Abstract

Session 1　Division of Metabolic Disorders

Atomic force microscopy – a versatile tool for imaging and probing microscopic mechanical properties of soft materials

Masami Kageshima (Biophysics)

Atomic force microscopy (AFM) has gained recognition as an imaging tool for surfaces of various materials ranging from solids to soft biological materials. Its force sensor is well-suited for spatially-resolved measurement of mechanical response in the form of a complex quantity, i.e. a viscoelasticity. During the course of research the author has developed a wide-band magnetic excitation AFM apparatus, with which viscoelastic response of the sample under study is obtained in a wide frequency range by exciting the force sensor with an well-controlled magnetic force. Features of the instrument will be briefly overviewed. By utilizing the potential of AFM it's possible to directly access folding/unfolding dynamics of single biopolymers through viscoelasticity. A single polymer chain is tethered between the AFM probe apex and the substrate. As it is stretched with a tensile force it undergoes structural deformation and corresponding features in its viscoelasticity profile are obtained. Examples of peptide in α-helix form, dextran, and titin, a giant protein molecule in muscle, will be shown[1]. In addition to single molecules, interest is also oriented to structure and dynamics of solid-liquid interfaces, especially those relevant to life phenomena. Response of water molecules on a hydrophilic surface will be shown. A novel pulse-response measurement technique will also be briefly introduced[2]. It should be noted that the potential of AFM as a tool for analyzing mechanical response also covers mesoscopic or macroscopic systems like cells or organisms. Proper understanding of its imaging mechanism and, if necessary, instrumentation technique will further enhance its applicability.

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[2] M. Kageshima, Beilstein J. Nanotech. **3** (2012) 260-266.

Immune regulation through the mTORC1 pathway

Satoshi Matsuda (Bioinformatics, IBS)

The mammalian target of rapamycin (mTOR) is a Ser/Thr kinase that affects broad aspects of cellular functions including immunity.　mTOR is the core component of the mTOR complex 1 (mTORC1), which contains the adapter protein Raptor, whereas Rictor and Sin1 classify mTOR complex 2 (mTORC2).　mTORC1 acts downstream of the PI3K-Akt pathway to phosphorylate p70 S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) in a rapamycin-sensitive manner.　Although the PI3K-Akt-mTORC1 axis is activated during T cell activation, its physiological role remains obscure.　Here, we demonstrate that the suppression of this axis by deletion of p85a, a predominantly expressed regulatory subunit of class IA PI3K, or Raptor, an essential component of mTORC1, impairs Th17 differentiation.　We further demonstrate that S6K is involved in the PI3K-Akt-mTORC1 axis-mediated nuclear translocation of RORg, a master transcriptional regulator for Th17 differentiation.　These results point toward a pivotal role of the PI3K-Akt-mTORC1-S6K axis in Th17 differentiation.

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2. Kurebayashi, Y., Nagai, S., Ikejiri, A., Ohtani, M., Ichiyama, K., Baba, Y., Yamada, T., Egami, S., Hoshii, T., Hirao, A., Matsuda, S., and Koyasu, S.　PI3K-Akt-mTORC1-S6K1/2 axis controls Th17 differentiation by regulating Gfi-1

expression and nuclear translocation of RORg. Cell Rep. 1: 360-373 (2012).

Analysis of JAK-STAT and cell death pathways induced by IFN-α in human adherent cancer cells

Takaya Tsuno (Radiology)

Human interferon (IFN)-α has been approved for the treatment of certain types of cancer. Nevertheless, its effectiveness has met with varying degrees of success. Currently, a number of tumors including solid carcinomas are still resistant to IFN-α. Therefore, we have investigated key molecules playing a significant role in cell viability regulated by IFN-α.

