Award Presentations（Oral）

MD Scientist Program（Oral）

Hiroshi and Aya Irisawa Memorial Award Symposium

Brain-gut association via peptides and amines

(March 22, 17:30～19:00, Room A)

IS-1

Effects of food deprivation on the hypothalamic feeding-regulating peptides gene expressions in serotonin depleted rats

Yoshimura, Mitsuhiro[1]; Hagimoto, Marina[1]; Matsuura, Takanori[1]; Ohkubo, Junichi[1]; Ohno, Motoko[1]; Maruyama, Takashi[1]; Ishikura, Toru[1]; Hashimoto, Hirofumi[1]; Kakuma, Tetsuya[2]; Yoshimatsu, Hironobu[2]; Terawaki, Kiyoshi[3]; Uezono, Yasuhito[3]; Toyohira, Yumiko[4]; Yanagihara, Nobuyuki[4]; Ueta, Yoichi[1] (*[1]Dept Physiol, Sch Med, UOEH, Kitakyushu, Japan; [2]Dept Internal Medicine 1, Sch Medicine, Oita Univ, Oita, Japan; [3]Div Cancer Pathophysiol, Gr Dev Mol diag and Ind Ther, Natl Cancer Cent Res Ins, Tokyo, Japan; [4]Dept Pharmacol, Sch Med, UOEH, Kitakyushu, Japan*)

We examined the effects of serotonin (5-HT) depletion induced by peripheral injection of 5-HT synthesis inhibitor p-chlorophenylalanine (PCPA) on the expression of feeding-regulating peptides expressions by using in situ hybridization histochemistry in adult male Wistar rats. PCPA pretreatment had no significant effect on basal levels of *oxytocin*, *corticotropin-releasing hormone* (*CRH*), *thyrotropin-releasing hormone* (*TRH*), *pro-opiomelanocortin* (*POMC*), *cocaine and amphetamine-regulated transcript* (*CART*), *neuropeptide-Y* (*NPY*), *agouti-related protein* (*AgRP*), *melanin-concentrating hormone* (*MCH*) or *orexin* in the hypothalamus. 48 h food deprivation caused a significant decrease in *CRH*, *TRH*, *POMC*, and *CART*, a significant increase in *NPY*, *AgRP* and *MCH*. After PCPA treatment, *POMC* and *CART* did not decrease despite food deprivation. *NPY* significantly increased by food deprivation with PCPA, but was attenuated compared to food deprivation without PCPA. These results suggest that the serotonergic system in the hypothalamus may be involved in the gene expression of *POMC*, *CART*, and *NPY* related to feeding behavior.

(COI: No)

IS-2

Roles of peptides and amines in the regulation of the colorectal motility via the spinal cord

Shiina, Takahiko[1]; Naitou, Kiyotada[1]; Nakamori, Hiroyuki[1]; Sano, Yuuki[1]; Ikeda, Azusa[1]; Hirayama, Haruko[2]; Shimizu, Yasutake[1] (*[1]Dept Basic Vet Sci, Lab Physiol, Unit Grad Sch Vet Sci, Gifu Univ, Gifu, Japan; [2]Dept. Animal Resources, Okayama Univ., Okayama, Japan*)

Colorectal motility is regulated by the enteric neurons locally and the defecation center systemically. The lumbosacral defecation center regulates colorectal motility via sympathetic lumbar colonic nerve and parasympathetic pelvic nerve. On the other hand, the brain stem defecation center modulates the lumbosacral defecation center through descending spinal neural pathway and then affects colorectal motor activity. Although it is speculated that various neurotransmitters including peptides and amines are involved in the regulation of colorectal motility by the defecation center, precise mechanisms remain to be clarified. Therefore, we have investigated regulatory mechanisms of colorectal motility by the lumbosacral defecation center. For assessing colorectal motility, we have used in vivo recording system. In brief, animals were anesthetized and their distal colon and anus were cannulated to measure the intracolorectal pressure and propelled intraluminal liquid volume. We have demonstrated that intrathecal injection (L6-S1 region of the spinal cord) of ghrelin enhances colorectal motility through activation of the lumbosacral defecation center. In addition, we found that several amines acting on the spinal cord enhance colorectal motility. In this presentation, we will summarize roles of peptides and amines in the regulation of the colorectal motility via the spinal cord.

