P-1

Study on the Neuronal Input for Establishing Proper Architecture for Islet Regeneration

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Self-derived regenerative islet is a thoroughly treatment to achieve blood sugar homeostasis, that would potentially overcome the limitation of scarcity of donor and immune-rejection. Thus, without a proper guidance to achieve correct architectural conformation of natural islets, would impair the survival and GSIS response of the regenerated organoids. We have designed a reconstitution assay to observe the behavior of islets cell organization in vitro. Observing separated islet cells reorganizing, different from human islet's natural arrangement, both β and α cells tend to organize by homeotropic aggregation. It reveals that there must be something helping them to build up correct constitution. In light that neuronal factors play an important role in islet organization during development, we reasoned that supplementation with these factors could also help to guide islet cells to achieve a more physiological structure in vitro. Besides of architectural examination, we will also assess the functional of organized islets in vivo. We believe that this study will help regeneration of islets to achieve functional architecture and improved performance in the future.

P-2

Type 2 Diabetes Mellitus Patients Impaired the Beige Adipocyte Differentiation Ability of Adipose Tissue-derived Mesenchymal Stem Cells

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In our body, brown adipose tissues (BAT) are responsible for the energy consumption while white adipose tissues are responsible for the energy storage. Recent studies have identified the new type of adipose tissues called beige adipose tissues which developed within the white adipose tissues and show the similar function in energy consumption as BAT. Therefore, the production of beige adipocytes from adipose-tissue derived mesenchymal stem cells (AT-MSCs) is considered as the novel therapeutic strategy for obesity and type 2 diabetes mellitus (T2DM) because of the ability in heat production. However, up to now, there is no report on how T2DM affect the beige adipocyte differentiation derived from AT-MSCs. In the present study, we aimed to evaluate the beige adipocyte differentiation ability of AT-MSCs derived from T2DM patients (dAT-MSCs), and compared to those from the healthy donors (nAT-MSCs).

After the induction of AT-MSCs to beige adipocyte, we found the lower number of adipocyte formed by dAT-MSCs than nAT-MSCs. In addition, the expression of beige adipocyte markers, such as UCP-1 and CIDEA, were decreased in beige adipocytes derived from dAT-MSCs compared to those from nAT-MSCs. Of note, we found beige adipocyte transcription factors, including PPARγ and PGC1α were downregulated in dAT-MSCs, compared to nAT-MSCs. Importantly, overexpression of PPARγ and PGC1α showed the rescued ability of dAT-MSCs into differentiate to beige adipocytes.

Further study is necessary to identify the molecular signaling pathways which are responsible for the impaired expression of PPARγ and PGC1α in dAT-MSCs. These findings are important to understand the relation between T2DM and beige adipocyte production in order to develop novel therapy for obesity and T2DM.

P-3

The role of Pbp1, the yeast ortholog of human Ataxin-2, in the cell growth on non-fermentable carbon source media

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The fate of a newly transcribed mRNA is highly dependent to post-transcriptional regulation in which 5' cap and 3' poly(A) tail of mRNA are important for mRNA stability and translation. Pbp1, the yeast ortholog of human Ataxin-2, is a protein that binds to poly(A)-binding protein (Pab1) and regulates the Pan2-Pan3 deadenylase complex, thereby modulating the mRNA stability and translation efficiency. However, since PBP1 deletion yeast strain (pbp1Δ) grows similarly to wild-type (WT) strain, the physiological function of Pbp1 remains unclear. Recently, our laboratory found that the pbp1Δ mutant grows similarly to WT cells on normal YPD medium containing glucose as a carbon source but shows slower growth on YPGL media containing non-fermentable carbon sources, glycerol and lactate. This result suggests that Pbp1 has a role on the growth on non-fermentable carbon sources. In this study, we aim at elucidating the regulatory role of Pbp1 in non-fermentable carbon source media. Microarray data, verified by quantitative PCR, is employed to compare gene expression profiles between WT and pbp1Δ in YPD and 4 hours after being transferred into YPGL. Deletion of PBP1 results in more than two-fold decrease in mRNA level of several genes involved in gluconeogenesis pathway (PCK1, FBP1, ICL1) and in mitochondria function (COX10, MRPL3, MRPS35, etc) in YPGL but not in YPD. These results indicate that Pbp1 regulates the expression of those genes involved in gluconeogenesis and mitochondria function in YPGL. To identify regulatory stages at which Pbp1 controls the level of each mRNA, the reporter plasmids harboring GFP gene driven by the promoter of each gene are constructed, transformed in to both WT and pbp1Δ. The level of GFP gene driven by PCK1 promoter shows similar decreased expression pattern with endogenous PCK1, suggesting that Pbp1 regulates PCK1 expression through the PCK1 promoter. On the other hand, the levels of GFP gene driven by COX10 promoter are similar between WT and pbp1Δ in YPGL, suggesting that Pbp1 regulates COX10 expression not through the COX10 promoter.

P-5

Contribution of Advanced Glycation End Products (AGEs) Formed on Lysine in BSA to binding to Receptor for AGEs

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Advanced Glycation End Products (AGEs) are formed by a reaction of sugars or di-carbonyl compounds with free amines/amino group of proteins involving the formation of Schiff's base as intermediate. AGEs are produced endogenously and can also be acquired from dietary sources. It is known that some AGEs bind to receptor of AGE (RAGE) inducing signal transduction linked with diabetic complications, inflammation and antioxidant defenses. AGEs are formed on lysine and arginine residues on a protein. For example, carboxymethyl lysine and methylglyoxal hydroimidazolone are formed on lysine and arginine, respectively, whereas pentosidine and pyralline are formed from crosslinking between lysine and arginine. Although elucidating a molecular mechanism of AGEs-RAGE binding grabs attention, a contribution of lysine- and arginine-derived AGEs on a protein binding to RAGE is still unclear.

In this study, native BSA and lysine-methylated BSA (Me-BSA) were modified with methylglyoxal to clarify an effect of lysine-derived AGEs on RAGE binding. Binding assay using purified RAGE clearly shows that lysine methylation in BSA resulted in loss of binding ability to RAGE, even after its modification with methylglyoxal. The modification of lysine and arginine in BSA was assessed through fluorometric quantification and LCMS/MS analysis, showing that arginine-derived AGEs were identified in methylglyoxal-modified BSA and Me-BSA. Lines of evidence indicate that lysine-derived AGEs would greatly contribute to binding of AGEs produced on BSA by methylglyoxal to RAGE.

P-6

Role of transcription factor MafB in adult β-cells under MafA deficient condition

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The large Maf protein family members, MafA and MafB, are known as islet enriched transcription factors, which play essential roles in pancreatic β-cell development and function. Their expression pattern in β-cells is distinct from each other. MafA is specifically expressed in β-cell in pancreas and is a key regulator factor for maintaining adult β-cell function. Whereas MafB plays important role in β-cell development during embryogenesis, and its expression in β-cell gradually decrease and restricted in α-cell after birth. However, in our previous study we observed that MafB started to reexpress in insulin^{+} β-cell in MafA-deficient adult mice. We have also found that MafA knock-out MafB heterozygote (A0B1) adult mice displayed more severe hyperglycemia than do A0 adult mice. According to those results, we hypothesize that MafB can take in part in maintenance of adult β-cell function under pathological conditions. To elucidate how MafB functions in adult β-cell activity under MafA- deficient conditions in the adult stage, we generated MafA, MafB double knock-out (A0B0) mice in which MafB was specifically deleted from β-cells, and compared its phenotype with those of A0 and wild type (WT) mice.

P-7

Effect of *Ophiocordyceps formosana* on adipocyte metabolism

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Cordyceps is a fungus containing several pharmacological compounds such as polysaccharides, cordycepin, mannitol, adenosine and ergosterol. It is commonly used as tonics that can be applied to cancer and diabetes treatments, as well as antioxidants among other uses.

