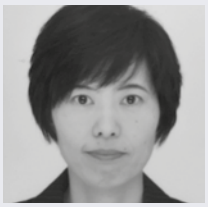
Impact Objectives

- Promote the elucidation of the physiological function of vitamin K by using UBIAD1 gene-deficient mice
- Analyse the function of MK-4 and the function of UBIAD1 at the cellular level using established cells and primary cultured cells

The mysterious mechanisms of vitamin K

In important research that could help advance disease prevention and treatment, ***Professor Kimie Nakagawa*** *is shedding light on the little understood roles of vitamin K in the body*



Can you briefly talk about your research background?

After graduating from Kobe Pharmaceutical University and obtaining a PhD in Pharmaceuticals, I was engaged in research on the physiological effects of vitamin D and vitamin K as an associate professor until March 2020. I subsequently became a professor at the Laboratory of Hygienic Sciences, Faculty of Pharmaceutical Sciences, Kobe Gakuin University. Currently, I have my own laboratory in the Faculty of Pharmaceutical Sciences, Kobe Gakuin University and I am actively promoting the elucidation of the physiological function of vitamin K by using UBIAD1 gene-deficient mice.

Could you tell us more about the research your team is currently engaged in?

The research that we are conducting in terms of UBIAD1, which is a vitamin K converting enzyme, includes the creation of several types of tissue-specific gene-deficient mice and investigation of their phenotypes to elucidate the new physiological functions of vitamin K and UBIAD1. We are analysing the function of MK-4 and the function of UBIAD1 at the cellular level using established cells and primary cultured cells.

What is the role of vitamin K and what is the importance of research on this topic?

Vitamin K plays an essential role as a cofactor for GGCX (γ-glutamylcarboxylase), an enzyme that activates blood coagulation factors and bone matrix proteins and promotes blood coagulation and bone formation. This action is common to vitamin K_1, vitamin K_2 and menaquinones, but vitamin K_2 (MK-4) has various other physiological activities. The functions that only MK-4 has, include action to promote differentiation and survival of brain nerve cells, action to suppress the growth of cancer cells and induce apoptosis, action to promote osteoblast differentiation and calcification, and action to control the transcription of various genes as a ligand for steroid and xenobiotic receptors (SXR).

In other words, the conversion and production of MK-4, which has many physiological functions, comes from vitamin K homologues in our body and is said to be an activation reaction of vitamin K in our body. However, the role of MK-4 in various tissues and the physiological significance of conversion and generation of MK-4 *in vivo* have not been fully elucidated. Clarifying the various roles of vitamin K *in vivo* will lead to the elucidation of its relevance to the prevention and treatment of various diseases.

Cardiovascular disease is a condition that progresses without symptoms, and when symptoms appear, it becomes severe with a high risk of death. Therefore, it is very important to prevent its development. Since cardiovascular disease is closely related to lifestyle and eating habits, if we can prevent the onset of the disease by taking vitamin K, which is a nutrient, we will be able to save lives. Through this study, it is possible to elucidate the role of vitamin K in cardiovascular diseases, that vitamin K plays an essential role in maintaining heart and blood vessels function and that vitamin K intake contributes to the prevention and treatment of cardiovascular diseases, which provides scientific evidence that vitamin K is required and effective for cardiovascular function.

How will your research results be applied in a healthcare setting?

If the results of this research reveal that MK-4, which is converted and produced by UBIAD1, is effective in preventing cardiovascular disease, I think that it will be possible for many people to benefit from widely disseminating that vitamin K intake helps prevent heart disease. Moreover, by developing a method that can enhance the function of UBIAD1 and a ligand that is efficiently converted to MK-4 by UBIAD1, I believe that it can be progressed into drug discovery research that leads to cardiovascular treatment. ▶

Conquering cardiovascular disease with vitamin K

*At **Kobe Gakuin University** researchers are unearthing important discoveries about vitamin K that will drive forward discoveries in cardiovascular treatment and prevention*

On a basic level, vitamin K is required by the human body for blood clotting and bone metabolism, helping to make the various proteins that are required for these processes. However, many unknowns remain about this group of vitamins, which means that decisions about vitamin K intake and bone health may not necessarily be effective as they can be. A researcher at Kobe Gakuin University in Japan is working to improve this situation by exploring vitamin K in granular detail, and her work could lead to important discoveries that may expedite breakthroughs in the prevention and treatment of cardiovascular disease.

Professor Kimie Nakagawa is based in the University's Laboratory of Hygienic Sciences in the Faculty of Pharmaceutical Sciences, where she is conducting studies on vitamin K to elucidate its various roles in the human body, ultimately helping to advance disease prevention and treatment research. Initially, she was engaged in research on vitamin D, exploring vitamin D derivatives effective for cancer treatment and prevention and shedding light on physiological functions. She later applied the knowledge she accumulated in this research to investigations on vitamin K which, similarly to vitamin D, is a fat-soluble vitamin. 'Vitamin K has not been sufficiently researched biochemically in Japan and worldwide,' Nakagawa highlights, which is a key motivation for her work.

UBIAD1 AND MK-4

Key foci for Nakagawa are UBIAD1 (UbiA prenyltransferase domain containing protein 1) and MK-4 (menaquinone-4). UBIAD1 is a prenyltransferase that mediates the formation of MK-4, while MK-4 is a homologue of vitamin K_2. 'The family of vitamin K (vitamin K_1, vitamin K_2 and menaquinones) that we ingest from the food are all converted to vitamin K_2 (MK-4) *in vivo* are analysing the function of MK-4 and the function of UBIAD1 at the cellular level using established cells and primary cultured cells. The key enzyme responsible for this conversion reaction is UBIAD1, and in 2010, I was able to identify UBIAD1 as an MK-4 synthase,' she comments. 'MK-4 is thought to exert various physiological actions in the body, but its role in each tissue has not been fully elucidated yet. UBIAD1 is also present in other tissues throughout the body, but it has been suggested that it has another function other than synthesising MK-4, and there are

many unclear points that remain about its function.' Nakagawa is now working to fill these gaps in knowledge, shedding greater light on the role of MK-4 in the human body and the unknown functions of UBIAD1.

