P-1

Longitudinal relationship between eating habits in pre-school children and social emotional development six years later

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Eating behavior is one of the key factors for the nutrient access and nutrient deficiency in the preschool age. Preschool age has a potent effect on the lifelong habits that includes health related behaviors. Prior studies of eating habits have focused its relationship with obesity or intelligence quotient. One of the domains that these measures do not provide detailed information is Social- emotional development. The objective of the study was to evaluate the eating habits and diet of preschool children as predictor of social emotional development six years later.

This study used a longitudinal design and parent-child dyads participated from a project named “Community Empowerment and Care for Well being and Health longevity” (CEC) that initiated in 1991 ata suburban area in central Japan. For this longitudinal study we studied preschool children at baseline year 2011 and followed till 2017. Main exposure was Choice of different food among children. Main outcome was social-emotional development, assessed by the Strength and Difficulties Questionnaire (SDQ) completed by parents. Higher score indicated greater behavior problems. Parents first provided details related to their child’s eating habits in the baseline year and then completed the Strengths and Difficulties Questionnaire 6 years later to assess social emotional development.

The results suggested that eating behavior such as eating vegetable, fruit, fish, egg, milk and soy are significantly associated with some aspects of the SDQ scoreHowever, no association was found between meat intake and small fish with any aspect of the SDQ. Thus, exploration of further in-depth association between eating habits in preschool age is required for their social-emotional development later.

P-2

Targeting AMPK to modulate B cell metabolism and treat Inflammatory Bowel Diseases

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AMPK integrates a group of metabolic sensors that control several intracellular processes such as metabolism, cell growth, autophagy and post-translational modifications. However, its role in B cell metabolism has been little assessed despite the relevance of these cells in both humoral and cellular responses. Thus, our aim was to evaluate the role of AMPK in B cell metabolism and how the B cell-specific metabolic changes could impact immune-mediated inflammatory diseases. Sorted splenic B cells from control (CT) and B cell-specific AMPK knockout ($AMPK^{\Delta B}$) mice were cultured with LPS. Both the lactate/glucose ratio and activation markers were mainly increased in $AMPK^{\Delta B}$ cells after LPS, while few or no differences were observed with other stimuli (CpG, anti-IgM or anti-CD40 + IL-4). Moreover, $AMPK^{\Delta B}$ cells presented a sharply increase in IL-10 and IL-6 synthesis, CD40/CD86 expression and glucose uptake compared to CT cells but decreased differentiation into plasmablasts. According to these results, CT B cells treated with AMPK agonist decreased IL-10 secretion and CD40/CD86 expression but increased plasmablast differentiation. Similar results were observed in mTORC1-deficient B cells, a downstream target negatively regulated by AMPK. $AMPK^{\Delta B}$ cells also increased mRNA expression of GLUT1, glycolytic enzymes and PDK1 as well as decreased mitochondrial activity, basal respiration and maximal respiratory capacity. DSS-induced colitis in $AMPK^{\Delta B}$ mice showed a marked improvement of the clinical signs and increased IL-10 levels in gut compared to CT mice. Finally, B cell-deficient μMT mice that received adoptively transferred $AMPK^{\Delta B}$ cells had more benign disease scores when compared to those that received CT B cells. These results provide evidence of a regulatory role of AMPK in glycolysis, activation threshold and plasmablast differentiation of B cells. We propose that B cell-specific AMPK inhibition is a potential target to treat inflammatory bowel diseases.

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P-3

Change Differences between Favorable and Unfavorable Eating Habits in Japanese Adults and Elderly over 15 Years

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The shift to older age is marked by several physiological and psychological changes that affect eating resulting in increased risk for chronic disorders. Several health problems in older age can be prevented or delayed by changes in lifestyle habits, including diet, at 55-65 years old, suggesting the importance of including middle age adults in programs supporting healthy aging. Insights on how eating habits evolve over time can help determine favorable and unfavorable eating patterns that lead to better or worst health. As a result, better health promotion programs can be created to improve overall intake and diet.

The aim of this study was to investigate differences in degree of change in favorable and unfavorable eating habits among a group of Japanese adults and elderly over 15 years.

Changes in eating habits of approximately 500 participants were evaluated through self-reported questionnaires between 2002 and 2017. Participants were adults aged 50 years and above at baseline and followed up again 15 years later at 65 years old and above. The questionnaire from the CEC Cohort study was used to evaluate participants' eating habits in terms of food-related behaviors and food consumption pattern. Each item was analyzed separately, and responses were organized into either "favorable" or "unfavorable" eating habits at baseline and endpoint. Mcnemar test was used to determine change status in both the favorable and unfavorable eating habits and investigate differences, if any, between such changes.

Results showed significant degree of change between favorable and unfavorable eating habits over 15 years in regard to reading food labels, eating breakfast, and controlling for salt, as well as small fish and seaweed consumption. Overall, some differences in change status between favorable and unfavorable eating habits were found. Further research is needed to the nature of such changes in more depth.

P-4

DOSE DEPENDENT TRANSCRIPTIONAL REGULATION OF KLF4

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Induction of pluripotency in somatic cells by four factors (OCT4, KLF4, SOX2, and c-MYC) suffers from low efficiency and poor quality. Only 1% of initial somatic cells can be fully reprogrammed to induced pluripotent stem cells (iPSCs), while remaining 99% remain as partially reprogrammed cells (partial iPSCs). These partial iPSCs pose a potential risk of tumorigenesis when used for medical applications. Hence, it is essential to increase the efficiency and quality of reprogramming. Our laboratory has developed 3S reprogramming system, which can generate iPSCs paused at various stages of reprogramming by controlling protein level of KLF4. At low protein level, KLF4 generates paused iPSCs (Low-K), and rescues paused iPSCs towards full pluripotency when a higher level of KLF4 (High-K) was supplied. Thus, we aim at identifying regulatory mechanisms that cooperate with KLF4 in progression of reprogramming

To identify mechanisms regulated by KLF4, we performed chromatin immunoprecipitation and sequencing (ChIP-seq) using Low-K and High-K iPSCs. Interestingly, only at high protein level; KLF4 occupied promoters of pluripotency inducing genes. Thus, we conclude that KLF4 binds to promoters of pluripotency genes in High-K iPSCs and regulate their expression. Therefore, we hypothesize that KLF4 at high dose recruit proteins that regulate pluripotency induction and reprogramming

To identify proteins that cooperate with KLF4, we chose *Nanog* promoter and applied CAPTURE (CRISPR affinity purification in situ of regulatory elements) which can isolate regulatory proteins bound to a specific genomic target. Proteomic analysis of *Nanog* promoter-CAPTURE revealed some known *Nanog* regulating proteins including TRIM28. And also, some splicing regulating proteins including THRAP3, and BCLAF1 whose roles on *Nanog* regulation remains unknown.

We now investigating the relationship between these splicing factors and *Nanog* gene regulation. Moreover, extending this CAPTURE to paused iPSCs, we will be able to identify the proteins that cooperate with KLF4 and have potential to increase efficiency of reprogramming.

P-5

Association between home-rearing environment and social skills among 6-year-old children in China

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Children's social skills are increasingly recognized as vital to their overall development in a number of areas, such as school enjoyment, academic achievement, and the development and maintenance of internalizing and externalizing behavior problems. According to previous studies, home-rearing environment predicts high levels of social skills and it is an important factor which is related to children's development. Contemporary Chinese parents with young children have increasingly recognized the importance of fostering children's social competence, perhaps as a result of radical economic and social changes underway in China. Therefore, the study of home-rearing environment and social skills are still needed in China. This study aims to examine the association between home-rearing environment and social skills among Chinese kindergarten children aged 6 years old. A cross-sectional study was implemented in one kindergarten, in a coastal city of Zhejiang Province, China.

Teachers rated children's social skills and caregivers rated the demographic characteristics and home-rearing environment. Teachers rated children's social skills using Social Skill Scale (SSS) and caregivers rated the demographic characteristics and home-rearing environment using Index of Child Care Environment (ICCE). We used Chi-square test, Fisher exact test and logistic regression model in this study. The results showed home-rearing environment is associated with children' social skills. It can be inferred that parents should increase interactions with their children in their free time to reduce the risk of social skills delay and during daily childcare, parents should more pay attention to social stimulation.

P-6

The influence of sleep conditions on health-related quality of life among Japanese adolescents three years later

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Adolescents' poor sleep conditions can lead to poor quality of life. As an important measurement, health-related quality of life (HRQoL) has recently attracted more and more attention, studies of HRQoL can be benefit for understanding people's physical and mental functioning. But study about adolescents' HRQoL is still lacking, especially which associated with sleep. This study aims to clarify the influence of sleep conditions on HRQoL among Japanese adolescents three years later.

The current study used about 200 adolescent participants aged 12 to 19 years from a suburban area located in the southwest part of Aichi-Ken, which is a typical community. Study set 2014 as the baseline year and examined how the sleep conditions of participants can effect adolescent's HRQoL score three years later, in 2017. 5 questions via the self-reported questionnaire are used to evaluate the sleep conditions. Demographic factors (gender, age, siblings, family structure, chronic disease, marital factors of parents) are also considered. KINDL^{R} is used to evaluate HRQoL score. Current results suggested that sleep satisfaction, duration, and regularity are all positively associated with HRQoL total score. Sleeping duration is found to be more influential than satisfaction and regularity. Further study will be applied to see the association between sleep conditions and HRQoL sub-scales. Discussing the results can figure out about how we can help to improve adolescents' HRQoL toward future research.

P-7

The effect of social interaction on Japanese elderly with depressive tendency

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This study aims to find out the impact of social interaction on recover depressive tendency among Japanese elderly.

Longitudinal study will be implemented with self-reported questionnaire in 2014 (baseline year) and 2017 (follow-up year). The subjects of this study will be the elderly over 65 years old with depressive tendency in 2014 Social interaction will be evaluated through the Index of Social Interaction (ISI). It contains 18 items in 5 subscales. Previous studies also demonstrated the measure's validity and reliability. ISI also has been used in the "Tobishima Cohort Study" in Japan for more than twenty years. Five questions will be used to measure the depressive tendency. There are two choices: Yes and No, choice of yes is rated 0 point, and choice of no is rated 1 point, and total points are 5. Lower than 3 points is regarded as risk.

Our result showed that taking an active approach, Having an active role, Participating in social groups, Trying to use new equipment, Having regular lifestyle, Having counsel, Having a hobby were found having a relationship with depressive tendency. In ISI subscales part, Independent, social curiosity, interaction, and total score were found having a relationship with depressive tendency.

This study found that depression tendencies are related to the total score of social interaction, which is consistent with the prior study also shows that social interaction has a positive effect not only in preventing depressive tendency, but also in the recovery of depressive tendency. We hope that this research can play a positive role in slowing down the tendency to depression.

P-8

Elucidation of the role of C1orf38 in human NK cells

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Natural killer (NK) cells are innate immune lymphocytes which comprise approximately 10% of peripheral blood mononuclear cells. NK cells play critical roles in anti-viral and anti-tumor immunity. When NK cells recognize virus-infected and tumor cells, NK cells exert cytotoxicity and produce interferon-γ (effector functions). Although NK cell function is regulated by the magnitude of activation signals through activating NK receptors, it has not been fully understood how the activating receptor signaling is integrated into the effector function. We performed a transcriptome analysis by using mouse NK cells after mouse cytomegalovirus infection and found that the mouse homolog of human *C1orf38* was upregulated specifically in a NK cell subset expressing an activating NK receptor which recognizes the infected cells. A previous study has shown that C1orf38 is predicted to regulate intracellular signaling by pathway analysis. However, the role of C1orf38 in NK cells is unknown.

The purpose of this study is to elucidate the role of C1orf38 in the effector function of human NK cells. Given the domains of C1orf38 protein, we hypothesized that C1orf38 would work as a signaling scaffold protein for signaling molecules for activating NK receptors, such as NKG2C, NKG2D, and DNAM-1. We found that primary human blood NK cells highly express *C1orf38*, while a human NK cell line NKL expresses the low levels of *C1orf38*. To perform gain-of-function experiments, *C1orf38* was cloned from primary human blood NK cells and constructed into an expression vector. To perform loss-of-function experiments, guide RNAs targeting *C1orf38* exons were designed and constructed into a CRISPR/Cas9 vector. To address the functional role of C1orf38 in NK cells, *C1orf38*-overexpressing and -knockdown NKL cell lines will be stimulated by cross-linking NKG2C, NKG2D, and DNAM-1, and degranulation and IFN-γ production will be evaluated by flow cytometry.

P-9

Role of sulfane sulfur in metallothionein-3 as a model of zinc-binding protein

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Persulfides/polysulfides in which contain sulfane sulfur atoms with six valence electrons and no charge (S^0) exhibit high nucleophilicity and play a role in redox homeostasis. For example, persulfides/polysulfides can scavenge hydrogen peroxide (H_2O_2) and also capture electrophiles such as methylmercury (MeHg), leading to formation of sulfur adducts. We previously reported that cysteinyl-tRNA synthetase 2 produces sulfane sulfur-binding proteins (SSBPs) during protein translation (Akaike T et al. Nature Commun 2017). We also found that there are a variety of SSBPs in hepatic cytosol of mouse and that GSTP1 is identified as a SSBP to capture MeHg, thereby yielding $(MeHg)_2S$ with less toxicity (Abiko Y et al. Chem Res Toxicol 2015). The purpose of present study is to identify and characterize SSBP from mouse brain, which is susceptible to oxidative stress. Separation of the cytosolic proteins by a variety of column chromatographies with derivatization of sulfane sulfur atom by addition of β-(4-hydroxyphenyl)ethyrl iodoacetamide, followed by LC-MS revealed that SSBPs extensively exist in mouse brain, and that metallothionein-3 (MT3) was identified as a SSBP. While this protein has been recognized as a zinc-binding protein through cysteine residues for a long time, recombinant MT3 was found to contain approximately 20 sulfane sulfurs per protein, thereby regulating oxidative, nitrosative and electrophilic stresses. Three-dimensional structure modeling analysis discovered that sulfane sulfur plays an important role in both protein thermostability and affinity to zinc without affecting the overall structure of MT3. The present study indicates an interdependent relationship between sulfane sulfur and zinc binding in MT3 and thus provides paradigm shift to a novel function of sulfane sulfur in cellular zinc regulation.

P-10

Regulation of the cardiovascular autonomic nervous system by the lateral habenula

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Neurons in the lateral habenula (LHb) are activated by stressful events such as pain, physical constraint, open field exposure and social defeat. Although these events induce cardiovascular responses, whether and how the lateral habenula regulates the cardiovascular autonomic nervous system remains unclear. To investigate this issue, here we electrically stimulated the LHb and examined the effect on cardiovascular responses in anesthetized rats. We used male Wistar rats (250-300g) with anesthetized by urethane (1-1.25 g/kg, *i.p.*). Mean arterial pressure (MAP) was monitored from a left femoral artery. Heart rate (HR) was calculated from electrocardiogram. Electrical stimulation with 300 μA intensity, 0.5 ms duration and 100 Hz frequency was delivered to the left LHb for 10 s. We found that the electrical stimulation of the LHb increased MAP and decreased HR, while stimulating the outside of the LHb at 0.25 mm distance did not affect MAP or HR. Notably, cutting the bilateral vagus nerves, which are the cardiac parasympathetic nerves, completely suppressed the effect of the LHb stimulation on HR but did not change that on MAP. On the other hand, systemic administration of propranolol (5-10 mg/kg, *i.v.*), a nonselective β adrenergic receptor antagonist mainly blocking the cardiac sympathetic system, partly attenuated the effect of the LHb stimulation on MAP but did not affect that on HR.

These data suggest that the LHb regulates cardiovascular responses through the cardiac parasympathetic nerve and cardiovascular sympathetic nerve. This system may work to induce cardiovascular responses when animals encounter stressful events.

