WILEY Microcirculation

ABSTRACT

ABSTRACTS FROM THE 43RD ANNUAL MEETING OF JAPANESE SOCIETY FOR MICROCIRCULATION JUNE 8TH – 9TH, 2018 HOKKAIDO, JAPAN

PRESIDENT'S LECTURE

PL | The importance of the development of methods to measure ocular circulation

A. Yoshida

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In this address, I am going to present my 38 years of research. We contributed to the development of two-point fluorophotometry and developed the Laser Doppler flowmeter, as well as the Doppler OCT flowmeter. I will also demonstrate how we have contributed to the international community through our experience and expertise in measuring ocular circulation.

SPECIAL LECTURES

SL-1 | Choroidal circulation changes associated with choroidal thickening

S. Ishida

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There are distinctly different types of abnormalities showing choroidal thickening with serous retinal detachment: Vogt-Koyanagi-Harada disease (VKH) as an inflammatory disease and central serous chorioretinopathy (CSC) as a non-inflammatory disorder, known as stemming from sympathetic (adrenergic) etiology or systemic corticosteroid use. On indocyanine green angiography, both clinical entities show choroidal vascular dilatation and hyperpermeability, leading presumably to choroidal thickening. However, we have recently reported laser speckle flowgraphy (LSFG) can distinguish these two disorders in regard to choroidal circulation changes. On LSFG, VKH and CSC showed opposite direction of changes in blood flow velocity, i.e., sequential increase and decrease, respectively, with regression of disease activity. These results may be reflected by the different pathogenesis of the two diseases. VKH at the acute stage has an inflammatory circulation disturbance in the thickened choroid with leukocyte adhesion to inner walls of vessels and massive infiltration into the stroma. In contrast, CSC at the acute stage has an increased hydrostatic pressure in the thickened choroid hyperperfused due to sympathetic vascular dysregulation. We have so far confirmed similar trends in several other clinical entities, such as serpiginous choroiditis and punctate inner choroidopathy as inflammatory diseases and hypertensive choroidopathy with non-inflammatory etiology. In this presentation, choroidal circulatory changes in inflammatory and non-inflammatory diseases with choroidal thickening will be highlighted and contrasted using LSFG.

SL-2 | Treatments for age-related macular degeneration: Current status and future perspective

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Age-related macular degeneration (AMD), a blinding disease that affects the center of the retina in the elderly, is the leading cause of legal blindness in developed countries. The number of AMD patients has increased exponentially in Japan, due to the aging of its society. Compared to Western countries, the proportion of exudative AMD is high in Asian countries including Japan. Exudative AMD is characterized by choroidal neovascularization (CNV). Recent studies disclosed the importance of chronic inflammation occurring at the retinal pigment epithelium (RPE) and Bruch membrane (RPE basement membrane) in the pathogenesis of AMD. Specifically, our studies, together with others, clarified that age-related accumulation of lipofuscin, an aging pigment, occurs in the RPE due to the overloading of debris derived from shed photoreceptor outer segments. Lipofuscin accumulation, together with hypoxia, induces RPE dysfunction and augments drusen deposits, a hallmark of early AMD, which thereby triggers chronic inflammation and CNV.

In this lecture, we will summarize the most important findings from our research and discuss the importance of inflammatory cells in the pathogenesis of AMD. We will also discuss our attempt to develop new treatments for this condition.

SL-3 | Vascular and lymphatic systems in health and disease

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Vascular and lymphatic systems are two major circulatory systems properly distributed throughout the body. While the former provides tissues oxygen and nutrients to sustain cellular metabolism and homeostasis, the later drains the interstitial fluid from the tissue spaces to return to the bloodstream. During development, both circulatory systems are properly distributed with an appropriate amount and patterning customized to the function of each organ. In this process, not only cell intrinsic properties of vascular/lymphatic endothelial cells but diverse interactions between those cells and other cell types contribute to the establishment of such tissue-specific vascular/lymphatic patternings. Here I discuss the knowledge about the cellular and molecular mechanisms of angiogenesis and lymphangiogenesis gained in our recent research mainly using genetically modified mouse lines. Disturbance in these developmental mechanisms may underlie the pathogenesis of vascular diseases such as cancers and ocular neovascular diseases.

