Abstract

Outline and prospect of Molecular Imaging Center of Diseases

Seiji Ito (Molecular and Functional Biology)

Molecular Imaging Center of Diseases was established in September 2011 upon adoption of the program for the strategic research foundation at private universities supported by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan. The Center aims to continue the former 5-year project “Interactive translational research toward the treatment of intractable neural diseases by restoration and regeneration” carried out in Brain Medical Research Center at Takii campus in 2006－2011 and expand translational research between basic and clinical departments in a wide-range field of medicine in Hirakata new campus which will open in April 2013. We plan to establish the Center on the 5th floor of the mid-rise building in Hirakata new campus and endeavor to make it a research foundation capable of pursuing interdisciplinary and strategic research. The objectives of the Center are (1) to systematically elucidate pathogenesis of various diseases by use of animal disease models and patient samples from molecular to individual levels, (2) to translate the basic research on diseases into clinical application, by making the most of state-of-the-art imaging systems including two-photon microscopy and imaging mass spectrometry, and (3) to cultivate young researchers. To accomplish them, the Center consists of three divisions of Neuroscience, Cancer, and Metabolic Disorders, each of which is managed by two division heads assigned from basic and clinical fields and a supporting division. The supporting division helps project members to promote research activities and cultivate young researchers. The research activities will be evaluated by advisory board members from outside. I will introduce examples of cooperative research and overview the prospect of the Center.

Session 1 Division of Metabolic Disorders

Development and regeneration of elastic fiber

Tomoyuki Nakamura (Molecular Pharmacology)

Elastic fibers are ubiquitous extracellular matrix (ECM) components responsible for tissue elasticity. Degradation of elastic fibers causes emphysema, arterial stiffness and loose skin. The turnover rate of elastic fibers is very low, and there is no method to effectively regenerate elastic fibers. The process of elastic fiber assembly involves elastin monomer (tropoelastin) deposition on microfibrils, followed by cross-linking of tropoelastin by lysyl oxidase. As the turnover rate of elastic fibers are very low, elastic fiber regeneration has been considered to be difficult.

We found that at least three secreted proteins, fibulin-4 and -5, and LTBP-4 (latent TGFβ-binding protein 4), play essential roles in the elastogenesis process. Mouse arteries that lack either of these gines were stiff, and the elastic lamellae were disorganized. We show independent roles of fibulin-4 and -5 in elastic fiber assembly that do not overlap each other, and an LTBP-4-dependent role of fibulin-5.

Genetic approaches to identify new targets of molecular imaging for kidney and neurodevelopmental disorders

.Hiroyasu Tsukaguchi (Internal Medicine 2)

Chronic kidney diseases and neurodevelopmental diseases (i.e., mental retardation, epilepsy) are major health concerns with an overall prevalence of ~ 1-2% worldwide. The development of simpler, non-invasive molecular imaging techniques, visualizing the spatio-temporal progression of disease processes, will greatly stimulate the generation of new therapeutic strategies for these common disorders. In this study, we reasoned that understanding the pathophysiological basis of rare mendelian disorders is the best way to select novel target molecules suitable for imaging studies in more common disorders.

To address this issue, as a first step, we searched as-yet-undefined genes causing a rare congenital disorder of Galloway-Mowat syndrome (GMS, MIM 251300). GMS is characterized by co-occurrence of malformation in central nerve systems (CNS) and renal glomeruli. Clinically, GMS patients typically exhibit two core manifestations consisting of (1) nephrotic syndrome progressing to end stage renal disease, and (2) psychomotor retardation and/or epilepsy. In ten Asian GMS patients, we screened the exomes and structural variations across the whole genome. Several suspicious candidate genes are now on the way of being validated for their biological significance. To gain more insights into the pathophysiology underlying GMS, we generated patient-specific iPS cells from one family (3 clones under 12 passages, in collaboration with Prof. Era's lab, Kumamoto University).

A neuron and glomerular podocyte share a common biological feature of terminally differentiated cells that exert their function through formation of specialized cell-cell junctions (i.e., synapse and slit diaphragm). Therefore, diseases pathways underlying GMS, e.g., metabolic activity, cell proliferation, apoptosis, and migration, etc. are likely shared by these two cellular lineages. Identification of GMS genes will open new avenue to create a real-time, noninvasive imaging system that detect an unique biological process indispensable for building up a proper tissue architecture of renal glomeruli and CNS.