Traditionally, the orthodox *Cordyceps* refers to *Ophiocordyceps sinensis*, and it is called 'Dong Chong Xia Cao' which means 'Worm in winter and grass in summer'. Due to the lack of natural resources and immature artificial cultivation, *O. sinensis* has become more precious and expensive. It is of utmost importance to find a substitution for cultivation and potential medicinal applications. Currently, the main constituents of *Cordyceps* products derive from *Cordyceps militaris*. Here, we elucidate the pharmacological use of an indigenous *Cordyceps* from Taiwan, *Ophiocordyceps formosana*. Phylogeny distance of *O. formosana* is shorter than *O. sinensis*, when compared to *C. militaris*. However, the pharmacological activity of *O. formosana* is similar to *O. sinensis*.

Previously, researchers had proved that *O. formosana*, can improve hyperglycemia in diabetic mouse. Here, we want to further investigate the effect of *O. formosana* on adipocyte metabolism. Our findings illustrated *O. formosana* extract (OFE) treatment with 6-days post-differentiation induction. Such results suggest that pre-adipocyte differentiation is reduced by the addition of OFE. Moreover, when 3T3-L1 cells are treated with OFE for 12 and 18 days, post-differentiation induction exhibit more fatty acid breaking down phenomena, conferring to an enhancement in adipocyte browning. These results propose positive effects of *O. formosana* extract on adipose metabolism and hold potentials for pharmacological application.

P-8

Differential surface protein modifications during epidermal stem cell aging

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Background: Aging in the skin epidermis is marked by a gradual decline in barrier function, impaired wound healing and an increased risk of cancer. This could be due to an age-related change in the property of epidermal stem cells or defective interactions with its microenvironment and/or other cell types in the skin. There have been conflicting reports for aged epidermal stem cells as there are no definitive markers available for these cells. In this regard, we need a new tool to dissect age-related changes in epidermal stem cells. The cellular glycosylation is known to have a role in cell-cell communications, cell to matrix adhesion in various physiological and pathological conditions. Herein, we explored the changes of glycans in epidermal stem cells during aging as a potential marker along with its functional implications.
Results: Using lectin array, a technique that utilizes lectins to recognize various glycan structures, we compared the cell surface proteins of epidermal stem cells isolated from young and old mice. We found several lectins were differentially identifying glycan structures in the young and old epidermal stem cells. The aged epidermal stem cells showed a significant decrease in mannose (Mana1-3Man, Mana1-6Man) and an increase in sialylation (α2-3 Sia) modifications. To further understand the molecular mechanism and biological significance of these glycan changes during aging, we are currently identifying the core protein(s) in which the glycan modifications take place by lectin pull-down assays followed by mass spectrometry.
Conclusion: During physiological aging, we found that epidermal stem cells showed changes in their cellular glycosylation patterns. These glycan modifications detected by lectins will serve as a molecular marker for aging, and further functional studies will lead us to a better understanding of the process of skin aging. Modification of these glycans may possibly be used as a strategy to reverse the aging phenotype.

P-10

A novel creation of reporter mouse model for assessing gastrulation and germ layer formation

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During embryogenesis, a number of genes are expressed and function spatiotemporally. Especially, gastrulation is most dynamic process in embryonic development. Differentiation into three germ layers occurs cooperatively in this stage. In developmental biology, a knock-out (KO) mouse model is often used for investigating a specific gene function and the knowledge from them has been helpful to understand developmental molecular mechanisms. However, functions of many genes expressed in gastrulation are still unclear. One major reason is the technical difficulty of gastrula embryo analysis for understanding germ layer location (e.g. their small size, fragility, many markers).

To solve this problem, we are attempting to generate a novel reporter mouse carrying MIERU (Multi-color Imaging of Embryonic Development by Reporter Units). In our plan, MIERU mouse will have three different fluorescent reporter genes; *EGFP*, *tdTomato*, *TagBFP* are knocked-in into *Sox17* (endoderm marker), *Otx2* (ectoderm marker), and *T* (kwon as mesoderm marker), respectively. Expectedly, each germ layer will be colored by different fluorescence in the gastrulation stage.

Now, we have generated each germ layer specific knock-in reporter mice using the CRISPR/Cas9 system. To express the reporter genes under the transcriptional regulation of the endogenous marker genes without their disruption, reporter genes following 2A peptide coding sequence were knocked-in into just before stop codon of each endogenous marker gene.

We constructed *pX330* plasmids expressing Cas9 and sgRNA, and knock-in donor plasmids and injected them into C57BL/6J mouse zygotes, respectively. In each reporter mouse, several founder mice were obtained, and fluorescent reporter expression was analyzed using each heterozygous gastrula embryos. Hereafter, we will generate MIERU mouse carrying three reporters by mating and analyze fluorescent reporter expression using gastrula embryos. We expect that the MIERU mouse system facilitates comprehensive analysis of gene function in gastrulation and germ layer formation.

P-12

Sik1, Sik2 point mutation mice
Paralog analysis of *Sik3* gene in *Sleepy* Mutant pedigree

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Through forward genetics research in which we screened randomly mutagenized mice for sleep abnormalities, we established a mutant pedigree which we termed *Sleepy. Sleepy* mutant pedigree shows short total wake time with a dominant inheritance pattern. We identified a splice mutation of *Sik3* gene in *Sleepy* mutant mice, resulting in the skipping of exon 13. SIK3 is a protein kinase which contains a well-conserved protein kinase A (PKA)-recognition site, serine 551. The skipping of exon 13 results in a deletion of 52 amino acids including S551. The genetic modulation of the PKA-recognition site equivalent to S551 in the *Sik3* orthologues of *Drosophila melanogaster* altered sleep-like behavior. Consistently, we recently found that SIK3 S551A knock-in mice showed short time spent in wakefulness, suggesting that the phosphorylation of S551 by PKA play a crucial role in determining sleep need. Importantly, other SIK family members, SIK1 and SIK2 have PKA-phosphorylated serine residues, S577 and S587, respectively, equivalent to SIK3 S551. Hence, SIK1 S577 and SIK2 S587 may be involved in sleep/wake regulation, similar to SIK3 S551.

Here, we examine the role of the phosphorylation of SIK1 S577 and SIK2 S587 in sleep/wakefulness using mice in which SIK1 S577 and SIK2 S587 were substituted by alanine using CRISPR/Cas9 method. We are now assessing the sleep/wake behavior of SIK1 S5771A mice and SIK2 S587 mice to examine whether the well-conserved PKA-phosphorylated serine resides in the SIK family commonly alters sleep need.

P-13

Effect of filaggrin loss of function mutations on atopic dermatitis in young age

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Atopic dermatitis (AD) is a common chronic inflammatory skin disease that cause considerable morbidity in both childhood and adulthood. Several studies have shown that skin barrier defect is often observed in patients with AD. An important key protein for skin barrier function is filaggrin (*FLG*), and it also acts as a moisturizing factor. The strong association between early onset AD and *FLG* loss-of-function mutations was reported previously, however, the association study between *FLG* loss-of-function mutations and onset of AD in the longitudinal study during early childhood have not been reported so far. In the present study, we performed association study to investigate the effect of *FLG* loss-of-function mutations on the status of AD in each age using birth cohort samples (720 participants). Genotyping was performed for five most common loss-of-function mutations in *FLG* (c.3321delA, p.Ser2554X, p.Ser2889X, p.Ser3296X, p.Lys4022X) in 720 participants of the birth cohort study. Sixty-eight (9.4%) children were heterozygote for *FLG* loss-of-function mutation, and 1 (0.1%) child was homozygote for the *FLG* loss-of-function mutation. Associations between these mutations and status of AD in each age were analyzed by logistic regression analysis. *FLG* loss-of-function mutations were significantly associated with very early onset (≤ 2 years of age) AD ($P<0.001$), but not with late-onset (> 2 years of age) AD ($P>0.05$). In conclusion, we observed the strong effect of *FLG* loss-of-function mutations for AD development in very early age.

