly regarded internationally. In particular, his research into the mechanisms of homeostasis based on the discovery of the Klotho gene family is especially noteworthy and has led to a new concept for calcium metabolism, brought about the discovery of a cholesterol/bile acid metabolic regulation mechanism and an understanding of the mechanisms of action of the metabolic FGF subfamily, advanced a new role for glycans in protein-protein interactions, and led to proposals for approaches to treating aging related syndromes. The research of second project can be summarized as follows.

(1) Discovery of α -Klotho mutant line and identification of α -Klotho gene

In 1997, Dr. Nabeshima found a mouse that displayed a variety of disorders that resembled the symptoms of human aging, and then identified α -*klotho* as the causative gene. α -*klotho* turned out to be a homolog of β -glycosidase, and was found to be expressed mainly in renal tubules, the parathyroid, and the choroid plexus, and to exist in cytoplasmic, membrane-bound, and secreted forms. He then identified patients in whom α -*klotho* gene expression was markedly increased, which together with another group's finding of a missense mutation, confirmed the existence of human diseases caused by changes in α -*klotho*. This research was the first to show that various aging-related disorders could be caused by the loss of function of a single gene, and had a major impact on the study of aging.

(2) Physiological function of α -Klotho

Establishing the mode of action of α -Klotho proved to be quite challenging, but Dr. Nabeshima's identification of α -Klotho's interaction with Na⁺K⁺-ATPase provided the first clue. This information enabled the finding that the α -Klotho/Na⁺K⁺-ATPase complex is transported through the ER and Golgi and accumulates in the endosomes, then in response to low extracellular calcium concentrations, quickly moves to the cell surface, increasing the number and function of Na⁺K⁺-ATPase molecules. Based on this finding, he

proposed a new molecular function for α -Klotho as a regulator of Na⁺K⁺-ATPase. He further proposed new mechanisms to explain the regulation of the secretion of parathyroid hormone in the parathyroid glands, the transepithelial transport of Ca²⁺ from blood to CSF in the choroid plexus, and Ca²⁺ reabsorption in the kidney. He then revealed that the membrane-bound form of α -Klotho can form a complex with FGF23 and FGFR1 that negatively regulates the production of vitamin D. Taking together, Dr. Nabeshima proposed a new concept of calcium homeostasis in which regulation is achieved through complicated reciprocal actions along with feedback mechanisms in the time course from seconds to minutes, minutes to hours, and hours to day(s). In this system, α -Klotho is critical for the rapid adjustment and continuous maintenance of extracellular Ca²⁺ levels within strictly narrow ranges. Together with the discovery of human α -klotho mutations, α -Klotho was concluded to be a key player that is involved in multi-step calcium control systems not only in mice but also in humans. Dr. Nabeshima advanced a new paradigm that changes the current concepts in mineral homeostasis and gives rise to new insights in this field.

(3) Molecular function of *a*-Klotho

 α -Klotho is a β -glycosidase homolog with a subtle β -glucuronidase activity and is thus predicted to act bimodally as an enzyme and as a carbohydrate-binding protein. Dr. Nabeshima found that a novel terminal glucuronidated O-glycan is affixed to FGF23 and that a certain proportion of N-glycans on all other α -Klotho binding proteins is likewise terminally glucuronidated. The glucuronide binding motif of α -Klotho recognizes these terminal glucuronidated N- and O-glycans to achieve a specific and selective interaction between α -Klotho and its binding partners. Interestingly, when the terminal glucuronidated O-glycan of FGF23 is docked to α -Klotho, the O-glycan induces a conformational change at the binding site and affects the conformation of α -Klotho, shifting α -Klotho toward a high-affinity state for FGF23. Based on these discoveries, he proposed that α -Klotho acts as a novel glucuronide-binding lectin and that the O-glycan works as a conformational-change initiator to facilitate an induced-fit type protein/protein interaction. In this way, α -Klotho causes circulating FGF23 to accumulate in the kidney and recruits the Na⁺K⁺-ATPase complex to cell-surface, where Na⁺K⁺-ATPase complex and FGF23 do their works.

(4) A therapeutic approach to aging related disorders

Dr. Nabeshima discovered that the various aging related phenotypes seen in the α -klotho mutant mice were caused by a marked increase in the activation of μ -calpain, and that by administering a μ -calpain inhibitor, he evaluated the effects of a μ -calpain inhibitor to investigate the importance of μ -calpain activation in the pathogenesis of α -klotho-deficiency phenotypes and found that daily administration of a μ -calpain inhibitor strikingly ameliorates many aging-related disorders of α -klotho knockout mice. He also found that the expression of FGF23 and osteogenesis-related genes are ectopically induced in the arteries of α -klotho knockout mice in accordance with the progression of cardiovascular calcification. These findings support the clinical evidence that FGF23 levels are highly associated with the cardiovascular mortality and allow us to propose that μ -calpain inhibition may prove useful in the alleviation of aging related syndromes.

(5) Identification of β -Klotho and its characterization

Dr. Nabeshima subsequently identified the β -klotho gene and found that it functions to negatively regulate the production of bile acids by the liver. He then showed that β -klotho forms a complex with Na⁺K⁺-ATPase, and in response to increases in the extracellular level of non-essential amino acids, the β -klotho/Na⁺K⁺-ATPase complex is induced to move to the plasma membrane, demonstrating an entirely new regulatory system for amino acid metabolism. Furthermore, with the two above studies, functions common to both the α -Klotho and β -Klotho systems have now been discovered, and the place and importance of the Klotho family genes in maintenance of homeostasis has been demonstrated.

In summary, Dr. Nabeshima's research stands at the forefront of aging science, and has opened up new fields of study in mechanisms of homeostasis, protein biology, and glycoscience. His global perspective and deep insights are truly outstanding and undoubtedly qualify him for the Japan Academy Prize.

Selected Publications

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Review

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