The specific linker phosphorylation of Smad2/3 indicates epithelial stem cells in colon; particularly increasing in the regeneration phase of murine model of dextran sulfate sodium-induced colitis

Toshiro Fukui (Internal Medicine 3)

Stem cells play roles in mucosal cell homeostasis and repair. Although they are supposed to be located around the crypt base of colon, useful markers have not been elucidated. Previously, we have identified the significant expression of Smad2/3, phosphorylated at specific linker threonine residues (pSmad2/3L-Thr), in specific epithelial cells of murine stomach and have suggested these cells to be stem cells. (J Gastroenterol 2011:46:456-68) In the present study, we explored whether pSmad2/3L-Thr could serve as a marker for stem cells of colon.

Normal colons from C57BL/6 or Lgr5-EGFP knock-in (Lgr5-EGFP-IRES-CreERT2) mice and colons from murine model of dextran sulfate sodium (DSS)-induced colitis were examined. In normal colons, pSmad2/3L-Thr immunostaining-positive cells were found around the crypt base and detected between Ki67 immunostaining-positive cells, but immunohistochemical co-localization of pSmad2/3L-Thr with Ki67 was never observed. pSmad2/3L-Thr immunostaining-positive cells showed co-localization with cytokeratin8 and CDK4, different localization with ChromograninA, and similar localization with doublecortin and calcium/calmodulin-dependent protein kinase-like-1 (DCAMKL1) immunostaining-positive cells. In DSS-induced colitis, both pSmad2/3L-Thr and Ki67 immunostaining-positive cells were significantly increased in the regeneration phase and decreased in the injury phase compared with those of normal C57BL/6 mice. In colon of Lgr5-EGFP knock-in

mice, most pSmad2/3L-Thr immunostaining-positive cells showed co-localization with Lgr5 (GFP), but a small number of pSmad2/3L-Thr immunostaining-positive cells showed different localization with Lgr5. After immunofluorescent staining, we stained the same sections with hematoxylin-eosin and observed pSmad2/3L-Thr immunostaining-positive cells indicated undifferentiated morphological features and were confirmed around the crypt base. 5-bromo-2-deoxyuridine (BrdU) labeling assay was used to examine label-retention (multiple BrdU pulses; three times a day for 2 days) of pSmad2/3L-Thr immunostaining-positive cells. Some pSmad2/3L-Thr immunostaining-positive cells showed co-localization with BrdU at 5 d, 10 d, and 15 d after BrdU administration.

We have identified the significant expression of pSmad2/3L-Thr in specific epithelial cells of murine colon and have suggested these cells to be stem cells.

Dendritic cells as therapeutic targets of autoimmune diseases

Tomoki Ito(Internal Medicine 1)

Dendritic cells (DCs) are widely distributed in the body to enhance the innate immune response by cytokine production through antigen recognition and initiate acquired immunity by priming naïve $CD4^+$ T cells. Therefore, DCs are the master cells in activating immune responses. Plasmacytoid dendritic cells (pDCs), one of the human DC subsets, play not only a central role in antiviral immune response in innate host defense but also a pathogenic role in the development of the autoimmune responses by vicious spiral based on the dysregulated type I IFN production through sensing immune complexes containing self-nucleic acids. Thus, type I IFNs and pDCs represent key molecular and cellular pathogenic components in autoimmune diseases such as systemic lupus erythematosus (SLE). Accordingly, control of the dysregulated pDC-derived type I IFN production provide an alternative treatment strategy for SLE. We focused on two agents, IκB kinase inhibitor (BAY11-7082) and HMG-CoA reductase inhibitors (statins), and investigated their immunomodulatory effects in targeting the IFN response on pDCs.

Our study identified that both BAY11 and statins have the ability to inhibit nuclear translocation of IRF7 and IFN-α production in human pDCs. We also found that these agents inhibited both in vitro IFN-α production induced by the SLE serum and the in vivo serum IFN-α level induced by injecting mice with TLR ligand. These findings suggest that BAY11 and statins have the therapeutic potential to attenuate the IFN environment by regulating the pDC function and thus provide the novel foundation for the development of an effective immunotherapeutic strategy against autoimmune disorders such as SLE.