SYMPOSIUM

SY1-1 | Understanding of human pluripotent stem cell metabolism for cardiac regenerative medicine

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Cardiac regenerative therapy using human induced pluripotent stem cells (hiPSCs) is a potentially promising strategy for patients with heart disease, whereas the inability to eliminate residual hiPSCs and generate a massive amount of pure cardiomyocytes has been a barrier for realizing this potential. Recently, we established a novel method for purifying hiPSC-derived cardiomyocytes by focusing on glucose, glutamine and lactate metabolism in hiPSCs and differentiated cardiomyocytes (Tohyama S, et al. Cell Stem Cell 2013, Cell Metab. 2016, Circ. Res. 2017). However, there are no efficient two-dimensional culture systems to obtain a large amount of pure cardiomyocytes. Therefore, we developed an advanced two-dimensional culture system using multilayer culture plates with active gas ventilation (AGV) that yielded a large number of hiPSCs and pure cardiomyocytes (Tohyama S, et al. Stem Cell Reports 2017). Approximately 6 × 10E8 cells (four-layer) and 1.5 × 10E9 cells (10-layer) were obtained with AGV. After metabolic purification with glucose- and glutamine-depleted and lactate-supplemented media, a large amount of pure cardiomyocytes was finally prepared. We also succeeded to develop a method to obtain mass producing homogeneous cardiac spheroids consisting of pure cardiomyocytes for transplantation (in revision). These advanced technologies will facilitate the clinical application of hiPSC-derived cardiomyocytes.

SY1-2 | Methionine metabolism regulates directional biotransformation of glucose via protein arginine methylation

T. Yamamoto

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Carbon monoxide (CO) is a multifunctional gaseous signaling molecule to regulate smooth muscle tonus, neurotransmission, intracellular metabolism, cell cycle, apoptosis, and proliferation/hypertrophy. Stress-inducible heme oxygenase-1 (HO-1)/CO system protects cells and tissues against oxidative stress, while the gas-responsive direct signaling mechanisms for the protection remain unknown. Since macromolecules possessing metal-centered prosthetic groups such as enzymes in metabolic enzymes might serve as targets for covalent binding of molecular oxygen or CO, we have recently attempted to mine the gas-responsive enzymes through reading out alterations in metabolites using metabolome analyses based on capillary electrophoresis assisted by mass spectrometry (CE-MS) in varied experimental models where intracellular O2 or CO level is largely altered. Metabolomics screening using several human cell lines allowed us to show that CO-sensitive methylation of PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3), a regulatory enzyme producing fructose 2,6-bisphosphate (F-2,6-BP), serves as an allosteric activator for phosphofructokinase-1, which is a rate-limiting glycolytic enzyme. In these cells, PFKFB3 is constitutively arginine di-methylation at R131 and R134 under support of protein arginine methyltransferase-1 (PRMT1). Either induction of HO-1 or exposure to CO reduces the methylation level of PFKFB3 in varied cancer cells to suppress F-2,6-BP, limiting glycolytic flux and shifting glucose utilization toward pentose phosphate pathway. The regulation of PFKFB3 methylation status depends on inhibitory effects of CO for the heme-containing enzyme, cystathionine β-synthase (CBS) that modulates remethylation metabolism. Once demethylated, PFKFB3 undergoes polyubiquitination to be degraded in proteasomes. Therefore, loss of PFKFB3 methylation increases PPP flux for NADPH production and reduced form of glutathione to ensure resistance against oxidative stress for cancer cell survival. These results suggest that the methylation status of PFKFB3, which is

regulated by CO/CBS-responsive pathway is determinant triggering directional glucose utilization for supporting anti-oxidative capacity in cancer cells.