To do this, Nakagawa and her team have created and are analysing UBIAD1 tissue-specific gene-deficient mice. Through this work, the researchers' goal is to clarify the significance of biosynthesis of MK-4 *in vivo* and elucidate the role of UBIAD1 and MK-4 in each tissue. This novel work could have important applications for cardiovascular disease prevention and treatment. Specifically, Nakagawa is engaged in research to elucidate vitamin K conversion mechanisms, shed light on the physiological function of the vitamin, identify vitamin K converting enzymes and explain more about the physiological function of this enzyme. The researchers have already made impressive strides in their work, uncovering an important discovery back in 2008. 'In a world first, we have succeeded in scientifically demonstrating that the vitamin K homologue is converted to MK-4.' Nakagawa reveals. 'Furthermore, we clarified that the enzyme responsible for the conversion reaction is UBIAD1. So far, the discovery that UBIAD1 is the enzyme responsible for its conversion to MK-4 is the greatest achievement of our research.'

> *'So far, the discovery that UBIAD1 is the enzyme responsible for its conversion to MK-4 is the greatest achievement of our research'*

FINDINGS FROM FUNCTIONAL ANALYSES

Currently, in a project funded by a Grant-in-Aid for Scientific Research (KAKENHI) entitled 'Elucidation of new vitamin K function aiming at application to cardiovascular disease prevention and treatment,' Nakagawa and her team have succeeded in producing cardiovascular-specific *Ubiad1* conditional knockout mice (Ubiad1-cKO) and have subsequently began phenotypic analysis. Now, the researchers are performing functional analyses of UBIAD1 and MK-4 in muscle cells and vascular endothelial cells. In the long term, this research could have far-reaching benefits. 'The results obtained from this study will contribute to maintaining the health of many people, reducing the risk of developing cardiovascular disease, and improving the effectiveness of treatments,' she observes.

Previous, interrelated projects of Nakagawa's that have led the team to important findings to date include: 'Identification of UBIAD1 as a novel human menaquinone-4 biosynthetic enzyme'; 'Vitamin K_2 Biosynthetic Enzyme, UBIAD1 Is Essential for Embryonic Development of Mice'; and 'UBIAD1 Plays an Essential Role in the Survival of Pancreatic Acinar Cells'. Nakagawa outlines what she considers to be some of the key findings her work has highlighted. 'I think that it is very interesting that vitamin K_1 and vitamin K_2 have the same basic skeletal structure, and despite the difference in the number of double bonds in the side chains, the role they play *in vivo* has changed completely, and only vitamin K_2 (MK-4) has a structure-specific function that has so many functions,' she states. 'I find it interesting to know that a compound with a molecular weight of only 450 has an important role in maintaining vital functions, and it is also useful to know the value in scientifically proving where and what is happening.'

DISCOVERY THROUGH INNOVATION

In their work, the researchers employ a number of innovative tools and methodologies, including LC-MS/MS, real-time PCR, RNA sequence analysis, siRNA, CRISPER/Cas system, immunohistochemical staining and CT analysis for animal use. Furthermore, Nakagawa and the team strive to obtain more reliable and grounded results by actively adopting new methods and utilising different combinations of methods. 'Alongside these devices and methods, collaboration plays an equally important role for our research,' highlights Nakagawa. Specifically, she collaborates with organic chemists, medical researchers and epidemiological researchers, adding even greater value to her work. 'By collaborating with researchers in various fields in this way, I believe that my research will develop into more clinically useful and meaningful work,' she says.

Nakagawa will continue to focus on the important functions of vitamin K and enhance understanding about its significance. Ultimately, her research has the potential to save lives. 'I think vitamin K has many physiological functions that are yet to be clarified. I would like to elucidate these as much as possible and widely disseminate the power of vitamin K in order to protect human lives and prevent diseases,' she underlines. Another focus for the team will be on the development of a new paradigm for fat soluble ligands, which involves looking at other vitamins. ●

Project Insights

FUNDING

- Ministry of Education, Culture, Sports, Science and Technology in Japan (MEXT), KAKENHI, Grants-in-Aid for Scientific Research, grant number 18K06644
- Japan Foundation for Applied Enzymology (2016 and 2019)
- Urakami Foundation for Food and Food Culture Promotion (2019)

COLLABORATORS

- Professor Naoko Tsugawa (Department of Health and Nutrition, Faculty of Health and Nutrition, Osaka Shoin Women's University)
- Professor Yoshitomo Suhara (Laboratory of Organic Synthesis and Medicinal Chemistry, Department of Bioscience and Engineering, College of Systems Engineering and Science, Shibaura Institute of Technology)
- Associate Professor Yoshihisa Hirota (Laboratory of Biochemistry, Faculty of Bioscience and Engineering, College of Systems Engineering and Science, Shibaura Institute of Technology)

TEAM MEMBERS

PhD student Shunsuke Hirashima and undergraduate students

CONTACT

Professor Kimie Nakagawa

T: +81 78 974 4624
E: kimie-naka@pharm.kobegakuin.ac.jp
W: http://kobegakuin-yakugaku.jp
W: https://www.kobegakuin.ac.jp/information/public/teacher/pharmacy/nakagawa_k.html

科研費 KAKENHI

KOBE GAKUIN UNIVERSITY