Session 2　Division of Neuroscience

The degradation of the inwardly rectifying potassium channel, Kir2.1, depends on the current level

Masayoshi Okada (Cellular and Molecular Physiology)

Protein expression is regulated by synthesis as well as degradation. Inwardly rectifying K^+ channel (Kir2.1) regulates the excitability of nervous, cardiac, and

vascular smooth muscular cells, and its expression level is accurately regulated. In fact, both loss- and gain-of-function mutations of Kir2.1 result in arrhythmia. But little is known about the degradation of Kir2.1. To examine the degradation of Kir2.1, we constructed Kir2.1 fusion proteins with SNAP-tag and fluorescent timer (FT). The SNAP-tag, which covalently binds to a specific membrane permeable fluorescent dye, allows us to conduct a pulse-chase experiment with fluorescence. Our pulse-chase experiments revealed the current-dependent degradation of Kir2.1 by following results: 1) shorter half-life of SNAP-Kir2.1 in high expression cells; 2) elongation of half-life by the blockade of Kir current; and 3) shorter half-life in a hyper-conductive mutant of Kir2.1. The fluorescence of FT changes spontaneously and slowly from green to red; thereby the green/red ratio allows us detect the changes in the half-life of the FT-fusion protein. Expectedly, the green/red ratio of FT-Kir2.1 was decreased by the channel blockade, confirming the current-dependent degradation. Our results suggest a presence of K^+ sensor and the usefulness of these two methods, which are in vivo applicable and do not need radioisotope or antibody, in studying neurodegenerative diseases.

We also introduce the outlines of other ongoing projects.

1. Compensation for the reduced activity in the hippocampal newborn neurons.
2. Enhancement of the titer of lentiviral vectors, which express K^+ channels.
3. High-though-put assay for the Kir2.1 using GFP.
4. Screening for Kir blocker and opener in Mexican Scorpion venom.
5. Lenti- and Retro-viral vectors specific for somatotroph.
6. Ion channels involved in the secretion of HCO_3^- from pancreatic duct cell.
7. Lentiviral vector specific for the secretin receptor expressing cells.
8. Ca^{2+} imaging and plasticity of the hippocampal GABAergic synapses.

Cell type specific expression of lamin subtypes in rat retinal neurons

Taketoshi Wakabayashi (Anatomy and Cell Science)

The nuclear lamins are type V intermediate filament proteins and the major component of the nuclear lamina. They are grouped into two subtypes, A-type, which include lamins A and C, and B-type, which include lamins B1 and B2. Lamins support nuclear membrane mechanically and are also essential for proper cellular functions, including DNA replication, gene expression and differentiation. Abnormal expression of lamins causes several inherited diseases including muscular dystrophy and neuropathy. However, the expression patterns of lamin subtypes have not been well studied, especially in the nervous system. In our laboratory, we have reported the expression patterns of lamin subtypes in neurons during adult neurogenesis.

Retina belongs to the central nervous systems. Because of its well-organized cellular architecture and relative simplicity, the retina has been used as a representative model for studying neuronal cells in the central nervous system. Here, we report the immunohistochemical expression patterns of lamin subtypes in retinal neurons of adult rat. All retinal neurons expressed lamin B1 and B2 in relatively equal amounts. By contrast, the expressions of A-type lamins were differentially regulated depending on the cell types. Photoreceptor cells did not show any immunoreactivity for anti-lamins A and C antibodies. Other retinal neurons, which include retinal ganglion cells, amacrine cells, bipolar cells and horizontal cells, were all positive for lamin C. Lamin A is expressed only in horizontal cells and small populations of retinal ganglion cells. The differential expression pattern of

lamin subtypes in retinal neurons suggests that they may be involved in cellular differentiation and expression of cell-specific genes in individual retinal neurons.

Analysis of the brain digitalis-like substance underlying hypertension

Hakuo Takahashi (Clinical Sciences and Laboratory Medicine)