(COI: No)

IS-3

5-HT$_4$ receptor-mediated facilitation of neurogenesis of enteric neurons from transplanted brain-derived neural stem cells

Takaki, Miyako (*Dept Mol Pathol, Sch Med, Nara Med Univ, Kashihara, Japan*)

Two photon-excited fluorescence microscopy (2PM), can provide deeper optical penetration (several hundred μm) in in vivo preparations. We have used this approach in Thy1-promoter YFP mouse after gut transection and anastomosis. The fetal brain-derived neural stem cells (NSC) were transplanted from the tail vein after treatment with red fluorescent cell linker, PKH26. We obtained clear three-dimensional imaging of newborn enteric neurons generated from enteric neural progenitors (mobilized resident NSC; green fluorescence) and those from transplanted NSC (red fluorescence). Neurogenesis was promoted by oral application of a 5-HT$_4$-receptor agonist, mosapride citrate (MOS: 100 μM) and this promotion was inhibited by simultaneous application of a 5-HT$_4$- receptor antagonist, SB-207266 (50 μM), indicating 5-HT4 receptor-mediated facilitation of neurogenesis. Number of new neurons from the transplanted NSC was much smaller (approximately 10%) than that from the mobilized resident NSC, but the facilitating effect of MOS was similar between the transplanted and resident NSC. The distribution pattern of new neurons from the transplanted NSC was similar to that of new neurons from the resident NSC. After in vivo imaging, PGP9.5 (+) cells (neurons), and PKH26 (+) and YFP (+) cells were compared by confocal microscope. New enteric neurons overlapped with PKH26 (+) or YFP (+) cells in the deep tissue of mouse small intestine.

(COI: No)

The Winning Lectures of Encouragement Award of the JAA

(March 22, 16:00～17:30, Room A)

AS-1 Functional organization of the neural circuit in the inferior colliculus

Ito, Tetsufumi — Division of Anatomy and Neuroscience, Faculty of Medical Sciences, University of Fukui

AS-2 Molecular imaging analysis of small GTPases in the regulation of macropinocytosis and phagocytosis

Egami, Youhei — Department of Histology and Cell Biology, School of Medicine, Kagawa University

AS-3 Application of cadavars embalmed by the saturated salt solution method for surgical training

Hayashi, Shogo — Department of Anatomy, Tokyo Medical University

AS-4 Molecular and anatomical evidence for input pathway- and target cell type-dependent regulation of glutamatergic synapses

Yamazaki, Miwako — Department of Anatomy, Graduate School of Medicine, Hokkaido University

MD Scientist Training Program

(March 21, 15:30～18:00, Room A)

MD-S1

Oncogenic Notch1 activities in esophageal squamous cell carcinoma

Otsuka, Yuki[1,2]; Tanaka, Koji[2]; Whelan, Kelly[2]; Kinugasa, Hideaki[3]; Rhoades, Ben[2]; Ikeda, Fusao[3]; Yamamoto, Kazuhide[3]; Nakagawa, Hiroshi[2] (*[1]Med.Sch.Okayama Univ., Okayama, Japan; [2]GI division, Univ.of Pennsylvania, Philadelphia, USA; [3]Dept. Gastroenterol & Hepatol, Okayama Univ., Okayama, Japan.*)