SY1-3 | Temporal dynamics of brain cells in cerebral development and ischemic injury

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Brain cells, including neurons, astrocytes, microglia, and circulating blood cells, reveal various characteristics in cerebral development and ischemic injury. Differentiation of neurons and astrocytes from neural stem cells is accurately regulated by essential transcription factors in brain development. Microglia differentiated from yolk-sac-derived macrophages is also implicated in cerebral development and maintains brain homeostasis. In the pathological condition, not only brain cells but also circulating immune cells triggers inflammation which promotes neuronal cell death, while these cells turn into neuroprotective and repairing phenotype in the recovery process after cerebral tissue injury.

We identified miRNA-153 (miR-153) as a modulator of the temporal regulation of NSPC differentiation. Overexpression of miR-153 delayed the onset of astrogliogenesis and maintained NSPCs in an undifferentiated state in vitro and in the developing cortex. The transcription factors nuclear factor I (NFI) A and B, essential regulators of the initiation of gliogenesis, were found to be targets of miR-153. These results indicate that miR-mediated fine control of NFIA/B expression is important in the molecular networks that regulate the acquisition of gliogenic competence by NSPCs in the developing CNS.

Neuronal cell death in brain tissue injury results in the release of damage-associated molecular patterns (DAMPs) that trigger the sterile inflammation. HMGB1 and peroxiredoxin family proteins are major DAMPs in ischemic brain injury. In the recovery phase of ischemic stroke, these DAMPs were internalized by microglia and infiltrating macrophages through MSR1, a scavenger receptor, for lysosomal degradation. We identified the transcription factor MAFB is a pivotal factor for the MSR1 expression in repairing macrophages and the resolution of cerebral post-ischemic inflammation.

These results indicate that temporal dynamics of brain cells is important in both brain development and regulation of inflammation after brain injury. Transcriptional regulation is thus a key for the maintenance of cerebral homeostasis.

SY1-4 | Glioma stem cells – metabolic characteristics and adaptation to hypoxic and nutrient challenges

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High grade malignant gliomas can be distinguished from the normal brain by several metabolic characteristics, such as an increased uptake of glucose and methionine. When oxygen and nutrients become scarce, rapidly dividing progeny at the center of the tumor cannot fulfil their metabolic requirements and undergo cell death. However, recent studies show that in several types of solid tumors, a select type of cell, the cancer stem cell, is characterized by a high ability to adapt to various forms of stress, including metabolic stress.

Here we present our findings from an induced-cancer stem cell model of glioma, based on orthotopic implantation of murine Ink4a/Arf$^{-/-}$ neural stem cells overexpressing HRasV12 into the brains of syngeneic mice.

Our results show that glioma stem cells (GSCs) have an increased energetic status when compared to their non-transformed counterparts, the neural stem cells. However, GSCs can use different fuels, including glutamine and lactate, and different energy pathways. They can also vary in their ability to adapt to changes in the environment, especially changes in oxygen and glucose. Of note, GSCs can also undergo metabolic changes as a result of therapeutic stress, such as a decrease in glycolysis after fractionated radiation. All of these patterns contribute to their survival and thereby to increased resistance to treatment.

Finally, we will discuss the implications of GSC metabolism in the biology of malignant gliomas and in GSC-targeted treatments.

SY2-1 | The effect of intravitreal anti-VEGF injections on systemic circulation

M. Sugimoto

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Molecular biological studies have shown that vascular endothelial growth factor (VEGF) is involved in the onset and progression of diabetic macular edema (DME), and clinical studies have shown that anti-VEGF therapy can resolve the DME. Nowadays, anti-VEGF therapy has become the first line therapy for DME because it improves the visual function and the effect is maintained for long periods. Although anti-VEGF agents are widely used, previous studies also reported that their use may also lead to vascular damage such as arterial thromboembolism and re-occlusion of new vessels. It is still unclear whether intravitreal injections of anti-VEGF agents can cause systemic side-effects.

To have systemic affects, the anti-VEGF agents must pass from the vitreous into the systemic circulation at high enough concentrations to be effective. To determine whether a unilateral intravitreal injection of ranibizumab, one of the major anti-VEGF agents, will affect the ocular circulation of the fellow eye, we measured the ocular circulation in the injected and fellow eye. The blood circulation on the optic nerve head of the treated and untreated eyes were determined by laser speckle flowgraphy (Softcare Co., Ltd) before, 1 day, and 1 week after the treatment. Our result revealed that the concentration is not high enough to affect the ocular circulation of the fellow eyes.