P-11

Mapping cortical brain activity associated with processing of recent and remote associative olfactory memories

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Initially encoded in the hippocampus, new associative memories, also referred to as memory engrams, are thought to become progressively dependent on a broadly distributed cortical network as they mature and consolidate over time. While acting as permanent repository sites for remote memories, converging findings point however to an earlier recruitment, upon encoding, of some cortical sites that participate actively to the generation of engram cells, albeit in an immature form. One such region is the prefrontal cortex (PFC) in which rapid functional and structural changes occur, raising the possibility that subsequent maturation of cortical engrams requires a complex and time-dependent interplay between dedicated brain regions. Here, by tracking the expression of the activity-dependent gene c-Fos, we sought to dissect the involvement of interconnected cortical regions (Prelimbic-Infralimbic cortices: mPFC; Anterior Cingulate Cortex: ACC; Posterior Cingulate Cortex: PCC; Orbitofrontal Cortex: OFC) that may participate to the generation of functionally mature engrams. Recent or remote associative olfactory memory was probed in rats submitted to the hippocampal-dependent social transmission of food preference paradigm. We identified 3 key brain regions that were co-activated upon remote memory recall (mPFC, ACC and OFC) and may act as critical “hub-like” nodes within the extended network of cortical regions supporting remote memory formation. By computing interregional correlations, we found that neuronal activity within mPFC and OFC were highly correlated although their recruitment was dependent on the age of memory. While OFC activation was delayed and detectable only remotely, that of mPFC occurred at both recent and remote delays. In contrast, PCC involvement was circumscribed to recent memory. Chemogenetic experiments aimed at examining causal relationships are ongoing. Altogether, these findings suggest that cortical engram maturation holds region-specificity but also involves complex cortical network connections and distinct kinetics of interactions that likely guide engram cells to their final maturation state to enable optimal remote memory storage and expression.

P-12

Odor exposure increased fat accumulation and enhanced motility on *C.elegans*

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Olfactory sensation is one of the most primitive senses. Unlike many other nerves, olfactory nerve, which is the shortest cranial nerve, does not join the brain stem. Although human have evolutionally reduced olfactory sense, this sense is still essential for our life. Therefore, we investigated effects of exposure to certain aromatic substances in *Caenorhabitis elegans*.

C.elegans, nematode in English, were used as an experimental model organism in this research. The reason of that is that nematodes can sense many chemical substances in their neurons to which they develop chemotactic behaviors.

To investigate the effect of odor exposure on fat accumulation, adults' nematodes were corrected and treated with aroma for 24 h before Nile Red staining. We found that fat accumulation significantly increased in nematodes with odor stimulation. There are mainly two possible reasons for the increase of fat accumulation; increase of food intake and metabolic change. To evaluate this, feeding movements was observed by measuring the pumping motion of nematodes with odor stimulation. Treated nematodes did not display the change of pumping motion compared with untreated group, indicating that lipid metabolism change causes the increase of fat accumulation by odor stimulation.

The effect of odor stimulation on motility was also measured after 35℃ for 4 h thermal stress. Motility restoration was increased significantly in treated nematodes. We thus explored whether Daf-16 is involved in motility restoration, because previous studies have shown that the transcription factor DAF-16 controls expression of various gene involved in lifespan, heat stress tolerance, and metabolism. daf-16 mutants with odor stimulation showed higher motility than daf-16 without odor, which could coincident with the results in N2, suggesting that DAF-16 involved in motility restoration after thermal stress.

This study has revealed that odor stimulation increased fat accumulation without the increase of food intake and improved motility after heat stress via DAF-16.

P-13

Transcription factor c-Maf is critical for renal glucose reabsorption through the direct regulation of *Sglt2*

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The renal proximal tubule reabsorbs 80-90% of the filtered glucose load through the sodium-glucose transporter SGLT2, and SGLT2 inhibitors are the most recent therapeutic drugs available to diabetes to increase urinary glucose excretion. Recent reports reveal that SGLT2 protein is expressed in the renal proximal tubules. Although the expression, the transcriptional regulation of SGLT2 *in vivo* have remained to be investigated. c-Maf is a basic leucine zipper transcription factor belonging to the large Maf family. Maf family shares the conserved basic region and leucine zipper motif that mediate dimer formation and DNA binding to the Maf recognition element (MARE) site. In addition to these domains, large Maf family is characterized by the acidic transactivation domain at their N-terminus. c-Maf is known to be responsible for lens formation, differentiation of T-cell subsets, terminal differentiation of hypertrophic chondrocytes during endochondral bone development, and development of the kidney in mouse embryo. However, since c-Maf deficient mouse is embryonic lethal, the roles of c-Maf in adult have remained unknown. For the purpose of investigating c-Maf functions in adult stage, we generated tamoxifen-inducible *c-Maf* knockout mice (*c-Maf* $^{del/del}$; CAG-Cre-ER^{TM}; called as *c-Maf* $^{\Delta TAM}$). Surprisingly, the *c-Maf* $^{\Delta TAM}$ mice treated with tamoxifen injection rapidly developed severe renal glycosuria. Furthermore, we found that the *c-Maf* $^{\Delta TAM}$ showed decreased expressions of *Sglt2* in the kidney of the *c-Maf* $^{\Delta TAM}$ mice. Lastly, we demonstrated c-Maf directly controls *Sglt2* expression using reporter assay. Thus, our results suggest that c-Maf is critical for glucose reabsorption by regulating Sglt2 in adult kidney and providing a platform for understanding renal glucose reabsorption under pathophysiologic conditions.

P-14

Physiological and psychological study on facial expression recognition in autism spectrum disorder

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Autism spectrum disorder (ASD) is one of the neurodevelopmental disorders with defect of social communication and interaction and restricted, repetitive patterns of behavior, interests or activities. In typical development (TD) people and ASD people, there may be differences in processes of facial expression recognition and brain activities. In this study, we examined the characteristics of facial expression recognition in TD adults and ASD adults by examining the coherence value of EEG during task execution using facial expression stimulation. The task was administered to 14 TD adults and 2 ASD adults. Before the task, participants were asked to answer the Japanese version Autism-Spectrum Quotient (AQ) test to examine the ASD tendency. Facial expression stimuli were used in the task and EEG recording was performed during the task. As a stimulation, four facial expressions (anger, happy, sadness, and surprised) and neutral one were used from the KDEF database. As a result, there was the main effect of facial condition in the value of coherence at Left frontal and Left frontposterior ROIs. So it was suggested that there was left-right difference in coherence value in facial expression recognition task. In addition, AQ score was correlated to the difference of coherence value between neutral face and expression face. By comparing with the previous study, there were conditions that may be related to age and conditions that may be related to ASD tendency. Since this study was based solely on the coherence values of EEG, it was only speculation on how to recognize facial expression. In the future study, it is considered that new findings can be obtained by recording gaze points and examining how stimuli are received.

P-15

Prevention of the prolongation of autoimmune arthritis by RORγt^{+}Foxp3^{+} regulatory T (Tr17) cells

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Objective: To clarify the role of RORγt^{+}Foxp3^{+} regulatory T cells (Treg), designated Tr17 cells in the development murine autoimmune arthritis model.

Methods:1) To analyze the cytokine production from Tr17 cells in the course of collagen induced arthritis (CIA), lymphocytes were harvested from Foxp3$^{IRES\text{-}gfp}$ reporter mice on 10 days after first collagen type II (CII) immunization. CD4^{+}GFP^{+} Treg and CD4^{+}GFP^{-} T cells were isolated and stimulated with anti-CD3 monoclonal antibody (mAb) and anti-CD28 mAb *in vitro*. The expression of IL-10 and IL-17 in RORγt^{+}GFP^{+} Tr17 cells was analyzed by flow cytometry (FCM) and compared with that in RORγt^{-}GFP^{+} cells or RORγt^{+}GFP^{-} cells. 2) After the induction of CIA, lymphocytes in inflamed ankle joints and lymph nodes (LN) were harvested from C57BL/6 mice. The expression of RORγt in both Foxp3^{+}Treg cells and Foxp3^{-} non-Treg cells were analyzed by FCM. 3) Foxp3^{cre}RORγt$^{fl/fl}$ (conditional knock out; cKO) mice which deficit Tr17 cells were immunized with CII on days 0 and 21. Incidence and severity of CIA were analyzed and compered with Foxp3^{wt}RORγt$^{fl/fl}$ (control) mice.

Results: 1) IL-10 producing cells were significantly increased in Tr17 cells compared with RORγt^{-}Treg cells. 2) RORγt^{+}Foxp3^{+} Tr17 cells and RORγt^{+}non-Treg cells were increased and Foxp3^{+}Treg cells were decreased in inflamed ankle joints compared with LN after the induction of CIA. 3) Although there was no difference in incidence and severity at peak of CIA, severe arthritis was significantly prolonged in cKO mice compared with control mice.

Conclusion: Our results raised the possibility that Tr17 cells might be related with the restoration of autoimmune arthritis.

P-16

Core 1−derived *O*-glycan is required for normal glomerular filtration and stable expression of podocalyxin on podocytes

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The glomerular filtration barrier is composed of the podocyte, glomerular basement membrane, and fenestrated endothelial cells. The disruptions of these structures cause several glomerular injuries such as focal segmental glomerular sclerosis (FSGS). The patients with FSGS reach end-stage renal disease in some cases and its incidence is rising around the world. Mice with a mutation of core 1 beta-1,3-galactosyltransferase (C1galt1) which elongated core 1-derived *O*-glycan in conjunction with C1galt1 specific molecular chaperone (Cosmc) showed thrombocytopenia and kidney disease (Alexander W.S. et al., PNAS., 2006). The apical membrane on the podocyte has a negatively charged surface coated by some proteins with core 1-derived *O*-glycan. Therefore, we hypothesized that core 1-derived *O*-glycan on podocytes is required for preventing the leakage of albumin from blood vessels. Then, we exploited the tamoxifen-inducible and podocyte-specific NPHS2-CreER^{T2} transgene to ablate a conditional mutant Cosmc allele (cKO).

cKO mice exhibited transient proteinuria and the foot process effacements of podocyte at 20 days after the final tamoxifen injection. Subsequently, cKO showed the pathogenesis of FSGS-like disease. To clarify the key molecules of the FSGS-like phenotype, we focused on podocalyxin and podoplanin which are sialylated and *O*-glycosylated glycoproteins and expressed on podocytes. As a result, western blot analysis using the isolated glomeruli revealed that the mature podocalyxin protein was significantly reduced in cKO compared to WT mice. Then, some signals of degraded podocalyxin appeared in cKO mice. However, the protein localization and mRNA expression of podocalyxin weren't changed between WT and cKO mice. On the other hand, the expression of podoplanin protein and mRNA were reduced at 20 days. Our results suggest that core 1-derived *O*-glycan on podocytes is required for normal glomerular filtration. The decreases of podocalyxin by the absence of core 1-derived *O*-glycan might be due to proteolytic attack and podoplanin might affect rather the prognosis than the cause of proteinuria because it was reported that podoplanin-deficient mice didn't show kidney disease.

P-17

Prevalence of Agr Phase Variants in *Staphylococcus aureus*

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Staphylococcus aureus is an important human pathogen whose success is largely attributed to its vast arsenal of virulence factors that facilitate its invasion into, and survival within, the human host. The expression of these virulence factors is controlled by the quorum sensing Accessory Gene Regulator (Agr) system. However, a large proportion of clinical *S. aureus* isolates are consistently found to have a mutationally inactivated Agr system. These mutants have a survival advantage in the host but are considered irreversible mutants. Here we show, for the first time, that a fraction of Agr-negative mutants can revert their Agr activity. By serially passaging Agr negative strains and screening for phenotypic reversion of haemolysis and subsequent sequencing, we identified two mutational events responsible for reversion: a genetic duplication plus inversion event and a poly(A) tract alteration. Additionally, we demonstrate that one clinical Agr-negative MRSA isolate could reproducibly generate Agr-revertant colonies with a poly(A) tract genetic mechanism. We also show that these revertants activate their Agr system upon phagocytosis. To assess the significance of our findings we screened 25 primary clinical isolates, which had undergone minimal handling post-isolation and are thus representative of the clinical setting, and identified that 28% of them were Agr phase variants. Taken together, we propose a model where a fraction of Agr-negative *S. aureus* strains are phase variants who can revert their Agr activity and may act as a cryptic insurance strategy against host-mediated stress.

P-18

The environmental stimulation during the postnatal stages regulates USP15 in neurons

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Environmental stimulation during the developmental period is critical for the establishment of neural circuits. A large number of genes regulated by environmental changes are identified and perturbed this machinery causes developmental disorder such as autism and ADHD. Recently, several groups have reported that mutations in *Usp15* gene increase the risk of developmental disorders. USP15 is a member of the deubiquitinating enzyme, which regulates several intracellular signaling, participating in alternative mRNA splicing. Although disrupted RNA splicing causes abnormal synaptogenesis associated with developmental disorders, molecular mechanisms how USP15 controls synaptogenesis mediated by environmental stimulation are not fully understood.

In this study, we found that the subcellular localization of USP15 was changed by light stimulation during postnatal stages. USP15 mainly expresses in layer V in the cerebral cortex and exists both in nucleus and cytoplasm. On the other hand, the mice reared in a dark chamber did not show an accumulation of USP15 in the nucleus. We also found that *Usp15* deficiency enhances chronical ER stress response pathways. These data demonstrate that USP15 subcellular localization is controlled by environmental stimulation and dysfunction of this system may cause abnormal stress signaling. Since USP15 affects RNA splicing, USP15 in the nucleus may control RNA splicing and specific protein synthesis, followed by regulation of synaptogenesis and neural circuit constructions.

P-19

Lipidomic Analysis Reveals Changes in Lipid Metabolism in a Mouse Model of Retinal Pathological Angiogenesis

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Lipid metabolism plays an essential role in retinal health and disease. It is associated with important ocular diseases, including retinopathy. The animal model of oxygen-induced retinopathy (OIR), in turn, is well accepted for the investigation of retinopathic disorders and hence molecular mechanisms of abnormal neovascularization (pathological angiogenesis). Here, we aim to characterize changes in lipid composition of mice retinas under pathological angiogenesis by performing a comprehensive lipidomic analysis, together with transcriptomics data.

In the OIR model, pathological angiogenesis is induced in mice through their exposure to variable oxygen levels. Thus, mouse pups with their nursing mothers (C57BL/6 strain) were kept at 75% O_2 from postnatal day 7 (P7) until day 12 (P12). Mice were then returned to ambient air (20.8% O_2) and retinas (N=6) were collected at different time points (P12, P12.5, P15 and P17) for lipid analysis. Retinas from mouse pups under physiological development were also collected. Total lipid extracts were then analyzed through non-targeted lipidomics by HPLC coupled to high-resolution mass spectrometry. In addition, high quality total RNA was obtained from all retinas and utilized for RNA-seq library construction. To enrich for exonic reads, all libraries were built using poly-A+ RNA and very high-depth sequencing was performed.

We identified and quantified 303 lipid species in all samples. Principal component analysis revealed alterations in retinal lipidome mainly according to time (postnatal day), but also to the condition (physiological or pathological angiogenesis). The most significantly altered lipids in pathological angiogenesis were storage lipids (i.e. cholesteryl esters and triacylglycerols) and membrane lipids (e.g. phospholipids) containing polyunsaturated fatty acids (PUFAs), particularly DHA, ARA and very long chain fatty acids. At all time points, we observed decreased concentrations of these PUFAs and an increase in phospholipids esterified to less unsaturated fatty acids. Pathological retinas at P17 have also presented a massive increase in triacylglycerol and cholesteryl ester levels.

In general, lipidomic analysis suggests that pathological angiogenesis leads to intense remodeling of membrane and storage lipids. Our findings also indicate that a decrease in polyunsaturated fatty acids, such as DHA and ARA, is linked to replacement by *de novo* synthesis of fatty acids or reflects impaired desaturase activity. The integration of these results with transcriptome analysis is ongoing and will provide a better characterization and understanding of lipid metabolism in pathological angiogenesis.

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P-20

Identifying the function of MafB expressed in macrophages during wound healing

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The skin has a system that repairs as soon as the body is injured. In wound healing process, macrophages are especially important because it does phagocytosis, promotes production of collagen and vascularization, but the mechanism of regulating the macrophages is unknown. In recent years, functional similarities of macrophages in wound healing and tumor-associated macrophages (TAMs) have been reported. TAMs are macrophages present in tumors, which promote tumor growth. We focus on the transcription factor MafB and our previous studies shows MafB expressed in macrophages is regulating the number of TAMs supplied from spleen. Therefore, we hypothesized that MafB affects wound healing via spleen and analyzed the function of MafB in wound healing using the mice that were deficient Mafb specifically in macrophages (CKO).

Comparing the wound healing speed in Control and CKO, it was significantly delayed in CKO. And FACS analysis suggested that MafB expressed in wound macrophages. Furthermore, it was revealed that the expression of ArgI in wound significantly decreased in CKO. Since there is a report that wound repair is delayed in ArgI-deficient mice, there is possible that expression of MafB in macrophages affects wound healing via ArgI.