First, in the Janus kinases/Signal Transducers and Activators of Transcription (JAK-STAT) pathway initiating IFN-α signal, IFN regulatory factor (IRF) 9-RNA interference (RNAi) completely restored cell viability regulated by IFN-α in human ovarian adenocarcinoma OVCAR3 cells sensitive to IFN-α (1). IRF9-RNAi followed by IFN-α treated in OVCAR3 cells inhibited gene expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) which is one of cell death ligands (1). IFN-α also upregulated TRAIL protein level in OVCAR3 cell–microenvironment (2). In cell death pathway after binding TRAIL to the receptors, BH3 interacting domain death agonist (BID)-RNAi significantly restored cell viability regulated by IFN-α in OVCAR3 cells (2). BID-RNAi prevented IFN-α from collapsing mitochondrial membrane potential (2). Furthermore, pIRES-hBID plasmid led to significant inhibition of cell viability regulated by IFN-α in human lung carcinoma A549 cells resistant to IFN-α (2). Thus, our *in vitro* studies suggest that BID is crucial for cell viability regulated by IFN-α which can induce mitochondria-mediated apoptosis.

To assess BID overexpression for the treatment of IFN-α-resistant tumors in a living body, *in vivo* study has also been performed. Intravenous and subcutaneous injections of pIRES-BID and pegylated human IFN-α were administered in nude mice bearing subcutaneous A549 xenografts, respectively. Electron microscopy findings revealed mitochondrial apoptosis in A549 xenografts treated with pIRES-BID and IFN-α. Live imaging study detecting near-infrared fluorescent tracers also indicated apoptosis induced by pIRES-BID and IFN-α in A549 xenografts. Consequently, our current study suggests that BID is a notable potential to be a targeted therapy for IFN-α resistant tumors.

1. IRF9 is a key factor for eliciting the antiproliferative activity of IFN-α. Tsuno T, Mejido J, Zhao T, Schmeisser H, Morrow A, Zoon KC. *J Immunother.* 2009;32:803-16
2. BID is a critical factor controlling cell viability regulated by IFN-α. Tsuno T, Mejido J, Zhao T, Phillips T, Myers TG, Bekisz J, Zoon KC. *J Immunother.* 2012;35:23-31

Session 2 Division of Neuroscience

Activation of TGFβ/Smad signaling reduces aggregate formation of mislocalized TDP-43

Satoshi Kaneko (Neurology)

Background: TAR DNA binding protein of 43 kDa (TDP-43) is naturally located in the nucleus and has been identified as the major component of cytoplasmic ubiquitinated inclusions in patients with amyotrophic lateral sclerosis (ALS). We have reported that TDP-43 and phosphorylated Smad2 (pSmad2), an intracellular mediator protein of Transforming Growth Factor-β (TGFβ) signaling, are co-localized within cytoplasmic inclusions in the anterior horn cells of sporadic ALS patients.

Objective: To investigate the possible pathophysiological linkage between pathologic cytoplasmic inclusions containing TDP-43 and TGFβ/Smad signaling.

Methods: We replicated cytoplasmic aggregates of TDP-43 in HEK293T cells by transfecting the

cells with a nuclear localization signal deletion mutant of TDP-43 and inhibiting proteasome activity, and assessed the effect of TGFβ/Smad signaling on the cytoplasmic aggregate formation.
Results: The aggregates contained ubiquitinated, phosphorylated, and fragmented TDP-43, consistent with the essential features of the human pathology. Moreover, the aggregates were co-localized with pSmad2 under continuous TGFβ stimulation. Overexpression of Smad2 reduced the amount of cytoplasmic aggregates in HEK293T cells, and TGFβ stimulation augmented this reduction effect in a dose-dependent manner.
Conclusion: Activation of TGFβ/Smad signaling system is protective against aggregate formation of cytoplasmically mislocalized TDP-43 and may be a potential therapeutic approach to delay progression of ALS.

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2. Nakamura M, Kaneko S, Wate R, Asayama S, Nakamura Y, Fujita K, Ito H, Kusaka H. Regionally different immunoreactivity for Smurf2 and pSmad2/3 in TDP-43-positive inclusions of amyotrophic lateral sclerosis. Neuropathol Appl Neurobiol. 2012 Mar 21. PMID: 22435645.

