# Chair Applied Biochemistry

# 2.3.1 Laboratory : Cellular Biochemistry

| Member: | Professor                      | Ueda, Kazumitsu, Ph.D.  |
|---------|--------------------------------|-------------------------|
|         | Associate Professor            | Kioka, Noriyuki, Ph.D.  |
|         | Assistant Professor            | Michinori Matsuo, Ph.D. |
|         | Assistant Professor            | Kimura, Yasuhisa, Ph.D. |
|         | Doctor's program               | 8                       |
|         | Master's Program               | 9                       |
|         | Undergraduate                  | 3                       |
|         | Other                          | 1                       |
|         | Program-Specific<br>Researcher | 4                       |

### A. Research Activities (2010.4-2011.3)

#### 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  $\beta$ -cell ATP-sensitive potassium (KATP) channels play an important role in the regulation of glucose-induced insulin secretion. The  $\beta$ -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(including book-reviews)

- Yamashita H, Ueda K, and Kioka N. WAVE2 forms a complex with cAMP-dependent protein kinase (PKA) and is involved in PKA enhancement of membrane protrusions. J Biol Chem. 286, 3907-3914, 2011

- Matsuo M, Campenot RB, Vance DE, Ueda K, and Vance JE. Involvement of low-density lipoprotein receptor-related protein and ABCG1 in stimulation of axonal extension by apoE-containing lipoproteins. Biochim Biophys Acta. 1811, 31-38, 2011

- Terakado, K., Kodan, A., Nakano, N., Kimura, Y., Ueda, K., Nakatsu, T., and Kato, H. Deleting two C-terminal alpha-helices is effective to crystallize the bacterial ABC transporter Escherichia coli MsbA complexed with AMP-PNP. Acta Cryst., D66, 319-323, 2010

- Kioka, N., Ito, T., Yamashita, H., Uekawa, N., Umemoto, T., Motoyoshi, S., Imai, H., Takahashi, K., Watanabe, H., Yamada, M., and Ueda, K. Crucial role of vinexin for keratinocyte migration in vitro and epidermal wound healing in vivo. Exp Cell Res, 316, 1728-1738, 2010

- Shichiri M, Takanezawa Y, Rotzoll DE, Yoshida Y, Kokubu T, Ueda K, Tamai H, and Arai H. ATP-Binding cassette transporter A1 is involved in hepatic alpha-tocopherol secretion. J Nutr Biochem. 21( 5), 451-456, 2010

- Yamazaki, T., N. Fujiwara, H. Yukinaga, M. Ebisuya, T. Shiki, T. Kurihara, N. Kioka, T. Kambe, M. Nagao, E. Nishida, and S. Masuda. The closely related RNA helicases, UAP56 and URH49, preferentially form distinct mRNA export machineries and coordinately regulate mitotic progression. Mol Biol Cell, 21, 2953-2965, 2010

Reviews

- Ueda, K. ABC proteins protect human body and maintain optimal health, Biosci Biotechnol Biochem 75, 401-409, 2011

- Nagao, K., Kimura, Y., Matsuo, M., and Ueda, K. Lipid outward translocation by ABC proteins, FEBS Lett. 584, 2717-2723, 2010

Reports, others

- Matsuo, M., Ueda, K. Transporters of nutritions and metabolites ~Lipids 191-199 KYOTOHIROKAWA (2011)

b) Conference and seminar papers presented

- The 62nd Annual meeting of the Japan Society for CellBiology (1presentation)

- Keystone Symposia Bioactive Lipids: Biochemistry and Diseases (1presentation)

- Gordon cenference for Lipoprotein Metabolism (1presentation)

- The 27th Naito Conference (1presentation)

- 6th IAS-sponsored workshop on HDL (1presentation)

- The 5th Transporter ---- (2presentations)

- Gordon Conferences "Signaling by adhesion receptor" (1presentation)

- The 69 th Annual Meeting of Japanese Cancer Association (1presentation)

- Japan Society for Bioscience, Biotechnology, and Agrochemistry, Kansai-branch Meeting (2 presentations)

- The 8th iCeMS symposium (2 presentations)

- BMB2010 (5 presentations)

- 2011 Annual meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (12 presentations)

# A-3.Off-campus activities 1

Membership in academic societies

- Ueda, Kazumitsu : the Japanese Biochemstry Society (Standing director), Japan Cancer Association (Councilor), Cancer Science (Editor)

Membership in Science Council of Japan, etc.

- Yasuhisa Kimura : Organizer of freshman's meeting(Japan society for bioscience, biotechnology and agrochemisitry

# A-3.Off-campus activities 2

Research grants

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

- Basic Research (S) : Ueda, Kazumitsu : Physiological Substrates and functions of ABC proteins involved in lipid transport

- Basic Research (B) : Kioka, Noriyuki : Physiological function of focal adhesion proteins as an environmental sencor and a environmental controller

- Grant-in-Aid for Young Scientists (A) : Matsuo, Michinori : Molecular basis of lipid efflux by ABCG proteins

2. Other Research Grants

- Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (BRAIN): Ueda, Kazumitsu: Development of omega-3 fatty acid material for inovating functional food

- Japan New Energy and Industrial Technology Development Organization (NEDO) : Kimura, Yasuhisa : Structural-ruided drug deveropment

#### A-4.International cooperation and overseas activities 1

Membership in academic societies

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

#### **B.Educational Activities**(2010.4-2011.3)

### **B-1.On-campus teaching**

a) Courses given

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

#### **B-2.Off-campus teaching etc.**

Part-time lecturer

- Ueda, K.: Nishinomiya High School special lecture

# **B-3.Overseas teaching 1**

International students

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

# **B-3.Overseas teaching 2**

Lectures and seminars

- Ueda, Kazumitsu

Mechanism and regulation of HDL formation by ABCA1(Invited Speaker) : Alberta University(Canada)