P-14

Labeling the Dorsal Root Ganglion Neurons in Neuropathic Pain Mouse Model

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Nociception is one of the responses produced by the sensory nervous system. It can be considered as a reflexive retraction from painful stimuli; meanwhile, it can also be regarded as a defense system that can effectively avoid harmful and detrimental situations in the future. Neuropathic pain is one of the chronic pain that originated from the pathology of nervous system. To further investigate the underlying mechanisms of neuropathic pain, several methods have been developed to induce neuropathic pain symptoms such as hyperalgesia and allodynia. The spared nerve injury (SNI) is one of the neuropathic pain animal model, which has been carried out in many studies with high reproducibility and robustness in the past few decades. Consistent with the idea that SNI inducing allodynia in the mouse model, it remains unclear about the underline neuronal mechanism for chronic neurophathic pain. Besides the injured neurons, do the adjacent intact neurons also contribute to allodynia? Furthermore, do any morphological changes happen in the axons of these neurons?

In this study, we first measured the withdrawal thresholds in C57BL/6J adult male mice before and after variant kinds of SNI surgeries (i.e. sparing one specific branch of the sciatic nerve as one group). Our results show that the spared tibial nerve and spared sural nerve groups appear to develop mechanical sensitization on specific areas of the hindpaw plantar skin compared to the sham control. On the contrary, the spared common peroneal nerve group does not show mechanical sensitization compared to the control as expected. By injecting DiI in the paw, a lipophilic tracer to label dorsal root ganglion (DRG) neurons that remain innervating to the paw after surgery, we can calculate the cell numbers in L3, L4, and L5 DRG, respectively, in each SNI variant. Afterwards, by identifying which DRG has the most cells after peripheral afferent neurons injuries, we can inject the pAAV-hSyn-FLEx-mGFP-2A-Synaptophysin-mRuby plasmid into the corresponded spinal cord segment to double-labeling the neurons we are interested in. Taking together, our data add to the growing evidence of SNI inducing allodynia. Most importantly, investigating the morphological changes of these DRG cells and their roles in neuropathic pain can provide novel insights and a step forward for understanding more broadly toward the underlying mechanisms of neuropathic pain and for developing more efficient and more accurate treatments on neuropathic pain.

P-15

Visualization of early inflammatory response with accumulated lymphocytes by using novel NIR imaging mice

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Experiments using animals are necessary to figure out processes of various diseases, because they are results of breakdown of higher-order life phenomena. Invasive methods such as surgery might disturb intravital circumstances and make it difficult to observe for long term. It is a powerful solution for the problem to visualize animals non-invasively with near-infrared (NIR) light. NIR light with wavelength from 650 nm to 900 nm is absorbed poorly by mammalian tissues, then it is easily transmitted into the body of mice. In 2009, the first NIR fluorescent protein (FP), IFP1.4 was reported by Tsien's group and in 2011, improved NIR FP, iRFP was reported by Verkhusha's group. Currently, we aim to establish NIR non-invasive imaging technique using iRFP.

So far, we established ROSA26 Halo-iRFP flox mice, by inserting the DNA fragment carrying *CAG-loxP-HaloTag7-loxP-iRFP* into the ROSA26 locus of mouse ES cells derived from C57BL/6J. Since these mice express the HaloTag protein in the whole body in the absence of Cre protein, the cells derived from them can be stained with any HaloTag ligands. Furthermore, iRFP can be expressed in specific tissues and organs in the presence of Cre protein by mating with various Cre-driver mice. Indeed, by crossing with Ayu-1-cre mice expressing Cre in fertilized eggs or Ins1-cre mice specifically expressing Cre in pancreatic β cells, we confirmed that Cre-loxP system functioned as expected in established mice.

Based on these results, we are investigating disease model mice non-invasively. In this study, at first, we obtained two mice strain expressing iRFP specifically in T cells or B cells by crossing ROSA26 Halo-iRFP flox mice with Lck-cre mice or mb1-cre mice, respectively. By using these mice, we analyzed lymphocyte accumulations associated with local inflammation and changes over time in the thymus and spleen associated with growth and atrophy by NIR fluorescence imaging noninvasively. Through these, we aimed to establish an experimental system that can be analyzed in the early stages of inflammatory responses.

P-17

Establishment of the neuron-type specific SIK3-deficient mice

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Sleep is a ubiquitous animal behavior conserved from vertebrates to invertebrates and time spent in sleep is determined by homeostatic sleep need which accumulates during wakefulness. SIK3, a member of AMP-activated protein kinase (AMPK) family, was recently identified as a novel sleep/wakefulness-regulating molecule through our forward genetic screening using mice. A single nucleotide substitution in the *Sik3* gene resulted in a longer NREM sleep time and increased NREM sleep delta density, which indicated the enhanced sleep need. Conversely, in case of flies and round worms, expression of hypomorphic SIK3 orthologue reduced their sleep-like behavior. Therefore, the long sleep phenotype of *Sik3* mutant mice is due to the gain-of-function effect of the mutant SIK3 protein. However, the role of the endogenous SIK3 protein on sleep/wakefulness regulation in mice remains unknown because most of *Sik3*-deficient mice died on the day of their birth and a few of survived mice suffered from growth retardation and malnutrition.

Here, we report newly developed *Sik3-flox* mice, whose exon 3 of *Sik3* gene is flanked by lox P sites. Cre recombinase excises the exon 3, which results in the amino acid reading frame shift and premature stop codon. *Sik3-flox* mice was produced by the combination of CRISPR/Cas9 system and the *Sik3* gene targeting vector. We confirmed that each loxP sequence was correctly inserted into the targeted regions within intron 2 and intron 3 in *Sik3* gene of F0 founder mice and their descendants. We then crossed *Sik3-flox* mouse with Vgat-Cre mouse and extracted genomic DNA from the brain and tail. As expected, brain DNA from $Sik3^{flox/+};Vgat^{Cre/+}$ mice precisely lost exon 3 region whereas the DNA from their tails did not. Furthermore, $Sik3^{flox/+};Vgat^{Cre/+}$ mice look healthy. Using *Sik3-flox* mice, we started examining the sleep/wakefulness behavior of the pan-neuronal or neuron-type specific SIK3-deficient mice.

P-18

Identified topoisomerase 2β-preferential catalytic inhibitor on reducing therapy-related side effects

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Topoisomerase has been demonstrated as an excellent anticancer target of chemotherapy for many years. Currently, many clinical drugs like Etoposide, Mitoxantrone and Doxorubicin target Type II DNA topoisomerase (Top2). Although they are very effective, many side effects especially cardiomyopathy and secondary leukemia have been reported which greatly limit their clinical usage. Top2 has two isozymes, Top2α and Top2β respectively. Some research has shown targeting Top2β is more relative to side effects and targeting Top2α is more relative to the ability of killing cancer cell. Dexrazoxane (ICRF-187) is the only FDA approved drug used as cardio-protectant during chemotherapy. As a Top2 inhibitor, it can reduce the formation of type II topoisomerase cleavable complex (Top2cc) and antagonize the Top2-poisoning-associated side effects. However, it doesn't have Top2 isozyme selectivity and target both Top2 isozyme, so it also affects the efficiency of killing cancer cell. Our lab has been focused on drug development and make effort to improving the efficacy of cytotoxicity and lowering down side effects by isozyme-specific targeting. In this regard, CL-14, a derivative of etoposide (VP-16), has a good Top2β preference and can reduce VP-16 induced DNA damage mediated by Top2β which can be developed as "first-in class" drug candidate. Our results also demonstrate the potential of CL-14 as a new cardio-protectant.

P-19

Neuronal representation of dynamically changing reward value in the primate striatum

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In our daily life, the value of rewards dynamically changes even by the second. For instance, when grilling a steak, its palatability (i.e., its value) changes depending on grilling time and we need to stop grilling at the best timing. Thus, the brain is required to monitor such dynamically changing value to maximize reward gain. To explore the neural mechanism in a macaque monkey, we used a classical conditioning in which a bar stimulus was presented as a conditioned stimulus (CS) and the length of the bar indicated the amount of a liquid reward (US, minimum: 0.1 ml, maximum: 0.3 ml). The bar was presented for 2900 ms and its length was dynamically changed. In one condition (value-increase condition), the length of the bar started from the minimum and gradually increased (0.082 ml/s). The gradual increase randomly stopped within 2450 ms. The monkey obtained the reward of which amount was corresponding to the final bar length. In another condition (value-decrease condition), the length of the bar started from the maximum and gradually decreased (0.082 ml/s). As a control (control condition), a bar was presented with a fixed length (minimum: 0.1 ml, medium: 0.2 ml or maximum: 0.3 ml). While the monkey was experiencing the three conditions, we recorded the single-unit activity of 139 neurons from the striatum in which neurons have been known to encode reward value. We found striatal neurons of which activity changed gradually while the reward value indicated by bar length was dynamically changing. A subset of these neurons gradually increased their activity as the reward value increased by the second (i.e., the value-increase condition), whereas another subset gradually decreased their activity as the reward value increased. Many of these neurons also encoded the reward value in other conditions. Our findings suggest that striatal neurons participate in online monitoring of dynamically changing reward value.