In addition, many individual molecules have been associated with a risk of systemic vascular infarctions or significant clinical effects. We hypothesized that vascular infarction-related molecules (VIRMs) and coagulation-related molecules (CoRMs) at downstream and other pathways of VEGF can be affected by anti-VEGF therapy. We also evaluate the expression of the VIRMs/CoRMs after intravitreal injections of anti-VEGF agents and no significant changes were seen for other VIRMs/CoRMs in the plasma and aqueous.

Both of our results confirm the safety of anti-VEGF therapy for DME.

SY2-2 | Ocular circulation in ocular tumor-related situation

S. Kase

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In this symposium, I would like to talk about ocular circulation in two ocular tumor-related situations: choroidal circulation following external beam radiation therapy in ocular adnexal lymphoma, and microvascular circulation in melanocytoma of the optic disc. In the former, mucosa-associated lymphoid tissue (MALT) lymphoma is a major malignancy among ocular adnexal lymphomas. Low-dose radiation therapy has contributed to control of the tumor and suppression of tumor recurrence. Moreover, the radiotherapy usually did not induce severe radiation-related phenomena such as vision loss caused by retinopathy or optic neuropathy. However, little is known about the influence of low-dose radiation to intraocular circulation. In this study, choroidal circulation and thickness were evaluated using laser-speckle flowgraphy (LSFG) and enhanced depth imaging optical coherence tomography (EDI-OCT). LSFG is a non-invasive and useful way to evaluate intraocular circulation. Mean blur rate (MBR) in the macula determined by LSFG reflects on relative values of choroidal circulation. Subfoveal choroidal thickness was determined using EDI-OCT which facilitates the elucidation of quantitative changes of the thickness. All patients examined in this study were histologically diagnosed with MALT lymphoma of the ocular adnexa. After informed consent was obtained, all patients received photon beam or electron beam radiation with total dosage of 30 Gy. In fact, severe ocular complications were not observed after radiation in each patient. There were no significant changes in terms of choroidal circulation and the thickness in patients who did not have fundus abnormalities. However, choroidal thickness was reduced and MBR decreased after radiation therapy in a patient who had aberration in retinal pigment epithelium. These findings suggest that choroidal circulation will be involved following radiation in ocular adnexal lymphoma. Next, I am going to talk about melanocytoma of the optic disc (MOD). MOD is a benign intraocular tumor, presenting with melanocytic proliferation taking place on the optic nerve head. Clinically, MOD can cause visual field defect and blurred vision. So far, the situation of ocular circulation and the correlation with visual field defect are largely unknown. In this study, we have conducted fluorescein angiography, indocyanine green angiography and OCT angiography in 5 patients with MOD. In melanocytoma, OCT angiography revealed abnormal radial vascular networks in selected patients which might be consistent with hyper-refractive lesions observed in the tumor detected by B-scan OCT. We also suggest that visual field defects associated with MOD might result from circulation disorders due to tumor cell invasion to the optic disc tissues. In this symposium, I would like to provide current data on ocular circulation in adnexal lymphoma and MOD, and future issues that should be resolved.

SY2-3 | Novel evaluation of diabetic eyes by optical coherence tomography angiography

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Optical coherence tomography (OCT) angiography provides in vivo, three-dimensional vascular information by the use of running red blood cells as natural contrast agents, enabling the visualization of functional vessel networks within chorioretinal tissue non-invasively, without a need of dye injection.

Using OCT angiography, we have reported that the enlargement of foveal avascular zone (FAZ) were noted even in the diabetic eyes without retinopathy (Takase N, et al. Retina 2015). Other reports also confirmed that the enlargement of FAZ in the diabetic eyes (Choi W, et al. Retina 2017), and the FAZ size were correlated with the severity of diabetic retinopathy (Bhanushali D, et al. Invest Ophthalmol Vis Sci, 2016).

