1. Introduction

This Course guidebook is designed for the exchange students who are to undertake their BSc projects at Tokyo Medical and Dental University, under the Exchange Student Programme between Imperial College London, hereafter IC, and Tokyo Medical and Dental University, hereafter TMDU. The projects listed in this course guide are available only for the students selected for this exchange programme.

2. Schedule

You are expected to arrive in Tokyo at the weekend of 28 February or 1 March, 2015. We ask that you all travel on the same flight, and that your arrival time does not occur late at night. This is because our students would like to meet you at the airport and show you to your accommodation. Also, the train system does not run 24 hours in Japan.

The exchange will start officially with Induction Week commencing 2 March, 2015. This week at Yushima Campus will include an introduction to Japanese life, an overview of this exchange programme given by our programme co-ordinator, and a welcome lunch with faculty. You will also receive a TMDU student ID card during this time, which you will need to access campus buildings. More details concerning Induction Week will be provided at a later date.

TMDU Project (your project and work lab) will commence on Monday, 9 March. You will work on your project from 9 March to 15 May for 10 weeks. During this period, you may take vacation for one week, as other IC students do in the UK, with permission of your supervisor. If you wish to do so, please discuss with your supervisor and your lab team members in advance. The last week of your exchange, 18 to 22 May, should be spent on finishing your project write-up. The final feedback session here at TMDU, a 10-minute oral presentation with slides, will also be arranged during that week (TBC).

You will return to London at the weekend of 23 -24 May, 2015. Oral presentations at IC will be held between 25 to 27 May, 2015.

The submission deadline for your project write-up is 5 pm (BST) on Thursday, 28 May, 2015. Please submit it via E-mail, to the following addresses:

a) TMDU supervisor - 1 copy,
b) Ms. Nicole Barnes, IC (nicole.barnes@imperial.ac.uk) - 1 copy,
c) Ms. Keiko Furuya, TMDU administrator (ryuugakusei.adm@cmn.tmd.ac.jp) - 1 copy.

In preparation for your project, we strongly suggest you contact your expected supervisor and discuss the details of your project before arrival. Please note, the circumstance of the laboratory and/or the content of your project might change after you make your choice. Some supervisors might also arrange a ‘custom-made’ project for you, depending on your experiences and expectations. Therefore, frequent communication before your arrival is crucial for a timely start to your project upon arrival to the lab. Even so, you may still spend the first 1-2 weeks in discussion to plan and refine your projects. Should you receive no reply from your supervisor, please contact Ms. Barnes.
### Course Map 2015 from IC

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**End of Part B exams:** Fri 27th Feb

**Arrival at TMDU, Tokyo, Japan:** Sat 28th Feb - Sun 1st Mar

**Induction at TMDU:** Week beginning Mon 2nd Mar

**Project 10 weeks:** Mon 9th Mar – Fri 15th May

**Write-up**: Mon 18th May – Fri 22nd May

**Return to Imperial College, London, UK:** Sat 23rd – Sun 24th May

**Oral Presentation of Project:** Mon 25th – Wed 27th May

*Note: In some BScs, the oral presentation of projects may be on Thurs 21st or Fri 22nd May – Students should check with the BSc Course Director(s) before booking a return flight and arrange their presentation to be held between Mon 25th – Fri 27th May, if possible.

**Write-up submission:** Thursday 28th May – 5pm
4. TMDU Application form (Sample) & CV

This form is only to be used for the Exchange Programme between IC and TMDU. This form and all the information regarding this exchange programme will be available from the Faculty Education Office (Ms. Nicole Barnes, nicole.barnes@imperial.ac.uk at IC). You must submit the completed application form and your CV (free-style) to Ms. Barnes. She will then carry out the necessary procedures for this exchange programme at IC.

Faculty of Medicine Tokyo Medical and Dental University Application Form for Exchange Student 2014

1. Personal Information

Family Name: __________________________ All Other Names: __________________________

Title: __________________________ Date of Birth: __________________________ (Month/ Day/ Year)

Sex: □ Male □ Female Marital Status: □ Married □ Single

Country of Birth: __________________________ Nationality: __________________________

Home address: __________________________

________________________________________

Phone Number: __________________________ Email Address: __________________________

Name and Address of Parent(s), guardian(s) or next of kin:

________________________________________

________________________________________

Special Dietary Needs: __________________________

Please state any medication(s) you are taking: __________________________

________________________________________

2. Project Details

Period of stay in Japan for the Project: from __________ to __________

Title of Project at TMDU: __________________________

________________________________________

Name of TMDU Supervisor: __________________________

Name of Home Supervisor: __________________________

3. Academic Qualifications

High School

from __________ to __________ (Month / Year)

/ / __________________________

Colleges, Universities

/ / __________________________

/ / __________________________
5. Accommodation

After your application procedure has been completed, you will receive an application form for the accommodation, named “International Student House”, located in Konodai, Ichikawa City, Chiba Prefecture. Chiba is located next to Tokyo, where you will work on your project at Yushima Campus (Main Campus). It takes about an hour from the accommodation to the lab at Yushima Campus, by bus and train. (http://www.tmd.ac.jp/english/outline/access/index.html).

It takes about 40 minutes on foot from your accommodation to the JR Ichikawa Station. If you take a bus (Keisei Bus), it will take 15-20 minutes. At JR Ichikawa Station, you must take the Sobu line local train (yellow) from Platform 1. It takes less than 25 minutes on the train to get to JR Ochanomizu Station, the nearest station to Yushima Campus.

● Monthly Accommodation Fee
You will be required to pay an accommodation fee after your arrival.

  Monthly payment: Room charge (single room): 5,900 yen per month
  We do not calculate the room charge by the days of your actual stay, but by the month.
  Therefore, we recommend that you stay here from 1 March even if you arrive in Japan on 28 February so that you will only have to pay for 3 months (March, April and May).

  Where you should pay: “Account Section, Finance Division”, 3 floor of Building 1 West (Payment by Credit card (Visa or MasterCard) is possible.)
You must also pay a Maintenance Charge of 500 yen per month. This fee must be paid at the administration office (1 floor) at International House in cash. Therefore, a one-month total would be 6,400 yen.

● Additional move-out charges
This fee must also be paid at the administration office (1 floor) of International House in cash.
・Security deposit (non-refundable): 19,000 yen
・Bed rental (futon and comforter): 8,000 yen

The approximate total amount of costs (i.e. monthly accommodation fee and move-out charge) for your accommodation would be around 50,000 yen for three months (this amount does NOT include utility fees or internet connection).

N.B. Utility fees, such as Gas, Electricity, Water and Internet (optional) are not included in the above room charge. You will be sent the bills for gas and electricity directly from the companies and can pay them at convenience stores or a post office. However, your water bills will be sent to you by the administration office at International House and you must pay the fee there in cash.
Please MAKE SURE to read the emergency guide and the rules provided by the residence; following the rules during your stay.

● Internet (optional)
The Internet service is optional. For more information, please ask at the administration office at International House.
6. Health Insurance

Please follow Ms. Nicole Barnes's instruction about health insurance, and bring a certificate showing your insurance coverage.