Two separate neural systems for understanding other's actions

Masaki Isoda (Cognitive Neuroscience)

Understanding the action of other individuals is of critical importance in the social world as it allows for a grasping of their mental states, such as beliefs, desires, and intentions. It has been proposed that the brain has two separate systems for such action understanding — the mirror system and the mentalizing system. The mirror system mainly consists of the ventral premotor cortex and inferior parietal lobule and is hypothesized to play a role in understanding *what* others are doing via embodied simulation. The mentalizing system, on the other hand, is mainly composed of the medial frontal cortex and temporoparietal junction and assumed to play a part in understanding *why* others are doing via mental simulation. These speculative roles are advocated on the basis of macro-level analyses of human neuroimaging data. Accordingly, it remains unclear whether and how response properties of *individual neurons* differ between the two systems during action observation. Answering this question requires use of experimental animals and methodologies with a much higher spatiotemporal resolution. In this talk, I will review single-neuron recording data obtained in the monkey brain, showing that mirror neurons nondifferentially respond to the actions of one's own and those of others, whereas mentalizing neurons can selectively respond to each of them. Thus, it seems likely that the brain is equipped with dual systems that complement each other for understanding others: the mirror system whereby one can view others as analogous to oneself and the mentalizing system whereby one can identify them as unique.

1. Yoshida K, Saito N, Iriki A, Isoda M (2012) Social error monitoring in macaque frontal cortex. ***Nature Neuroscience*** 15: 1307-1312.
2. Yoshida K, Saito N, Iriki A, Isoda M (2011) Representation of others' action by neurons in monkey medial frontal cortex. ***Current Biology*** 21: 249-253.

Visualization of "hypoxia" *in vivo*: imaging of HIF-1 activation

Kiichi Hirota (Anesthesiology)

For all organisms, changes in oxygen (O_2) concentration represent a fundamental physiologic stimulus. Particularly in vertebrate animals, either deficiency or excess of O_2 elicits acute (rapid-onset and short-term) and chronic (delayed- onset and long-term) responses.
Oxygen plays a critical role in cellular homeostasis and many human diseases, including ischemic

diseases and cancer, are characterized by inadequate tissue oxygenation. In the former, lack of oxygen contributes to cell death and, in the latter, lack of oxygen is an early signature of disease that might also affect malignant cell behavior. Oxygen deprivation in human tissue leads to the up-regulation of a complex array of genes that exert adaptive functions, particularly with regard to cellular metabolism and improvement in O_2 delivery.

One of the most important transcription factors controlling O_2-tension regulated gene expression is hypoxia-inducible factor 1 (HIF-1). HIF-1 is a heterodimer containing the constitutively expressed HIF-1β subunit and the inducible HIF-1α subunit, which binds the consensus sequence hypoxia responsive element (HRE) in the expression regulatory region. Regulation of HIF-1 activity occurs at multiple levels in cells, and mechanisms regulating HIF-1α protein expression and transcriptional activity have been extensively analyzed. The von Hippel–Lindau tumor suppressor protein has been identified as a HIF-1α-binding component of an ubiquitin–protein ligase that targets HIF-1α for proteasomal degradation in non-hypoxic cells. Under hypoxic conditions, hydroxylation of HIF-1-specific proline and asparagine residues is inhibited due to substrate (O_2) limitation, resulting in HIF-1α protein stabilization and transcriptional activation.

This time, I will talk about the transgenic mice that carry HRE/ODD(oxygen-dependent degradation domain)-luciferase (HOL) gene that generates bioluminescence in an HIF-1-dependent manner and its successful usage to monitor HIF-1 activity in ischemic tissues and postnatal hypoxia.

1.Harada H et al. .Cancer cells that survive radiation therapy acquire HIF-1 activity andtranslocate towards tumour blood vessels. Nat Commun 2012;3:783.

2.Kadonosono T, et al. Detection of the onset of ischemia andcarcinogenesis by hypoxia-inducible transcription factor-based in vivo bioluminescence imaging. PLoS ONE 2011;6:e26640.