To confirm whether spleen-derived cells are present at the wound site, we did spleen transplantation and FACS analysis with the wound. The result shows spleen-derived cells expressing macrophage marker existed in wound. In addition, comparing the wound healing speed in WT or CKO spleen transplanted mice, the healing delayed in CKO.

From these results, it was revealed that macrophages at the wound sites express MafB, and deletion of MafB specifically in macrophages reduces the expression of ArgI and delays the speed of wound healing. In addition, it was suggested that macrophages derived from spleen exist in the wound, and the cause of healing delay in CKO may be spleen-derived macrophages.

P-21

Exploring mental fatigue by passive induction: from rise to fall

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The neural underpinnings of human mental fatigue are largely unknown. Different theories have been proposed on the causes of the feeling of weariness experienced when performing cognitive tasks over a prolonged period of time. An enticing possibility is that when we are engaged in a specific task, the sustained activity of the involved neuronal populations leads to local alterations in neural activity, giving rise to the feelings of fatigue. This would signal the draining of necessary resources to carry out the task optimally, resulting in deteriorating performance.

The proposed work aims at addressing this question by means of passive stimulation of visual neuronal populations, in order to assess its consequences. To do so, we estimate changes in performance in a task that employs the same stimuli, which is carried out pre and post stimulation. Concurrently, we expect that physiological alterations in the brain, measured by means of Electroencephalography (EEG), occur precisely in the brain regions that are recruited during the stimulation.

Furthermore, a set of auditory distractor task are presented to the subject while undergoing the passive visual stimulation, thus engaging a different sensory modality. We therefore verify the interaction of arousal modulation induced by the different difficulties in these auditory tasks with the behavioural and neural consequences of the prolonged activity of specific neuronal assemblies in the visual domain.

If preliminary results are confirmed, this experiment would show that prolonged recruitment of brain resources has local consequences on neural processing. Alternatively, if the consequences are global, rather than local, this would suggest that fatigue impacts on network properties of the brain and not on the local circuits recruited during the task. We expect that the subjective (feeling of fatigue) and objective (decreased performance) manifestations of fatigue will relate to different aspects of the measured brain alterations.

P-22

Development of novel tools to monitor neuron-microglia communication

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Microglia, the resident immune cells in the central nervous system (CNS), engage in various CNS-specific functions that are critical for development, homeostasis, and plasticity of neuronal circuits in the healthy brain. Recent studies have suggested that microglia communicate with neurons to regulate synaptogenesis and neural circuits under the postnatal stages. However, the precise mechanisms of how microglia communicate with neurons have not been elucidated. In this study, we have developed probes that monitor microglial activation. We cloned the microglial activity-related promoters such as *Cx3cr1, Cd68,* and *P2ry12*, and inserted them into the luciferase reporter plasmids. Inducing these plasmids into microglial cell line BV2 enhances the luciferase activity compared to another cell line, HEK293 cells. We are also developing the chemogenetic probes to control the neural activity connecting to the microglial activity using the reporter plasmids with microglia specific promoters. Using these plasmids, we pursue the mechanisms that link neural activity to microglial functions. Thus, our research suggests that these tools become useful for analyzing the microglial diversity *in vitro* and *in vivo.*

P-23

MafB regulates thermogenesis through controlling innervation to brown adipose tissue

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Keeping body temperature is important for homoiotherms to maintain their function of metabolism. There are two ways of thermogenesis in mammals, one is shivering and the other is non-shivering. Skeletal muscle does the main role in shivering thermogenesis. On the other hand, Brown Adipose Tissue (BAT) does in non-shivering thermogenesis. Mitochondria in BAT resolves fatty acid and UCP1 at its inner membrane uses this energy for producing heat responding to the catecholamine binding to β3 receptor on brown adipocytes. Therefore, BAT can release extra energy as a heat. Concerning this non-shivering thermogenesis, Recent study has suggested that macrophages could control innervation and homeostatic energy expenditure in BAT. However, the precise mechanism is not cleared in detail. To investigate it, we focused on transcription factor MafB, which expresses selectively in monocyte-macrophage lineage. It is said that MafB is important for homeostasis function of macrophage like regulating apoptosis of macrophage and inflammation. Therefore, we suspected that MafB in macrophage is also related to energy homeostasis.

To examine this hypothesis, *Mafb*$^{f/f}$*::LysM-Cre* (Macrophage specific *Mafb* deficient, cKO) mice and *Mafb*$^{f/f}$ (Control) mice are exposed to 4 ℃ for 10 days. After that, the lower body temperature could be seen in cKO mice than control mice. Moreover, immunohistochemistry results showed the lower UCP1 expression and less axons of sympathetic fibers in cKO BAT than in control. Corresponding with these results, the expression of Nerve Growth Factor (NGF), which is important for arborization of sympathetic nerves, was reduced in cKO than control. From these results, we thought that MafB has an important role for thermogenesis through controlling BAT innervation by regulating the expression of nerve growth factor.

P-24

Longitudinal Relationship between Changes in Social Interaction and Physical Function among Older People

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The present study examined the association between changes in the social relationship and physical function among older adults aged 65 years and over in Japan.

We used the data from a longitudinal study conducted for all community-dwellings aged 65 years who were living in a suburban area in Japan. Questionnaires were used to collect data in 2011 (baseline) and 2017 (endpoint), respectively. The social relationship was assessed by the index of social interaction scale. Changes of social relationship were calculated by the endpoint score minus the baseline score. Physical function was evaluated by the subscale (physical strength) of the Kihon checklist and was categorized to normal physical function and lower physical function. Approximately 250 older adults with functionally independent in baseline year were included in analysis. Age, gender, instrumental activities of daily living, physical activity, and history of disease were considered as confounding variables based on results of previous studies. Logistic regression analysis was used to examine the association between a change in social relationship and the physical function for adjusting covariates.

As the result, an increase or no decrease in social relationship was positive factors related to the risk of developing lower physical function after controlling for covariates.

Overall, we suggest that keeping social relationships may be a positive factor to physical disfunction in older adults.

P-25

Characterization of stress induced cell wall deficient bacterial cells

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Abstract

Bacteria are clones and thought to show similar cell morphology in the same population of a species. By single cell analysis, we found that sub-population cells can convert to round cells (R-cells) from rod cells in Pseudomonas aeruginosa. Conversion to R-cell is induced by endolysin which is an enzyme that hydrolyze the cell wall. The expression of endolysin is controlled by DNA damage, which can accumulate under certain environments including, anoxic conditions and biofilms. Endolysins is one of the most conserved gene suggesting that many bacteria can convert to R-cells. Because R-cells lose their cell wall, it can be speculated that R-cells have distinct characteristic features compared to the normal rod-shape cell, but has not been examined so far.

To characterize R-cells, we first established a method and condition to induce R-cell formation. In this method, endolysin expression was controlled under an inducible promoter or DNA damaging agent was added to the cell culture. To understand the fate of the R-cells, we used live-cell imaging, and found many of the R-cells undergo explosive cell lysis that leads to membrane vesicle formation. Interestingly, some of the R-cells were stable and reverted to rod-shape cells, starting to divide again. We propose that bacteria can show transient cell morphotype, and further analysis are expected to reveal the biological impact of this type of cell.

P-26

New insights into the functions of RNF19b in microglia

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Microglia are resident macrophages in the central nervous system (CNS). Microglia play important roles in scavenging damaged or dispensable neurons and synapses. Their functions have been extensively studied in various brain pathologies. Previous studies have reported that a deleterious activation of microglia induces aberrant neuroinflammation. It has been suggested that neuroinflammation in the CNS affects ER homeostasis and proteostasis. Although the mechanisms that underlie the ER-associated degradation (ERAD) pathway in microglia is a crucial process for regulating neuroinflammation, the mechanisms by which neuroinflammation affected by ERAD is controlled in neurons remain poorly understood despite its importance. In this study, we investigate the mechanisms that link ERAD in microglia to neuroinflammation. We first looked for the specific E3 ubiquitin ligase because E3 ubiquitin ligase is essential for regulating the ERAD system. We identified the E3 ubiquitin ligase, RNF19b, which is highly expressed in microglia. We also identified that the potential target of this protein. Our data may suggest that RNF19b in microglia is a potential candidate that control neuroinflammation. Since neuroinflammation triggers the neurodegenerative disorder, our research may provide a clue with their pathogenetic mechanisms of neurodegenerative disorder, including Alzheimer's diseases.

P-27

PA200$^{-/-}$/Ecm29$^{-/-}$ Male Mice are Infertile Due to Mitochondrial Malformation Caused by Abnormal Proteostasis of Lipin 1 in Mice Epididymis

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Proteasomes are the indispensable components of ubiquitin-proteasome system (UPS), a main selective and recycling process of degrading polyubiquitinated-proteins to maintain a metabolic system in eukaryotic cells. The extensive involvement of UPS in cellular metabolic system includes spermatogenesis, which is a complex process that initiates with differentiation of spermatogonial germ cells and ends with the formation of mature spermatozoa. In testis, PA200, one of the four proteasome activators, is required during spermatogenesis as PA200 deficiency results in defective spermatogenesis with histological abnormalities caused by failure in histone degradation. Furthermore, Ecm29, a proteasome accessory protein, is necessitated to maintain the regulation of proteasome assembly.

This study aims to investigate the essential physiological role of proteasome-associated proteins by generating PA200$^{-/-}$/Ecm29$^{-/-}$ dKO mice. We found that loss of both PA200 and Ecm29 leads to male infertility, suggesting their essentiality in producing normal spermatogenesis. Therefore, proteome analysis of key regulatory proteins expressed in epididymis was done to inspect the exact molecular mechanism. In this research, we focused on one specific regulatory protein, lipin 1. Lipin 1 is a protein encoded by LPIN1, which plays a pivotal role in mitochondrial morphology homeostasis by activating mitochondrial fission, and germline specification by producing mitochondrial nuage. The overexpression of lipin 1 was detected in the testes of dKO male mice through western blot and immunofluorescence analysis. Furthermore, the mRNA level of LPIN1 did not show a significant difference, which indicates that lipin 1 accumulated due to a failure in protein degradation caused by the impairment of proteasome activity. Lastly, histological analysis revealed defective morphological development of mitochondria, especially nuage malformation in PA200$^{-/-}$/Ecm29$^{-/-}$ mice testes. Hence, given the regulatory function of lipin 1, this investigation attempts to demonstrate linkages between the abnormal proteostasis of lipin 1 and the malformation of mitochondria under defective spermatogenesis.

P-28

The micronuclei induced by damaged neurons activates the cGAS-STING pathway in microglia

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Microglia are immune cells of the central nervous system. Microglia significantly contribute to efficient clearance of cell debris, which is crucial to the maintenance of brain functions. The extracellular factors released from damaged neurons trigger microglial migration into the damage sites. Microglia subsequently recognize “eat-me” signals attached to the targets and engulf them. However, the precise mechanisms of how microglia are activated in response to the signals from damaged neurons are not fully understood. In this study, we show that micronuclei induced by the damaged neurons promote innate immune response in microglia. Micronuclei have known to be small nuclei comprising chromosomal DNA and trigger innate immune responses through the cGAS-STING pathway. Our preliminary data suggest that the number of micronuclei in microglia is increased in response to the conditioned medium from damaged neurons. We found that treatment with conditioned medium increased the amount of phosphorylated STING in microglial cell line BV2. Since phosphorylated STING activates NFκB and IRF3 responsible for inflammatory responses, the conditioned medium derived from damaged neurons contain the unknown factors, which activate inflammatory signaling, leading to microglial activation. Taken together, our data suggest that micronucleus is a potential factor that controls microglial activation associated with sensing damaged neurons via cGAS-STING pathway.

P-29

Identification of Novel Factors for Diet Induced NASH Susceptibility

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Introduction: Nonalcoholic fatty liver disease (NAFLD) is an alarmingly rising metabolic disorder with a high worldwide prevalence. Nonalcoholic steatohepatitis (NASH) is the progressive liver damage from NAFLD with inflammation, which progress in to liver fibrosis and carcinogenesis. Exact underlying factors for NASH pathophysiology is unclear.

Interestingly, we observed that high cholesterol diet (HCD) fed *tyrosinase* point mutated mice (B6 Albino) are highly susceptible for NASH compared to Wild type mice (WT), a phenotype not reported before.

Objectives: To understand the underline mechanism of elevated NASH susceptibility of B6 Albino mice. Based on this understanding, develop a simple and fast diet induced NASH mouse model using B6 Albino mice.

Method and Results: B6 Albino mice carries a point mutation in *tyrosinase* and this is the only genetic difference compared to WT mice. B6 Albino mice and WT were fed with HCD for 10 weeks. Normal diet fed mice used as controls. Body weights, Blood indices and NASH related serum parameters were monitored. Liver samples were histologically analyzed.

Surprisingly, liver injury was observed in B6 Albino mice from post day 1 HCD feeding, with elevated serum ALT and AST levels. 2 weeks of HCD induced NASH in B6 Albino mice, but no symptom was observed in WT mice even after 10 weeks of diet. HCD fed B6 Albino mice showed significantly high mortality rate. Histological analysis of liver revealed significant inflammatory cell and lipid infiltration, and severe fibrosis. Serum cholesterol species analysis showed significantly high chylomicron and VLDL levels in B6 Albino mice.

Conclusion: We believe that our work will advantage the identification of susceptible genetical factors for NASH development and to expand the understanding on NASH pathophysiology.

P-30

Neuronal control of reproduction dormancy under a cold condition in the fruit fly *Drosophila melanogaster*

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Animals have the flexibility to adapt to environmental change. One such example is the adaptation of physiological conditions in response to seasonal changes. In some insects, they have evolved a reproductive dormancy that significantly shrinks the ovary and saves energy in winter when it is not suitable for reproduction. This response also downregulates the biosynthesis of the indispensable gonadotropic humoral factor, called juvenile hormone (JH), which is produced in the corpora allata (CA). However, it is still largely unclear which neuronal circuits are responsible for receiving such environmental stimuli and regulating JH biosynthesis in the CA. In this study, we investigated the neuronal system that regulates reproductive dormancy in response to seasonal change using the genetic model species *Drosophila melanogaster*. We found the neurons that project directly to the CA in adult *Drosophila*. These neurons produce a certain type of neuropeptide, and a specific receptor for the neuropeptide is highly expressed in the CA. Furthermore, a genetic null mutant flies of this neuropeptide exhibited a reduced dormancy response under cold conditions. Moreover, forced activation of these neurons prevented oocyte maturation in non-dormancy conditions, and this phenotype was rescued by the feeding of JH analog. As far as we know, this is the first study to imply the direct neuronal regulation of the biosynthesis of the gonadotropic hormone, controlling cold temperature-induced reproductive dormancy.

P-31

The association between child development and home-rearing environment among Japanese children aged 0-6 years old

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Early childhood is an essential period for child development in physical, social and language skill which has a lasting effect on the children’ s later growth. According to the studies of National Institute of Child Health and Human Development (NICHD), the wide range longitudinal study in America, have indicated that the quality of the child care plays an important role in child development. Caregivers influence children not only by their care behaviors but also by how they create the child’s growth environment. The child care behaviors of parents and other caregivers are critically important to child development in the early childhood. It is very urgent for us to explore the association between the home-rearing environment and child development.

The current study recruited parents with children from the centers for early childhood education and care across Japan in 2014. Data was collected from approximately 1300 participants by self-reported questionnaire regarding home-rearing environment and child development. Investigations contained three parts: 1) demographic information, such as age, gender and family structure; 2) Child development was evaluated with Child Development Scales by child care professionals from Centers for Early Childhood Education and Care; 3) Home-rearing environment was evaluated with Index of Child Care Environment (ICCE) by caregivers.

The chi-square tests showed a significant association between child development and parenting behavior. Current study showed that human stimulation and social support are related to child development. Child care behavior is very essential for child development in the early childhood. Family is the first place which can give early childhood children more stimulation and help them develop their motor, social and vocabulary ability. Understanding the child care related factors can help parents create a better home-rearing environment for the growth of early childhood children.

P-32

The role of an inhibitory immunoreceptor in food-induced anaphylaxis model

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Food-induced anaphylaxis is a hypersensitivity reaction characterized by multi-organ dysfunction, hypothermia and fatal outcome. Although numerous studies have shown the effector function of mast cells in IgE-dependent food-induced anaphylaxis, contribution of basophil is poorly described.