For a minor illness, you may walk in to our Health Service Centre at TMDU Yushima Campus without an appointment. The Centre provides the minimum amount of care and will not write a prescription. For a more serious illness, you may go directly (or by referral from the Health Service Centre) to our affiliated hospital or any other hospital of your choice. In this case, you will be charged for consultation and treatments at those hospitals. Please note that our Health Service Centre does not provide any dental services.
Contents of Projects

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※ Due to the length of your project period, there is a possibility the contents of the project may slightly change from what is described.

※ Only one student per project. If two or more students choose the same project, the students are expected to negotiate and solve.
Project title: “Molecular cell biological analysis of morphological dynamics in neurons through innovative spectroscopies”

Supervisor: Prof. TERADA Sumio –terada.nana@tmd.ac.jp
http://www.tmd.ac.jp/cgi-bin/nana/index.cgi

Key words: cytoskeleton, neuron, intracellular transport, microscopy, biophysics, chemical screening, MS (Mass Spectrometry) imaging

Background:
Neuronal cells such as neurons and glial cells are atypical and asymmetric in their morphology; both of them having long processes. They have to endure the burden of energy-consuming long-distance intracellular transport, and develop specialized cytoskeletal structures. Both intracellular transport and cytoskeletal dynamics are inseparably interrelated, and essential for the cellular homeostasis and function. One of the main interests of our laboratory is to understand how their dynamics are regulated and how these dynamics define neuronal morphologies and functions. We are marshaling our forces to elucidate the process with a multidisciplinary approach, such as gene engineering, transgenic animals, molecular genetics, fluorescence correlation spectroscopy, AFM (Atomic Force Microscope), and other cutting-edge innovative spectroscopies. Among them included are devices for a biomolecular network analysis, a visualization of small drugs in biological systems (Raman spectroscopy), and proteomic analysis with high sensitivity (MS imaging).

Subject 1: Screening and identification of novel functional molecules influencing cytoskeletal dynamics in neurons
We have developed a new experimental system for screening functional molecules that influence cytoskeletal dynamics in neurons. Through elaborate cell-based chemical screening trials, we have found several candidate chemicals disrupting specific cytoskeletal protein dynamics. Molecular mechanisms responsible for these processes will be studied extensively by various molecular and cell biological experiments.

Subject 2: Development of a new, innovative MS imaging device for highly sensitive proteomic analysis
A MS imaging is a powerful technique for detecting and visualizing biomolecules in situ, but regarding proteomic analysis, its sensitivity is far below the level for a practical usage. We have been working on the molecular cell biological and chemical technologies to improve the MS sensitivity through collaboration with MS specialists, chemists and company engineers.

No prior technical backgrounds are necessary but active and enthusiastic participation is indispensable for both subjects. For details, please contact Prof. TERADA by e-mail.

Animal Experiment:
Concerning Subject 1, we might use neuronal tissue and/or primary cultured neurons from mice. Concerning Subject 2, we usually use mice and rats for neuronal tissue sections.
Project title: Analyzing genome network on tissue development and pathogenesis of inflammatory diseases using a novel "systems approach"

Supervisor: Prof. Hiroshi Asahara - asahara.syst@tmd.ac.jp

Key words: musculoskeletal systems, transcriptional network, non-coding RNA, microRNA, Cell-based high throughput screening, inflammatory diseases, regenerative medicine

Background:
Now is the exciting duration for scientists when new strategies and paradigm, including systems biology, non-coding RNA and epigenetics, are open to researchers in any medical fields. We medical researchers are strongly encouraged to jump into this hot field with our own specific interests. Our recent accomplishment of a whole-mount in situ hybridization (WISH) database, termed EMBRYS, containing expression data of 1520 transcription factors and cofactors expressed in E9.5, E10.5, and E11.5 mouse embryos led us to identify critical cascade for myogenesis (Rp58; Dev Cell, 2009) and tendon development (Mkx, PNAS 2011). Also, our current study on non-coding RNA provides evidence that microRNA can act not only for cartilage development but also for its homeostasis against arthritis (Genes Dev, 2011).

Subject1: The function of non-coding RNA in development and diseases will be examined.
Subject2: Genome dynamics during embryogenesis will be monitored by new technique.
Subject3: Novel systems approaches will be established and applied for developmental biology and medicine.

Animal Experiment:
No animal experiments are planned. Cell and molecular biological experiments are performed with cultured cells. Students are expected to learn how to culture cells in vitro, infect them with adenovirus and analyze them with molecular biological techniques.

References:
**03 Department of Systems Neurophysiology**

**Project title**: Organization of axonal projections in the cerebellum

**Supervisor**: Prof. Izumi Sugihara – isugihara.phy1@tmd.ac.jp

**Key words**: light microscopy, rodents, neuronal labelling, axonal reconstruction, cerebellum

**Background**:  
Axons allow nerve cells (neurons) to signal each other over long distances. Axonal projection patterns determine the organization of the central nervous system. We have developed a computer-aided light microscopy system with the camera lucida apparatus to trace labelled single axons in serial sections. We have been studying afferent and efferent axonal connections in the cerebellum with this system (Refs. 1, 3), since they are essential in understanding the cerebellar function.

**Subject**:  
We propose you to study the axonal projection patterns from the pontine nucleus, which still remain generally unknown. The pontine nucleus conveys cerebral information to the cerebellum and is essential in control of voluntary and fine movements. A microinjection of the neuronal tracer to the pontine nucleus in anesthetized rodents can label pontoocerebellar single axons. Labelled axons can be reconstructed completely in three-dimensional space. The branching pattern of reconstructed axons will be compared with the striped molecular expression pattern of the cerebellum (Ref. 2). We expect a single axon has as many as 100 branches that terminate in multiple interrelated areas in the cerebellum. The interrelationship between areas in which axonal collaterals terminate may be essential in understanding the cerebellar organization.

**References**:  
**Project title**: Identifying the mechanisms for the regulation of bone metabolism

**Supervisor**: Prof. Shu Takeda - takeda.phy2@tmd.ac.jp

**Key words**: bone metabolism, osteoblast, osteoclast, osteocyte, cross talk between bone and other organs, neuronal control, microRNA, bone metastasis

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**Subject 1**: Studies on the regulatory network of metabolism between bone and other internal organs

It has been believed that bone is controlled by local environment through the action of hormone and cytokines, independently of other organs. However, our discovery that leptin regulates bone formation through the central nervous system shed light on a new regulatory system of bone metabolism, i.e., neuronal control (Takeda S, Cell, 2002, Nature, 2005). In addition, we have also demonstrated that neuromedin U, an anorexigenic neuropeptide, regulates bone formation through the central nervous system (Sato S, Nat Med, 2007). Moreover, recent studies have revealed that FGF23 or osteocalcin, which is secreted by bone, regulates the metabolism of kidney or pancreas. Thus, bone is now considered as a major player for the whole body homeostasis, and forms a regulatory network of metabolism together with other organs. We are now conducting further experiments to clarify a comprehensive network between bone and other organs.

**Subject 2**: Studies on the regulation of bone metabolism by sensory nerves

We have recently revealed that sensory nerves inside bones have a crucial role in regulating bone mass, and that the penetration of sensory nerves into bones is necessary for normal bone development or fracture healing (Fukuda T, Nature, 2013). Based on these findings, we are now conducting further experiments to develop novel therapeutic approaches to osteoporosis.