P-20

Evaluation of anti-oxidative and anti-arsenic potential of phytochemicals derived from spice plants using zebrafish

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From ancient times, spices have been expected to exert a variety of health promoting effects. For example, curcumin, a phytochemical extracted from the curry spice turmeric, have been shown to protect cells and animals from various diseases. Among these health promoting effects, anti-oxidative and anti-arsenic potential may be important activities for phytochemicals to extend healthy life span of humans. We previously developed an assay system to evaluate these potential of small molecules using zebrafish. In this study, we utilized this zebrafish assay system to evaluate anti-oxidative and anti-arsenic potential of phytochemicals derived from various spice plants.

In this study, we analyzed 10 spice-derived phytochemicals: capsaicin from red peppers, carnosic acid from rosemary, cinnamaldehyde from cinnamon, curcumin from turmeric, diallyl trisulfide from garlic, eugenol from cloves, gingerol from ginger, isoeugenol from nutmeg, quercetin from onion, and 6-(methylsulfinyl)hexyl isothiocyanate from Wasabi. Sulforaphane, a phytochemical constituent of cruciferous vegetables, was used as a control, which we previously demonstrated to have strong anti-oxidative and anti-arsenic activities. First, anti-oxidative effects of each phytochemical were investigated. Three-and-half days zebrafish embryos were pre-treated with each phytochemical for 12 hours and then treated with hydrogen peroxide for 48 hours, and survival rates were measured. Second, anti-arsenic activities were examined in a similar manner. Some phytochemicals attenuated the toxic effects of oxidative stress induced by hydrogen peroxide and others inhibited the toxicity of arsenite. Since protective effects of sulforaphane have been shown to be mediated by the Keap1-Nrf2 pathway, we are now testing whether or not anti-oxidative and anti-arsenic potential of these spice-derived phytochemicals was mediated by the Keap1-Nrf2 pathway using Nrf2 mutant zebrafish.

P-21

Serotonin 5-HT4 receptor mediated expression of Collapsin response mediator protein-2 in dendrite and axon formation of mice hippocampus neurons in vitro.

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Recent studies have shown that serotonin (5-HT) is involved in various aspects of the development of hippocampal neurons. However, the specific role of 5-HT4 receptor (5-HT4R) is poorly understood. We investigated the role of 5-HT4R in the formation of dendrites and axons of the mouse hippocampal neurons in vitro using 5-HT4R agonists (RS67333, BIMU8) and antagonist (GR125487). Neurons from the mouse hippocampus at embryonic day 18 were dissociated and treated for 4 days with 1 nM, 10 nM, 100 nM of RS67333. The treatment increased the number of primary dendrites and the branching of dendrites and axons. Scanning electron microscopic (SEM) measurement showed the increase of axon diameter. We also investigated the role of 5-HT and BIMU8, and confirmed the effects of RS67333 in the development of axons and dendrites. Next, we found that treatment of GR125487 neutralized the effects of RS67333 on the primary dendrites and branching of dendrites and axons, which confirmed the specific effects through 5-HT4R.

Now we are investigating the involvement of collapsin response mediator protein 2 (CRMP2) in the role of 5-HT4R. CRMP2 is an important therapeutic target in various psychiatric and neurodegenerative diseases. CRMP2 is widely expressed in embryonic brain, and plays important roles in axon formation-through the interactions with microtubules. Recent studies have shown that c-terminus of 5-HT4R also affects the CRMP2 expression via direct or indirect molecular pathways which are not well studied. We investigated the localization of CRMP2 in developing hippocampal neurons in vitro. We are now examining the effect of RS67333 on *crmp2* mRNA expression, and also the involvement of *crmp2* in RS67333-mediated formation of dendrites and axons.

P-22

Functional analysis of unsaturated fatty acids in brown adipocytes

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Mammals have two different types of adipose tissues, white and brown adipose tissues. Brown adipocytes express a unique mitochondrial protein UCP1 (uncoupling protein 1) that plays a central role in thermogenesis. Thermogenesis is an energy-consuming process and is induced by adrenergic stimuli or cold exposure in brown adipocytes. Due to the energy-consuming feature, brown adipocytes have received a lot of attention in recent years because of their anti-obesity potential. Previous studies have shown that cold acclimation increases unsaturated fatty acids in the phospholipid fraction of brown adipocytes, which implies that unsaturated fatty acids may regulate thermogenesis through their effect on the membrane structure. However, the role of unsaturated fatty acids for regulating metabolism in brown adipocytes is poorly understood. In this study, we used a Raman microscope to observe the lipid droplets in brown and white adipocytes and found that the amount of carbon double bonds, possibly of unsaturated fatty acids, is higher in brown adipocytes than in white adipocytes. Quantitative RT-PCR analysis showed that Stearoyl CoA desaturase-1(SCD1), which catalyzes formation of monounsaturated fatty acids from stearoyl-CoA and palmitoyl-CoA, is mainly expressed in brown adipose tissue. To analyze the role of unsaturated fatty acids in brown adipocytes, we tried to reduce the amount of unsaturated fatty acids by treating the cells with an SCD1 inhibitor PluriSIn. After treatment of brown adipocytes with PluriSIn, reduction of unsaturated fatty acids was confirmed by a Raman microscope. We then analyzed the effect of PluriSIn on cell morphology, expression of brown-specific genes and oxygen consumption. We also overexpressed SCD1 in brown adipocytes to increase unsaturated fatty acids. Expression of exogeneous SCD1 was confirmed by western blotting, and the alteration of the amount of unsaturated fatty acids was analyzed by a Raman microscope. The results of these analyses will be presented in the poster.

P-23

Nucleoid dynamics in *Staphylococcus aureus*

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Staphylococcus aureus is a gram-positive bacterium which inhabits the human nasal cavity and skin flora. It has unique adaptive potential to harsh environments and stresses. Once inside the host, *S. aureus* encounters the innate immune system including phagocytes such as neutrophils and macrophages. Remarkably, it is able to survive in the phagosome for several days, where it can cope with reactive oxygen species (ROS) by anti-oxidant factors such as SOD, catalase, carotenoid pigments, and the metallo regulon gene A (MrgA). MrgA belongs to the Dps protein family, and has ferroxidase activity, which contributes to the oxidative stress resistance by reducing the concentration of ferrous iron required for the Fenton reaction. Additionally, MrgA can bind and clog nucleoid under oxidative stress, while maintaining proliferation of *S. aureus*. The nucleoid is a complex structure consisting of genome DNA, RNA, and hundreds of nucleoid-associated proteins (NAPs). In *Escherichia coli,* unlike *S. aureus,* nucleoid is clogged towards the stationary phase, replacing most log-phase NAPs with the major stationary-phase nucleoid protein, Dps. Recently, the composition of clogged staphylococcal nucleoid was deciphered by using sucrose gradient centrifugation and liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) techniques to isolate the nucleoid and analyze the proteins respectively. 299 proteins were identified in the nucleoid structure under oxidative stress, including 113 csNAPs (contaminant-subtracted NAPs). The results indicate the nucleoid constitutively holds global regulators such as Hu, SarA, FabG, and ribosomal proteins even under the oxidative stress, demonstrating active functions of the clogged *S. aureus* nucleoid, unlike the dormant clogged *E. coli* nucleoid. Further progress in our research will be discussed in terms of mechanism and physiological roles of nucleoid clogging in *S. aureus.*

P-24

The analysis of whole body of *Mafb* p.Leu239Pro variant mouse

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Background: MafB is the transcriptional factor that binds to Maf recognition elements (MAREs) in the promoter region and regulates the expression of downstream genes. MafB plays important roles in the differentiation and function maintenance of various tissues and cells. In kidney development, MafB is expressed in podocyte and regulate nephrine and podocine that constitute slit film. *Mafb* knock out mice also showed severe kidney development. By using next generation sequencer technology, it was identified that a rare heterozygous substitution (p.Leu239Pro) of *Mafb* is the cause of Focal segmental glomerulosclerosis (FSGS) associated with Duane retraction syndrome (DRS), characterized by impaired horizontal eye movement due to cranial nerve maldevelopment n human.