Our group has reported that an inhibitory immunoreceptor, which has an immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in its cytoplasmic portion, is highly expressed on mast cells and basophils and inhibits high affinity receptor for IgE (FcεRI)-mediated signaling in mast cells through recruiting SHP-1 phosphatase, resulting in suppression of IgE-mediated type-I allergic reactions. However, the function of this inhibitory receptor on basophils remained elusive.

To elucidate the role of this inhibitory receptor in food allergy, wild-type (WT) and the receptor deficient mice (KO) were sensitized with intraperitoneal injection of ovalbumin (OVA) with alum, followed by oral challenge with OVA. In agreement with a previous report, this model induced diarrhea but not hypothermia in WT mice. However, KO mice showed significantly increased mortality and hypothermia compare to WT mice, as well as increased diarrhea incidence. Nevertheless, serum levels of antigen-specific IgE and IgG were comparable between WT and KO mice, suggesting that this receptor is involved in the effector phase in this food allergy model. To examine the effector function of this receptor on basophils, anti-CD200R3 antibody was used to deplete basophils by intravenous injection one day prior the oral challenge. Basophil-depleted mice showed no hypothermia symptom in KO mice, although diarrhea symptom was still observed. These results indicate that this receptor deficiency on basophils promotes food-induced anaphylaxis (detected as hypothermia), but not diarrhea. Since platelet-activating factor (PAF) can be released by activated basophils and induces anaphylaxis with 1,000- to 10,000-fold more potency than histamine, PAF receptor antagonist was injected intraperitoneally 1.5 hours before oral challenge. We found that no hypothermia symptom was observed in KO mice.

Thus, our finding shows that basophils are key drivers for food-induced anaphylaxis, in which this receptor has a pivotal role in the suppression of basophil activation in food-induced anaphylaxis.

P-33

Abnormal Sleep and Cognitive ability in an *App* Knock-in Mouse Model of Alzheimer's Disease

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Alzheimer's disease (AD) is the most common neurodegenerative disease. It is a slow progressing disease characterized by aggregation of amyloid-beta (Aβ) peptides. In addition to cognitive decline, AD patients often suffer from poor sleep. These symptoms not only affect their QOL but also those of the primary caregivers. Increasing studies support that sleep deficits can contribute to the progression of AD, consistent with the roles of sleep in memory and brain homeostasis. Thus, it is crucial to understand the underlying mechanisms of sleep disorders in AD.

Here, we unveil the sleep abnormalities exhibited by an AD mouse model. We chose the *App* knock-in mouse (Saito et al., Nature Neuroscience, 2014). In this AD mouse model, Aβ accumulates in a manner similar to human AD via mutation in the gene encoding amyloid-beta precursor protein (APP), and thus, various aspects of human AD are faithfully recapitulated. In these mice, at 6 months of age, when cognitive deficits become apparent, impairment in various aspects of the sleep architecture were detected. These phenotypes became more severe at 12 months of age. Consistent with the notion that sleep is important for memory consolidation, the AD mouse model also exhibited learning deficits. We expect that our results provide a start point for addressing how sleep is impaired in AD and how it can affect cognitive functions.

P-34

Involvement of two-component systems in competence development in *Staphylococcus aureus*

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Staphylococcus aureus is a major human pathogen responsible for various illnesses ranging from mild to potentially fatal. It has high adaptive capability to harsh environments and stresses in addition to rapidly acquire antibiotic resistance leading to the rise of multidrug resistant strains.

Previously our group demonstrated that *S. aureus* strain N315 is able to develop competence and natural transformation for DNA, however, the role of natural transformation in *S. aureus* evolution has been regarded to extremely limited due the low transformation frequencies in wild-type cells ($<10^{-11}$) and in clinical isolates.

Development of natural transformation is generally tightly regulated in bacteria. However, the environmental cues required for the development of natural transformation differs among bacterial species and is unknown in *S. aureus*. Among distinct sensory mechanisms, two-component systems (TCSs) are major means for mediating the extracellular signals and modifying the cellular response. TCSs are composed of a histidine kinase sensor embedded in the membrane and a cognate response regulator, which upon phosphorylation regulates gene expression.

Here we aimed to clarify the unknown condition(s) / signal(s) for competence development in unmodified cells, and we investigated the involvement of *S. aureus* TCSs in the activation of the competence operon *ComG* by using the reporter PcomG.

We found that deletion of two TCSs significantly reduced the reporter activity in N315. Consistently, the natural transformation efficiency was significantly reduced upon the deletion of these TCSs. Preliminary data in three clinical isolates showed similar reduction effect in natural transformation frequencies following the deletion of these TCSs.

These TCSs are responsible for quorum sensing and cell-wall stress sensory. Significance of these findings will be discussed in terms of physiology of this important human pathogen.

P-35

The role of DNAM-1 in Concanavalin A-induced acute liver injury

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【Introduction】

Acute liver injury is a life-threating disease. Although liver T cells are involved in the pathogenesis, the role is not completely understood. Concanavalin A (ConA)-induced liver injury is a T cell-dependent animal model of acute liver injury. ConA bridges glycosylated receptors including T cell receptors and induces activation and cytokine production of T cells. DNAM-1 (CD226) is a highly glycosylated activating receptor for T cells. However, the role of DNAM-1 on liver T cells in the pathogenesis of acute liver injury is unknown.

【Purpose】

We aimed to elucidate the role of DNAM-1 on liver T cells in ConA-induced liver injury.

【Material and methods】

ConA injection

ConA was intravenously injected into wild-type (WT) and DNAM-1-deficient (*Cd226*$^{-/-}$) mice and the survival was monitored. A neutralizing antibody against IL-10 was intraperitoneally injected after ConA injection.

Cytometric beads array (CBA)

Plasma concentrations of IL-10 after ConA injection were quantified.

Flow cytometry

Expression of activation markers CD25 and CD69 on liver T cells were evaluated after ConA injection.

Quantitative RT-PCR (Q-RT-PCR)

Liver T cell subsets were sorted after ConA injection and *Il10* transcripts were quantified by Q-RT-PCR.

In vitro assay

Liver lymphocytes were stimulated with ConA and activation and IFN-γ production were evaluated.

【Results】

Cd226$^{-/-}$ mice exhibited the lower survival rate and IL-10 concentrations than WT mice after ConA injection. Neutralization of IL-10 abolished the difference in the survival between WT and *Cd226*$^{-/-}$ mice. *Cd226*$^{-/-}$ liver T cells expressed the higher levels of CD25 and CD69 than WT liver T cells, and *Cd226*$^{-/-}$ liver regulatory T cells (Treg) expressed the low levels of *Il10* after ConA injection. *Cd226*$^{-/-}$ liver T cells were activated and produced IFN-γ more efficiently than WT liver T cells after ConA stimulation *in vitro*.

【Conclusion】

These results suggest that DNAM-1 on liver Treg inhibits excessive immune responses through IL-10 production after ConA injection. Further studies are needed to clarify that DNAM-1 is required for the optimal IL-10 production by liver Treg.

P-36

In silico structural analysis of the interaction between house dust mite and HLA class II molecules

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Background: Human leukocyte antigen (HLA) plays an important role in type I allergic diseases. The binding of HLA to allergen-derived peptides is essential for initiation of the immunologic reaction. The genetic polymorphisms of HLA class II have been reported to be associated with the development of type I allergy. Recent studies reported *HLA* alleles associated with allergen sensitization and allergic diseases, however, most of the antigen epitopes have not been discovered yet. The aim of the present study is to predict the binding epitope of Der f 2, the major allergen components of house dust mite by using *in silico* structural analysis.

Method: To select risk alleles of sensitization against mite, we performed logistic regression analysis using 544 Japanese population. The amino-acid sequences of *HLA* alleles were downloaded from IPD-IMGT/HLA database. The three-dimensional structures of *HLA* alleles were constructed by homology modeling using HLA-Modeller (Amari et al., 2013) implemented in molecular operating environment (MOE, Chemical Computing Group, Canada). The template of HLA and antigen for homology modeling were downloaded from Protein Data Bank (PDBID: 4OV5, 4D8P, 3LQZ). The binding modes and affinities between various allergen-derived peptide sequences and *HLA* alleles were simulated by HLA-BAP implemented in MOE. Then, we calculated docking potential and U total score calculated by ASEdock.

Results: *HLA-DRB1*15:02*, *DQB1*06:01*, and *DPB1*09:01* alleles were associated with sensitization against house dust mite ($P < 0.05$). *HLA-DRB1*15:02* had the lowest value and showed strong binding regarding the docking potential score. We detected several peptide sequences of Der f 2 that strongly bind to HLA class II molecules.

Conclusions: The present study shows the potential of *in silico* analysis to detect binding epitope of HLA. Further studies is need to optimize the length of the peptides and the statistic modeling for the determination of epitope.

P-37

Analysis of Hybrid Sterility genes on Hstx2 locus.

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Reproductive isolation is the barrier distinguishing between related species by hybrid sterility (HS) and/or hybrid inviability. HS predominantly appeared on the heterogametic sex. The molecular mechanism underlying this phenomenon is unclear. Recent studies identified the critical genomic region named *Hstx2* on chromosome X (Chr.X) causing HS by forward genetics analysis used two subspecies that *Mus musculus domesticus* (*Mmd*) and *Mus musculus musculus* (*Mmm*). There are 30 genes on *Hstx2*. However, it has not been clear which genes regulate HS. I speculated that HS caused gene was disrupted their function in hybrid F1 male. Therefore, I investigated these genes by knocking out each gene. At first, I screened these genes for testis expression and non-synonymous SNPs between *Mmd* and *Mmm*. Eleven candidate genes have testis expression and polymorphism. Only 2 out of 11 candidate genes knock-out (KO) mice were reported, but the other 9 were not. To confirm whether 9 target genes were essential for sperm formation, we generated 9 KO strains on *Mmd* genetic background by deleting each target's genomic region. We then mated various mutant male mice to wild type female mice, respectively. Unexpectedly, all 9 mutation strains didn't show sterility. Hence, I hypothesized that not single but several genes are involved in sperm formation. I thus focused on two gene, *Mirror-like structure on Chr.X 1* (*Moli1*) and *Moli2*. These genes have completely same coding sequence which suggests that these genes have same function. To confirm whether at least *Moli1* or *Moli2* was necessary for sperm formation, I tried to generate *Moli1* and *Moli2* double KO mice by deleting all genomic region of two genes. However, the double KO mice implied incomplete embryonic lethality. These data suggest that *Moli1*/*Moli2* are necessary for viability and/or development. Therefore, I proposed this hypothesis; in hybrid F1 male, the functions of *Moli1*/*Moli2* were lost at sperm formation stage but not at embryonic development.

P-38

Pro-inflammatory gene programming of histamine H3 receptor agonism in a mouse model of cardio-renal association

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Heart failure and chronic kidney disease are major causes of morbidity and mortality. Although these dysfunctions are common and frequently coexist, the factors and molecular targets involved in their relationship in cardiorenal regulation are still largely unknown. Here, we found the increased plasma histamine in a mouse model of severe cardiac dysfunction, that had been co-treated with angiotensin II, nephrectomy, and salt (ANS). The ANS mice exhibited impaired renal function accompanied with heart failure, and histamine depletion by the genetic inactivation of histidine decarboxylase in mice exacerbated the ANS-induced cardiac and renal abnormalities, including the reduction of left ventricular fractional shortening and renal glomerular and tubular injuries. Interestingly, while the pharmacological inhibition of the histamine H3 receptor facilitated heart failure and kidney injury in ANS mice, administration of the H3 receptor agonist was protective against cardiorenal damages.

In the present study, in order to elucidate the protective mechanism of H3 agonists, we performed transcriptome analysis and biochemical examinations. Transcriptome and gene ontology analyses using renal mRNA from ANS mice identified the increased expression of inflammation-related genes, those of which were found to be suppressed by H3 agonist administration. As biomarkers of inflammation, blood levels of CRP and SAA were markedly increased in ANS mice but decreased by H3 agonist administration. Furthermore, by qPCR analysis, we found that the elevated expression levels of acute-phase inflammatory response regulator genes such as IL6, TNF and C3 were decreased by H3 agonist administration in the kidney of ANS mice. In addition, expression of these genes was also elevated in the heart and liver of ANS mice and reduced by H3 agonist administration. This study suggests that histamine H3 receptor agonist inhibits the systemic inflammatory response in ANS mice by transcriptional repression of proinflammatory genes.

P-39

Foreign Workers in Taiwan: A Literature Review

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Object and Method

Taiwan has adopted a national health insurance system like Japan, and actively accepted foreigners as blue-collar workers in advance of Japan. The purpose of this literature review is to describe the overview of foreign workers in Taiwan. Researchers searched electronic databases; *Ichushi* and PubMed from 2010 to 2019 using the following key word: *foreign, Taiwan, care, and hospital*.

Result

From 30 articles initially extracted, researchers selected two original articles under inclusion criteria, additionally, searched two books and four reviews from these references.

1. Acceptance of foreign workers

In Taiwan, there are about 760,000 foreigners in 2018, of which 630,000 are blue-collar workers. Acceptance of blue-collar foreign workers has been started from 1989. Foreign blue-collar workers can be divided into shipping business, household services, manufacturing, care business, and so on. Among them, the manufacturing is the largest number of foreigners approximately 410,000 people (60.4%).

2. Working condition

Acceptant period of stay is the maximum of 12 years. During stay, it is impossible for foreign workers to change the type of work and workplace although they don't require special qualifications. Taiwan has been accepting blue-collar foreign workers under strict management resulting in a vicious cycle that irregular workers increase and regulations have been are further tightened.

3. Health insurance system

The national health insurer in Taiwan is operated by a single insurer, unlike Japan. Insured persons should be reresidents in Taiwan, which include foreigners. Foreigners need to have residence permits and stay for six months (183 days) to take part in the insurance. However, the foreign employees can obtain health insurance from the first day of working.

Discussion

Recently, the number of foreign patients has rapidly increased in Japan; furthermore, Japanese government will accept approximately 340,000 foreign blue-workers from Southeast Asian countries within five-years. However, the countermeasures for foreign patients such as the medical insurance system and working conditions have not been enough prepared. It would be effective for Japan to learn from Taiwan's history of accepting foreigners, nearly 30 years ahead of Japan.

P-40

The autophagy in the central nervous system is essential for the homeostasis of body temperature

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The body temperature of mammals is controlled by the hypothalamus in the brain. Hypothalamus also maintains biogenic homeostasis. Interestingly, previous studies have reported that impairment of autophagy in hypothalamus causes abnormality of neuronal circuit formation involved in thermoregulation. Although autophagy is thought to be essential for the maintenance of brain homeostasis, the mechanisms that underlie autophagy-regulated thermoregulations is not well understood. Here, we report that autophagy plays important roles in maintaining body temperature, especially with cold exposure. The body temperature of NestinCre:ATG7$^{flox/flox}$ (Atg7 cKO) mice is slightly lower than of wild-type (WT) mice in room temperature. Moreover, we found that the body temperature of Atg7 cKO mice is rapidly decreased with chronic cold exposure (4℃). In addition, microarray analysis has revealed that the expression level of RNA binding motif protein 3 (RBM3) is increased in Atg7 cKO mice brain. Normally, RBM3 is upregulated in response to cold shock stimulation. This protein has been considered to protect neurons from their toxicity. We found that the expression of RBM3 is increased at hypothalamic arcuate nucleus (ARC) in Atg7 cKO mice. These findings suggest that impairment of autophagy in brain disrupts thermoregulation at cold temperature and increase of expression of RBM3 in ARC. Our study may shed light on the novel mechanisms by which RBM3 expression in ARC is a novel candidate linking autophagy defect to thermoregulation.

P-41

Sleep/wake in mice expressing a constitutively active form of SIK3

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Sleep is a ubiquitous behavior conserved in vertebrates as well as invertebrates. Although neural circuits switching sleep/wakefulness have been elucidated, intracellular signaling regulating sleep/wake behaviors is still unknown. *Sik3*, which belongs to AMP-activated protein kinase (AMPK) family, is a newly identified gene regulating sleep/wakefulness. *Sleepy* mutant mice, whose *Sik3* exon 13 is skipped, shows prolonged NREM sleep time and increased sleep need. The exon 13-encoded region of SIK3 contains a PKA phosphorylation site, Ser551 (S551). The 14-3-3, scaffold protein, binds to the SIK3 S551 in a phosphorylation-dependent manner, and our previous report showed that the deletion of exon 13-encoded region resulted in abolishment of binding of 14-3-3, suggesting that 14-3-3 binding is important for sleep/wake regulation. Since our result showed that a hypomorphic *Sik3* mutation in *Drosophila* resulted in a decrease in sleep time, the nature of the *Sleepy* mutation seems to be gain-of-function. Moreover, it has been reported that SIK3 kinase activity is tightly linked with phosphorylation of Thr221 (T221) in the kinase domain T-loop of SIK3. Interestingly, the phosphorylation of T221 was increased in wild-type mice after sleep deprivation, suggesting that SIK3 kinase activity increases in mice which have a higher sleep need. However, whether the SIK3 kinase activity is involved in sleep/wake regulation remains unveiled.