**Subject 3**: Studies on the regulation of bone metabolism by microRNA

microRNA (miRNA) is a small non-coding RNA molecule, and regulates various developmental and homeostatic events in vertebrates and invertebrates. Aberrant expression of miRNA has been implicated in numerous disease states, and miRNA-based therapies are under investigation. We have previously demonstrated the physiological role of miRNA in osteoblast differentiation (Inose H, PNAS, 2009). We are now conducting further experiments to identify novel bone-specific miRNAs and analyse the function of these miRNAs.

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**Animal Experiments**:

We use a lot of knockout mice and transgenic mice for the above subjects. Students are expected to learn various animal experiments.

**References**:

The Hippo pathway was originally identified in *Drosophila melanogaster* as the tumor suppressor signals. The pathway comprises the upstream regulators (cell adhesion molecules, receptors, membrane-associated proteins, and cytoskeletons), the core kinase cascade (serine/threonine kinases, activators, inhibitors, and adaptors), and the downstream targets (transcriptional co-activators). All components of the pathway are conserved in mammals. Its dysfunction is frequently detected in human cancers, and correlates with malignant properties of cancer (metastasis, invasiveness, and recurrence) and poor prognosis. Therefore, the reagents that recover and stimulate the Hippo pathway should be useful in the cancer treatment. The Hippo pathway also plays important roles in organogenesis, cell differentiation, and tissue regeneration. The inhibition of the pathway suppresses adipogenesis and enhances osteogenesis and myogenesis. In this point of view, the reagents that suppress the pathway should be useful to treat obesity, osteoporosis, and sarcopenia (age-related skeletal muscle reduction in the elderly). We develop several novel cell-based assay systems and perform the chemical library screenings. We have obtained candidate compounds that stimulate and inhibit the Hippo pathway. We now test whether and how these compounds are useful to suppress cancer metastasis and to enhance myogenesis. For further details, please contact with Prof. Hata by e-mail. We use mice, rats, and rabbits in the experiments, but it is possible to plan the study without using animals.
Project title: Roles of basophils in health and disease

Supervisor: Prof. Hajime Karasuyama – karasuyama.mbch@tmd.ac.jp

Key words: basophils, allergy, inflammation, parasitic infections, animal models

Subject:

The study in our laboratory mainly focuses on the physiological and pathological roles of basophils. Basophils are the least common granulocytes, and represent less than 1% of peripheral blood leukocytes. They were first documented by Paul Ehrlich more than 120 years ago, much earlier than the discovery of T and B cells, however their functional significance has remained an enigma for a long time. Basophils have often been considered to be a minor relative or precursor of tissue-resident mast cells.

We have recently succeeded in generating novel tools suitable for analysis of basophil functions, namely a basophil-depleting antibody, engineered mice for selective and inducible ablation of basophils in vivo, and engineered mice expressing green fluorescence protein only in basophils. Taking advantage of these tools, we identified previously unrecognized, non-redundant roles for basophils in vivo. Basophils are crucially involved in the protective immunity to parasitic infections, that is, the acquired resistance against repeated infections with blood-sucking ticks and intestinal helminths. Basophils also play crucial roles in the development of allergic disorders, including an atopic dermatitis-like skin disease. Further roles for basophils in health and disease are currently under investigation.

Animal experiments:

Laboratory mice including genetically engineered mice may be examined in some experiments. However, experiments with no use of animals can be planned.

HP:  (http://immune-regulation.org/index.php?id=27)

References:

07 Department of Immunotherapeutics

Project title: Immunological understanding of virus-mediated diseases and approaches for immunotherapy

Supervisor: Prof. Mari Kannagi – kann.impt@tmd.ac.jp

Key words: Retrovirus, Pathogenesis, Host defense, T-cell immunity, Innate immunity, Immune suppression, Adult T-cell leukemia, AIDS.

Background:
We investigate the disease mechanisms mediated by human retroviruses, such as human T-cell leukemia virus type 1 (HTLV-1) and human immunodeficiency virus (HIV). HTLV-1 causes adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), and HIV causes AIDS. These diseases are not simply explained by the direct pathogenic effects of the viruses, but influenced by a complex interplay between viruses and the host immune system. The aim of our research is the understanding disease mechanisms and the development of prophylactic and therapeutic strategies in these viruses infection.

Specific research subjects:
Subject #1: Development of anti-tumor vaccines for adult T-cell leukemia.
We identified major epitopes of HTLV-1-specific cytotoxic T lymphocytes (CTL) in the target molecule Tax. By using these CTL epitopes, we investigate the differences in the host T-cell responses among diseases, and develop therapeutic vaccine for ATL. Animal experiments (mice and rats) can be included if desired.

References:

Subject #2: Molecular mechanism of HTLV-1 gene suppression.
The status of HTLV-1 expression in vivo is another critical determinant for the diseases. We found that the host innate immunity especially type-I interferon is involved in the control of viral expression, and investigate its mechanisms.

References:

Subject #3: Host factors required for HIV-1 replication especially for HIV-1 integrase.
Anti-viral therapy for HIV has markedly improved the prognosis of HIV infection. We found that HIV-1 integrase, the third target molecule for anti-viral drug, plays critical roles in the reverse transcription step as well as the integration step of HIV-1 replication, and investigate its molecular mechanisms.

References:
Department of Molecular Virology (Virology)

Project title: Mechanism and therapeutic intervention of human retrovirus infection

Supervisor: Professor Shoji Yamaoka – shojmmb@tmd.ac.jp

Key words: human retrovirus, virus replication, host factors, NF-kappaB, cell survival.

We mainly deal with retrovirus-mediated oncogenesis and immunodeficiency in humans; Human T-cell Leukemia Virus type 1 (HTLV-1) causes Adult T-cell Leukemia (ATL), and Human Immunodeficiency Virus type 1 (HIV-1) causes Acquired Immunodeficiency Syndrome (AIDS).

Subject 1: Studies on host cell factors regulating HIV-1 replication
HIV-1 has long been studied virologically, but little is known about cellular factors required for HIV-1 replication. Because HIV-1 rapidly undergoes mutations under current drug therapies, it is necessary to establish therapeutic strategies that target host factors required for its replication. To identify such cellular factors, we employ unique genome-wide screens in which cells genetically modified by lentivirus-mediated expression of a cDNA library or shRNA library are infected with recombinant HIV-1, followed by recovery of genetic information from cells resistant to HIV-1 infection. The HIV-1 virus used in this study is safe because it can infect target cells, but is incapable of producing progeny infectious virus.

Subject 2: Mechanism of cancer cell survival
One of the prominent features of cancer is aberrant activation of cellular transcription factors and their target gene expression, and we are focusing on host cell proteins that are pivotal for the survival and growth of HTLV-1-infected cells. It is believed that constitutive activation of transcription factor NF-kappaB contributes to the manifestation of malignant phenotype such as uncontrolled proliferation, resistance to anti-cancer drugs and invasion, but its molecular mechanism remains poorly understood. One of cellular proteins whose expression is profoundly induced by constitutive NF-kappaB activity is A20, a dual ubiquitin-editing enzyme regulating NF-kappaB activity and cell survival. We have found that depletion of A20 induces apoptotic cell death in a variety of cancer cells. Studies are underway to elucidate the molecular mechanism, particularly focusing on its dual enzymatic activities.