The central nervous system has a key role in regulating the circulatory system by modulating the sympathetic and parasympathetic nervous systems, pituitary hormone release, and the baroreceptor reflex. Digoxin- and ouabain-like immunoreactive materials were found more than 20 years ago in the hypothalamic nuclei. These factors appeared to localize to the paraventricular and supraoptic nuclei and the nerve fibers at the circumventricular organs and supposed to affect electrolyte balance and blood pressure. The turnover rate of these materials increases with increasing sodium intake. As intracerebroventricular injection of ouabain increases blood pressure via sympathetic activation, an endogenous digitalislike factor (EDLF) was thought to regulate cardiovascular system-related functions in the brain, particularly after sodium loading. Experiments conducted mainly in rats revealed that the mechanism of action of ouabain in the brain involves sodium ions, epithelial sodium channels (ENaCs), the renin–angiotensin–aldosterone system (RAAS) and oxidative stress, all of which are affected by sodium loading. Rats fed a high-sodium diet develop elevated sodium levels in their cerebrospinal fluid, which activates ENaCs. Activated ENaCs and/or increased intracellular sodium in neurons activate the RAAS; this releases EDLF in the brain, activating the sympathetic nervous system. The RAAS promotes oxidative stress in the brain, further activating the RAAS and augmenting sympathetic outflow. Angiotensin II and aldosterone of peripheral origin act in the brain to activate this cascade, increasing sympathetic outflow and leading to hypertension. Thus, the brain Na+–ENaC–RAAS–EDLF axis activates sympathetic outflow and has a crucial role in essential and secondary hypertension. Major antihypertensive agents available recently are all acting at the central sites to reduce sympathetic activity and blood pressure. However, the exact site of actions and the cascade operating in the brain are left undetermined. Our project aims to anatomically visualize the digitalislike substance and the related bioactive materials in the brain to elucidate the mechanism of actions in hypertension.

Analysis of intracytoplasmic inclusions of neurodegenerative diseases by imaging mass spectrometry

Satoshi Kaneko (Neurology)

The presence of insoluble protein aggregates in the cytoplasm of neurons is a key pathological feature of most neurodegenerative diseases. In several disorders, the disease-specific pathological proteins were identified. However, the normal and pathological functions of these proteins are largely unknown. Through this project we are planning to analyze biomolecules in relation to intracytoplasmic inclusions using imaging mass spectrometry (IMS). Prior to analysis of human brain section by IMS, we performed immunohistochemical studies on intacytoplasmic inclusions of neurodegenerative diseases to obtain further preliminary findings.

1) We investigated a family manifesting amyotrophic lateral sclerosis (ALS) with a heterozygous E478G mutation in the optineurin (*OPTN*) gene. TAR DNA-binding protein 43 (TDP-43)-positive neuronal intracytoplasmic inclusions were reactive with anti-ubiquitin and anti-p62 antibodies, but negative for anti-OPTN antibody. Immunoreactivity of nuclear TDP-43 was reduced in inclusion-bearing cells. Fragmentation of Golgi apparatus was identified in 70% of the

anterior horn cells. The presence of anterior horn cells with preserved nuclear TDP-43 and a fragmented Golgi apparatus indicates that patients with the E478G *OPTN* mutation would manifest fragmentation of Golgi apparatus before loss of nuclear TDP-43.

2) Immunohistochemical analysis revealed that all the basophilic inclusions (BIs) were positive for OPTN, *Fused* in *sarcoma* (FUS) and myosin VI in sporadic basophilic inclusion body disease (BIBD) patients and familial ALS with FUS mutation. However, the BIs showed no immunoreactivity for TDP-43 or SOD1. Therefore, OPTN associates with each of the 3 major ALS-related proteins, i.e., TDP-43, SOD1, and FUS. FUS is known to act as a co-activator of NF-κB, and OPTN negatively regulates NF-κB activation. Therefore, sequestration of both FUS and OPTN in BIs may induce dysregulation of NF-κB activation, leading to neurodegeneration. Moreover, OPTN and myosin VI play a role in the maintenance of Golgi apparatus. Their sequestration in BIs may be an underlying pathomechanism of fragmented Golgi apparatus.

3) We demonstrated for first time that Smad ubiquitination regulatory factor-2 (Smurf-2) is co-localized in the phosphor-tau inclusions in progressive supranuclear palsy (PSP). As Smurf-2 is an E3 ligase of Smad2, intracellular signaling component of TGFβ, dysregulation of TGFβ/Smad signaling may be an underlying pathomechanism of PSP.

These findings suggest that fragmentation of Golgi apparatus, impairment of the RNA quality control system due to mislocalized RNA binding proteins, dysregulation of NFκB activation and/or disturbed TGFβ/Smad signaling may take part in pathological processes of neurodegeneration.