Here, we investigated the role of SIK3 kinase activity in its phosphorylation status and established genetically modified *Sik3* T221E mice whose SIK3 is constitutively active. First, we examined the phosphorylation status and physical interaction of mutant SIK3 proteins using HEK293T cells. Next, we have made *Sik3* T221E mice using CRISPR/Cas9 system. To confirm the introduction of *Sik3* T221E in mice, we performed genotyping and direct sequencing. Moreover, we confirmed the expression of protein of SIK3 by western blot using brain samples. These results suggest that we successfully produced *Sik3* T221E mice.

P-42

In vitro and in vivo pharmacology of small-molecule orexin agonists for treatment of narcolepsy

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Sleep/wakefulness is regulated by orexin, a neuropeptide produced by neurons exclusively localized in the lateral hypothalamus. Orexin deficiency causes narcolepsy-cataplexy characterized by excessive sleepiness, sleep/wake fragmentation and cataplexy, a sudden loss of muscle tone caused by strong emotional trigger such as laughing. Orexin acts on two receptors, OX_1R and OX_2R, and previous studies suggest that OX_2R is the main receptor regulating sleep; the role of OX_1R is less clear. As orexin cannot pass the blood-brain barrier, the peptide is difficult to use as a clinical drug. Therefore, small-molecule orexin receptor agonists, especially OX_2R agonists, are expected to be a novels therapy for narcolepsy-cataplexy. We previously showed that YNT-185, an OX_2R-selective receptor agonist, ameliorates narcolepsy-cataplexy symptoms in mouse models when peripherally (i.p.) administered. However, the effective dose of this compound for oral administration (p.o.) was too high.

Here we further optimized YNT-185 (EC_{50} for OX_2R ≈ 28 nM) and produced 2 different types of agonists; a selective OX_2R agonist, YNT-X (EC_{50} for OX_2R ≈ 1.1 nM by intracellular Ca assay) and a dual OX_1R/OX_2R agonist, YNT-Y2 (EC_{50} for OX_1R ≈ 3.7 nM, EC_{50} for OX_2R ≈ 0.9 nM). Oral administration of these compounds increased wake time in wild-type mice in a dose-dependent manner. Their effective oral doses are several hundred times lower than YNT-185. These effects were not detected in orexin receptor-deficient mice. Now we are conducting experiments to confirm that they suppress narcolepsy-cataplexy symptoms in orexin-deficient mouse models. Previous reports suggest the possibility that OX_1R signal may be important for consolidation of wakefulness. We will be able to dissect the role of orexin receptor subtypes in narcolepsy treatment by using these two types of orexin agonists.

P-43

TBC1D24 regulates tubular recycling endosome formation through the small GTPase Rab22a

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TBC1D24 was initially identified as a novel causative gene for familial infantile myoclonic epilepsy. TBC1D24 has the TBC domain which is a common structure found in regulators of Rab small GTPases. TBC1D24 has been shown to bind to the small GTPase Arf6, through which TBC1D24 regulates neuronal migration and maturation. Skywalker, the *Drosophila melanogaster* homologue of TBC1D24, regulates synaptic endocytic vesicle trafficking through the small GTPase Rab35. However, detailed functions of TBC1D24 in mammalian cells are still unclear.

Plasma membrane proteins internalize into cells through either clathrin-mediated endocytosis (CME) or clathrin-independent endocytosis (CIE). Following endocytosis, cargo proteins are transported to lysosomes for degradation or recycled back to the plasma membrane. Since Arf6 and Rab proteins play pivotal roles in endocytosis and following intracellular membrane trafficking of plasma membrane proteins, TBC1D24 is likely to be involved in the regulation of these processes. In this research, we show that CRISPR/Cas9-based deletion of *TBC1D24* in HeLa cells delayed the recycling of CIE cargo proteins back to the plasma membrane through the impairment of tubular recycling endosome (TRE) formation. We also find that TBC1D24 binds to the small GTPase Rab22a, the key regulator of TRE formation as well as recycling of CIE cargo proteins. Knockdown of Rab22a reverses the effect of TBC1D24 overexpression on the trafficking of CIE cargo proteins, while the decrease of CIE-positive TRE in *TBC1D24*-deleted cells is rescued by overexpression of Rab22a. These results indicate that TBC1D24 regulates TRE-mediated CIE cargo recycling through Rab22a.

P-44

Knockdown of *nup98* and leukemia-associated Nup98 fusion proteins interrupt the nucleocytoplasmic transport

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The exchange of macromolecules between the nucleus and cytoplasm is carried out by nucleocytoplasmic transport system, and the gateway for the nucleocytoplasmic transport at the nuclear membrane is the nuclear pore complexes (NPCs). NPC, which is embedded in the nuclear envelop to form a channel, comprises of about thirty different proteins called nucleoporins (Nups). It has been reported that qualitative or quantitative changes of Nups commonly cause nucleocytoplasmic transport abnormalities that closely link to diseases. *Nup98* gene encoding an NPCs component is known to be fused to at least 28 different partner genes in hematopoietic malignancies. Although it has been reported that expressions of several *nup98* fusion genes lead to the onset of leukemia in mice, the detailed functions of these fusion proteins remain unclear. In this study, we have examined the effect of *nup98* knockdown and Nup98 fusion proteins on nucleocytoplasmic transport by focusing on nuclear export receptors including exportins and nuclear RNA export factor 1 (NXF1). Knockdown of *nup98* caused accumulation of exportins and their cargoes while NXF1 and mRNA formed dots in the nucleus. Similarly, when Nup98 fusion proteins were overexpressed, subcellular mislocalization of nuclear export receptors and their cargoes were found. In addition, we found that EGFP-Nup98 colocalized with overexpressed Nup98 fusion proteins in the nucleus. These results suggest that Nup98 is required for the nuclear export of cargoes through the proper localization of nuclear export receptors and Nup98 fusion proteins may inhibit endogenous Nup98 function by changing Nup98 localization, leading to the impairment of nucleocytoplasmic transport. More interestingly, treatment of exportin 1 inhibitor also caused the accumulation of other exportins, suggesting that exportin 1 plays a role in subcellular localization of other nuclear export receptors.

P-45

The role of IFN-beta in the regulation of regulatory T cell proliferation and function

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Regulatory T cells (Treg cells) are one of the $CD4^+$ T cell subsets that maintain immune homeostasis by suppressing excessive immune responses. The deficit of Treg cells causes severe inflammatory diseases while Treg cells prolong virus infection and enhance the tumor progression. Thus, it is important to regulate the appropriate number of Treg cells according to the situations. Interferon-β (IFNβ) is one of the Type-I interferons (IFNs) which has an important role in anti-viral or anti-tumor immune responses. However, the function of IFNβ in Treg cells remains controversial. The purpose of this study is to elucidate the role of IFNβ in Treg cell proliferation and function. We induced Treg cells in the presence or presence of IFNβ from naïve T cells and then tried to elucidate whether IFNβ promotes or inhibits the proliferation and the function of Treg cells in time-dependent manner. Our data suggested that the IFNβ showed different effects on Treg cells in early induction phase and late survival state. We will discuss the implications of our findings in this meeting.

P-46

Ubiquitin-specific protease TRE17/USP6 promotes cancer cell invasion through the regulation of plasma membrane protein recycling

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Metastasis begins with the invasion of cancer cells into surrounding tissues. Key processes for cancer cell invasion are signal transduction mediated by plasma membrane proteins and following extracellular matrix (ECM) degradation. Plasma membrane proteins that enter cells by endocytosis are sorted either to lysosomes for degradation or recycled back to the plasma membrane, thereby cells regulate localization and expression levels of the proteins at the cell surface. The cycle of ubiquitylation and deubiquitylation is an important modification which determines this sorting process: cargo proteins tagged with ubiquitin are transported to and degraded in lysosomes. We have previously demonstrated that TRE17/USP6, a member of the ubiquitin-specific proteases (USPs), promotes recycling of plasma membrane proteins that enter cells through clathrin-independent endocytosis (CIE) by deubiquitylating them. TRE17 is highly expressed in several cancers and tumor-like lesions, which show high invasive ability, and it has been shown that TRE17 contributes to tumorigenesis and metastasis in a deubiquitylating (DUB) activity-dependent manner. However, the functional link between TRE17-mediated recycling of plasma membrane proteins and cancer cell invasion remains unknown. In this study, we investigated the role of TRE17 in cancer cell invasion.

In the highly invasive breast cancer cell line MDA-MB-231 cells, overexpression of TRE17 significantly promoted cancer cell invasion in a manner dependent on its DUB activity, while knockdown of TRE17 suppressed their invasion. To elucidate the mechanism how TRE17 promotes cancer cell invasion, we focused on a CIE cargo protein CD147, which is involved in induction of Matrix metalloproteinases (MMPs), the enzymes responsible for ECM degradation. Knockdown of TRE17 decreased the level of CD147 at the cell surface. Correlated with the reduction of cell surface CD147, MMP2 production was suppressed by TRE17 knockdown. We also found that overexpression of TRE17 decreases accumulation of CD147 at lysosomes, indicating that TRE17 promotes recycling of CD147 to the cell surface. Furthermore, inhibition of CD147 by a specific inhibitor attenuated the TRE17-promted cancer cell invasion. These results suggest that TRE17 increases cell surface CD147 by promoting its recycling, which in turn increases MMP synthesis, thereby promoting cancer cell invasion.

P-47

Morphological and functional adaptation of pancreatic islet blood vessels to insulin resistance is impaired in diabetic *db/db* mice.

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In obesity-associated insulin resistance, pancreatic β cells expand and increase insulin secretion to keep normoglycemia. When this compensation fails, type 2 diabetes (T2D) develops. Pancreatic islet vasculature plays an important role in this β-cell adaptive response.

To investigate the islet vasculature in the development of T2D, we studied two diabetic models; *ob/ob* mice and *db/db* mice. *ob/ob* mice develop only mild hyperglycemia with remarkably elevated serum insulin levels. On the other hand, *db/db* mice develop severe hyperglycemia due to failure in the compensatory insulin secretion.

We used tomato lectin staining to investigate the islet vascular morphology from young to old ages. Compensatory islet expansion and islet capillary dilation were observed both in *ob/ob* mice and in *db/db* mice. However, while this adaptive change was progressive over time associated with developing obesity and insulin resistance in *ob/ob* mice, this adaptation reached a plateau and declined at older ages in *db/db* mice, when hyperglycemia exacerbated further.

Islet blood flow was investigated using in vivo live imaging. Islet blood flow volume was increased in *ob/ob* mice whereas it was decreased in *db/db* mice compared with control *db/+* mice at the old age.

Islet parasympathetic innervation, pericyte coverage of islet capillaries, and hyperinsulinemia in *db/db* mice by insulin supplementation were verified, none of which were associated with the difference in islet vasculature between *ob/ob* and *db/db* mice.

The protein ratio of phosphorylated eNOS at Ser1177 per total eNOS was preserved in *ob/ob* islets whereas it was diminished in *db/db* mice, suggesting decreased eNOS activity in *db/db* islets.

Amelioration of T2D by Elovl6 deficiency involved restoration in capillary dilation, blood flow and eNOS phosphorylation in *db/db* islets.

Our data suggest that impaired ability of islet capillary dilation due to endothelial dysfunction impairs islet local blood flow, which may contribute to loss of β-cell function and further exacerbation of T2D.

P-48

Possibility of corticosteroids to induce premature rapture of membrane in patients with systemic lupus erythematosus

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Introduction: It is mentioned that premature rapture of membrane (PROM) occurs with higher rate in women with systemic lupus erythematosus (SLE), when compared to the general population. Most women who have SLE need to continue corticosteroids during their pregnancies. The relationships between PROM and corticosteroids are not well known, but a recent report in mice showed that corticosteroids weakened fetal membranes, which induced PROM.

Hypothesis: Here we hypothesize that corticosteroids induce PROM.

Methods: We reviewed all pregnancies delivered after 22 completed weeks' gestation in patients with systemic lupus erythematosus (SLE) who were seen at the National Center for Child Health and Development from 2005 to 2016. We summed up the dosage of corticosteroids given during pregnancy, and evaluated the relationship with pregnancy outcomes by univariable analyses.

Results: Forty-eight pregnancies in 48 women were identified. 47 women except one took corticosteroids during their pregnancy. 31 % women received immunosuppressants at the same time. PROM occurred in 11 (22.9 percent). The sum of corticosteroids during the pregnancy was significantly bigger in the PROM group than that of the non-PROM group (central value (interquartile range)) (2730 (1862, 3570) mg v.s. 1890 (1259, 2590) mg ; p=0.031). Furthermore, the risk of PROM was increased in frequency with the increased corticosteroids dose. The amount of corticosteroids at the beginning of pregnancy and immunosuppressants didn't show any relationships with PROM. The rate of chorioamnionitis didn't show significant difference between the PROM group and the non-PROM group.

Discussion: The possibility of risk that the more corticosteroids during pregnancy induce the more PROM was identified, and PROM in our study showed no relationship with chorioamnionitis. The possibility of corticosteroids to induce PROM in non-infectious etiology is suggested. Further evaluation is needed to seek out better management for pregnancies with SLE.

P-49

Electrophysiological investigation into roles of the lateral habenula in autonomic cardiovascular control

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The habenula is a pair of small nuclei which is located above the thalamus and divided into the medial and lateral nuclei (LHb). It is known that exogenous stress events activate neurons in the LHb. Although the stress events induce various autonomic physiological responses such as changes in blood pressure and heart rate, little is known about whether and how the LHb regulates the cardiovascular responses to the stress event. To understand the roles of the LHb in the cardiovascular responses to stress events, here we investigated the effect of the electrical stimulation of the LHb on blood pressure and heart rate. We used Wistar male rats that were anesthesia by urethane (1 g/kg, *i.p.*). Arterial pressure was recorded from the femoral artery via a catheter. Heart rate was analyzed from R-R intervals of the electrocardiograph. The LHb was electrically stimulated with a coaxial electrode (300 μA, 0.5 ms duration, 100 Hz, for 10 s). As a result, electrical stimulation of the LHb significantly increased the mean arterial pressure. Electrical stimulation of the LHb also significantly decreased heart rate. The onset latency of heart rate was significantly earlier than that of blood pressure. These results suggest that the excitation of LHb neurons produced the pressor response and bradycardia with directly controlling the autonomic nervous system because the bradycardia was not induced by baroreflex which is triggered by the pressor response. The LHb stimulation might evoke the cardiovascular changes by affecting the same neural mechanism that regulates autonomic cardiovascular responses under stressful environments.

P-50

Characterization of a Novel Knock-in Reporter Mouse for investigating the role of Cables2 *in vivo*

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CDK5 and ABL1 enzyme substrate1 (Cables1) and CDK5 and ABL1 enzyme substrate2 (Cables2) are the members of Cables family. The analogy between Cables1 and Cables2 is observed in their common C-terminal cyclin box-like domain and their PXXP motif, it is observed likewise in their shared interacting proteins such as Cdk3 and Cdk5 and their involvement in apoptotic pathways. Although Cables1 was deeply investigated in several aspects like functions and proteins interactions, Cables2 was not. To boost our understanding of Cables2 expression pattern, localization, protein-protein binding network, and functions in vivo, a novel knock-in mouse was generated in which 3xFLAG, 2A, and a fluorescent reporter gene (tdTomato) were knocked in before the stop codon of Cables2 gene. Hereafter, the knock-in reporter mouse is referred to as Cables2-tdTomato. The knock-in gene is integrated into the endogenous Cables2 locus and its expression is controlled by the Cables2 promotor. In this study, I investigated whether the Cables2-tdTomato mice can provide characteristics of Cables2 by the several different analyses. RNA analysis by RT-PCR showed that mRNA transcripts encoding tdTomato were expressed in the brain, lung, liver, kidney, spleen, colon, testis, and ovaries. Due to rabbit globin poly-A, Cables2 mRNA expression was higher than that in the wild-type when it was measured by RTqPCR. Flag-tagged protein was detectable only in Cables2-tdTomato mice by Western blot. Moreover, the difference in tdTomato fluorescent signals was clear between homozygous knock-in and wild-type in the brain, testis, and ovary in which Cables2 is expressed highly. These results suggest that the Cables2-tdTomato reporter mouse is useful for investigating the role of Cables2 in vivo. Interestingly, a unique Cables2 expression pattern was discovered in a sole tissue. This unique expression may elucidate some of Cables2 functions and protein interactions. More about the unique expression will be discussed in the conference.