Animal Experiments:
Students are expected to learn how to culture cells in vitro, infect them with retrovirus or lentivirus and analyse them with molecular biological techniques. Animal experiments are not planned in these subjects.
09 Department of Comprehensive Pathology

**Project title**: Pathogenesis of haematological malignancies: regulatory mechanisms of proliferative/apoptotic signals

**Supervisor**: Prof. Masanobu Kitagawa – masa.pth2@tmd.ac.jp

**Key words**: haematological malignancies, bone marrow, lymph node, proliferation, apoptosis, oncogenesis, angiogenesis, real-time PCR, cell line

**Subject**:

We study the molecular mechanisms of the regulation of proliferative/apoptotic signals in tumour cells from haematological malignancies. We can prepare the fresh samples and cDNA samples from the bone marrow/lymph node of normal individuals and cases with various haematological diseases such as acute leukaemia, myelodysplastic syndromes, multiple myeloma, and malignant lymphoma. We also have the samples of pre-treated and post-treated subjects of these cases. You can design the real-time PCR primers and probes of genes of your own interest to analyze the proliferative/apoptotic signals in tumour cells from haematological malignancies. You can also design the experiments using cell lines to confirm the effects of treatment in case samples on proliferative/apoptotic signals.
10 Department of Nephrology

Project title: Novel Regulatory Mechanisms of Blood Pressure through Phosphorylation of Ion Transporters by WNK Signaling

Supervisor: Prof. Shinichi Uchida – suchida.kid@tmd.ac.jp

Key words: ion channel, ion transporter, kidney epithelial cell, electrolytic disorder, blood pressure disorder, pseudohypoaldosteronism type II (PHA II), WNK (with no lysine (K) kinase), thiazide-sensitive Na–Cl cotransporter (NCC), phosphorylation, Kelch-like 3 (KLHL3), ubiquitination

Background:
We have worked on the mechanism of pseudohypoaldosteronism type II (PHA II), an autosomal-dominant disorder characterized by hyperkalemia and salt-sensitive hypertension. Mutations in with-no-lysine kinase 1 (WNK1) and WNK4 genes were reported to be responsible for PHAII. Mutant WNK kinases lead to over-activation of Na–Cl cotransporter (NCC), due to increased phosphorylation, resulting in abnormally increased sodium reabsorption in the kidney. Moreover, two additional novel genes, Kelch-like 3 (KLHL3) and Cullin3, were identified as responsible for PHAII. Recently, we have demonstrated that Cullin3-KLHL3 E3 ligase complex induces ubiquitination of WNK kinases and reduces their protein levels.

Subject 1: Novel regulatory mechanism of sodium reabsorption by WNK signal in kidney
In this subject, we will investigate the mechanisms of physiological regulation of WNK signaling in the kidney, by several physiological regulators that are known to regulate WNK signal, such as aldosterone, angiotensin II, and insulin. We will focus on degradation pathway of WNK kinases by Cullin3-KLHL3 E3 ligase complex, as well as transcripts of the components of WNK signaling.

Subject 2: Regulation of vascular tonus by WNK signal in vascular smooth muscle cells (VSMC)
Recently, the WNK signal in vascular smooth muscle cells was also found to be important in the regulation of vascular tone through the phosphorylation of Na–K–Cl co-transporter isoform 1 (NKCC1), another down-stream component of WNK signal.
In this subject, we will investigate what physiologically regulates WNK signaling in cultured VSMC cell lines. We will examine mouse aorta, as well.

Animal Experiments:
Students are expected to learn how to culture cells in vitro, transfet over-expression vector and analyze them with molecular biological techniques in both subjects. If student wants to learn, animal experiments can be planned in both subjects.

References:
**11 Department of Life Science and Bioethics Research Center (Yoshida Laboratory)**

**Project title**: Dive into vascular biology: Exciting Journey to the heart of cardiovascular disease.

**Supervisor**: Prof. Masayuki Yoshida – masa.vasc@tmd.ac.jp

**Key Words**: vascular biology, atherosclerosis, chronic kidney disease, gut immunology

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**Subject 1 : Mechanistic link between nutrient and vascular inflammation**

Inflammatory responses consists critical part of vascular diseases such as atherosclerosis. Although traditional risk factors including high fat diet clinically associates with development and onset of cardiovascular diseases, the molecular cascade via which" bad meal" leads to atherosclerosis is not fully understood. We have utilized a well established flow chamber model as well as recently developed intravital imaging system to reveal this interesting question. Some of our achievements were already published as follows. A student can learn and conduct some of these topics under a hand-to-hand instruction by our staff.

**Subject 2 : Uremic toxins and its role in cardiovascular diseases**

Our laboratory focuses on kidney function and its influence on inflammation in atherosclerosis. One of our targets in this context is uremic toxins, which are elevated in renal insufficiency and are not removed by hemodialysis. We hypothesized that high level of uremic toxins may explain higher cardiovascular risks of CKD patients even after hemodialysis. We utilize a unique technique to introduce kidney dysfunction in mice and try to see an effect of uremic toxins in mediating vascular inflammation in vivo and in vitro. A student can learn and conduct these animal and biochemical experiments using genetically engineered mice model.

**Animal Experiments**

In some projects, we use rodent models (mouse and rat) under permission from the Animal Research Ethical Committee of the TMDU. In animal studies, animals are euthanized after the experiments to collect tissue and blood samples.

**Meeting and Conferences**

In addition to actual experiments, it is also important to present and discuss one’s own data in various opportunities. To this end, we encourage students, if applicable, to submit their abstract to relevant international meetings, such as European Heart Society Meeting or American Heart Association Meeting to further enhance their scientific exposure.

**Recent relevant publications from our laboratory**:

12 Department of Gastroenterology and Hepatology

Project Title: Characterization of Intestinal Epithelial Stem Cells in IBD.

Supervisor: Prof. Mamoru Watanabe – mamoru.gast@tmd.ac.jp

Key Words: intestinal stem cells, mucosal regeneration, inflammatory bowel disease

Subject: Characterization of Intestinal Epithelial Stem Cells in IBD.

The intestinal epithelium is continuously replenished for a lifetime from stem cells located at the base of crypts. Recent rapid advances in the basic research have identified many properties of intestinal stem cells. Our laboratory has been working on in this field for many years, and developed a novel culture method for murine colonic stem cells (Yui et al., Nature Medicine 2012).

Inflammatory bowel disease (IBD) is chronic remittent or progressive inflammatory conditions that may affect the entire gastrointestinal tract and the colonic mucosa, respectively, and are associated with an increased risk for colon cancer. However, the pathogenesis of intestinal epithelial stem cells in IBD and colitis associated colon cancer has not been elucidated.

Taking advantages of the novel culture technology that we developed, applicants to our proposal will be able to investigate unique features of the intestinal stem cells in inflammatory condition.