Molecular Imaging for Diseases Using Mass Spectrometry

Ikuko Yao (Molecular and Functional Biology)

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) provides the means for analysis of a wide variety of biomolecules. The technology, a combination of MALDI-MS and microscopy which is known as imaging mass spectrometry (IMS), provides us a novel opportunity to visualize molecules directly on tissue surfaces without the need for target-specific reagents such as antibodies. As most of previous IMS studies analyzed the relative abundances of metabolite species, it is important to expand its application to various biomolecules for exploring molecular basis of diseases. Here, we will show some examples of IMS application to diverse types of biomolecules.

We applied IMS technique to directly detect neurotransmitters in the central nervous system (CNS) and visualize their localization in tissue samples. In MS/MS imaging, the transmitter could be visualized with a high signal/noise ratio (S/N) by elimination of matrix interference. In the tandem MS-imaging analysis of ACh in the mouse spinal cord, reconstructed ion intensity maps for ACh ion showed that the ion which corresponds to ACh distributed around large motor neurons in the spinal ventral horn. Similarly, we analyzed mouse brain sections with tandem MS imaging. In comparison with the previous reports, it was revealed that ACh was localized in the region abundant in acetylcholinestelase. These results show that ACh is the suitable molecular species to detect in positive ion detection mode by IMS, and that tandem MS imaging is useful for the analyses of cholinergic neurons in the postmortem brain of neurodegenerative patients.

We also applied the IMS technique to formalin-fixed paraffin-embedded (FFPE) samples to identify a substance(s) responsible for the intestinal obstruction caused by an unidentified foreign body. In advance of IMS analysis, some pre-treatments were applied. After deparaffinization of sections, samples were subjected to enzyme digestion. The sections co-crystallized with matrix were desorbed and ionized by a laser pulse with scanning. A combination of enzyme digestion and the 2,5-dihydroxybenzoic acid (DHB) matrix gave best mass spectrum. These results for the first time show that MALDI-IMS is an accurate technique with high sensitivity for direct analysis of

oligosaccharides in histopathological samples.

Our study shows that the direct detection of biomolecules will be useful for assessments such as pathogenesis and evaluations of therapeutic strategies.

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2) Yamada M, Yao I, Hayasaka T, Ushijima M, Matsuura M, Takada H, Shikata N, Setou M, Kwon AH, Ito S. Identification of oligosaccharides from histopathological sections by MALDI imaging mass spectrometry. Anal Bioanal Chem. 2012 Feb;402(5):1921-30.

Session 3　Division of Cancer

Clinical applications of indocyanine green-fluorescence imaging to liver surgery

Morihiko Ishizaki (Surgery)

BACKGROUND: Recently, some studies have reported the usefulness of intraoperative fluorescent imaging using indocyanine green (ICG) for the detection of sentinel nodes in breast and gastric cancer. These techniques are based on the finding that ICG binds to plasma proteins and protein-bound ICG emits near-infrared light. Fluorescent imaging using ICG also has the potential to detect liver cancers through the visualization of the disordered biliary excretion of ICG in liver cancer tissues compressed by the tumor, and to preventing postoperative bile leakage by using ICG fluorescent cholangiography.

METHODS: Intravenous injection of the ICG reagent was performed as a liver function test 1 to 2 weeks prior to surgery. ICG fluorescent imaging was performed using the Photodynamic Eye (PDE) infrared camera (Hamamatsu Photonics k. k., Shizuoka, Japan). 102 patients who underwent hepatic resection without biliary reconstruction were divided into 2 groups. The control group (n = 50) underwent a leak test with ICG dye alone, and the experimental group underwent a leak test with ICG dye, followed by ICG fluorescent cholangiography using the PDE (PDE group, n = 52).