P-51

The analyzation of anti-aging effects induced by dead *Bifidobacterium longum* in *Caenorhabditis elegans*

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We have microbes in our intestine. These microbes form our intestinal flora and are called Probiotics. They are beneficial for our health. *Bifidobacterium longum* (BL) is a typical Probiotics. It is one of the most famous microbes and the number of BL in the intestine is 100–1000 times higher than that of Lactobacillus. BL is known to promote the health of the host. Recent studies have described several physiological effects of BL, such as to regulate intestinal function and its beneficial effects in immunity and allergy reduction. Despite the potential benefits of Bifidobacterium mentioned above, its physiological effects on longevity and anti-aging and their mechanisms are not well described. The aim of this study is to analyze the physiological effects of BL for stress tolerance, anti-aging and longevity in *C. elegans*.

C. elegans is a kind of worms and it is easy to culture. It has many kinds of orthologue genes with higher animals. Hence it is widely used as a model organism. In general *C. elegans* eat *E. coli* as food. Therefore we fed them on BL with *E. coli*. Here, we evaluated the effects of dead BL (BR-108). Dead bacteria were used in this study considering bacteria ingested by human are likely to be killed by digestive juice. To be more practical we adopted dead microbes.

As a result, dead BL prolonged the lifespan of *C. elegans*. BL also increased oxidative stress tolerance in nematodes. Consequently upregulation of stress tolerance resulted in the prolongation of life span. In general the movement of worms decrease age-dependently. However *C. elegans* fed on BL prevented the age-related retardation of motility. Regarding the increased motility, we analyzed the amount of muscle and mitochondria time-dependently. As the result of analyzation, BL prevented the age-related retardation of muscle and mitochondria in nematodes day by day. These results suggested dead *Bifidobacterium longum* increased the amount of muscle and mitochondria in *C. elegans* and induced the anti-aging effects.

P-52

Effect of RNA methylations on mRNA 5'-degradation pathway

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The messenger RNA turnover plays an important role to control gene expression by modulating the level of translatable mRNA. All mRNAs are subject to degradation by either a 5′-to-3′ or a 3′-to-5′ exonucleolytic cleavage, which is initiated by shortening of the poly(A) tail. In the 5′-to-3′ pathway, the 5' cap of mRNA is removed by the RNA decapping enzyme, to produce a 5′ monophosphorylated RNA (pRNA) which is subsequently degraded by a 5′-to-3′ exonuclease (Xrn1/Rat1). In this study, we examine whether methyl-modifications at 5' terminus of the RNA affect Trypanosoma brucei decapping and 5'-exonuclease activities. In Trypanosome, the 5'-end of the mRNA is hypermethylated by N6, N6 dimethyladenosine (^{m6,6}A) on the first adenine, N3 methyluridine (^{m3}U) on the fourth uracil, and 2'-O methylations ($A_mA_mC_mU_m$) on the first four nucleosides adjacent to N7 methlyguanosine (^{m7}G), to form a cap 4 structure. We hypothesize that cap 4 methylation may protect the mRNA from degradation to regulate the amount of steady-state level of mRNA. By utilize the synthetic methylated RNA derived from cap 4, we demonstrate methylation at 5' terminus can influence the decapping and 5'-exonuclease activities in vitro. These finding suggested that RNA methylation at 5' terminus can regulate 5'-to-3' mRNA degradation process that indirectly control mRNA homeostasis.

P-53

Generation of bicistronic reporter knockin mice for analyzing germ layers

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Knockout mouse models are commonly used in developmental biology to investigate the functions of specific genes, and the knowledge obtained in such models has yielded insights into the molecular mechanisms underlying developmental processes. Gastrulation is the most dynamic process in embryogenesis. During this stage, a number of genes are expressed spatiotemporally, and differentiation into three germ layers occurs. However, the functions of genes involved in gastrulation are not completely understood. One major reason for this is the technical difficulty of embryo analysis to understand germ layer location.

To solve this problem, we are attempting to generate a novel reporter mouse strains in which the germ layers are distinguished by different fluorescent reporters. The fluorescent reporter genes, *EGFP*, *tdTomato*, and *TagBFP* including 2A peptide sequences were knocked into the appropriate sites before the stop codon of the *Sox17* (endoderm marker), *Otx2* (ectoderm marker), and *T* (mesoderm marker) genes, respectively.

So far, the *Sox17-2A-EGFP*, *Otx2-2A-tdTomato*, and *T-2A-TagBFP* knockin reporter strains were properly generated using CRISPR/Cas9 genome editing in C57BL/6J mouse zygotes. Further, homozygous knockin mice of all strains appeared viable and were fertile. On stereomicroscopic analysis, fluorescent signals were detected in a germ layer-specific manner from heterozygous embryos at embryonic day (E) 6.5–8.5 in all strains, and were immunohistochemically demonstrated to match their respective germ layer-specific marker protein at E7.5. Moreover, we successfully created multiple knockin reporter mice by mating, enabling us to distinguish each germ layer and to visualize overlapped cells in an embryo.

Taken together, these observations suggest that the *Sox17-2A-EGFP*, *Otx2-2A-tdTomato*, and *T-2A-TagBFP* knockin reporter mice may accelerate comprehensive analysis of gene function in germ layer formation combined with genome editing technologies.

P-54

Is There Any Difference in Efficacy to Conduct Suicide Prevention Education Program for Japanese University Students Before or After Summer Vacation?

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University students face risk of suicide in Japan. We developed the Crisis management, Anti-stigma, and Mental health literacy Program for University Students (CAMPUS), a 3-4 hour education program for suicide prevention. The present study evaluates the difference of the efficacy of the program which enforced for university medical students before or after the summer long vacation.

The CAMPUS is composed of (1) a lecture about mental health literacy, self-stigma and gatekeeper, (2) watching a gatekeeper movie, and (3) role-play based on scripts about issues of self-stigma and suicide. One-hundred and forty sophomore medical students of the University of Tsukuba participated in this program. They attended the CAMPUS as a required class either before or after the summer vacation. 134 students answered the questionnaires before and immediately after the class. We analyzed the data to compare with suicide behaviors, depression, and mental health before the class, and to evaluate the change of self-efficacy of the gatekeeper, help-seeking intentions, self-acceptance.

Mean scores of suicide behaviors, mental health and self-acceptance were different between the students who attended in CAMPUS before the vacation and the ones who did after the vacation. However, mean scores of self-efficacy of the gatekeeper, help-seeking intentions, and self-acceptance significantly improved regardless when the students attended the program. Accordingly, although the students had differences in mental health before and after the summer vacation, the efficacy of CAMPUS was observed similarly. It concludes that the efficacy of CAMPUS does not matter at least before or after the summer vacation.

P-55

Two-Dimensional Architecture In Bacteria Biofilm

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Bacteria exist primarily as planktonic cells or members of dense social communities, attached to wet surfaces; these aggregates are called biofilms. About 80% of all known bacteria are known to form biofilms. Biofilms can be either beneficial or harmful to human interests. For example, they can play useful functions in the human microbiome or cause serious chronic infections. Biofilms begin with the attachment of a bacterium to a surface followed by cell division, which eventually generates a community of bacteria encased in extracellular polymeric substances (EPS). Since cells are both self-sticky and secrete EPS, as they divide, they adhere to each other creating amorphous 3D structures. In these systems, how order is self-imposed is unknown. Clarifying the underlying intercellular interactions may lead to a deeper understanding of the forces driving biofilm formation.

We present a biofilm that self-assembles with a unique ordered morphology, grown in a microfluidic device. We analyze the external and internal forces exerted by the cells on their environment and analyze the biofilm structure to investigate the mechanical driving forces for the order we observe. External forces were determined by calculated the adhesion force of individual cells as a function of shear stress in a microfluidic, then analyzing biofilm structure. We estimate the internal forces by measuring cellular growth-pressure to determine how young biofilms spread. We accomplish this by measuring elongation rate of bacteria embedded in hydrogels of tunable elasticity, which is proportional to the cell stiffness. We are analyzing these driving forces to determine the fundamental forces driving biofilm formation. Finally, we are characterizing the structure factor of this highly ordered biofilm. The principles directing biofilm development in this environmental bacterium may be applicable to control biofilm development in other bacteria.

P-56

The defects in USP15 perturbs SPARCL1 trafficking by changing RNA processing

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Autism spectrum disorder (ASD) is a mental developmental disability that occurs in the developmental stage. Ubiquitin specific peptidase 15 (USP15), a deubiquitinating enzyme, has been identified as a high-risk factor in ASD. Previously, we found that impaired USP15 changes the global mRNA processing; however, the molecular mechanisms that link USP15 to ASD is not clarified.

Here we found that secreted protein acidic and rich in cysteine-like 1 (SPARCL1) is a potential splicing target of USP15. SPARCL1 is a secretory protein expressed in astrocyte and neuron and also known as a risk factor for ASD. This protein plays important roles in synaptogenesis by enforcing synaptic connections. We found that the novel SPARCl1 mutants, which fused the respiration chain NADH dehydrogenase [ubiquinone] 1 alpha subunit 11 (Ndufa11) to SPARCL1 C-terminal (SPARCL1-Ndufa11) and SPARCL1 without EF-hand motif (ΔEF) in USP15 knock out (KO) mouse brain. Overexpressed SPARCL1-Ndufa11 and ΔEF SPARCL1 in HEK293T cells were not secreted and accumulated in endoplasmic reticular (ER).

These results suggest that SPARCl1 mutants are not secreted to extracellular region, causing dysfunctions of synaptogenesis in UPS15 KO brain. Our results imply that defects in USP15 associates with ASD via abnormal SPARCL1 mutant. Since mRNA splicing-changed genes caused by impaired USP15 is not only SPARCL1, our data provide the possibility that other protein might be also accumulated in ER that leads to ER stress, linking neuronal survival and synaptogenesis.

P-57

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

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Beige adipocytes have been identified as a new type of adipocyte which developed within the white adipose tissues of adult body. It is also known that beige adipocytes have the function in energy consumption as well as brown adipocytes. Beige adipocytes can be generated by the progenitors such as adipose tissue-derived mesenchymal stem cells (AT-MSCs), and considered as the novel cell therapy for obesity and type 2 diabetes mellitus (T2DM). However, there is no report on how T2DM affects beige adipogenesis ability of AT-MSCs. In this study, we aimed to evaluate the beige adipogenesis ability of AT-MSCs derived from T2DM patients (dAT-MSCs) and non-T2DM donors (nAT-MSCs).

After the induction of AT-MSCs to beige adipocytes, we found the lower number of adipocytes differentiated from dAT-MSCs rather 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. Intriguingly, inflammation cytokines, such as IL1β and TNFα were highly expressed in dAT-MSCs, which led to the increase of nuclear accumulation of p50, resulting in the decrease of beige adipogenesis.

The above results suggested that the increased nuclear accumulation of p50 promoted the suppression of PPARγ and UCP1, which might affect the impaired beige adipogenesis in dAT-MSCs.

P-58

Abnormalities of bone marrow microenvironment in acute myeloid leukemia

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Acute myeloid leukemia (AML) is a hematologic cancer characterized by the proliferation of malignant myeloid cells and the impairment of normal hematopoiesis. Normal hematopoietic system is supported by several components of bone marrow microenvironmental cells, the function of which has been most vigorously characterized as an HSC niche. The HSC niche is composed of several types of hematopoietic/non hematopoietic cells including macrophages, megakaryocytes, endothelial cells, mesenchymal stromal cells, osteoblasts and sympathetic nerves. Several studies in various organs suggested that proliferation of malignant cells induce the functional impairment of normal tissues. According to these backgrounds, we investigate how leukemic cells affect normal hematopoiesis through niche cell dysfunction, possibly in addition to direct leukemic-hematopoietic cell interaction.

We firstly made an AML mouse model using MLL-AF9 retrovirus and then, established the non-irradiated AML mouse model by serial transplantation to avoid irradiation effect on bone marrow environments. By using this mouse model, we analyzed the residual normal hematopoietic cells and the component of HSC niche cells in bone marrow.

In apoptosis analyses using annexin V staining, residual normal hematopoietic cells in AML mice increased the frequencies of apoptotic cells, compared with those in normal mice ($p=0.006$). Interestingly, the frequencies of HSC/hematopoietic progenitor cells were significantly reduced in residual normal hematopoietic cells ($p<0.001$), suggesting that the maintenance system for normal HSC is specifically impaired in leukemic bone marrow. Next, we analyzed each niche component of leukemic bone marrow. In immunofluorescence of leukemic bone marrow, leukemic cells tended to specifically accumulate near vessels and abnormal proliferation of vascular structures was observed. These data imply that leukemic cells might affect the function of endothelial cells or perivascular stromal cells. Then, we isolated endothelial cells, perivascular stromal cells, mesenchymal stem cells (MSC) by multicolor flow cytometry and analyzed gene expression of each component. Perivascular stromal/osteogenic cells (CD45-/TER119-/CD31-/ICAM1+) from AML mice demonstrated significantly decreased expression level of essential hematopoietic cytokine/chemokine, C-X-C motif chemokine ligand 12 (cxcl12) and stem cell factor (scf), compared with control ($p<0.001$). We also found that MSC from AML mice showed the decreased proliferation/differentiation capacity, resulting in the osteoporosis in leukemic mice. These results suggested that leukemic cells induce dysfunction of HSC niche component, resulting in the impairment of maintenance of normal HSC/HSPC, rather than direct impairment of normal hematopoietic systems.

P-59

The relationship between social interaction and health-related quality of life among middle school students in China

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This study aims at investigating the association between social interaction and health-related quality of life (HRQoL) among middle school students in China. We conducted a cross-sectional middle school survey in Nantong, China, using a cluster sampling procedure. The research conducted a questionnaire for adolescents aged from 12 to 17 years old in a public secondary school located in the center area of Nantong, which is a typical city in China. The research will be explained to junior high school students aged from 12 to 17 and their guardians to recruit participants. Students who got consent form from parents are subject to survey and question paper is distributed to them. T-test was implemented to explore the association among gender, family structure, siblings, living arrangement and health-related quality of life. A simple regression model was applied to test the association between mother's educational background, father's educational background, family income and health-related quality of life. Finally, a multiple regression model was used to indicate the association between social interaction and health-related quality of life.

All procedures in this study were proved by ethic committee of the University of Tsukuba. The final sample comprised 295 students' self-reports. The main outcome was HRQoL measured with the KINDL^{R}. Social interaction was evaluated by the Index of Social Interaction (ISI). Age, gender, family structure, siblings, living arrangement, parents' marital status, mother's educational background, father's educational background and family income were also measured.

P-60

Molecular genetic analysis of the role of interleukin 17A in CNS

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Th17 cells, which produce the proinflammatory cytokine IL-17, play important roles in the induction of inflammation. A previous study has reported that an increase in IL-17A contributes to disruption of blood brain barrier integrity, which leads to memory disturbance in mice. Furthermore, elevated levels of IL-17A have been detected in the serum of a subset of ASD patients. However, to date, the role of IL-17A in the pathogenesis of neuropsychiatric disorders remains to be determined.

To evaluate the effect of long-term upregulation of IL-17A to function and development of CNS in vivo, and elucidate the significance of IL-17A in pathogenesis of neuropsychiatric diseases, we used transgenic mice with C57BL/6 background in which retinoic acid related orphan receptor gamma-t (RORγt) is overexpressed under the control of human CD2 gene (RORγt Tg mice). RORγt is known to be a transcription factor required for the differentiation of Th17 cells. In this study, we confirmed that serum IL-17A level is markedly upregulated in the RORγt Tg mice compared to WT mice.

To obtain a first clue of the effect of IL-17A upregulation in CNS, we performed immunohistochemical analysis of RORγt Tg mice using various neuronal and glial markers. We also carried out an analysis of behaviors that could relate to higher brain functions. In this presentation, we will show the results of histological, behavioral, and other data of the RORγt Tg mice, and discuss their significance in the pathogenesis of neuropsychiatric disorders such as Autism Spectrum Disorder and schizophrenia.