Animal Experiments:

Students are expected to learn how to culture cells in vitro, infect them with lentivirus and analyze them with molecular biological techniques. Animal experiments are also planned in these subjects.
Subject 1 : Dramatically effective third class oligonucleotide drug for gene therapy of Alzheimer disease

RNA interference (RNAi) is a powerful tool for the post-transcriptional gene silencing. Small interfering RNA (siRNA) binds and cleaves the targeted RNA in sequence-specific manner. We had demonstrated that such an RNAi therapy is effective to cure the familial ALS using RNAi transgenic mouse (Saito Y. et al. J Biol Chem 2005; Arch Neurol 2006), and developed the mutant allele-specific gene targeting strategy to escape the side effect (Kubodera T. et al. Hum Gene Ther, 2011). Furthermore, we had published efficient systemic delivery of siRNA to the liver by conjugation of vitamin E (Nishina K. et al., Mol Ther, 2008), and using this patented method, we succeeded to inhibit neuronal gene with intra-ventricular and intravenous injection of siRNA (Uno Y. et al. Hum Gene Ther, 2011; Kuwahara H. et al, Mol Ther, 2011).

Recently, we innovated a third class of oligonucleotide, double-stranded ASO, which is different from antisense or siRNA in their structure and its mechanism of gene silencing. The dsASO achieves dramatically increased gene silencing effect which is 20-1,000 times more effective than siRNA or antisense oligonucleotide. In this program we can develop this third class oligonucleotide and apply to deliver to hypocampus for Alzheimer disease and DRG neurons for neuropathic pain. This project has been studied by four Imperial College students in 2009-2013.

Subject 2: Propagation in vitro and in vivo model of amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive motoneuron loss. With overexpression of wild-type TDP-43 in spinal cord of cynomolgus monkeys, last year, we show that monkeys developed progressive motor weakness and muscle atrophy with fasciculation initiated by distal hand muscles, reminiscent of ALS patients. This monkey model, but not rat model, could produce the neuropathological hallmarks of ALS, frequent cytoplasmic mislocalization of TDP-43 associated with loss of its nuclear staining. There is species difference in TDP-43 pathology, and our monkey model recapitulates ALS pathology much better than rodent model, providing a valuable tool to study pathogenesis of sporadic ALS. Excitingly, this is the first non-human primate model of ALS better recapitulated ALS features than rodent model, and is published in Brain 135:833-46, 2012.

Cytoplasmic mislocalization and various type of inclusions of TDP-43 are seen in motor neurons in ALS patients, which is related to motor neuron death. By our previous review of ALS pathology, it is considered that TDP-43 pathology might spreads by contiguous and non-contiguous propagation (Kanouchi T et al. JNNP 2012, Sekiguchi T et al. 2014). In non-contiguous propagation, two possible mechanisms are supposed to be existed, that are trans-synaptic spread among neural networks and non-synaptic remote spread. We propose a hypothesis that the full-length or the toxic fragments of wild-type TDP-43 might become incorporated into exosome and they spread in blood and/or cerebrospinal fluid (CSF), which will cause TDP-43 pathology propagation.

We are now engaged in elucidating propagation mechanism of TDP-43 in ALS using in vitro and in vivo model.

You can experience any experiments using in vitro and in vivo materials in our lab. No prior experience is required, however, we expect your enthusiastic participation and discussion during your stay. We remind you that your research experience here will be applicable to many other fields of science. We hope you will achieve something that lives up to publication or find your original theme even after you go back to UK.

Animal experiment:
Many mice and rats are used in our laboratories. No experiments using animals, however, are planned in this particular project for foreign students.
Subject 1: Development and evaluation of a novel artificial bone – porous hydroxyapatite/collagen composite.

We have been developing porous hydroxyapatite/collagen (HAp/Col) composites since 2002. HAp/Col is an artificial bone material with a nano-structure similar to natural bone and has been undergoing clinical trials since 2006. Currently under development is porous HAp/Col with unidirectional pores. This next generation HAp/Col has been confirmed to augment the tissue penetration rate compared to the original porous HAp/Col. The next step is to develop treatments for bone defects and osteochondral defects using porous HAp/Col as a scaffold and a drug delivery system for BMPs or FGFs.

Subject 2: Reconstruction of large bone defects using bone marrow stromal cells (MSC).

We are trying to optimise the use of bone marrow stromal cells (MSCs) for bone reconstruction. In culturing MSCs, we have already confirmed that continuous dexamethasone treatment during the culture period is effective for in vitro osteogenic differentiation and in vivo bone formation capability.

We have developed a novel cell induction method for introducing MSCs into a porous cell scaffold. Briefly, the scaffold is soaked in the cell suspension under low pressure in a chamber, and thereafter the valve connected to the chamber is opened to recover the normal pressure. The procedure and the device were very simple, and the cell-induction efficiency was significantly higher than those in previous reports which also used pressure control techniques.

We have tested the effects of fibrin as a three-dimensional scaffold of MSCs. MSCs were suspended in plasma or culture medium and introduced into porous β-tricalcium phosphate (β-TCP) blocks. Following induction, fibrin network formation of the cell suspension using plasma was initiated, and then the blocks were implanted into extra-skeletal sites. The transplantation of the culture medium group was performed after 3 hours incubation to allow cell attachment within the porous β-TCP blocks. The implants were harvested after 3-6 weeks later, and the quantification of the bone formation showed more abundant bone formation in the plasma group. Now, we have started to develop methods for reconstruction of large bone defects using these improved procedures.

Subject 3: Cell therapy to promote spinal cord regeneration

We focus on spinal cord injury repair using 1) biomaterials across injury sites, 2) cell transplantation, and 3) pharmacological neuroprotectants. The combination therapy including cell transplantation is recommended for repairing injured spinal cord. However, functional recovery is still limited even in animal models. We are trying to find the best therapeutic approach for SCI. We have developed collagen biomaterials, and applied into a fully transected lesion site in rat spinal cord. The implant replaced neural tissue, and guided regenerating axons. We have also established rat SCI models for genetically modified cell transplantation. Transplanted Schwann cells expressing eGFP survived in the host spinal cord and increased regenerated axons. To enhance efficacy of cell transplantation, we have to improve cell survival in host spinal cord. The combination of biomaterial scaffolds and some neurotrophins with cell transplantation is one of the promising approaches for better cell survival. In summary, our studies evaluate all aspects of SCI mechanisms and experimental treatment to improved motor and sensory deficit after SCI. We hope to improve the basic understanding of SCI and the best strategy will be explored in human trials.

We are currently doing clinical trial for cartilage regeneration. All patients have their cartilage defects filled with synovial MSCs arthroscopically. Favorable results are obtained by MRI imaging in many cases, by second look arthroscopies, and by biopsies. Our method has such advantages that no periosteal coverage or scaffold were required and that transplantation is possible arthroscopically. We are doing another clinical trial for meniscus treatment with synovial MSCs.