We hypothesized that longitudinal FAZ area analysis might predict worsening of diabetic retinopathy, we conducted retrospective study. During the 22 months of follow-up, the FAZ area of diabetic retinopathy eyes in the deep capillary plexus significantly enlarged (5.1% per year). No significant changes were observed during the study period for FAZ areas in the deep capillary plexus of controls and diabetic patients without retinopathy. And enlargement of FAZ in the deep capillary plexus was significantly greater in eyes with diabetic retinopathy progression versus those without progression. Our data suggested that FAZ enlargement in deep capillary plexus was correlated with diabetic retinopathy progression.

We also speculated that there might be correlation between peripheral retinal ischemia (ischemic index) and FAZ area and irregularity (acircularity index) in diabetic retinopathy. FAZ acircularity index was correlated with severity of diabetic retinopathy. And ischemic index was significantly associated with FAZ area and FAZ acircularity index. From our findings, OCT angiography is useful noninvasive tools to follow the FAZ area of patients with diabetes to detect retinopathy progression and peripheral ischemia.

SY2-4 | Evaluating retinal blood flow in eyes with healthy subjects and retinal diseases using doppler optical coherence tomography flowmeter

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Evaluating the ocular circulation is of paramount important for understanding physiologic and pathologic features in many retinal diseases. Though multiple devices are used to measure ocular blood flow such as the blue field entoptic technique, microsphere method, laser Doppler flowmeter, laser Doppler velocimetry (LDV), oxygen measurements, retinal vessel analyzer, color Doppler imaging and laser speckle technique, we have shown that LDV is a reliable, noninvasive, and clinically useful tool for evaluating the retinal circulation in humans based on the absolute values of vessel diameter and blood velocity. Considering these advantages, we have measured the retinal blood flow (RBF) in several retinal diseases such as diabetic retinopathy (DR) in type 1 and type 2 diabetes mellitus, branch retinal vein occlusion (BRVO), and age-related macular degeneration. However, the RBF measurements acquired by LDV have a number of disadvantages, i.e., the RBF measurements need a condition of the parabolic flow distribution to calculate the absolute values of blood velocity, fine alignment of the laser beam, and good eye fixations. Therefore, it is challenging to measure the RBF at the bifurcation or the margin of the optic nerve head because it is not assumed the parabolic flow distribution there.

In recent years, Doppler Optical Coherence Tomography (DOCT), a novel velocimetry technique using OCT, has been developed. OCT can detect not only retinal morphologic images but also a Doppler shift of reflected light, which provides the information of the RBF. We developed a DOCT instrument with novel software, referred to as a segmental-scanning method, which enables simultaneous measurement of the RBF in the retinal arterioles and the adjacent venules during one cardiac cycle. We previously reported the accuracy of the measurements in in vitro glass capillaries and the RBF of the anesthetized cats.

Recently, we revamped the DOCT instrument, namely DOCT flowmeter that can be used more in a clinical setting and examined the usefulness of DOCT flowmeter. Firstly, we confirmed the repeatability and reproducibility of RBF measurements in healthy subjects, and arteriosclerosis of healthy subjects was determined using DOCT flowmeter. Second, we evaluated the RBF of several eye diseases such as DR, Glaucoma and BRVO. Finally, abnormality of retinal arterial velocity profiles, a novel technique to image the velocity distribution of each red blood cell circulating in a blood vessel, in a case of Takayasu's arteritis with aortic regurgitation. Several pathological mechanisms of eye diseases could be elucidated by DOCT flowmeter.

YOUNG INVESTIGATORS AWARD SYMPOSIUM

Y-01 | Altered component of group 3 innate lymphoid cells in lymph from indomethacin-induced inflamed intestinal mucosa in rat

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Introduction: Innate lymphoid cells (ILC) play a role in the maintenance of intestinal homeostasis by contributing to the protective immune response toward intestinal pathogens. Among ILC, RORγt+ILC (ILC3) is the most abundant subset in the intestine and mesenteric lymph nodes (MLNs). Since no study about indomethacin (IND)-induced intestinal inflammation has been reported about ILC, we aimed to clarify how IND-induced intestinal inflammation would affect on each population of ILC.