In this research, we generated mice carrying a *Mafb* p.Leu239Pro variant and compared the phenotype of this mice and *Mafb* knock out mice. The aim of this study is elucidating the mechanisms of diseases and developing new treatment strategies by analyzing *Mafb* p.Leu239Pro variant mice.

Methods: We generated *Mafb* p.Leu239Pro variant mice by use of CRISPR/Cas9-mediated genome editing. The homozygous p.Leu239Pro (*mt/mt*) mice were generated by mating between female and male of heterozygous p.Lew239Pro (*mt/+*) mice. We analyzed 18.5 newborn mice. The phenotype of mt/mt mice were compared with control mice (wild-type or *mt/+*)

Results: Electron micrography of inner ear demonstrated that *mt/mt* mice show abnormal development. In HE staining of kidney, we observed abnormal development of tubules and glomerular in *mt/mt* mice. Although the percentage of non-mature glomerular was not different between control and *mt/mt* mice, the size of mature glomerular was smaller in *mt/mt* mice than in control mice. This result indicated that in *mt/mt* mice, developmental abnormality is occurred when non-mature glomerular become mature glomerular. I also conducted immunostaining by using nephrin and podocin antibody. In *mt/mt* mice, both nephrin and podocin weren't stained, on the other hand these were stained clearly in control mice. The immunostaining using caspase-3 antibody showed that more caspase-3 positive cells were observed in *mt/mt* mice than in control mice. This result indicates that apoptosis was induced more in *mt/mt* mice than in control mice. These results indicate that some developmental abnormalities are occurred in *mt/mt* mice. Thus, this *Mafb* mutant mice may contribute for the analysis of disease derived by human MAFB mutation.

P-25

Analysis of collagen secretion and fiber formation by new imaging technology

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Currently there are few effective therapies for fibrosis, because the detailed mechanism of fibrosis has not been elucidated enough. Therefore, new methods for evaluating the progress of fibrosis are required. In our laboratory, we have been trying to develop a new imaging method that enables us to observe collagen secretion and fiber formation directly.

So far, we established a new method for visualization of collagen fibers. When collagen molecule is secreted extracellularly, the N-terminal and C-terminal domains of collagen are cleaved and remaining central part which is called Tropocollagen has a tight helical structure. Therefore, it is expected to difficult to insert fluorescent proteins. However, we found that several exceptional types of collagen can be used for imaging and we have developed collagen probes by incorporating fluorescent protein. Actually, we succeeded in imaging the collagen fiber formation.

In this research, to investigate whether collagen probes we developed form the same morphology and functions as physiological collagen fibers in vivo, we tried to develop a method for recovering collagen fibers without changing the structure of them. It is known that collagen not only supports the animal's body structure but also affects cells in cell development, differentiation, and morphogenesis. If we can collect collagen fibers, it might be applied establishment of new cell culture method on collagen fibers and analysis of collagen fiber degradation mechanism.

P-27

Light influence Time Perception through intrinsically photosensitive retinal ganglion cells

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Light has a strong effect on our lives and physiological functions. In addition to image-forming visual function for animal to perceive our environment, ambient light could also influence many non-imaging-forming functions such as resetting the circadian clock, controlling the pupil size and modulating emotion and cognition functions. It has been shown that intrinsically photosensitive retinal ganglion cells (ipRGCs) play an important role in non-imaging-forming functions. These ipRGC express melanopsin, a special photopigment with principal absorption wavelength around 470 nm (blue light). Recent evidence also indicates that ipRGCs innervate the SCN and deep layer of the superior colliculus, which is involved in the regulation of circadian and the multisensory integration respectively. Our preliminary data showed that blue light can affects the perception of time in human. However, whether different wavelength of light could directly influence the time perception, or which kind of photoreceptor is involved in this kind of perception modulation remain unclear. To test our hypothesis, we setup a training protocol for mice to perform time perception test using two-alternative forced choice task. Mice were first learn to poke the middle hole to start the trial and hold the position under two beeping sounds plays with interview ranging from 0.6 to 2.4 sec. They then next learn to approach the specific choice point to obtain water reward. After 3 weeks of training, we can test the time perception of mice under different background of light. This training protocol showed that we can used two - alternative forced choice task to study time perception of mice model.

P-28

The attentional capture to emotional human stimulus and the disengagement of attention from it

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Previous researches have demonstrated that an emotional stimulus often captures observers' attention. Furthermore, it is also known that the presence of human image in an emotional stimulus facilitates the attentional capture. The attentional capture to the emotional stimulus including other human(s) (emotional human stimulus) is considered to be derived from an innate sensitivity to other humans. This study investigated the nature of the attentional capture to an emotional human stimulus and the disengagement of attention from it.

Study 1 examined the effect of available attentional resources on attentional capture to an emotional human stimulus. Because the attentional capture was reduced when attentional resources were deployed to another task, it is possible that the decrease of available attentional resources interferes with the attentional processing to other humans in emotional stimuli. Participants were required to perform an orientation matching task, in which two bars were presented peripherally concurrent with the central presentation of task-irrelevant emotional human/non-human stimulus. The available attentional resources to emotional stimuli were manipulated by the difficulty of the orientation matching task. The results showed that the pleasant human stimuli captured attention when available attentional resources were sufficient, whereas the unpleasant human stimuli captured attention when less attentional resources were available. These results indicated that available attentional resources determined the type of emotions that elicit the sensitivity to other humans.

In Study 2, to examine the speed of disengagement of attention from emotional human stimuli, the emotional human/non-human stimulus was presented 200 ms prior to the onset of the peripheral bars by using an easy task (i.e., sufficient attentional resources were available). The results indicated that reaction times were faster when the unpleasant human stimulus was presented than when the unpleasant non-human stimulus was presented. This implies that the processing of unpleasant stimuli was faster when they included other human images, resulted in the faster disengagement of attention from the unpleasant stimuli.

P-29

Identification of the Responsible Genetic Factors Involved in Enhanced Susceptibility of NASH in Tyrosinase Deficient Albino Mice

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Introduction: Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide with a prevalence estimated at 20-30% of total population. Nonalcoholic steatohepatitis (NASH) is the progressive liver damage from NAFLD with hepatic inflammation and fibrosis progression. In recently years, the multiple parallel hits hypothesis has been proposed, which indicates NASH is caused by various factors acting together. Such factors include insulin resistance, oxidative stress, gut microbiota and genetic and epigenetic factors. But the exact underlying pathophysiology remain unclear. Therefore, approaches to understand the disease mechanism is in high demands. Interestingly, we observed that high cholesterol diet (HCD) fed C57BL6(CG)-Tyr$^{c\text{-}2J}$ mice (B6 Albino) are highly susceptible for NASH compared to WT C57BL6 mice (B6 Black) given the same conditions.

Objectives: Here we aim to elucidate the cause of elevated NASH susceptibility in B6 albino mice.