Introduction: Notch signaling regulates stem cells and differentiation; however, its role in tumor biology remain elusive. We investigated the functional consequences of Notch activation in esophageal squamous cell carcinoma (ESCC).
Methods: Immunohistochemistry was performed using primary tumors from ESCC patients and a 4-Nitroquinoline 1-oxide-induced mouse model of ESCC as well as xenograft tumors. ICN1, the active form of Notch1, was ectopically expressed in ESCC cells (TE11) in a tetracycline-inducible fashion to determine how ICN1 affects sphere formation (self-renewal), anchorage-independent growth in soft agar, and tumor growth in immunodeficient mice.
Results: ICN1 was expressed at the invasive tumor fronts in primary ESCC and was associated with poor prognosis in patients. Immunohistochemistry suggested epithelial-mesenchymal transition in murine ESCC cells expressing ICN1. In TE11 cells, ICN1 stimulated sphere formation and increased colony size and number in soft agar. In xenograft tumors, ICN1 promoted tumor growth with increased cell proliferation and decreased squamous-cell differentiation.
Conclusions: Our data suggest that Notch1 may be activated in invasive ESCC cells with increased malignant properties. Notch signaling may have oncogenic activities in the pathogenesis of ESCC.

(COI: No)

MD-S2

Analysis of Arhgef2 phosphorylation at Ser885

Oda, Kaishu (*Nagoya Univ., Nagoya, Japan*)

abstractRhoA is one of the small GTP-binding proteins (small G proteins) which mediates F-actin polymerization and regulates cytoskeleton and cell adhesion. The small G proteins function as molecular switches, cycling between inactive GDP-bound state and active GTP-bound state. Arhgef2 (Lfc or GEF-H1) is a major Rho guanine exchange factor (RhoGEF), that activates RhoA by exchanging GDP for GTP. Depending on PKA, phosphorylation at Ser885 of Arhgef2 inactivates its GEF activity. In vitro investigation of the Ser885 phosphorylation has been conducted, however, the effect of such phosphorylation in vivo yet remains unknown. Assuming that phosphorylation of Arhgef2 at Ser885 alters cell morphology, we tried to produce Ala885 mutant Arhgef2 mouse with CRISPR/Cas9 system targeting Arhgef2 Ser885. We checked F0 mice and have detected knock-in allele in some mice. In the future, we will examine RhoA activity, cell morphology and behavior in knock-in mice in order to analyze the functions of Arhgef2 phosphorylation at Ser885.

(COI: No)

MD-S3

The remodeling of somatotopic area in intact hemisphere was improved by application of dehydroepiandrosterone after focal infarction in somatosensory cortex

Obi, Kisho; Takatsuru, Yuusuke; Amano, Izuki; Koibuchi, Noriyuki (*Dept. Integrative Physiol., Gunma Univ. Grad. Sch. Med, Gunma, Japan*)

The contralateral hemisphere of infarction play an important roles for functional compensation in somatosensory cortex (SSC) after focal ischemia (Takatsuru et al., J.Neurosci., 2009). Recently, it is reported that infarction of the unilateral SSC changes the somatotopic area in the intact contralateral SSC (Takatsuru et al., J.Neurosci., 2013). In this study, we examined the changes of somatotopic area in contralateral (left) SSC during recovery phase by using electrophysiological technique. Sensory responses from the right paw are innately process in the left SSC. When the right SSC is infarcted, sensory responses from left paw became also processed in the left SSC. However, the size of the receptive field which responded from right and left limb somatosensory stimulation in 4 weeks (wks) after stroke significantly increased compared with those in 2wks after the stroke. This change was improved by repeated application of dehydroepiandrosterone (DHEA), which is demonstrated to have a neuroprotective effects, during 2 to 3wks after stroke. We next aim to clarify the underlying mechanisms by using electrophysiological and molecular biological techniques. Previously reported that DHEA is metabolized to estradiol in a brain (Elkihel et al., Steroids., 2012). Thus, next we will measure the amount of estradiol in a brain by ELISA and determine the expression of estradiol receptor by Western blotting.