The challenges of neurodegenerative disease modeling using patient-specific iPSCs

Masahito Shimojo (Molecular and Functional Biology)

Neurodegenerative diseases such as Huntington's disease (HD), Alzheimer's disease, and amyotrophic lateral sclerosis are progressive diseases involving the degeneration of selected neurons. Molecular biological analyses are advancing our understanding of intractable neurodegenerative diseases, but translating them to the treatment of diseases is slow. For example, HD is a choreic psychiatric neurodegenerative disease caused by a mutation in the huntingtin protein (Htt) in which expansion of CAG tract at the 5'-end of the gene is translated into a toxic polyglutamine (polyQ) stretch. The pathogenic mechanisms induced by polyQ-Htt are still not clearly understood and lack adequate models to investigate. It has been reported that Htt interacts with a key transcription factor, RE1-silencing transcription factor (REST). REST is a major regulator of many neuronal genes repressing their expression. The interaction between Htt and REST was found to retain REST in the cytosol, thereby preventing REST target gene repression. Several lines of evidence indicate that polyQ-Htt increases transcriptional repression by REST in HD. This results in increased occupancy of multiple RE1s in different genes and would cause a number of REST target microRNAs and neuronal genes to be aberrantly repressed, which now provides us with a target for developing treatments for HD. However we are lacking adequate systems for further advancing these studies in human-derived material.

The development of therapeutic strategies for treating diseases has relied heavily on the use of animal models. However they are limited in their ability to recapitulate the human disease. Recent molecular technologies could provide human materials for studying human disease through differentiating human stem cells into disease-specific cell types; especially disease-specific induced pluripotent stem cells (iPSCs). The iPSCs can be generated by reprogramming fibroblasts derived from individuals with a specific neurodegenerative disease. Generation and characterization of

iPSCs from patients have real advantages due to their ability to be differentiated into specific cell types such as neurons and retain the defect causing the disease. In neurodegenerative diseases, such affected cells cannot be collected from patients but can be generated from disease-specific iPSCs. By using iPSCs for disease modeling, the genetic information contributing to neurodegeneration, is retained in in vitro studies.

Thus the generation of disease-specific iPSCs from patients with currently incurable diseases is a promising approach for studying the mechanism of a particular disease at the cellular and molecular levels and for developing new drugs and therapeutic targets.

Session 3 Division of Cancer

Prospectively isolated novel PDGFRα and Sca-1 double positive mouse dental pulp stem cells exist in the tooth-forming niche

Yoshiaki Sonoda, (Stem Cell Biology and Regenerative Medicine)

Mouse incisors erupt continuously throughout the life of the animal. Tooth-derived stem cells are thought to exist in the apical end of the mouse incisor to mediate this process. Dental pulp stem cells (DPSCs) are considered to be one of the tooth-derived stem cells. The isolation of DPSCs was formerly carried out by cell attachment to cell culture dishes, thus resulting in the isolation of unpurified heterogeneous DPSCs. On the other hand, a previous study showed that mouse bone marrow-derived mesenchymal stem cells (MSCs) could be prospectively isolated by fluorescence activated cell sorting (FACS) using MSC markers. In this study, we successfully isolated mouse DPSCs from mandibular incisors by FACS using the MSC markers, PDGFRa and Sca-1. Mouse PDGFRa and Sca-1 double positive DPSCs exhibited a high growth potential in vitro and in vivo. In addition, the DPSCs expressed odontoblast progenitor cell markers and they showed the ability to differentiate into osteocytes, chondrocytes and adipocytes. An immunofluorescence analysis of the apical end of mouse incisors revealed that PDGFRα^{+}Sca-1^{+} cells are present in the cervical loop (CL) of the incisors and the region surrounding the CL. In conclusion, these results revealed that the mouse PDGFRα^{+}Sca-1^{+} DPSCs possess the potential to differentiate into osteocytes, chondrocytes and adipocytes. Furthermore, our findings suggest that PDGFRα^{+}Sca-1^{+} DPSCs exist in the niche for tooth-forming cells, because these cells localized in the CL of the incisors, and exhibited high growth potential.

Analysis of ATL development in HTLV-1 infected humanized mouse model

Jun-ichi Fujisawa (Viral Oncology)

Adult T-cell leukemia (ATL) is an aggressive malignancy of mature CD4+ CD25+ T cells that affects about 5% of individuals infected with HTLV-1 thirty to fifty years after neonate infection. The molecular and genetic factors induced by HTLV-1 during such a long latent period remain unclear, in part from the lack of an animal model that accurately recapitulates leukemogenesis. To understand the mechanism of ATL development, we established a mouse model of HTLV-1 infection by using the humanized mouse established by the intra-bone marrow transplantation of NOG-SCID mouse (IBMI-huNOG) with CD133+ hematopoietic stem cells purified from human cord blood.

