Development of Small-Molecule Tools for Cell Therapy

Cell Biology empowered by small molecules

Professor Motonari Uesugi – Institute for Chemical Research *and* iCeMS

Taking advantage of technology available in fields ranging from organic chemistry to clinical medicine, Prof. Motonari Uesugi and his research team seek to discover and use synthetic small molecules that modulate fundamental processes in human cells, specifically small-molecule adhesion factors and small-molecule growth factors. Their goals extend beyond the discovery of clinically useful molecules, to include design innovations and chemical synthesis that will lead to new, broad, and cost-effective general applications of synthetic organic molecules.

Prof. Uesugi's discoveries have made possible the investigation of complex cellular events and the improvement of cell therapies. The team has already discovered a synthetic small molecule, "adhesamine," which boosts adhesion and growth of cultured human cells. Using this molecule as a seed, Prof. Uesugi and colleagues designed a synthetic molecule that behaves just like fibronectin, a naturally-occurring protein commonly used in a range of fields from basic biology to cosmetics. The team has additionally shown that it is possible to replace fibronectin, a large protein, with a synthetic compound appropriate in size for mass chemical production. The development of this "small molecule fibronectin" is a groundbreaking contribution to chemistry, biology, and medicine.

www.icems.kyoto-u.ac.jp/e/pr/2011/05/12-tp.html

Receptor Behaviors Observed in Living Cell Membranes

Expanding drug development horizons

Professor Akihiro Kusumi [left] – iCeMS
Rinshi Kasai PhD [right]

Unprecedented single molecule imaging movies of living cell membranes, taken by a research team based at Kyoto University and the University of New Mexico, have clarified a decades-old enigma surrounding receptor molecule behaviors. The work focuses on G protein-coupled receptors (GPCRs), a class of molecules in cell membranes that comprise the largest superfamily in the human genome. According to iCeMS Prof. Akihiro Kusumi, "We obtained a parameter called the dissociation constant, which will allow us to predict numbers of monomers and dimers if the total number of GPCRs in a cell is known. The ability of scientists to obtain such key numbers will be essential for understanding GPCR signaling, as well as defects leading to diseases from the neuronal to the immune systems. The blocking of signal amplifications by monomer-dimer interconversions and its implications for drug design are profoundly important."

www.icems.kyoto-u.ac.jp/e/pr/2011/02/08-nr.html

Epigenetic Regulation of the TGF-beta Pathway in Ovarian Cancer

The cancer advances when genes are silenced

Professor Ikuo Konishi – Graduate School of Medicine
Assistant Professor Noriomi Matsumura – Graduate School of Medicine

Researchers at Kyoto University and Duke Cancer Institute have found evidence of epigenetics at work on a genome-wide scale in cases of ovarian cancer. The researchers performed a series of studies on cancer cell lines and primary tumor specimens from ovarian cancer patients by comparing the genome-wide gene expression profiles of cells that were treated or mock-treated with drugs that inhibit DNA methylation. From these studies they identified 378 candidate methylated genes. From this group, all 43 of the predicted genes the researchers analyzed showed methylation in ovarian cancers. The researchers found that many of these genes were part of one pathway, the TGF-beta signaling pathway. When the researchers treated tumor cells with methylation inhibitors, the TGF-beta pathway showed increased activity. In addition, the genes they studied included a cluster of genes that strongly correlated with TGF-beta pathway activity in specimens from older women, which suggested that age-related epigenetic changes can accumulate and may contribute to cancer. Two different groups of patients the team identified might need different approaches. Some women with ovarian cancer have a lower expression of these tumor-suppressing genes and may be amenable to epigenetic therapies that lead to gene reactivation. Another group of women with ovarian cancer have a higher expression of these genes, suggesting it may be possible to specifically inhibit particular components in this pathway to stop tumor development or progression.

www.kyoto-u.ac.jp/en/news_data/h/h1/news6/2011/101214_1.htm
www.med.kyoto-u.ac.jp/E/grad_school/introduction/1404/

Imaging Cell-Cell Communications in Living Animals

To flow, or not to flow,
——that is the question for the first red blood cells

Professor Atsuko Sehara [left] – Institute for Frontier Medical Sciences
and Graduate School of Medicine
Assistant Professor Atsuo Iida [right] – Institute for Frontier Medical Sciences