(COI: No)

MD-S4

Identities of FoxP2-positive retinal ganglion cells in the visual system of ferrets and mice

Sato, Chihiro[1,2,3]; Ebisu, Haruka[2,3]; Ichikawa, Yoshie[2,3]; Kawasaki, Hiroshi[2,3] (*[1]Fac Sch Med, Univ of Tokyo, Tokyo, Japan; [2]Dept Biophys Genet, Grad Sch Med Sci, Kanazawa Univ, Ishikawa, Japan; [3]Brain/Liver Interface Med Res Ctr, Kanazawa Univ, Ishikawa, Japan*)

The mammalian retina contains principally two subtypes of retinal ganglion cells (RGCs). Magnocellular RGCs (M-RGCs) send visual information about object motion to M cells in the LGN in the thalamus, and parvocellular RGCs (P-RGCs) transmit information about form discrimination and color recognition to the lateral geniculate nucleus (LGN) along by parallel visual pathways. These RGC subtypes and parallel pathways are believed to be a fundamental basis of visual recognition. However, because the RGC subtypes are not well-developed in mice, the molecular properties of these RGC subtypes are still unknown. Using the ferret, which has M cells and P cells, we recently uncovered that FoxP2 is selectively expressed in P cells in the LGN of ferrets and monkeys (Iwai et al., 2013). This previously study led us to hypothesize that FoxP2 is selectively expressed in P cells also in the retina. We examined FoxP2 expression patterns in the ferret retina and found that FoxP2 was expressed in a small subset of RGCs and amacrine cells. Our morphological analyses suggested that FoxP2-positive RGCs were X cells (P-RGCs in ferrets). Interestingly, as in the case of ferrets, Foxp2 was also expressed in a small subset of RGCs in mice. We are currently examining the role of Foxp2 in the development of RGC identities using mice. Our results should be helpful for understanding the molecular mechanisms underlying RGC development. COI:NO

(COI: No)

MD-S5

Identification of novel interacting partner for SHP-1

Sugimoto, Ayano; Fujita, Yuki; Kimura, Yuriko; Yamashita, Toshihide (*Dept.Mol. Neurosci.Grad.Sch.Med.Osaka Univ.Osaka, Japan*)

Once the adult central nervous system (CNS) is damaged, it is difficult to regenerate. Under the existence of myelin-derived inhibitors of axonal regeneration, it is known that paired immunoglobulin-like receptor B (PIR-B) binds to tropomyosin receptor kinase (Trk), which enhances regeneration of the injured CNS. Then, Src homology 2-containing protein tyrosine phosphatase (SHP) is recruited to PIR-B, and inhibits axonal regeneration by dephosphorylation and inactivation of Trk receptor. Furthermore, under the circumstances of reduced expression of SHP-1 using small interfering RNA (siRNA), axon regeneration is enhanced in mice optic nerve after injury. Hence, it is suggested that SHP-1 may be an effective molecular target for CNS injury. However, as SHP-1 is expressed ubiquitously, inhibition of SHP presumably causes severe adverse event. Thus, we aimed to clarify the molecular mechanism of inhibition of axonal regeneration by SHP, and identify appropriate therapeutic target molecule.We adopted a mass spectrometry (MS)-based approach to identify proteins that associate with SHP-1. Among the SHP-1-associated proteins, we focused on microtubule-associated protein 1B (MAP1B) and filamin A (FLNA). MAP1B is abundantly expressed in neurons, and regulates the organization of microtubules in neurites. FLNA is a cytoskeleton protein that crosslinks actin filaments. We examined the physiological functions of the interactions between SHP-1 and these proteins.

(COI: No)

MD-S6

A novel protein in the central pair apparatus of *Chlamydomonas* flagella

Tani, Yuma; Yagi, Toshiki; Oda, Toshiyuki; Kikkawa, Masahide (*Grad. Sch. Med., The Univ. of Tokyo, Tokyo, Japan*)

Eukaryotic cilia and flagella are highly intricate cell organelle that plays important roles in various organs as propellers or sensors. Therefore, impaired cilia/flagella in human causes various diseases, which are called ciliopathies. Motile cilia/flagella share a well-conserved "9+2" structure, which consists of nine doublet and two central pair microtubules. In this structure, the central pair apparatus is considered as a mechanical regulator, which turns on or off the activity of dynein arms on the doublet microtubules via radial spokes. However, the functions or components of central pair apparatus are not fully understood.