Method and Results: B6 albino mice have spontaneous c.291G<T mutation in *Tyrosinase* gene. B6 Albino and Black mice were fed with HCD for 10 weeks. Surprisingly, around half number of B6 albino mice showed the steatosis and died due to the liver dysfunction much earlier than B6 black mice. To verify NASH was occurred due to that mutation in *Tyrosinase* gene, we performed 2 weeks HCD trial with other two kinds of albino mice. One is the mice have the same mutation in *Tyrosinase* gene artificially by CRISPR Cas9 system (CRISPR albino), the other one is BALB/c mice which have c.369G<C mutation in *Tyrosinase* gene. All CRISPR albino mice showed NASH-like phenotype with elevation of liver injury markers, inflammatory cells, extensive fibrosis and cholesterol crystals in liver, but BALB/c mice did not show any liver damage symptoms after 2 weeks of HCD induction.

Conclusion: Results suggest that only certain mutations in *Tyrosinase* are responsible for the enhanced susceptibility of NASH. We believe that our work will advantage the identification of susceptible genetic factors for NASH development and expand the understanding on NASH pathophysiology.

P-30

MafB promotes osteoclastogenesis: Relevance for the osteolysis mechanism in Multicentric Carpal Tarsal Osteolysis

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Background: Multicentric carpal tarsal osteolysis (MCTO) is a rare disease with progressive osteolysis of the carpal and tarsal bones. Zankl A *et al.,* defined an autosomal missense mutation in the transactivation domain of transcription factor *MAFB* as the cause of MCTO. The osteolysis is predicted to occur from the loss of MAFB function causing increased osteoclast resorption. However, there is no definite treatment today, since the function of MAFB in osteoclastogenesis has not been examined *in vivo*.

Aim: In this study, we aim to clarify the function of MafB in osteoclast differentiation by utilizing myeloid specific *Mafb* knock-out mice (cKO) and relate it to the pathogenesis of MCTO by using a human point mutation integrated MCTO model mice ($Mafb^{MCTO/MCTO}$).

Results: μCT analyses of the femur bone of cKO mice showed an increase in bone density compared to control, indicating that MafB promotes osteoclast differentiation *in vivo*. Culturing osteoclasts from bone marrow of cKO mice showed lower numbers of multinucleated cells compared to control. From this, it was confirmed that MafB promotes osteolastogenesis, however, this contradicted to the symptoms of MCTO patients which shows excessive bone resorption. Therefore, we analyzed the osteoclastogenic ability of MCTO mutated MafB by analyzing $Mafb^{MCTO/MCTO}$ mice. $Mafb^{MCTO/MCTO}$ mice showed reduced bone density with smaller carpal and tarsal bones, like MCTO patients. The function of MCTO mutated MafB were also examined by luciferase assay against reported target genes of MafB. Interestingly, MCTO mutated MafB increased transcriptional ability compared to MafB containing the intact protein sequence. This suggested an increased osteoclast differentiation in $Mafb^{MCTO/MCTO}$ mice, which was confirmed by culturing bone marrow derived osteoclasts. As expected, $Mafb^{MCTO/MCTO}$ mice showed an increased multinucleated cell number compared to control.

Conclusion: Our study show that MafB promotes osteoclast differentiation and MCTO mutation causes osteolysis by promoting osteoclast differentiation. These findings may provide new insights for the molecular regulation of osteoclastogenesis and possible treatments for MCTO.

P-32

Analysis of the efferent projection pattern of ERα and ERβ expressing neurons in the medial preoptic area

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Estrogen plays an important role to initiate and maintain maternal behaviors. The medial preoptic area (MPOA) has dense expression of estrogen receptors, and it has been identified as a responsible region of the effect of estrogen on maternal behaviors. There are two types of estrogen receptors, the estrogen receptor α (ERα) and the estrogen receptor β(ERβ). It has shown that expression pattern of these two types of estrogen receptors are different in the MPOA. Previously, we examined the effect of region-specific knockdown (KD) of ERα or ERβ in the MPOA on the maternal behaviors, and found that both ERα KD and ERβ KD remarkably extended latency to retrieve pup in lactating female mice. These results indicated that both ERα and ERβ in the MPOA are necessary for maternal behavior. Here we investigate the patterns of the efferent projections of ERα positive neurons ($MPOA^{ER\alpha+}$) and ERβ positive neurons ($MPOA^{ER\beta+}$) in the MPOA. We virally expressed Cre-inducible Green fluorescent protein (GFP) in the MPOA of ERα-Cre mice and ERβ-Cre mice. A week after viral injection, we analyzed the expression pattern of GFP in several brain areas to compare projection patterns of $MPOA^{ER\alpha+}$ and $MPOA^{ER\beta+}$ neurons. So far, our data showed dense projections of $MPOA^{ER\alpha+}$ neurons in the ventromedial and dorsomedial nuclei of the hypothalamus, ventral tegmental area and, periaqueductal gray. This study provides a detailed insight into anatomical feature of ERs positive neurons in the MPOA. (Supported by Grant-in-Aid for Scientific Research 15H05724 to SO.)

P-33

The PPARα Modulator Pemafibrate Protects against Diet-induced Obesity in Mice

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Peroxisome proliferator-activated receptor α (PPARα) is a therapeutic target for hyperlipidemia. Pemafibrate is a new selective PPARα modulator that activates PPARα transcriptional activity. We assumed that Pemafibrate have a beneficial effect on adipose tissue and conducted experiment to determine the effect of Pemafibrate on diet-induced obesity. Wild-type mice were fed a high-fat diet (HFD) containing Pemafibrate or Fenofibrate for 12 weeks from 6 weeks old. Pemafibrate and Fenofibrate significantly suppressed HFD-induced body weight gain, decreased plasma triglyceride (TG) levels, and increased plasma fibroblast growth factor 21 (FGF21). Pemafibrate had the comparable effects with Fenofibrate in 1/500 amount of Fenofibrate. Both agonists activated PPARα transcriptional activity in the liver, increasing both hepatic expression and plasma levels of FGF21. In addition, both agonists increased the expression of genes involved in thermogenesis, including *Ucp1*, *Cidea* and *Cpt1b* in inguinal adipose tissue (iWAT) and the mitochondrial maker *Elovl3* in brown adipose tissue (BAT). These results indicate that both agonists activate thermogenesis in iWAT and BAT by increasing plasma level of FGF21. Additionally, only Pemafibrate induced the expression of *Atgl* and *Hsl* in epididymal white adipose tissue (eWAT), leading to activation of lipolysis. Taken together, these findings indicate that Pemafibrate suppressed diet-induced obesity in mice and improved their obesity-related abnormalities more efficiently than Fenofibrate. Conventional fibrates have made it difficult to improve human obesity. However, since Pemafibrate had stronger lipolysis in mice, we expect that Pemafibrate might improve human obesity.

P-34

Association between HLA class II alleles and allergen sensitization: a case control study of 544 Japanese adolescent

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Background: Type I allergic diseases including allergic rhinitis, bronchial asthma, and atopic dermatitis are considered to be multifactorial inheritance diseases that is caused by both environmental and genetic factors. The development of type I allergic diseases is related with the sensitization against allergen such as Japanese cedar pollen (*Criptomeria japonica*) and house dust mite (HDM). Human leukocyte antigen (HLA) class II genes have been reported to be associated with the development of type I allergy. Several studies have investigated the relationship between *HLA-DRB1*, *-DQB1*, *-DPB1* alleles and allergy-related phenotypes, however, the details of these associations remains unclear. The aim of the present study is to examine the relationship between sensitization against HDM and *HLA-DRB1*, *-DQB1*, and *-DPB1* alleles.
Methods: Subjects in the present study were 544 students who belonged to University of Tsukuba between 2013 and 2015. Sensitization against allergen was defined as an allergen specific IgE level ≥ 0.7 U_A/ml (RAST score of 2). Each individual's *HLA* allele including *HLA-DRB1*, *HLA-DQB1*, and *HLA-DPB1* was determined by PCR-SSOP. Logistic regression analysis was performed to examine relationships between allergy-related phenotypes and *HLA* alleles.
Results: Association was observed between *HLA-DPB1*09:01*/*HLA-DRB1*15:02* /*HLA-DQB1*06:01* and sensitization against HDM ($p < 0.05$). None of *HLA* alleles was associated with sensitization against both HDM and Japanese cedar pollen ($p > 0.05$).
Conclusions: The present study showed genetic association of HDM sensitization with HLA class II genes, suggesting the importance of the molecular interactions between HLA class II gene and HDM.