Cells produce different kinds of cell-to-cell signaling and cell adhesion molecules. Such molecules are often generated as transmembrane proteins, and their extracellular domains are cleaved off when cells send messages or detach. This process, called “ectodomain shedding”, has come into focus since the discovery of proteases that possess this ability . Questions are in what physiological contexts do these proteases play roles and how do they manage to control shedding spatiotemporally. In order to address these questions, Prof. Atsuko Sehara and her colleagues utilize transparent zebrafish embryos, in which dynamic cell behaviors can be visualized as 3D movies. Assistant Prof. Atsuo Iida succeeded in capturing the onset of blood circulation by monitoring fluorescently labeled erythrocytes and blood vessels. Unexpectedly, the earliest circulation of blood began synchronously. This synchrony was achieved by the retention of erythrocytes in the lumen of blood vessels, and then, by their simultaneous release into the plasma flow. Erythrocytes remained attached when they were devoid of a metalloprotease named ADAM8. ADAM8 shed ectodomains of cell adhesion molecules, which caused synchronous detachment of erythrocytes from blood vessels. Thus, this study demonstrated that the first erythrocytes require both heartbeat passive and proteolysis-dependent active processes to enter the circulation.

www.frontier.kyoto-u.ac.jp/rc03/index-j.html

Production of High Quality Human Embryonic Stem Cell Lines at Kyoto University

Genetic changes mapped in a diverse sample of ES Cells by international collaboration as a major step toward medical applications

Associate Professor Hirofumi Suemori – Institute for Frontier Medical Sciences
Professor Norio Nakatsuji – Director, Institute for Integrated Cell-Material Sciences

Human embryonic stem (hES) cell lines can proliferate indefinitely and differentiate into all kinds of tissues in the body. Therefore, they are considered to have great potential in medical research and applications such as cell transplantation therapy and drug discovery. Since they began deriving hES cell lines in 2003 at the Institute for Frontier Medical Sciences, they have established five hES cell lines, named KhES-1, KhES-2, KhES-3, KhES-4 and KhES-5. They have been studied extensively and characterized in detail, and so far distributed for use in over 50 research projects. They have now started a project to produce higher quality hES cell lines which can be used in clinical applications.

However, genetic changes can occur during prolonged proliferation in culture, and they cause potential risks in clinical applications. Thus, an international collaboration by the International Stem Cell Initiative (ISCI) including our group at Kyoto University began in 2008 to identify genetic changes that occur during the culture of many ethnically diverse hES cell lines. The effort, led by Prof. Peter Andrews of the University of Sheffield, analyzed 125 human ES cell lines including our five hES cell lines and 11 human induced pluripotent stem (iPS) cell lines collected from 38 laboratories across 19 countries. The study published online in *Nature Biotechnology* on Nov 27, 2011 (vol. 29, 1132-1144) revealed that most cell lines remained karyotypically normal, but change in karyotype during prolonged culture, especially in chromosomes 1, 12, 17 and 20. Copy number analysis using SNP arrays also showed that amplification of a small genomic region on chromosome 20 was found in over 20% of cell lines. These findings can be used for the accurate and cost-effective quality control of cell lines, which are needed in the application of stem cell technologies to regenerative medicine.

www.icems.kyoto-u.ac.jp/e/pr/2011/11/28-nr1.html
www.frontier.kyoto-u.ac.jp/es01/topE.htm

Mouse Genome Protection

Understanding the role of the miwi protein

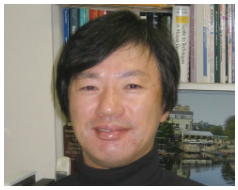
Professor Shinichiro Chuma – Institute for Frontier Medical Sciences

The germline is the cell lineage that transmits genetic information to the next generation. Genetic and epigenetic changes in the germline affect embryonic development and subsequent offspring, so the genomic stability of germ cells is a critical requirement for maintaining both the individual and the species. Damage to Genomic DNA generally occurs as a consequence of physical or chemical attacks, such as from exposure to ionizing radiation, genotoxic reagents and oxidative stress. Another threat to the genome is the encoding mechanism of the genome itself, namely in the form of mobile transposable elements, which move or duplicate themselves and transpose into new genome positions. They recently discovered, in an international collaboration led by Dr. Ramesh S. Pillai at EMBL, France, the role that a particular protein plays in protecting the genome: specifically, the means by which the Miwi protein silences transposon RNAs in male mice. Among other findings, They demonstrated conclusively that disrupting the mice's capacity to produce the functional Miwi protein with RNA slicer activity leads to an inability to create viable sperm. They provide evidence for Miwi slicer activity directly cleaving transposon messenger RNAs, offering an explanation for the continued maintenance of repeat-derived Piwi proteins interacting with small RNAs (piRNAs) long after transposon silencing is established in germline stem cells. This work builds on earlier studies of our own and by other groups, investigating genomic protection, nearly completing the picture of the Miwi-based transposon silencing mechanism, and proposes that Piwi proteins act in a two-pronged mammalian transposon silencing strategy: one promotes transcriptional repression in the embryo, the other reinforces silencing at the post-transcriptional level after birth, both of which are critical for normal male fertility.

