

2.3.1 Laboratory : Cellular Biochemistry

Member :	Professor	Ueda, Kazumitsu, D. Agric.Sci
	Associate Professor	Kioka, Noriyuki, D. Agric.Sci
	Assistant Professor	Matsuo, Michinori, D. Agric.Sci
	Assistant Professor	Kimura, Yasuhisa, D. Agric.Sci
	Doctor's program	5
	Master's Program	12
	Undergraduate	4
	Program-Specific Researcher	4

A. Research Activities (2009.4-2010.3)

A-1. Main Subjects

a) ABC proteins: their physiological functions and molecular mechanisms

ATP-binding cassette superfamily proteins (ABC proteins) are membrane protein family, which have two highly conserved ATP binding domains in a molecule. ABC proteins are important for various cellular functions, which are involved in host defense mechanisms, glucose homeostasis, and lipid homeostasis. ABC proteins have divergent functions and can be classified as transporters, channels, and receptors, although their predicted secondary structures are very much alike. We are studying physiological functions of ABC proteins and molecular mechanisms of their functional diversity.

b) Molecular mechanism of xenobiotic efflux pumps MDR1, MRP1, and MRP2

MDR1/P-glycoprotein is a physiologically important ABC protein in limiting the uptake of toxic compounds from the gastrointestinal tract, stimulating their excretion from the liver, kidney, and intestine, and moreover protecting the brain by functioning as a blood-brain barrier. MRP1 and MRP2 are also physiologically important ABC proteins, which extrude xenobiotics after conjugated with glutathione and glucuronate. To understand the mechanism of drug efflux by these ABC proteins and to overcome multidrug resistance of cancer cells by preventing their function, we are studying molecular mechanisms how these ABC proteins transport a wide variety of compounds and how they carry their substrates across membranes by using the energy of ATP hydrolysis.

c) Molecular mechanism of ATP-sensitive potassium channels

Pancreatic β -cell ATP-sensitive potassium (KATP) channels play an important role in the regulation of glucose-induced insulin secretion. The β -cell KATP channel comprises two subunits, the sulfonylurea receptor SUR1, a member of ABC proteins, and Kir6.2, a channel pore subunit. We have analyzed properties of the two NBFs of SURs and proposed that SUR1 is not a transporter but a switch, like a G-protein, and is a sensor monitoring changes in intracellular ADP concentration. We are analyzing ATP hydrolysis properties of SURs and comparing with those of other ABC proteins to reveal how KATP channels are regulated by intracellular ATP and ADP concentrations.

d) ABC proteins involved in fatty acid and cholesterol homeostasis

Many ABC proteins are involved in lipid homeostasis. ABCA1 mediates release of cellular cholesterol and phospholipids to form high density lipoprotein (HDL). Cholesterol is not catabolized in the peripheral cells and therefore mostly released and transported to the liver for conversion to bile acids to maintain cholesterol homeostasis. Although it is clear that ABCA1 plays a critical role in HDL generation, the molecular mechanism of ABCA1 remains unclear. We are analyzing ATP hydrolysis properties and post-transcriptional regulation of ABC proteins involved in lipid homeostasis to reveal physiological roles of ABC proteins in lipid homeostasis.

e) Functional analysis of focal adhesion proteins on cell migration, cell proliferation and tumor metastasis.

Cell adhesion to extracellular matrix regulates various cellular events, including cell proliferation, survival, differentiation, and migration, in a coordinated manner with growth factor signaling. We have shown that a focal adhesion protein vinexin is involved in regulation of cell adhesion, cytoskeletal organization, and anchorage-dependent cell signaling. Our goal is to understand this coordination of cell adhesion and growth factor signaling using methods of molecular biology and cell biology.

A-2.Publications and presentations

a) Publications

Original Papers

- Saeki, T., Mizushima, S., Ueda, K., Iwami, K. and Kanamoto, R. Mutational analysis of uncharged polar residues and proline in the distal one-third (Thr130-Pro142) of the highly conserved region of mouse Slc10a2. *Biosci Biotechnol Biochem* 73, 1535-40 (2009)