According to our previous study, the more we transplanted synovial MSCs onto the cartilage defect, the better the cartilage defect is filled with the regenerated cartilage. To gain more synovial MSCs in the limited period, addition of some cytokines or modification of culture condition may be effective. We are going to examine the effect of cytokines or oxygen condition on proliferation of synovial MSCs.
**16 Department of Dermatology**

**Project title**: Induction of keratinocytes from transgene-free but not transgene-residual human induced pluripotent stem cells

**Supervisor**: Professor Hiroo Yokozeki – [3064derm@tmd.ac.jp](mailto:3064derm@tmd.ac.jp)
Associate Professor Ken Igawa

**Subject**: Recently, some defined factors which are highly expressed in embryonic stem cells (ESCs) have been shown to reprogram human somatic cells into ESC-like pluripotent cells, named induced pluripotent stem cells (iPSCs).

Induction of reprogramming by the defined factors is mostly carried out by co-infection with retroviral vectors. Using the retrovirus-based method, we cannot overlook its oncogenicity and mutagenesis. The reactivation of residual transgenes could be one of the reasons for oncogenicity of iPSCs. Furthermore, retroviral integration itself causes insertional mutagenesis and also may alter the expression pattern of nearby genes. Therefore, transgene integration–free iPSCs are necessary for their clinical use.

To achieve this, we used the piggyBac (PB) transposon system to deliver the reprogramming factors. The PB transposon is a moth-derived DNA transposon and has been used for gene delivery and mutagenesis. The advantage over viral integration is that transposon can be easily removed from host genome. Among various DNA transposons, PB does not leave 'footprint' mutations upon excision. This means removal of transposons from the host genome without changing any nucleotide sequences.

We, in this study, using the PB transposon system, successfully generated transgene-free human iPSCs from human dermal fibroblasts. Moreover, we differentiated these iPSCs into epidermal keratinocytes. Intriguingly, keratinocytes from transgene-free but not transgene-residual iPSCs seemed to be morphologically and functionally more relevant ones. These phenomena could be related to the reactivation of residual transgenes upon induction of differentiation.

From our present study, we should use transgene-free hiPSCs in clinical application, after all. And our PB transposon system for a creation of hiPSCs can be one of the powerful candidates for the purpose of clinical use.
Subject 1: Inhibition of formation of biofilm on metals

Biofilm formation inducing infectious desease is a major cause for retrieval of medical implants in orthopedics. To prevent the formation of biofilm, we attempt the following approaches. We could control bonding manner of poly(ethylene glycol), PEG, to metal surfaces with electrodeposition at present and experiments to evaluate biofilm formation are designed on trial and error with collaborating a laboratory in the dental school. We have investigated factors influencing bonding strength and durability at the interface between metals and polymer. In the next stage, we attempt surface modification of polymer with ion beam and immobilization of functional radicals to inhibit the formation of biofilm.

Subject 2: Prevention of artifact under MRI

Metals show a low magnetic susceptibility or antimagnetic materials are required for implant devices form the viewpoint of the imaging of MRI. In addition, these materials are required for medical devices and instruments for operations and treatments used under open MRI. We are attempting the following approaches. We have found that the addition of Nb decrease magnetic susceptibility of Zr which shows originally low magnetic susceptibility and that the metallurgical structure governs magnetic susceptibility. Therefore, we must investigate more detail about the effect of alloying elements and structure.

Subject 3: Control of hard tissue compatibility

Both high bone conductivity and inhibition of bone formation are required according to implants’ purpose. Therefore, we attempt to add both properties to metals with the following techniques. We have found that more RGD is immobilized on titanium through electrodeposited PEG than without PEG and calcification on the RGD/PEG/Ti material is much larger than those on Ti and RGD/Ti.. Therefore, we deeply investigate this phenomenon to improve hard tissue compatibility of Ti alloys. Zr does not form calcium phosphate on itself that is a excellent property for intraosseous bone fixators. Here, we attempt the bone formation on Zr to develop materials having both high bone conductivity and inhibition of bone formation according to the parts. With cathodic polarization, we could obtain alkaline near the surface and calcium phosphate easily formed there. We have to examine the condition of the polarization.
Project title: Extracellular and intracellular approaches for biomedical applications of supramolecular biomaterials

Supervisor: Prof. Nobuhiko YUI - yui.org@tmd.ac.jp
Assis. Prof. Ji-Hun SEO - seo.org@tmd.ac.jp
Assis. Prof. Atsushi TAMURA - tamura.org@tmd.ac.jp

Keywords: polyrotaxanes, stem cell differentiation, protein adsorption

Subject 1: Directing stem cell differentiation on dynamic biomaterials
We have clarified that directing mesenchymal stem cell differentiation is controlled by surface properties of biomaterials such as molecular mobility of supramolecular polyrotaxanes. Marker protein expression from cultured stem cells will be analysed on a variety of polyrotaxane surfaces by using a real-time PCR technique.

Subject 2: QCM-D/ELISA analysis of protein adsorption onto dynamic biomaterials in relation to cellular proliferation and differentiation
Integrins on plasma membranes play an important role in cellular adhesion, proliferation, and differentiation. Presentation of integrin-binding motifs in adhesion proteins such as fibronectin and vitronectin adsorbed on biomaterials will be analysed by using physical and biochemical techniques including a quartz cell microbalance with energy dissipation (QCM-D) and enzyme-linked immunosorbent assay (ELISA).

Subject 3: Promoting tissue regeneration using protein/polyrotaxanes complexes
A family of tissue growth factors (TGF) is highly expected to promote osteoblast differentiation in bone regeneration therapy, however, its short activity in vivo is problematic. We have clarified the complex of the TGF with polyrotaxanes is promising to promote osteogenic differentiation in cultured cells. Relation of the polyrotaxanes structures with osteogenic differentiation will be characterized in cell culture.

Animal Experiments:
Students are expected to learn how to culture cells in vitro with molecular biological techniques. Animal experiments are not planned in these subjects.
**19 Department of Material-based Medical Engineering**

**Project Title**: Tissue regeneration using decellularized biological tissues

**Supervisor**: Prof. Akio Kishida – kishida.mbme@tmd.ac.jp  
Assoc. Prof. Tsuyoshi Kimura – kimurat.mbme@tmd.ac.jp  
Assoc. Prof. Kwangoo Nam – nam.mbme@tmd.ac.jp

**Key words**: decellularized tissue, stem cell differentiation, tissue regeneration, stem cell niche

**Background**:  
Decellularized tissues become the key material for developing novel tissue engineering and regenerative medicine. In our laboratory, a unique decellularization process, which applies high-hydrostatic pressure (HHP), was developed. We have reported that the various tissues decellularized by HHP method showed good regeneration activity for cornea, blood vessel and bone marrow. Nowadays, decellularized tissues gathered interest from the point of view of regeneration of not only tissues but also organs. Moreover, decellularized tissue powder and/or paste are expected to have activation of regeneration function of living body.

**Project**:  
1) Functionalization of decellularized tissue by incorporating biological substances, such as drugs, peptides, proteins and polysaccharides.  
2) Regeneration of soft tissues using various decellularized tissues in various shape and morphology (sheet, fiber, paste and powder).  
3) Regeneration of bone marrow tissue in vitro for production of blood and studying hematopoiesis niche.  
4) Regulation of stem cell differentiation using decellularized tissues as a biomodulator or a niche for differentiation.