Human T-lymphocytes differentiate normally in the spleen and the peripheral blood of IBMI-huNOG mice in several months after transplantation and peritoneal injection of HTLV-1 producing cells resulted in the leukemic growth of CD4+ CD25+ T-lymphocytes and led to the hepatosplenomegaly three to four months after infection. In the late period of infection, cells with highly lobulated or flower-shaped nuclei appeared in the peripheral blood as seen in ATL patient.

Oligoclonal expansion of infected T cells was observed and common T-cell clones with identical integration sites were identified in CD25- and CD25+ populations, indicating the conversion from CD25- to CD25+ in infected CD4+ T-cell populations in the course of selective growth. Although Tax protein of HTLV-1 has been thought to be associated with the leukemogenesis, expression of Tax was observed in CD25- population but not in CD25+ population. It was therefore suggested that Tax might enhance the proliferation of CD25- CD4+ T-lymphocytes in early phase of infection but that some epigenetic change should be involved in the outgrowth of CD25+ CD4+ T-lymphocytes in the late phase.

Thus, the HTLV-1 infected huNOG mouse model should provide a valuable system for the analysis of ATL pathogenesis and the development of treatments against various HTLV-1 associated diseases.

Application of MALDI-imaging mass spectrometry to esophageal and gastric cancer research

Masanori Yamada (Surgery)

Acute abdomen obstruction is a common disease but may threat life occasionally. In the present case, she had severe abdominal pain and deteriorated rapidly in a shorter period. Abdominal CT scanning demonstrated a high density mass in the dilated small intestine and air-fluid level formation without free air. We did not know what caused acute abdomen obstruction. Laparoscopic operation was performed to excise the inflamed small intestine and the obstructive material. For imaging MS analysis, the surgically isolated intestinal portion and the foreign body were fixed and paraffin embedded as performed routinely at Kansai Medical University Hospital. With direct MALDI-TOF MS imaging, we identified *mochi* as the obstructing material in a small intestinal obstruction. This is the first case of identification of obstructing material in direct MS analyses of histopathological samples. MALDI-IMS is a very rapid and accurate technique with high sensitivity for direct analysis of materials in histopathological samples.

The liver is a frequent site of metastasis from gastric cancer and its rate is from 5 to 10%. The hepatic resection or re-resection is thought to be a benefit for patients if gastric cancer and its metastasis are well controlled, and liver metastasis is solitary or restricted to one lobe. However, hepatic resection is carried out in less than 10% of patients with metastasis because of multiple hepatic nodules or associated peritoneal dissemination. Our prospect of research in Molecular Imaging Center of Diseases is to detect a biomarker for liver metastasis with direct MALDI-TOF MS imaging, which can help us perform appropriate therapy for patients by early diagnosis of metastasis.

Live imaging of high-speed lymphocyte migration in lymph node stromal network by two-photon microscopy

Tomoya Katakai (Molecular Genetics, IBS)

Lymph node (LN) is a small organ for immuno-surveillance filtrating and monitoring foreign substances (antigens) in lymph drainage from peripheral tissues. Live imaging using two-photon laser scanning microscope (TP-LSM) is a valuable technique for addressing the behavior of fluorescent-labeled motile cells inside the tissues. Applying TP-LSM to live LN has revealed that lymphocytes within the tissue parenchyma robustly migrate searching for antigens. Especially, T cells are moving at more than 10mm/min on average velocity. Tissue structure of LN is supported by the three-dimensional (3D) network of non-hematopoietic stromal cells, which has been suggested to be involved in the interstitial T cell migration. However, details of the interaction between T cells and stromal cells, as well as underling molecular mechanism for the efficient, high-speed movement of T cells remain unclear. To address these issues, we set out to establish a TP-LSM-based imaging system using LN tissue slice. T cells showed a complicated migratory behavior in close association with stromal network. Antibody blockage of the major lymphocyte integrin LFA-1 and the ligand ICAM-1 partially but significantly reduced T cell motility within LN slice. T cell migration was also decreased in LN from ICAM-1 deficient mice compared with the wild type LN. Moreover, the inhibition of LFA-1 led to T cells' slip on and detachment from stromal network but still left substantial motility. Taken together, our observations indicate that LFA-1/ICAM-1-dependent and -independent motilities enables T cells to exhibit high-speed migration within 3D tissue environment. In addition, stromal network functions as scaffold for the efficient forward movement of T cells.