In this study, we identified a new flagellar-associated protein (FAP) as a component of central pair apparatus of *Chlamydomonas reinhardtii* flagellar axoneme. From an analysis of FAP-deficient mutants, we showed that the deficiency of FAP in flagellar axonemes reduces the flagellar motility and affects the bridge structure between the two microtubules in the central pair apparatus. These results suggest that FAP is a component of C1-C2 bridge structure in *Chlamydomonas* central pair apparatus and the structural defect by its deficiency affects the flagellar motility.

(COI: No)

MD-S7

Systems pharmacology of teratogenic action by valproic acid

Murakami, Soichiro[1]; Nishimura, Yuhei[1,2,3,4,5]; Sasagawa, Shota[1]; Ashikawa, Yoshihumi[1]; Kawabata, Miko[1]; Cho, Beibei[1]; Umemoto, Noriko[1,2,3,4,5]; Tanaka, Toshio[1,2,3,4,5] ([1]*Grad. Sch. of Med, Mie Univ, Japan;* [2]*Systems Pharmacology, Grad. Sch. of Med, Mie Univ, Japan;* [3]*Medical Zebrafish Research Center, Mie Univ, Japan;* [4]*Omics Medicine, Industrial Technology Innovation Institute, Mie Univ, Japan;* [5]*Bioinfo, Life Sci. Res. Center, Mie Univ, Japan*)

It is well known that valproic acid (VPA) has a histone deacetylase inhibition activity and exposure to the chemical during developmental period causes fetal valproate syndrome. However, the detailed mechanism has been unknown. To elucidate the molecular mechanism, we exposed VPA to fertilized eggs of zebrafish and analyzed the gene expression variations comprehensively using DNA microarray. Besides, we compared the transcriptome data in Gene Expression Omnibus data repository. The microarray data included the gene expression variations in embryos of zebrafish or mouse, and mouse embryonic stem cell exposed to VPA. As a result, we discovered that the expression of epc2 was significantly decreased by VPA exposure. EPC2 is related to 2q23.1 microdeletion syndrome characterized by psychomotor retardation, seizure, and stereotypic repetitive behavior. Then, we knocked out epc2 gene in zebrafish using TALEN to analyze the functional role of epc2 downregulation by VPA exposure. We were able to demonstrate that the expression of several genes were similarly dysregulated between zebrafish exposed to VPA and epc2-KO zebrafish, suggesting that epc2 may be involved at least partly in the neurotoxicity of VPA.

(COI: No)

MD-S8

A novel role of Regnase-1 in the iron homeostasis and anemia

Yoshinaga, Masanori; Takeuchi, Osamu (*Inst. Virus Res., Kyoto Univ., Kyoto, Japan*)

The coordinate regulation of iron homeostasis is largely dependent on the post-transcriptional control. However, the players and mechanisms of post-transcriptonal regulation in the iron homeostasis have not been fully understood. Our group previously found a ribonuclease, named Regnase-1, which can destabilize a set of mRNAs of pro-inflammatory cytokines, including *Il-6* and *Il-12p40*. In this study, we investigated the role of Regnase-1 in the control of iron homeostasis and anemia through the analysis of the Regnase-1-deficient mice. We found that the Regnase-1-deficient mice showed severe iron deficiency and anemia, which is partly rescued by the intraperitoneal iron supplementation. High levels of leukocytes, but not iron deficiency, observed in Regnase-1-deficient mice was rescued by the lack of lymphocytes. These findings suggest that Regnase-1 is critical for dietary iron absorption, and that this function is largely independent of the control of inflammation. To identify Regnase-1 target mRNAs responsible for the iron uptake, we conducted the transcriptome analysis in duodenum, where iron uptake takes place, and found that several iron-controlling genes, including Egln3, were up-regulated under Regnase-1 deficiency. The overexpression of Regnase-1 accelerated the decay of the Egln3 mRNA via its 3′ untranslated region. Furthermore, the administration of an Egln3 inhibitor rescued the iron deficiency in the Regnase-1-deficient mice. Taken together, these results indicate that Regnase-1 prevents the development of anemia, by regulating the duodenal iron uptake.

(COI: No)