**Animal Experiments**: It depends on the research theme and progress. Possible animal experiment is the subcutaneous implant of the materials to rat or mouse.

**References**:
20 Department of Biomedical Information (Biosystems)

Project Title: On-chip Cellomics Technology Development for Predictive in-vitro Drug Discovery

Supervisor: Prof. Kenji YASUDA - yasuda.bmi@tmd.ac.jp

Key words: hES cells, hiPS cells, cardiomyocyte cells, neuronal cells, on-chip screening, MEMS, nanobiomedicine, drug discovery, toxicology

Subject 1: Studies on Epigenetic Information Stored in Living System
In this subject, we have examined a series of studies to analyze emergence of order in the spatiotemporal structures of cell network to expand our understanding of how the emergence of the order in living systems is determined. As cells are minimum units reflecting epigenetic information, which is considered to map the history of a parallel-processing recurrent network of biochemical reactions, their behaviors cannot be explained by considering only conventional simple one-way 'self-organization' process regulated by DNA information, especially during the cell division process. The role of emergence of order in the higher complexity of cellular groups, which complements their genetic information, is inferred by comparing predictions from genetic information with cell behaviour observed under conditions chosen to reveal adaptation processes and community effects. A system for analyzing emergence of order will be developed starting from the twin complementary viewpoints of cell regulation as an 'algebraic' system (emphasis on temporal aspects; adaptation among generation) and as a 'geometric' system (emphasis on spatial aspects; spatial pattern-dependent community effect). The acquired knowledge may lead not only to understand the mechanism of the inheritable epigenetic memory but also to be able to control the epigenetic information by the designed sequence of the external stimulation. In practice, students will measure the epigenetic information in living systems such as brain (neuronal network system), immune system.

Subject 2: Constructing “On-chip Quasi-in vivo Model” using Nano-Bio Technology
Using constructive approach, we are developing artificial organ model on chip for drug discovery and toxicology use. Especially, using hES/hiPS cell-delivered cardiomyocyte cells, we are developing the preclinical cardiotoxicology screening system on a chip. This system has an potential to measure the risk of TdP occurrence.

Animal Experiment:
Students are expected to learn how to culture hES/hiPS cell-delivered cells on the biochips. Animal experiments are not planned in these subjects.
21 Department of Bioelectronics

Project Title: Sensing methodology for bio-molecular recognition and cell functions

Supervisor: Prof. Yuji Miyahara - miyahara.bsr@tmd.ac.jp

Key words: biosensors, point of care testing, biomarkers, intelligent polymers

Subject 1: Detection of Circulating MicroRNAs and Exosomes for Cancer Diagnosis

We have been investigating direct interaction between biomolecules and a solid-state substrate. We proposed novel concept of biologically coupled field effect transistors (FET) which is based on direct transduction of charge density change of biomolecules into electrical signal by the field effect. We are currently investigating a new method to detect circulating microRNAs and exosome simply using the bio-FET, since microRNAs and exosomes may serve as a diagnostic marker for cancer.

Subject 2: Non-destructive Monitoring of molecular interaction at Cell Membrane

We investigate a cell-based field effect transistor (cell-based FET) for detecting various electrical phenomena at cell membrane, such as drug transport analysis, in which target transporters are expressed at the cell membrane. Non-destructive and real-time monitoring of the uptake kinetics of substrates mediated by membrane-bound transporters can be realized with oocyte-based FET. Discrimination of transporting ability among genotypes of the transporters can be achieved just by placing the oocyte on the gate surface. The platform based on the cell-based FETs is suitable for high-throughput screening in pharmaceutical lead discovery.

Subject 3: Point-of-care-testing Devices for Nucleic Acid Quantification

Many efforts have been made to provide simpler and more cost-effective methods for nucleic acid quantification. Electrochemical measurement is proven a promising method to serve as devices suitable for point-of-care diagnostics because of its miniaturized instrumentation, simplicity and safety. Quantitative analyses of nucleic acids have been used for infectious disease diagnostics and monitoring of recovery after treatment. We have been investigating several methods for nucleic acid quantification based on electrical or electrochemical approaches.

Animal Experiments:
None

References:
22 Department of Biomedical Devices and Instrumentation

Project title : Advanced Biomedical Sensors and Bioinstrumentation.

Supervisor : Prof. Kohji MITSUBAYASHI - m.bdi@tmd.ac.jp

Key words : Volatile biomarker analysis, Bioelectronic-sniffers, Fiber-optic biosensor, Artificial pancreas, Human-MEMS sensors

Subject 1 : Biochemical gas sensors for volatile biomarker analysis
Biochemical gas sensors (bio-sniffers) were developed for the continuous analysis of volatile information of human bodies. The bio-sniffers incorporate biological molecules such as a drug-metabolizing enzyme in human liver for highly selective gas detection. Previously we demonstrated a variety of potential applications of the bio-sniffer including objective halitosis diagnosis, highly selective monitoring of aldehyde and breath alcohol. Recently we have started to develop the bio-sniffer for the detection of acetone which is a causative agent of diabetes.

Subject 2 : Fiber-optic biosensor for sensitive detection of influenza virus
Fiber-optic biosensors were developed for the sensitive and selective detection of airborne influenza virus. The biosensor relies on such a biorecognition molecule as an antibody which has a high affinity to a target protein on the virus. By modifying the sensor surface with the antibody, only the virus of interest is captured on the surface, resulting in the highly selective detection. With the use of fluorophores, binding of the virus on the surface induces high signal intensity and the virus at very low concentration could be detected. In our previous study, direct analysis of an airborne mite allergen was performed in conjunction with a bioaerosol sampler.

Subject 3 : Artificial pancreas based on chemo-mechanical energy conversion
A novel autonomous drug release system with pancreas-like function based on chemo-mechanical energy conversion was developed. The system consists of two units: organic engine and drug release unit. The organic engine senses glucose concentration and produces decompression to release drug autonomously in the drug release unit. The system was evaluated in closed loop system. As a result, the possibility of feedback control of glucose concentration without external energy was confirmed. Currently, we are enhancing the system to actuate it at the physiologically relevant glucose concentration (10~25 mmol/L).

Subject 4: Wearable chemical sensors for non-invasive bio-monitoring
Flexible and wearable chemical sensors were developed for non-invasive biomonitoring. This type of sensors incorporates functional polymers and is fabricated using Soft-MEMS (Micro Electro Mechanical System) technology. We applied these flexible sensors to continuous in vivo monitoring of glucose in tear fluid. In our previous study, the change of tear glucose level induced by oral administration of glucose was successfully monitored in animal experiments. We are currently conducting research on non-invasive transcutaneous gas monitoring with these flexible sensors.

Animal Experiments:
Concerning Subject 4, animal experiments are planned with Japanese white rabbits. No animal experiment is planned in Subject 1, 2, 3.
Department of Neuropathology

Project title: Mechanisms of Neurodegeneration

Supervisor: Prof. Hitoshi Okazawa – okazawa.npat@mri.tmd.ac.jp

Background:
Neurodegenerative diseases are caused by aggregation of misfiled disease proteins inside or outside of neurons. The disease proteins are suspected to interact with physiological cellular proteins before aggregation, and to dysfunction or decrease the normal proteins. We have searched for the key molecules mediating the toxicity through multiple omics approaches and have shown that several key molecules are involved in DNA damage repair. We are currently focusing on several new mechanisms connecting to DNA damage repair.