P-35

Comparison of the assessment of fetal heart rate during labor in low risk women between Japan and Mongolia

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The aim of this study is to clarify the similarity and difference of the assessment method of fetal heart rates during labor in low risk women between Japan and Mongolia.

We had the panel discussion that involved Japanese midwifery professors (n=4) and graduate students (n=2), and two Mongolian professionals, a general physician and an obstetrician leading the midwifery education in Mongolia. We discussed and reviewed each nationally gold standard guideline about how the fetal condition should be assessed during labor.

As a result, we found that the way of the assessment of fetal heart rate was regulated by different guideline in each country. Mongolian clinicians use a global standardized guideline, the Managing Complications in Pregnancy and Childbirth; A guide for Midwives and Doctors (World Health Organization: WHO, 2017), while Japanese obstetricians and midwives use the Guideline for Obstetrical Practice in Japan 2017.

In Mongolia, based on the WHO guideline, the fetal heartrate is counted for a full minute at least once every hour during the latent phase, once every 30 minutes during the active phase, and every five minutes during the second stage. On the other hand, in Japan, it is recommended that the cardiotocography (CTG) should be conducted within 6 hours after the previous observation based on the Japanese guideline. The continuous CTG is also allowed to be conducted to low risk women during the first stage in Japan. Furthermore, it is recommended that the continuous CTG should be conducted to all women during the second stage in Japan.

In summary, based on guidelines, the solid and updated methods of the assessment of fetal heart rates which provide mothers and infants with safety perinatal cares have been established in both countries, even though the guidelines are different. We need more field surveys in clinical situations to further clarify the assessment of fetal heart rates in both countries.

P-36

Development of Live Imaging Technology for Collagen Fibers

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Currently, there is few effective therapy for fibrosis, because the detailed mechanism of fibrosis has not been elucidated. The molecular size of collagen is over 300 nm, which is larger than common transport COPII vesicles. Therefore, it is known that the transport system of collagen is different from other proteins. Recently, it was found that TANGO1, an integral membrane protein localized to endoplasmic reticulum (ER) exit sites, load collagen VII into COPII carriers (Saito *et al*., 2009). Due to the discovery of this unique mechanism, collagen exportation from the ER has been actively analyzed. However, there are still many unknown points in the process of collagen secretion. In this study, we tried to develop a new imaging technology that can observe collagen fiber formation over time, in order to analyze the mechanism of fibrosis, the excessive collagen secretion, easily.

Collagen is unsuitable protein for imaging. There is no established method for visualization of collagen fibers directly. Since the N-terminal and C-terminal domains of pro-collagens are cleaved off, fluorescent protein fused to its termini is also removed. Since central domain of collagen has a tight structure of triple helix, it is difficult to insert fluorescent protein in the middle. However, we found that several types of collagen might be applied for imaging. We have developed collagen probes and transfected to MC3T3-E1 cells derived from mouse calvarias. We established stable expressing cell lines. However, collagens were not secreted in normal dish culture.

In this study, we transplanted the cells to C57BL/6 mouse in order to evaluate our collagen probe. When cells were transplanted subcutaneously into the dorsal region of the mouse, fibrous fluorescence was observed. Moreover, the fluorescence was consistent with collagenous fibers observed in tissue specimens. According to these *in vivo* experiments, we have found that the collagen probes can be secreted from cells and form fibers. Then, we examined methods which can reproduce fiber formation *in vitro*. As a results of testing various culture methods, we have found that some special culture conditions might be needed. We have also found that gravitational stress may be involved in the fiber formation. By advancing analysis of these special factors, there is a possibility of finding therapeutic targets for fibrosis. We will improve the culture method aiming for establishing the High Throughput Screening system using this probe.

P-37

Towards the design of artificial islets chip: study on the spatial relationship between vasculature and pancreatic islet cells

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Despite the development of 3D-printing technology and stem cell research, it is possible to achieve artificial pancreatic islet production and transplantation for fundamentally curing of diabetes mellitus. For developing artificial pancreatic islets, 3D structure of vasculature in physical pancreatic islets needs to be clarified due to the important role of blood vessels in metabolites delivering, sufficient nutrients supplying, and signals transduction. Recent knowledges of pancreatic islet architecture are basically built from analyzing immune- or chemical- staining of flat-slices which might loss the original morphology and stereo- architecture messages. Here we propose the technique to preserve the authentic pancreatic stereo-structure in mice, and using immunostaining to identify the original arrangement in murine pancreatic islets surroundings. We hope this strategy would figure out the original physical 3D structure of vasculature and be the basic scheme for designing artificial pancreatic islets.

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Myosin Id localizes in dendritic spines through the tail homology domain 1

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Dendritic spines are postsynaptic compartments at excitatory synapses of neurons, which receive excitatory neurotransmitters such as glutamate. Dendritic spines are capable of changing their shape and size to modulate synaptic transmission in response to environmental stimuli. The actin cytoskeleton and a variety of actin-binding proteins play a critical role in the dynamics of dendritic spines. It has been suggested that abnormal spine morphogenesis may cause neurodevelopmental disorders such as autism spectrum disorder (ASD) and schizophrenia.

Class I myosins are monomeric motor proteins that move along actin filaments using the energy of ATP hydrolysis. Myosin Id, a member of class I myosins, is the mammalian homolog of Drosophila *Myo31DF* and has been reported to be expressed in mammalian neurons. A linkage analysis has suggested that single nucleotide polymorphism of myosin Id gene is associated with increased risk of ASD. Although myosin Id has been expected to play a role in neurons, neither its function nor its subcellular localization in neurons has been studied so far.

Here, we investigated the subcellular localization of myosin Id and determined the domain responsible for it. First, we found that myosin Id is enriched in synaptosomal fraction of mouse cerebra. *In vitro* analyses have revealed that myosin Id is accumulated in the F-actin-rich pseudopodia of HEK293T cells and in the dendritic spines of primary hippocampal neurons. Both deletion and substitution of the tail homology 1 (TH1) domain drastically diminishes its colocalization with F-actin. In addition, the mutant form lacking the TH1 domain is less distributed in dendritic spines than is the full-length form. Unexpectedly, the conserved sequence corresponding to actin binding domain of *Myo31DF* is dispensable for actin-binding capacity of myosin Id. Taken together, our findings reveal that myosin Id localizes in dendritic spines through the TH1 domain.

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Analysis of adipose tissue-derived mesenchymal stem cells (AT-MSCs) under the atherosclerosis microenvironment

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During the atherosclerosis (AS) progression, perivascular adipose tissue-derived mesenchymal stem cells (PVAT-MSCs) are exposed to hypoxic environment due to the oxygenic deprivation which might influence the adipose tissue-derived mesenchymal stem cells (AT-MSCs) function. Additionally, it has been reported that the angiogenic ability of subcutaneous AT-MSCs (SAT-MSCs) was impaired in the AS patients. However, up to now, the effects of AS on the characteristics and function of PVAT-MSCs have not been clarified yet.

In the present study, we analyzed the AS microenvironment effects on the characteristics and function of AT-MSCs. We found that there was no significant difference in cellular morphology and differentiation ability between SAT-MSCs and PVAT-MSCs in AS patients. However, the proliferation and migration ability of AS-derived PVAT-MSCs were less than those of AS-derived SAT-MSCs. Of note, AS-derived PVAT-MSCs showed the upregulation of SDF1, which is related to the homing, and VEGF, which is related to the angiogenesis compared to those of AS-derived SAT-MSCs. Consistent with these results, AS-derived PVAT-MSCs showed the higher ability to recruit EPCs and ECs than AS-derived SAT-MSCs. In addition, EPCs and ECs which cultured in the presence of AS-derived PVAT-MSC conditioned medium showed the higher angiogenic function of the tube formation compared to those cultured in AS-derived SAT-MSC conditioned medium. This result suggests that the higher paracrine effects of AS-derived PVAT-MSCs to support the angiogenic function of the target cells.