Since it has been suggested that IL-17 play important role in dysregulation of fetal brain development caused by maternal immune activation, we examined pregnant female RORγt Tg mice, and found a significant increase in abortion rate after maternal immune activation induced by poly(I:C) administration. This suggests an alteration of intrauterine environments in RORγt Tg mice, which could alter fetal brain development.

P-61

Novel pattern of cerebral blood flow across sleep/wake cycle revealed by *in vivo* imaging

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Cerebral blood flow (CBF) is critical in maintaining energy-dependent processes, clearing metabolic byproducts generated by neuronal activity, and predicting neurotransmitter activities (Dukart *et al*., Sci Rep, 2018). Impaired CBF regulation can affect protein and ATP synthesis, disrupt action potential generation, or cause ischemic neuronal death (Hossmann *et al.*, Ann Neurol, 1994). CBF dysregulation is associated with neurodegenerative disorders such as Alzheimer's Disease (Kassandra *et al.*, Nature Rev Neurosci, 2017). CBF is regulated strictly and independently from the peripheral circulation. Here, I aimed to investigate the role of sleep in the regulation of CBF. Till now, various approaches have been taken to investigate how CBF changes during wake, NREM sleep and REM sleep. However, conflicting conclusions have been drawn from multiple approaches. According to studies using positron emission tomography, CBF is decreased during NREM sleep and comparable to wake during REM sleep (Braun *et al.*, Brain, 1997), whereas according to studies using ultrasonic techniques, CBF is highest during REM sleep (Grant *et al.*, J Physiol, 2005; Bergel *et al.*, 2019). These conflicts likely stem from differences in data processing, normalization procedures, and from differences in the type of blood vessels that are observed.

We hypothesized that the problem with the above approaches is that they cannot directly observe blood flow in individual capillaries, where the actual substances exchange between blood and glia/neurons occurs. Therefore, we developed an alternative approach using two-photon microscopy (2PM) imaging to directly measure the velocity of red blood cells in capillaries while monitoring sleep/wake stages. This technique would help us draw a final conclusion on how CBF changes in sleep/wake. Analyzing CBF via 2PM is not only able to achieve high spatio-temporal resolution, but also provides a novel method for studying other physiological activities.

P-62

Proteasome-associated proteins, PA200 and Ecm29, contribute to sperm motility and normal spermatogenesis

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Proteasomes are large enzyme complexes that degrade unneeded or abnormal proteins for the protein homeostasis. Various proteasome activators regulate the proteasome function. PA200 is one of the proteasome activator, that specifically recognizes acetylated histones and promotes ATP- and ubiquitin-independent degradation of core histones during DNA damage response. PA200 is highly expressed in testis and *PSME4* (gene for PA200)-deficient mice are subfertile due to defects in normal spermatogenesis. Spermatogenesis is a complex process by which sperm is produced from male primordial germ cells through mitosis and meiosis. In this process, the Sertoli cells, which have a supportive and nutrient function, are also involved and plays essential roles for spermatogenesis.

Ecm29 is a protein which associates with the proteasome. Ecm29 is involved in the maintenance and regulation of proteasome assembly and promotes proteasome dissociation under oxidative stress. Although Ecm29 and PA200 have different function, they have similar HEAT repeat motifs raising the possibility that they may have redundant roles on the regulation of the proteasomes.

Here, we generated *PSME4*$^{-/-}$ *ECPAS*$^{-/-}$ (gene for Ecm29) double knockout(dKO) mice. Each single knockout mice were fertile, however, dKO mice were completely infertile. Histological analysis revealed that dKO mice sperms have abnormal structure such as double head, midpiece defect. Furthermore, the sperm motility of dKO mice were markedly decreased. These abnormalities can be attributable to the defects in either or both germ stem cells and Sertoli cells. Therefore, to clarify this issue, we established germ stem cells from dKO mice and injected into the wild type mouse testis in which the seminiferous tubules were disrupted.

P-63

Visual Cognition Reconstruction using a Single-channel EEG

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Real-time decoding of brain activity by brain computer interface has not been fully realized despite many efforts in the field. That said, we are closer than ever before in turning that into a reality. Kamitani and others have succeeded in visualizing fMRI signal as an intermediate layer of a convolutional neural network to decode what is being seen. Although the spatial resolution of fMRI is of the highest caliber, it is limited by high cost and operational skill requirement. Another method that is widely used to measure and record brain activity is via electroencephalogram (EEG). It can detect different frequency waves from brain signals. Compared to fMRI, EEG may be inferior in terms of spatial resolution, but it does have advantages over fMRI. This includes better temporal resolution, low-cost, easier to use, and some are even portable. These features allow data collection from large number of individuals possible.

To achieve higher spatial resolution in EEG, more channels are opted as a dense network of electrodes creates a spatial map to a degree. However, this results to higher cost while decreased ease-of-use and comfort. As firm believers that we primarily need to address the lack of data in brain imaging, we used single-channel EEG headsets which are powerful yet underexploited tools in neuroscience. This follows the ideology of bigger quantity over quality. In this study, we attempted to reconstruct images based on the detected brain signals from 15 healthy subjects. This was done by creating a library of the images and the associated brain waves that were detected upon exposure to the image and during image recalling phase.

P-64

Babyula: Smart Baby Tracker

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Babyula is a wearable device that integrates different sensors to monitor the baby's condition. It is capable of longtime monitoring, especially during sleep. Its purpose is to detect unusual states in infants, which could help prevent Sudden Infant Death Syndrome (SIDS). It sends a notification to primary caregivers in case of emergency. It also records and interprets data for improved healthcare and diagnosis. This device implements machine learning to predict susceptibility to SIDS.

Epilepsy is another issue affecting infants. It is a treatable disease when diagnosed early and correctly. However, the misdiagnosis rate is as high as 20-30%. Babyula can be used to monitor and record seizures continuously. Seizures come in different forms (not all involves convulsions), but the most dangerous ones are the ones left unattended. Being able to track seizures, especially during sleep, can prevent detrimental consequences.

Babyula is for infants (0-3 years old). It will be equipped with accelerometers and microphones to monitor the baby's motion and the ambient sound. We are currently developing Babyula 1.0, using a sound recorder attached in baby's clothing. Long-term recording can be used to monitor different activities such as breathing (heartbeat), crying, excretion, vomiting, movement, and external noises. We created a sound-activity library from the initial set of volunteers. In the future, this data will also be used for epilepsy monitoring and predicting SIDS susceptibility. We are currently building data libraries and conduct real-time tracking to a small sample size of babies in Japan.

Our long-term vision is to create a cost-effective platform for reducing infant mortality worldwide, especially in developing countries. It could be achieved by initially targeting countries having adequate healthcare capacity and purchasing power (East Asia, North America, Europe). These countries could provide a suitable environment for product development and optimization.

P-65

Non-invasive *in vivo* Murine Atherosclerosis Visualization with One-time DiR Liposome Injection

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Introduction: Quantitative, non-invasive analysis of atherosclerosis progression in animal models has a great importance in better understanding of the disease, validating current therapeutic measures and establishing novel effective therapeutic interventions. Here, we address an *in vivo* non-invasive animal atherosclerosis visualizing system, which is effective and user friendly in disease surveillances, frequently used in preclinical animal experiments in novel drug discovery procedures.

Methods: Our murine atherosclerosis imaging system targets the macrophages phagocyting DiR liposome in the atheroma, which emits Near Infrared Fluorescent **(**excitation 748 nm / emission 780 nm**)**. DiR liposome was injected through tail vein after induction of atherosclerosis. LDL receptor-deficient mice (*LDLR*$^{-/-}$) were used throughout the experiment as an inducible atherosclerosis model. Atherosclerosis was induced by high cholesterol diet (HCD). Two groups as HCD, Normal Diet (ND) were maintained for the comparison. *In vivo, ex vivo* IVIS® imaging were carried out every day for a week. *LDLR*$^{-/-}$ mice fed with ND and PBS injected *LDLR*$^{-/-}$ mice were used as controls. Oil Red O and histological staining methods were used to confirm coexistence of DiR signal and aortic plaque.

Results: *In vivo* and *ex vivo* IVIS® images of the test group exhibited a specific DiR signal enhancement in the aorta, while DiR signals were not observed in the aortic area of control groups. Time course imaging showed a stable signal of the aortic area throughout experiments. Oil Red O staining of aortas showed that our detected signal is strictly emitted from plaque positive areas of the aorta.

Conclusion: One-time DiR Liposome injection is able to noninvasively image atheroma positive areas in aorta. And signals are stable throughout experiment and coexist with Oil Red O positive area whereas it is injected only once. We believe that this imaging system can be used as the easiest system for *in vivo* live atherosclerosis imaging, which has a great potential to accelerate effective drug development procedures for atherosclerosis.

P-66

Associations of *GTF2I-NCF1* region polymorphisms with SLE and systemic sclerosis.

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【Background】Genome-wide association studies (GWAS) of systemic lupus erythematosus (SLE) in Chinese and Korean populations demonstrated strong association of a single nucleotide polymorphism (SNP) rs73366469, located upstream of *GTF2I,* encoding a transcription factor. Other two SNPs around the *GTF2I* region, rs117026326 and rs80346167, have also been associated with SLE. In addition, a missense SNP rs201802880 of *NCF1* encoding a subunit of NADPH oxidase, is in linkage disequilibrium (LD) with *GTF2I* SNPs and has been associated with SLE. However, association studies with systemic sclerosis (SSc) have not been reported. Here we examined whether these SNPs are associated with SLE and SSc in a Japanese population and made an attempt to identify the primarily associated SNP.

【Method】Genotyping was performed on 842 SLE, 467 SSc patients and 934 healthy controls using TaqMan SNP Genotyping Assay. In the case of rs201802880, nested PCR was performed before SNP genotyping because of the presence of highly homologous *NCF1* pseudogenes. Association studies were performed by logistic regression test using R. Correction for multiple testing was performed by calculating FDR q values.

【Results】Striking associations of rs73366469 C, rs117026326 T and rs201802880 A were confirmed in Japanese SLE, and the same alleles were also found to be significantly associated with SSc. rs80346167 A was also associated with SLE and SSc but its association was weaker than the other SNPs. The association of *NCF1* rs201802880 was remarkably strong (SLE : $p=3.77\times10^{-44}$, $q=4.52\times10^{-43}$, Odds Ratio (OR)=3.57, SSc : $p=2.40\times10^{-4}$, $q=5.76\times10^{-4}$, OR=1.50). Conditional logistic regression analysis using each SNP as independent variable revealed significant residual association only in rs201802880.

【Conclusion】Associations of *GTF2I-NCF1* SNPs with SLE were confirmed in the Japanese population, and associations with SSc were detected for the first time. *NCF1* rs201802880 was suggested to be the primary association, and the association of other SNPs to be caused by LD.

P-67

MAIR-II deficiency promotes favorable cardiac remodeling post-myocardial infarction

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[Background] MAIR-II (Myeloid-Associated Immunoglobulin-Like Receptor II) is expressed on myeloid cells and involved in Toll-like receptor-4 (TLR4)-mediated inflammatory monocyte migration from the blood to sites of infection as part of the host response in bacterial infections. In myocardial infarction (MI), monocytes are known to be recruited to infarcted myocardium followed by a response of inflammatory M1 and anti-inflammatory M2 macrophages. However, the role of MAIR-II in MI remains elusive.

[Purpose] To determine the role of MAIR-II in MI pathophysiology.

[Methods/Results] MAIR-II$^+$ myeloid cells were abundant from post-MI days 3 to 5 in infarcted hearts of C57BL/6 (WT) mice induced by permanent ligation of the left coronary artery in flow cytometric analysis. To address MAIR-II's role in myeloid cell function *in vivo*, effects from MAIR-II knockout (KO) mice were investigated. In post-MI survival, MAIR-II KO mice lived longer than WT mice ($p = 0.043$). In echocardiography, MAIR-II KO mice had thicker left ventricle posterior walls and higher ejection fractions compared to WT mice. After further investigation, we found that MAIR-II KO hearts had less IL-1β, less TGF-β, and collagen expressions, and more CD206$^+$ M2 macrophages in infarcted hearts compared to WT. To elucidate MAIR-II's role in macrophages, we analyzed bone marrow-derived macrophages (BMDM) from WT and MAIR-II KO mice polarized to M1 and M2 using HMGB1 or IL-4 respectively. We found that M1 and M2-polarized BMDM from MAIR-II KO expressed less M1 and M2-related gene expressions compared to WT mice. Furthermore, RNA sequencing pathway analysis of MI day 3 CD11b$^+$ cells from MAIR-II KO and WT revealed up-regulated extracellular matrix receptor interaction and down-regulated T cell receptor signaling in MAIR-II KO.

[Conclusion] MAIR-II deficiency suppresses exacerbated inflammation and fibrosis, promoting favorable cardiac remodeling post-MI.

P-68

Signal dynamics corresponding to value-to-choice transformation in midbrain dopamine neurons and orbitofrontal neurons in monkeys performing an economic decision-making

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In economic decision-making, individuals evaluate the value of options, and then decide to choose or not to choose the options. Although several cortical and subcortical areas are known to signal value information and contribute to decision-making, neural mechanisms that transform the value information into a choice command still remains unknown. Here we focused on midbrain dopamine (DA) neurons that are well-known to represent value information, and examined whether and how these neurons contribute to the value-to-choice transformation process. We recorded single-unit activity from DA neurons in monkeys performing an economic decision-making task in which the monkey was required to decide to choose or not to choose an option based on its value immediately after the option was offered. We found that DA neurons represented diverse signals related not only to the option's value but also to the animal's choice; some neurons represented the value of the offered option, some represented whether the animal would choose or not choose the option, and some represented the value of the option only when the option was chosen by the monkey -we therefore called this activity pattern as *choice-dependent value* signal that was influenced by both value and choice. We next analyzed the time course of these DA signals and found that the order of signal representations corresponded to the value-to-choice transformation. Shortly after the onset of the option, the value signal rapidly appeared, which was followed by the choice-dependent value signal. The choice signal arose at last. For comparison, we also recorded single-unit activity from the orbitofrontal cortex (OFC) that has been implicated in economic decision-making, and found that OFC neurons also represented the three signals and exhibited the same order of signal representations. Notably, the last-arising choice signal appeared before the monkey executed a motor action to choose the option in both DA and OFC neurons. Thus, both neurons were temporally capable of regulating the monkey's choice behavior. Our findings provide evidence that not only prefrontal regions but also the subcortical DA system plays a crucial role in value-based choice formation.

P-69

The role of p62/Sqstm1 in leptomycin B cytotoxicity

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Even in animal cells, the antibiotic leptomycin B (LMB) against fungi specifically binds to CRM1, which exports nuclear proteins to cytoplasm, and causes cell death by inhibiting nuclear export. One of the proteins whose nuclear export is inhibited by LMB treatment is p62 (Sqstm1). p62 has nuclear localization signals (NLS) and a nuclear export signal (NES), and accumulates in the nucleus by LMB treatment. Since p62 is mainly localized in the cytoplasm under steady conditions, it is considered that p62 is always pumped from the nucleus to the cytoplasm via CRM1, but its significance is unknown. there are many unclear points regarding the relationship between p62 subcellular localization control and function.

The purpose of this study is to investigate the role of p62 in LMB cytotoxicity using mouse embryonic fibroblasts (MEF) derived from wild-type (WT) and p62 knockout mice (p62-KO).

p62 accumulated in the nucleus from 1 hour after addition of LMB, and more p62 stored in the nucleus after 24 hours. When viable cell activity was examined with MTT reagent, that of WT were significantly reduced by LMB, but that of p62-KO MEF showed almost no decrease even at high concentrations. LMB strongly induced Cleaved-Caspase3 in WT, but in p62-KO MEF, the degree was weak.

When p62 was expressed in p62-KO MEF by transfection of a p62 expression vector, the cytotoxicity of LMB recovered. Furthermore, TUNEL-positive cells and p62 expression were consistent in some cells.

Taken together, we revealed that p62-KO cells are resistant to LMB. The accumulation of nuclear-cytoplasmic shuttling protein p62 in the nucleus may play important role of cytotoxicity of LMB.

P-70

Establishment of a model mouse for diabetes mellitus using MafA and MafB conditional KO mouse

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Diabetes mellitus (DM) is characterized by a relative or absolute lack of insulin. Because DM patients have been steadily increasing over the past few decades, the establishment of model mice is crucial for developing the treatment for DM.