- Umemoto, T., Tanaka, K., Ueda, K. and Kioka, N. Tyrosine phosphorylation of vinexin in v-Src-transformed cells attenuates the affinity for vinculin. *Biochem Biophys Res Commun* 387, 191-5 (2009)
- Sato, T., Kodan, A., Kimura, Y., Ueda, K., Nakatsu, T. and Kato, H. Functional role of the linker region in purified human P-glycoprotein. *FEBS J.* 276(13), 3504-16. (2009)
- Morita, S-y., Ueda, K. and Kitagawa, S. Enzymatic measurement of phosphatidic acid in cultured cells. *J. Lipid Res.* 50, 1945-1952 (2009)
- Hozoji, M., Kimura, Y., Kioka, N., and Ueda, K. Formation of two intramolecular disulfide bonds is necessary for apoA-I-dependent cholesterol efflux mediated by ABCA1. *J Biol Chem.* 284, 11293 - 11300 (2009)
- Azuma, Y., Takada M., Maeda, M., Kioka, N., and Ueda, K., The COP9 signalosome controls ubiquitinylation of ABCA1. *Biochem. Biophys. Res. Commun.* 382(1), 145-148 (2009)
- Maekawa, M., Kikuchi, J., Kotani, K., Nagao, K., Odgerel, T., Ueda, K., Kawano, M., Yusuke Furukawa, Y., and Sakurabayashi, I. A Novel Missense Mutation of ABCA1 in Transmembrane α -Helix in a Japanese Patient with Tangier Disease. *Atherosclerosis* 206, 216-222 (2009)
- Kodan, A., Shibata, H., Matsumot, T., Terakado, K., Sakiyama, K., Matsuo, M., Ueda, K., and Kato, H., Improved expression and purification of human multidrug resistance protein MDR1 from baculovirus-infected insect cells. *Protein Expr. Purif.* 66, 7-14 (2009)
- Nagao, K. Zhao, Y., Takahashi, K., Kimura, Y., and Ueda, K. Sodium taurocholate-dependent lipid efflux by ABCA1-Effects of W590S mutation on lipid translocation and apoA-I dissociation. *J. Lipid Res.* 50, 1165-1172 (2009)
- Azuma, Y., Takada, M., Shin, H-W., Kioka, N., Nakayama, K., Ueda, K. The retroendocytosis pathway of ABCA1/apoA-I contributes to HDL formation. *Genes to Cells* 14, 191-204 (2009)
- Hirata, T, Okabe, M., Kobayashi, A., Ueda, K., Matsuo, M. Molecular mechanisms of subcellular localization of ABCG5 and ABCG8. *Biosci Biotechnol Biochem.* 73(3), 619-626 (2009)
- Umemoto, T., Inomoto, T., Ueda, K., Hamaguchi, M. Kioka, N. v-Src-mediated transformation suppresses the expression of focal adhesion protein vinexin. *Cancer Letters* 279, 22-29 (2009)

Reviews

- Ueda, K. Transcriptional- and post-translational regulation in cholesterol homeostasis, *Seitaino Kagaku* 60, 570-578 (2009)
- Ueda, K. Functions and regulation of ABC proteins, *MOOK12 Recent transporter research* 42-47 (2009)
- Kimura, Y. Ueda, K. Functions and transport mechanisms of ABC transporters *Library of drug transporters* 122-126 (2009)
- Kimura, Y. Ueda, K. MDR1 *Library of drug transporters* 122-126 (2009)

b) Conference and seminar papers presented

- The 82th Annual meeting of the Japanese Biochemistry Society: 7 Presentations
- 2009 Annual meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 6 Presentations
- The 5th Takeda Science Foundation Symposium on PharmaSciences: 9 Presentations
- The 5th Annual meeting of the Japanese Lipid Biochemistry Society: 1 Presentation
- The Annual meeting of the Pharmaceutical Society of Japan: 1 Presentation
- Gordon Conference “Mechanism of Membrane Transport” 1 Presentation
- Gordon Conference “Molecular & Cellular Biology of Lipids” 1 Presentation
- The 13th Annual meeting of the Japanese Association for Molecular target Therapy: 1 Presentation
- The 4th iCeMS International meeting “Integrated Physical/Chemical Biology of the Cell: from Genes to Membrane Systems”: 9 Presentations
- The 49th Annual Meeting of American Society for Cell Biology: 3 Presentations

A-3.Off-campus activities

Membership in academic societies

- Ueda, Kazumitsu, D. Agric.Sci : the Japanese Biochemistry Society (Standing director), Japan Cancer Association (Councilor), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Public Information Committee member), Cancer Science (Editor)

Research grants

1. Grants-in-aid for Scientific Research(KAKENHI)

- Scientific Research (S) : Ueda, Kazumitsu, D. Agric.Sci : Physiological Substrates and functions of ABC proteins involved in lipid transport

- Scientific Research (B) : Kioka, Noriyuki, D. Agric.Sci : Physiological function of focal adhesion proteins as an environmental sensor and an environmental controller
- Scientific Research on Priority Areas : Matsuo, Michinori, D. Agric. Sci. : Mutual regulation of lipid transporters and membrane environments
- Young Scientists (A) : Matsuo, Michinori, D. Agric. Sci. : Molecular basis of lipid efflux by ABCG proteins

2. Other Research Grants

- NEDO : Kimura, Yasuhisa, D. Agric. Sci. : Structural-guided drug development

A-4. International cooperation and overseas activities

Membership in academic societies

- Ueda, Kazumitsu, D. Agric.Sci: The American Cancer Society (member)
- Kioka, Noriyuki, D. Agric.Sci: The American Society for Cell Biology (member)

B. Educational Activities(2009.4-2010.3)

B-1. On-campus teaching

a) Courses given

- Undergraduate level: New Strategy of Agricultural Biotechnology (Ueda, K.), Basic Course of Applied Life Sciences III (Ueda, K), Molecular cellular Biology I (Kioka, N), Molecular Biology II (Ueda, K.), Introductory lecture and laboratory course in Molecular Biology (Kioka, N. Matsuo, M. Kimura, Y.)
- Graduate level: Advanced Cellular Biochemistry (Ueda, K., Kioka, N, Kimura, Y), Biochemistry Seminar (Ueda, K. Kioka, N), Experimental Course of Biochemistry (Ueda, K. Kioka, N).

B-2. Off-campus teaching etc.

Part-time lecturer

- Ueda, Kazumitsu, D. Agric.Sci: Tezukayama Junior High School (Special Lecture)

B-3. Overseas teaching

International students

- International students : Master 2 (Brazil 1, China 1)

Lectures and seminars

- Ueda, Kazumitsu, D. Agric.Sci

Special Lecture(Invited Lecturer) : Columbia University(USA)