Project:
1) Molecular mechanisms of neurodegeneration
2) Metabolism and neurodegeneration
Department of Immunology

Project title: Molecular and cellular pathogenesis of autoimmune diseases

Supervisor: Prof. Takeshi Tsubata – tsubata.imm@mri.tmd.ac.jp

Key words: autoimmune disease, SLE, Guillain-Barre syndrome, autoantibody, self-tolerance, cell signaling

Subject 1: Molecular and cellular mechanisms for the production of pathogenic autoantibodies in systemic lupus erythematosus.
Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmune diseases, whose prevalence (40-50 in 100,000) is high among various autoimmune diseases. SLE is characterized by production of autoantibodies to various nuclear components, which appear to play a crucial role in the development of the disease. To elucidate the pathogenesis of SLE, we have been studying how self-reactive B cells that produce such autoantibodies are regulated in healthy individuals but are generated in SLE. Recently, we demonstrated that B cells reactive to the RNA-related nuclear antigen Sm is regulated by a novel mechanism, i.e., elimination of self-reactive B cells at the Marginal Zone B cell compartment (Kishi et al. PNAS 2012). In this project, the role of this tolerance mechanism in prevention of SLE will be addressed using various transgenic and knock out mice.

Subject 2: Genetic and biochemical studies on autoantibody production to gangliosides in Guillain-Barre syndrome (GBS)
Immune response to glycan antigens including gangliosides is different from that to protein antigens. The immune system expresses molecules that recognize glycans abundantly expressed in mammalian cells, and suppress immune cell activation to prevent immune responses to these glycans. A well characterized example is the Siglec family molecules that suppress immune responses to sialic acids-containing glycans. Nonetheless, patients with GBS produce autoantibodies to various gangliosides, glycolipid containing sialic acids. We hypothesized that defect in Siglecs may disrupt immunological tolerance to gangliosides, resulting in production of anti-ganglioside antibody responsible for neurological symptoms in GBS as gangliosides are rich in neuronal system. In this project, genetic and biochemical analysis will be done to address whether there is any mutations or other abnormalities in Siglec family molecules in patients with GBS.

Animal Experiments:
Project #1 but not #2 requires experiments using mice.
Project title: Structure and function of human genome diversities involved in the pathogenesis of cardiomyopathy and autoimmunity

Supervisor: Prof. Akinori Kimura – akitis@mri.tmd.ac.jp

Key words: Human genome, Cardiomyopathy, Heart failure, HLA, Autoimmunity

Subject 1: Molecular pathogenesis of cardiomyopathy and heart failure
Primary cardiomyopathy is a disease condition caused by functional abnormalities of cardiac muscle resulting in heart failure. There are two major clinical types of primary cardiomyopathy: hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). Since 50-70% of HCM cases and 20-35% of DCM cases have apparent family history of the disease, primary cardiomyopathy can be caused by gene mutations. We have identified various gene mutations causative for HCM or DCM. However, the disease-causing mutations can be identified in about 60% of familial HCM cases and 30% of familial DCM cases, indicating there are still unknown disease-causing genes to be deciphered. In this project, discovery of novel disease genes and/or investigation of functional alterations caused by the disease-causing mutations will be done to find-out a strategy for prevention of cardiomyopathies.

Subject 2: Molecular mechanisms of HLA-linked susceptibility to autoimmune or chronic inflammatory diseases
Human leukocyte antigen complex (HLA) is a locus controlling the immune-responsiveness to foreign as well as self (auto) antigens. There are many genes in the HLA region, including HLA class I (HLA-A, B, C) and class II (HLA-DR, DQ DP) genes, which are the most polymorphic genes in human genome, but other genes within the HLA region also showed remarkable diversities. HLA genes are well known to be associated with autoimmune diseases and chronic inflammatory diseases, but it remains to be elucidated the contribution of other genes in the HLA to the diseases. Recently, we have revealed that NFKBIL1 mapped near HLA-B controls alternative splicing of various immune-related genes and viral genes. In this project, the functional role of NFKBIL1 in the diseases will be investigated.

Animal Experiment:
Model animals of cardiomyopathy (transgenic mice and knock-in mice) will be analyzed for the pathological features. Mice will be sacrificed following the guidelines for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH Publication 85-23, revised 1985)
26 Department of Molecular Genetics

Project title: Molecular mechanism for breast carcinogenesis

Supervisor: Prof. Yoshio MIKI – miki.mgen@mri.tmd.ac.jp

Key Words: Breast cancer, DNA damage repair, apoptosis, BRCA1, BRCA2

Background:
The failure of DNA damage repair function causes many kinds of disease including cancer. Especially, the breast cancer occurs due to mutations of BRCA1 and BRCA2 genes related to double stranded break repair mechanism, and the explication of the signal transduction pathway by these two molecules is indispensable to clarify the mechanism of breast carcinogenesis. To clarify the mechanism of breast carcinogenesis, we are analyzing the DNA damage repair and cell death induction.

Subject 1: Investigation of a molecular mechanism for breast carcinogenesis
The breast cancer susceptibility protein, BRCA2, preserves chromosomal stability through roles in the repair of DNA double-strand breaks and cell division. We have previously reported that BRCA2 may regulate the positioning of the centrosome. However, molecular mechanisms of BRCA2-based functions in centrosomes are not fully understood. Here we analyzed BRCA2 co-sedimented proteins from centrosomes of HeLa cells by mass spectrometry. We will analyze the function of the gene responsible for hereditary breast cancer, BRCA2 and its related genes.

Subject 2: The intracellular signaling transduction and cell death in DNA damage
- Analysis of the cell cycle control mechanism by protein kinase C delta
- The investigation of the mechanism of apoptosis induction by c-Abl

Animal experiment: None
**Subject 1 : Gene-environmental interaction in metabolic syndrome**

Metabolic syndrome is a typical multifactorial disease characterized by diabetes, hypertension, hyperlipidemia, and obesity. The etiology involves both genetic and environmental factors, but the detail is still unknown. Systemic and low grade inflammation and following insulin resistance are known to be instrumental for the pathophisiology. The aim of this project is decipher genes and gene-environment interactions that lead to the development of metabolic syndrome on the bases of a cohort study in Japan. Genotyping of SNPs in the human genome as well as biostatistical analysis will be employed in this project.

**Subject 2 : Genetic factors in atherosclerosis development**

Atherosclerosis is a silent disease, whose progression is not usually recognized until the outbreak of cardiovascular or celebrovascular events. The development of atherosclerosis involves both environmental and genetic factors, where the latter is estimated to contribute to 30%–50% of the risks of atherosclerosis. The aim of this project is to decipher genetic factors that risks and protects systemic atherosclerosis. More than 1,500 consecutive autopsy cases, which are evaluated with pathological atherosclerosis is employed for the study. Candidate genes are selected from the recent genome wide association studies. Involvement of epigenetic changes such as DNA methylation will also be in the scope of the research.

**Animal experiment :**

None