Transcription factor MafA and MafB play important role in development of pancreatic endocrine cells and maintaining their functions. Previous studies reported MafA and MafB deficient newborn mice showed significantly reduced insulin and glucagon cell population and developed DM symptoms. This result suggests that MafA and MafB control insulin and glucagon cell formation and control glucose homeostasis. However, because these mice die soon after birth, they cannot be applicable to DM model. Thus, we evaluated that induction of MafA and MafB deletion in adult stage can become the DM model mice.

In this study, we generated *MafA*$^{-/-}$*;MafB*$^{flox/flox}$*;Pdx1*-CreER^{TM} mice (*A0B*$^{\Delta panc}$), which MafB is specifically deleted in pancreas after tamoxifen injection to investigate the function of MafA and MafB in the pancreatic endocrine cells at adult stages. The *A0B*$^{\Delta panc}$ male mice developed higher blood glucose level after intraperitoneal glucose injection than wild-type (WT). In addition, urine volume significantly increased in male *A0B*$^{\Delta panc}$ mice. In histological analysis, pancreatic islets in male *A0B*$^{\Delta panc}$ mice tended to be impaired in their structures and decreased the numbers.

In conclusion, the deficiency of MafB in pancreatic cells under the MafA deficient condition causes severe diabetic phenotype in male mice. Because *A0B*$^{\Delta panc}$ male mice can rapidly and reproducibly develop DM after tamoxifen injection, the mice line can be utilized as DM model mice.

P-71

SHED-EV* on Temporomandibular Joint Osteoarthritis Repair

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Temporomandibular joint osteoarthritis (TMJOA) is a common disease affecting the adult, causing a myriad of symptoms including pain, headache, difficulty in mouth opening etc., which greatly affect the life quality of the patient. Current treatments for TMJOA include administration of NSAIDs, physical therapy, filler injection, that aim to alleviate the pain and slow down bone damage. However, a radical treatment that could stop the inflammatory process and repairs the eroded bone is still lacking. In this study, we explore the potential of *SHED-EVs, i.e. extracellular vesicles derived from stem cells of human exfoliated deciduous teeth origin, for the treatment of TMJOA. We will isolate EVs via ultracentrifugation from SHED cultures established upon clinical extraction, and inject into the inferior joint cavity of a mechanical-induced TMJOA rat model. We will pair micro-computer tomography analysis together with immunocytochemistry staining to assess the progression of bone repair. We hope that combining these macro- and molecular level results, we would be able to provide with new evidence on future directions of TMJOA treatment.

P-72

Calorie Restriction Feeding Induces Memory Enhancement with Modification on Gut Microbiota

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Since the light/dark cycle in the world is about 24 hours, in order to act normally throughout every single day, almost all organisms have developed the endogenous circadian clock system to match the outside environmental fluctuation, and to collaborate the physiological functions of different organs. In addition to daily light-dark cycle, daily fed calorie is one of the external cues, which affecting body weight, immune system as well as gut microbiota of an organism. Previous studies showed that calorie restriction (CR) feeding, i.e. only 60% energy intake compared to *ad libitum* (AL) feeding groups, is one of the powerful regime to improve animal's physiological condition, including anti-inflammation, longevity, cognitive function as well as memory. This improvement were reported in multiple model animals. However, the underlying mechanism of CR and improved memory is still not clear.

In this study, using wild-type mice as model animals, we combined 16s ribosomal RNA Amplicon Next Generation Sequencing analysis and several behavior tests, including novel object memory task and three chamber social test, and found that CR resulted in the alteration of gut microbiota, as well as improved memory ability of mice. Furthermore, using multiple antibiotic to remove the gut microbiota eliminated these beneficial effects of CR. Moreover, transplantation of microbiota from CR mice to AL mice elevated the memory ability of the recipient mice. Overall, calorie-restriction feeding enhances the memory ability of mice via modification on gut microbiota.

P-73

Topoisomerase 2β contributes to the inflammation-associated DNA damage in ulcerative colitis and colon tumorigenesis

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Inflammatory bowel disease is chronic inflammation disease of colon and small intestine, including Crohn's disease (CD) and ulcerative colitis (UC). Many studies demonstrated that inflammatory bowel disease (IBD) is highly associated with a 20-fold higher incidence of colorectal cancer, whose underlying molecular mechanism(s) might related to the generation of oxidative stress which includes reactive nitrogen and oxygen species (RNOS), pro-inflammatory cytokine, and microbiota dysbiosis. Moreover, also from literature reviews, we hypothesize that the inflammation–associated oxidative stress leads to DNA damage and results in genome instability, then this is one of the major contributory factors affecting tumor initiation as well as development. Topoisomerase 2β (Top2□) is one of the two isozyme of type II DNA topoisomerase. It is important in regulating chromatin DNA topology and related processes through its catalytic function cause transient DNA double-stranded break (DSB). Interestingly, in addition to studying topoisomerases in chromatin biology and our laboratory also investigates and showed that the pathogenic involvement of Top2β in DSB caused by inflammation-associated protein nitrosylation and oxidation, trying to understand the mechanism of inflammatory disease leading to tumor formation. Toward this end, we use dextran sodium sulfate (DSS) induce UC mice as animal model, and took advantage of our established genetic and pharmacological platforms and approaches (e.g. ICRF-193, the Top2 catalytic inhibitor) to study the potential tumorigenic mechanism(s) using further evaluation of the tumor formation of Top2β knockout mice under DSS-AOM treatment. We observed that DNA DSB signaling is decreased when Top2β function was inhibited/compromised, indicating the potential involvement of Top2β in the generation mechanism of inflammation- associated DNA damage formation and genome instability, then causing tumor formation.

P-74

Using the Exotic Plant *Cassia fistula* to Biologically Control the Invasive Animal *Pontoscolex corethrurus*

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This research investigates the application of *Cassia fistula* to expelling *Pontoscolex corethrurus*. *P. corethrurus* is an invasive species that has caused negative influence on native biodiversity and soil structure in many areas around the world (Barros, Curmi, Hallaire, Chauvel, & Lavelle, 2001; Chapuis-Lardy et al., 2010; Hendrix et al., 2008). However, there have been only scant studies exploring the control of earthworms, and most strategies are mainly based on chemical pesticides and land management. To bridge this research gap, the present study devises a *P. corethrurus*-targeted biological control method using *C. fistula*. Previous research has indicated that *P. corethrurus* would keep away from *C. fistula* by nature (Chen, 2015). Based on this instinct of *P. corethrurus*, we carried out a secession of experiments to identify the ingredients of *C. fistula* and observe the behavior of the earthworm when treated with substances extracted from the plant. The results demonstrate that alkaloids in *C. fistula* would produce a marked effect on preventing the activeness of *P. corethrurus*. That is, when dry leaf powder of *C. fistula* is spread to the habitat of *C. fistula*, within one to two weeks, the density of the earthworm turned out to reduce significantly. However, the populations of native earthworms remain stable and unaffected. We found that the key reason for the phenomenon is that *P. corethrurus* is specifically susceptible to *C. fistula*, which would cause laxative effect on it. Since the *C. fistula*-treatment test reflects a significant interspecies difference, we suggest that it is ideal to use *C. fistula* as a biological control agent against *P. corethrurus*. Moreover, since leaves of *C. fistula* are easily available in Taiwan, related control programs could be eco- friendly and cost effective. This study hence contributes to solution of earthworm invasion and contemporary investigation of biological control.

Keywords: *Cassia fistula*, *Pontoscolex corethrurus*, exotic species, invasive species, biological control, sennoside

P-75

Establishment of High Throughput Drug Screening System of Hematopoietic Drugs in Human Leukemia Cell Line K562

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Erythrocytes and platelets account for 99% of the total human blood cells, therefore disruption of these differentiation processes would lead to severe acute and chronic diseases, including anemia and Thalassemia for erythroid lineage, hemophilia for the other. Erythrocytes and platelets are differentiated from the same progenitor cells, megakaryocyte-erythroid progenitors(MEPs). Thus, by analyzing the different differential surface markers of K562, which has similar differential potential as MEP, can be used to screen the effective drugs for these two sorts of diseases. Nowadays, the observations of cell differentiation are mostly performed by flow cytometry to discriminate distinct differential stages of cells by recognizing various surface markers with specific antibodies. However, the drawbacks of flow cytometry are time-consuming and highly dependent on specificity of antibodies. As a results, this study aims to set up a luciferase-based drug screening system for hematopoietic diseases. By establishment of K562 cells carrying luciferase gene driven by promoter of erythrocyte marker glycophorin A (GlyA), we can real-time detect the differential of erythrocyte with treatment of candidate drug. Then we can assess the level of differentiation by the activity of luciferase to easily select the potential therapeutic drugs. To sum up, this system can not only improve the observation of hematopoietic cell differentiation currently, but can be used as a novel high throughput drug screening model for hematopoietic diseases.

P-76

Function-Position Relationship of the Intrapancreatic Islet Network

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Pancreatic islets of Langherhans are the sole organoid capable of lowering blood glucose in the human body, and thus the epicenter of *Diabetes Mellitus* progression. With the advent of single cell analysis techniques, complex cellular heterogeneity within the islets is being unraveled, evoking novel understanding on pancreatic development and disease progression. Nevertheless, how the pancreatic islets intercommunicate to concert their output as a whole, remains largely unmasked. Here we propose a novel surgical isolation of pancreatic islets from the mice mesentery *in situ*, which enables the mapping of the islets under study in relationship to the pancreatic circuitry. We will use single cell transcriptome analysis to address functional and compositional differences within the pancreas, and how positional effect translates into network subdivision. We envision our study to bring novel understandings of the endocrine pancreas as a system, as well as to give insights for the design of artificial pancreas to combat *Diabetes Mellitus*.

P-77

The effects of water managements on the arsenic accumulation and speciation in water spinach grown in As-contaminated soils

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Arsenic (As) is considered as one of the most dangerous carcinogens in natural environment. In general, vegetables are grown in upland field which availablility of As in soil is low, so it is not easy to accumulate As in plants. However, due to water spinach can be planted in both flooding and upland field, which may cause the differences in As accumulation in plants between these two water managements. Therefore, the objective of the present study is to compare the distribution of As species and total As concentration in water spinach grown in As-contaminated soils with different water managements (flooding and upland conditions). In this study, the As concentration of the tested soils in Guandu(Gd) are 18.40 mg As kg^{-1}(GdL) and 103.30 mg As kg^{-1}(GdH), and those in Minsyong(Ms) are 22.86 mg As kg^{-1}(MsL) and 72.10 mg As kg^{-1}(MsH). The results show that biomass of the water spinach grown in Gd-F is the highest, and MsH-U is the lowest, which is related to the amounts of Fe plaque and water management conditions. In the tests, biomass of the water spinach grown in upland field is lower, and due to the higher contents of free Fe oxides in Gd soils, the amounts of Fe plaque on the root surface and biomass of the water spinach grown in Gd soils are higher than Ms soils. Besides, the amounts of Fe plaque and As concentration in aboveground parts show negative correlativity, so the As concentration in plants grown in MsH-F was higher than other tested soils. Finally, inorganic As was the predominant species in the soil pore water under two water managements and in water spinach, especially iAs^{III}, while the proportion of arsenate (iAs^{V}) in the upland field was slightly increased. Based on the results of this study, it suggests the As species concentration in water spinach is highly related to those in soil pore water, and the As accumulation in edible parts are significantly decreased under upland condition, so the water spinach planting in the upland field can significantly reduce the health risk.

P-78

Placebo response in randomized trials of biologics and small molecules for psoriatic arthritis: a systemic review and meta-analysis

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Abstract

Background: Biologics and small molecules are used for treating psoriatic arthritis. This study is aimed to examine the placebo response in clinical trials of biologics and small molecules in treating psoriatic arthritis.

Method: We searched MEDLINE, Embase, and CENTRAL databases for randomized controlled trials that evaluated the effects of biologics and small molecules in treating psoriatic arthritis on June 9, 2018. We performed meta-analysis for studies reporting American College of Rheumatology 20% (ACR 20) response. We also conducted a logistic regression to examine possible associated factors.

Results: We included 34 studies with a total of 10,973 participants with psoriatic arthritis. The meta-analysis showed 20% (95% CI 19%–21%) of participants receiving placebo reached ACR 20. Multivariate logistic regression analysis found no correlation between placebo response and the phase of trial, characters of the included participants (i.e. disease-modifying antirheumatic drug (DMARD) unresponsiveness, biologics-experienced, tumor necrosis factor-inhibitor failure), co-treatment with DMARDs, sample size, the length of follow up and route of administration (all $P > 0.05$). However, a binomial classification and regression tree model displayed a lower placebo response for biologic naïve patients.

Conclusions: A considerable placebo response exists across clinical trials on biologics and small molecules in treating psoriatic arthritis. The prevalent placebo effect should be considered when evaluating the efficacy of interventions for psoriatic arthritis. Objective outcome measures and patient stratifications should be implemented in future trials for psoriatic arthritis.

P-79

Exosomal surface biomarker discovery of brest cancer early detection

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Liquid biopsies bear numerous biological molecules in relation to tumorigenesis and cancer progression. These molecules are abundantly expressed and are dynamic biomarkers that exist within a variety of body fluids including blood, thereby providing valuable information for cancer diagnosis and prognosis. Thus, liquid biopsies serve as a solid foundation for precision medicine due to rapid and highly sensitive characteristic. Breast cancer is the most commonly diagnosed cancer among women. High proportion of women diagnosed with breast cancer at the earlier stage survive. Through effective biomarker, breast cancer can be early detected. Herein, we attempt to analyze the protein content of exosomes from breast cancer blood to discover potential biomarkers. We collected serum from patients of different subtypes and phases of breast cancers and patients with benign tumor and ductal carcinoma *in situ* (DCIS), which in our studies we defined as non-cancer controls. Exosomes of these sera were isolated by the EVSecond column. In addition, exosomes from breast cancer cell lines were also collected. The cell-lines which were used included MDA-MB-231, MCF7, SK-BR-3, representing triple negative, Her2+ and ER+/PR+ respectively, and a breast luminal epithelial cell line MCF10A were used as a non-cancerous control. Exosomes from cell-lines were purified by the differential centrifugation protocol. The protein identification and quantification of exosomes from patients and cell-lines were analyzed by LC-MS/MS. The results from patients' sera and cell-lines were analyzed separately. The proteins overlapped between the results from serum and cell-lines would then further be verified their expression and topological orientation by immuno-blotting and dot-blotting. The candidates for early detection biomarkers of breast cancer would also be validated using a cohort of breast cancer plasma/sera, validating the translational possibility of this study. Through our studies, we intend to find out potential biomarkers which may be applied in clinics, providing a rapid, precise early diagnosis for breast cancer.

P-80

The development defect of serotonergic neuron induced by maternal immune activation

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Maternal immune activation (MIA) in pregnancy induces abnormal development of fetal brain, and could cause neuropsychiatric diseases such as schizophrenia and autism spectrum disorder (ASD). To model MIA, poly(I:C) was injected into pregnant mice. Serotonergic neurons project their axons throughout the central nervous system. Anterior part of their fibers, originated from the dorsal and median raphe nuclei, passes through diencenphalon and send terminals to ventral tegmental area, globus pallidus, hippocampus, septal nuclei, and cerebral cortex. These areas are implicated in mental illnesses. Previous studies report that the mouse embryos exposed to MIA at E12.5 showed abnormal development of serotonergic fibers. Importantly, disturbed development of serotonergic fibers is considered to be related to ASD.

Serotonergic neurons release glutamate as well as serotonin, and that specific deletion of vesicular glutamate transporter type 3 (VGLUT3) in serotonergic neurons induces defects in axonal transport of 5-HT.The development of raphe nuclei begins at E10.5, and serotonergic fibers enter diencephalon and frontal cortex at E12-17. Previous works use a protocol that induce MIA at E12.5. In this study, I plan to determine stage-specific effect of MIA on the development of serotonergic fibers by inducing MIA at different developmental stages.

In this study, we used pregnant C57BL/6J mice to generate a MIA model. These mice were divide into 3 groups and poly(I:C) or PBS was injected to them at different developmental stage. The offspring were perfused by paraformaldehyde. Brain cryosections were prepared. Hippocampus, lateral septum, habenular nucleus, Globus pallidus, basal lateral amygdala, and raphe nuclei were observed. Fluorescent or ABC-DAB labeling was used to detect 5-HT and VGLUT3 signals. We are now making efforts to determine the optimal method for quantitative analysis of SERT-positive fibers and VGLUT3-positive structures. We will present data showing the effect of MIA, induced at differential stages, on SERT- and VGLUT3-positive structures.