www.kyoto-u.ac.jp/en/news_data/h/h1/news6/2011/111128_1.htm

Toward Clinical Application of iPS Cells from Patients with Genetic Disorders

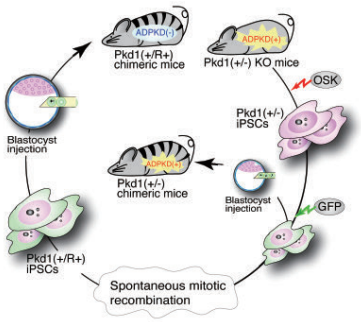
Genome Editing in Pluripotent Stem Cells



Associate Professor Takashi Tada – Institute for Frontier Medical Sciences

Induced pluripotent stem (iPS) cells, which are pluripotent stem cells reprogrammed from individual somatic cells, are anticipated to contribute to regenerative medicine as a cell source for generating replacement cells and tissues. In iPS cells, an epigenotype, but not a genotype, can be reprogrammed into a pluripotent cell type. Therefore, mutations that cause genetic disorders are not restored in iPS cells generated from patients. For therapeutic treatment of genetic disorders with iPS cells, development of a technique of genome editing in disease-specific iPS cells is required.

To demonstrate proof of principle for spontaneous genetic correction of disease-related mutation alleles through mitotic recombination, Associate Prof. Takashi Tada investigated a prevalent inherited disorder, autosomal dominant polycystic kidney disease (ADPKD), which is caused by genetic mutation of the PKD1 in 85% and PKD2 in 15% of cases clinically diagnosed by intrarenal cystogenesis. Large-scale screening for the PKD1 mutation in heterozygous iPS clones demonstrated that restoration of genetic mutation occurred spontaneously through mitotic cell divisions. Genetically restored iPS cells generated no intrarenal cysts, while parental (genetically mutated) iPS cells induced cystogenesis in chimeric mice. The mitotic recombination-mediated genetic correction approach will open a new path to clinical application for human iPS cells that is relevant to patient groups.



Dr. Tada believes that further development of the new technologies and understanding mechanisms involved in epigenetic reprogramming will advance the shift in iPS cell technology application from bench to bed.

www.frontier.kyoto-u.ac.jp/es03/index.html

Genome Features of "Dark-fly"

Molecular Mechanisms Underlying Environmental Adaptation

Research Fellow Naoyuki Fuse – Graduate School of Science

Organisms are remarkably adapted to diverse environments by specialized metabolisms, morphologies or behaviors. How organisms come to possess adaptive traits is a fundamental question for evolutionary biology. Experimental evolution studies have provided insights into the molecular mechanisms underlying environmental adaptation, but were limited mostly to bacteria that carry a small genome. Recently, next-generation sequencing technology has enabled researchers to determine the whole genome sequences of sexual organisms and is beginning to be applied to experimental evolution studies. Research Fellow Naoyuki Fuse and his research team are studying the environmental adaptation using an unusual Drosophila melanogaster line, termed "Dark-fly," which has been maintained in constant dark conditions for 57 years (1400 generations). They have found high fecundity rates of Dark-fly in darkness, and determined the whole genome sequence of Dark-fly using a next-generation sequencer. They have identified many genomic alterations and obtained a list of the potential candidate genes involved in the Dark-fly's traits. These included genes related to detoxification and light perception. Although functional analysis of each mutation remains a future issue, they are able to present a framework for linking genomic alterations to environmental adaptation. This finding was published on the PLoS ONE Website.

(www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0033288).

www.kyoto-u.ac.jp/en/news_data/h/h1/news6/2011/120315_1.htm

Figure legend:
The Dark-fly [right] looks similar to normal fly [left], but they carry many mutations in their genome.