Our data showed the different characteristics and functions of AT-MSCs derived from different sources of tissues. Under the AS microenvironment, it seems that the characteristics and functions of PVAT-MSCs might reflect the progression of AS. Further study will be necessary to clarify the mechanism in future.

Key words: atherosclerosis, mesenchymal stem cells, perivascular adipose tissue

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Identification and analysis of slow-proliferating cells in colon cancer cell line

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Many types of tissue stem cell are thought to have slower cell cycle compare with other proliferating cells. Tissue stem cells are required for maintenance of tissue structure and homeostasis. It is reasonable to delay cell division rate for reducing the possibility of mutation, inappropriate chromosomal distribution, and telomere shortening. In hair follicle stem cells, there are two types of stem cells and one of them proliferate slower than the other. In hematopoietic stem cells, there is the population that proliferate slower than average.

We previously investigated the cell proliferation kinetics of colon epithelial cells in wild type C57BL/6 mouse and $Apc^{min/+}$ mouse using BrdU with continuous administration by subcutaneous osmotic pomp. In these experiment, we observed a few number of cells at the bottom of crypt were not proliferated during 7 days of BrdU continuous administration in normal colon. So, we thought that BrdU negative cells might have stem cell properties. In addition, the number of BrdU negative cell in adenoma of $Apc^{min/+}$ mouse was dramatically increased compared with normal colon crypt. These results indicated that the regulation of BrdU negative cell may affect an adenoma progression. Dormant cell in small intestine is reported to play an important role in the maintenance and redundancy of tissue stem cell. However, there is no report about colon epithelial dormant cell.

Based on our previous findings, we sought to reproduce those results and isolate the slowly proliferating cells without fixation and *in vitro* using human colon cancer cell lines. To trace the number of cell division, we utilized lipophilic carbocyanine dye(NeuroDiO), which reflected the number of cell division by cell division-dependent dilution to distinguish slow proliferating cells. We stained three different colon cancer cell lines in 2D(normal) and 3D(sphere) culture condition with NeuroDiO, and analyzed the intensity of NeuroDiO by FACS sorting. In this experiment, we observed the shapes of histograms in 3D culture are wider and more uneven than it in 2D culture. 3D culture is a common method to culture cancer cells and thought to be closer to *in vivo* condition. This result indicates that the pattern of cell division in 3D culture is more diverse and restore hierarchy of cell population. As a next step, we plan to sort slower proliferating cells from those cell line in 3D culture condition, and analyze the gene expression by RNA-seq. Furthermore, we are trying to culture organoid of colon epithelium and adenoma to isolate slow proliferating cells and analysis the property of these cells.

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Establishment of iRFP Based *in vivo* Liver Inflammation Imaging System

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Introduction: Liver is a major vital organ in the body, maintaining numerous biochemical functions and diverse metabolic processes essential for life. Any condition that causes liver inflammation (hepatitis) or damage and that may affect liver function is recognized as liver disease. Liver diseases are a global health issue with high morbidity and mortality worldwide. Assessing liver inflammation has a great importance to predict about liver health. In mouse models, traditional histopathological hepatitis assessing methods are much labor and time intensive, which is disadvantageous in pre-clinical settings. In this study, we establish the Fluorescence based *in vivo* imaging systems that can be used to non-invasively examine the livers of living animal.

Methods: Near Infra-red Fluorescent Protein iRFP is a nontoxic, stable protein (excitation/emission wavelengths 690nm/ 713nm) with great deep-tissue penetration and minimal autofluorescence. C57BL6 albino mice reconstituted with beta-actin promoter derived iRFP transgenic mice bone marrow cells were generated. This system targets the signal created by the infiltrated iRFP expressing immune cells in liver damage. Liver damage was induced by feeding high cholesterol diet (HCD). IVIS® imaging system was used for *in vivo* imaging. Serum liver damage markers ALT and AST were measured to assess the liver damage.

Results: *In vivo* and *ex vivo* IVIS® images of the HCD fed group exhibited a specific iRFP signal enhancement in the liver, compared to normal chow diet fed control groups. Time course imaging showed a gradual increase of the signal intensity, which was correlated with serum ALT and AST levels and symptoms of liver failure in mice. Histological analysis of livers showed accumulation of iRFP positive inflammatory cells.

Conclusion: This mouse hepatitis imaging system is able to noninvasively image and generate longitudinal data of hepatitis progression. Moreover, it can be used as a novel tool for *in vivo* live liver imaging in preclinical set up to simply assess liver inflammation.

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Functional analysis of SOX9 variants in chondrocyte differentiation

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Hyaline cartilage plays important roles in shock absorption and smooth movement of diarthrodial joints as articular cartilage. Hyaline cartilage consists of chondrocytes and cartilage extracellular matrix including types II, IX, and XI collagen, proteoglycans, and other proteins. Due to the low self-repair ability of hyaline cartilage, its focal defects or degeneration caused by trauma and aging lead to osteoarthritis, which is the most common pathological condition of joints. Cartilage damage is sometimes treated with fibrocartilage, which differs from hyaline cartilage with respect to the composition and localization. Fibrocartilage, which appears in scarred tissues, is composed of mainly types I and II collagen. Since type I collagen impairs, rather than repairs, the matrix architecture and mechanical function of cartilage, fibrocartilage cannot replace hyaline cartilage without some degree of functional impairment. One treatment of cartilage defects is autologous chondrocyte implantation (ACI), which requires healthy articular hyaline cartilage from the same patient. The patient needs to sacrifice a small piece of articular cartilage from a healthy joint, and thus the availability of autologous hyaline cartilage limits the effectiveness of ACI.

Recent studies have shown that transduction of a chondrogenic transcription factor SOX9 and two reprogramming factors, KLF4 and c-MYC, can directly induce chondrogenic cells from dermal fibroblasts or adipose tissue-derived stromal cells. Although this method is potentially promising for preparing sufficient amounts of chondrocytes, the efficiency of chondrocyte induction is still very low (<0.3%).

To improve the efficiency of chondrocyte differentiation, we plan to manipulate the key transcription factor SOX9. A previous study has reported that the efficiency of reprogramming can be enhanced by a variant of transcription factor NANOG, suggesting that a mutation in a key reprogramming factor may, in rare cases, enhance rather than compromise its reprogramming ability. We therefore created various SOX9 variants and tested their ability to induce chondrocyte differentiation. We also developed an improved system for chondrocyte differentiation by constructing a vector that expresses KLF4 and c-MYC simultaneously at a relatively constant stoichiometry.

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Biomimetic PDMS-Gum Arabic hybrid biopolymer adhesive for drug delivery

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There has been an increasing demand in surgery and clinical medicine to have effective tissue adhesives, which are biocompatible, structurally robust and able to operate in the wet environment. Inspired by the examples from nature in adhesion, we are developing a hybrid adhesive, that consist of an array of micro-fabricated pillars to mimic the sticky mucilage, secreted by Sundew plant to catch preys. The micropillars are made of PDMS and Gum-Arabic, a synthetic polymer with complex and branched structures, to confer adhesive property, despite in wet condition. In addition, Kanamycin A - an antibiotic for treating severe bacterial infections - is employed in our adhesives for transdermal drug delivery demonstration.

The micro-fabrication procedure was based on soft lithography. Afterwards, the force curves were obtained by controlling the AFM probe to perform an approach−retract cycle in the vertical direction on the adhesive substrates. The adhesion forces were calculated from the retract curve and equal to the magnitude of the peak. The adhesion force was generally at the order of ~10 nN - this value similar to the strength of gecko's setae, 10nN/each setae. The adhesives with micropillars had larger adhesion forces than the flat adhesives without pillars. The sample with PDMS-Gum-Arabic (9x9μm2 pillars) provide twice higher adhesive forces than the one with PDMS-only.

The use of hybrid biopolymers, made of PDMS and Gum-Arabic, is successfully demonstrated as an adhesive with the potential to deliver antibiotics to treat severe bacterial infections. The hybrid biopolymer is found to provide higher adhesion forces, or stickier, than the adhesive made of PDMS-only. The technology developed from this study therefore can provide a new platform with high adhesion and biocompatibility for drug delivery system.