RESULTS: ICG fluorescent imaging was useful for liver surgical navigation as follows: three cases of recurrent hepatocellular carcinoma(HCC) after transcatheter arterial chemoembolization or thermal ablation therapy; three cases of colorectal metastatic liver tumors after chemotherapy as conversion therapy; two cases of intrahepatic cholangiocellular carcinoma (ICC); and two cases of surface HCC that were not detected by preoperative computed tomography (CT) scan. In the ICC cases, although dilatation of the bile duct could be detected, the tumor margins were ambiguous by ultrasonography(US). ICG fluorescent imaging clearly described the dilatation of the bile duct including the tumor. Among 42 patients with fluorescence in the PDE group, 25 patients had insufficient closure of bile ducts on the cut surface of the liver, which were closed by suture or ligation. There were 5 patients who developed postoperative bile leakage in the control group versus no bile leakage in the PDE group (10% vs 0%, P = .019).

CONCLUSIONS: ICG fluorescent imaging was useful for liver surgical navigation including selection of the mode of hepatic resection. ICG fluorescent cholangiography may have useful potential for prevention of bile leakage after hepatic resection.

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2. Kitai T, et al. Fluorescence navigation with ICG for detecting sentinel lymph nodes in breast cancer. Breast Cancer 2005; 12: 211-215.

Mst1 regulates integrin-dependent thymocyte trafficking and antigen recognition in the thymus

Yoshihiro Ueda (Molecular Genetics, IBS)

Thymocyte trafficking has an important role in thymic selection. Here we show that the Hippo homologue Mst1 is required for thymocyte migration and antigen recognition by LFA-1 and ICAM-1 within the medulla. Using two-photon imaging of thymic tissues, we found that highly motile mature thymocytes arrest and are activated in the vicinity of rare populations of Aire(+) ICAM-1(hi) medullary thymic epithelia in a negatively selecting environment. Notably, Mst1 deficiency or blocking the cell adhesion molecules LFA-1 and ICAM-1 results in inefficient migration and antigen recognition of CD4(+) thymocytes within the medulla. Consistent with these defects, thymocyte selection is impaired in Mst1(-/-) mice, which display T cell-dependent inflammatory infiltrates in multiple organs and develop autoantibodies. Our results suggest that Mst1 has a key role in regulating thymocyte self-antigen recognition in the medulla.

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2) Katagiri K, Katakai T, Ebisuno Y, Ueda Y, Okada T, Kinashi T. Mst1 controls lymphocyte trafficking and interstitial motility within lymph nodes. EMBO J. 2009 May 6;28(9):1319-31.

Development of a highly efficient method for isolating bone-derived small stem cells identified in adult mouse bone

Ryusuke Nakatsuka (Stem Cell Biology)

(Background) Ratajczak and his colleagues identified a unique population of very small embryonic-like (VSEL) stem cells in adult mouse bone marrow (BM) (Leukemia 2006:20;857). However, it is difficult to isolate these small stem cells (SSC) in BM very effectively. This study describes our recently developed highly efficient method for isolating SSCs using enzymatic treatment of murine bone. **(Materials and Methods)** The bone tissue from 8 week-old C57BL/6 mice were crushed in a mortar and then incubated in cell dissociation buffer. Next, the BM nucleated cells (BMNC) and bone-derived nucleated cells (BDNCs) were stained with various monoclonal antibodies and then were used for subsequent FACS analyses. **(Results)** The incidences of SSCs in the BMNCs and BDNCs were 0.001% and 0.1%, respectively. Therefore, the enzymatic treatment of bone tissues yielded about 100 times the efficiency for the isolation of SSCs. The bone-derived (BD) SSCs were small and possessed a relatively large nucleus surrounded by a narrow rim of cytoplasm. However, the gene expression profile of the BD-SSCs was clearly distinct from the well-defined populations of ES cells, KSL cells, and MSCs. The number of these BD-SSCs significantly increased after the induction of liver injury by carbon tetrachloride administration. They were then most likely mobilized into the peripheral blood. **(Conclusion)** The present data suggest that the majority of the Lin-Sca-1+CD45- cells reside in the bone tissue. The gene expression profile of BD-SSCs was different from those of the previously reported BM-derived SSCs (VSELs) while BD-SSCs resemble BM-derived VSELs.

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2. A population of very small embryonic-like (VSEL) CXCR4+SSEA-1+Oct-4+ stem cells identified in adult bone marrow Leukemia, 20, 857-869, 2006.