Methods: To obtain lymph directly drained from intestinal mucosa, Wistar male rat (5 weeks) was mesenteric lymphadenectomized and thoracic duct was cannulated 4 weeks later. Some rats were administered IND (10 mg/kg, i.p.) just after cannulation. Lymphatic fluid was collected for a day. We depleted T cells by magnetic cell sorting system with Pan T Cell MicroBeads and stained the collection of T cell-depleted lymphatic fluid by IL-7Rα, T-bet, GATA-3 and RORγ. After gating population of lymphocytes and IL-7Rα-positive cells, we compared the expression of T-bet, GATA-3 and RORγ to examine how IND-induced inflammation affect the population of ILC1, ILC2 and ILC3.

Results: We collected ~106 cells/rat after depletion of T cells with or without IND. Among IL-7Rα+ lymphocytes, ILC3 were observed in 1.6% in steady state, whose 55% strongly expressed T-bet (T-bethighRORγ+ILC). IND administration increased percentage of ILC3 to 2.0%, but surprisingly T-bethighRORγ+ILC decreased to 5.1% (T-betlowRORγ+ILC increased contrarily). The populations of ILC1 (T-bet+RORγ-ILC) and ILC2 (GATA-3+ILC) were small in both steady and inflamed states compared with the population of ILC3.

Conclusions: This is the first study showing that ILC3 flow intestinal lymph from intestinal mucosa toward mesenteric lymph nodes. In

addition, IND administration increase total ILC3 drained from intestinal mucosa and alter the component of ILC3. Increased T-betlowICL3 might be involved in pathogenesis of IND-induced inflammation.

Y-02 | Endothelium-derived FLRT2 is selectively required for expansion of actively growing immature vessels in tumors

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Angiogenic factors represented by Vascular endothelial growth factor (VEGF) cause growth of blood vessels which deliver nutrients and oxygen to support the proliferation of tumor cells. Although VEGF inhibitors are currently used for the treatment of some types of human cancers, the resistance to those drugs caused by other angiogenic factors is the obstacle to sufficiently suppress tumor growth, suggesting the need for comprehensively uncovering other angiogenic pathways in tumors. Here, we found that an axon guidance molecule, FLRT2, is specifically expressed in endothelial cells of human colorectal cancers, particularly in advanced ones with poor prognosis. Endothelial-specific deletion of FLRT2 in mice selectively ablated actively growing immature vessels in tumors resulting in suppression of tumor growth and metastasis. In accordance with the concept of the tumor vessel normalization, we found a synergistic effect between FLRT2 deletion and administration of cytotoxic anti-tumor drugs. Mechanistically, this angiogenic effect of endothelium-derived FLRT2 is attributed to its homophilic adhesive action but not its heterophilic repulsive effects on the cognate receptor UNC5B. Taken together, our data indicate that endothelial FLRT2 could be a novel prognostic biomarker and a new anti-angiogenic target in human cancers.

Y-03 | Aberrant innate immune responses result in autistic-like features

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Autism spectrum disorder (ASD) refers to a complex developmental disorder that impairs social interaction, communication, and behavioral flexibility. Although ASD is highly heritable, its etiology is complex and involves multiple cellular and molecular mechanisms. Here we show that aberrant activation of microglia, cells responsible for innate immunity in the central nervous system, caused by conventional or myeloid-specific deletion of Leucine-rich repeat containing 33 (Lrrc33) in mice leads to severe ASD-like behavioral abnormalities. Mechanistically, massively secreted inflammatory cytokines due to constitutive activation of TLR3 in Lrrc33-deficient microglia persistently damage brain neurons. Moreover, genomic analysis of human samples found novel SNPs in the Lrrc33 gene specific to ASD patients. Knock-in mice of Lrrc33 recapitulating such mutations confirmed those significance in behavioral abnormalities. Taken together, our results suggest that innate immunity is highly associated with human ASD pathology and immune modulatory drugs could ameliorate a population of human ASD.

Y-04 | Perivascular passage and accumulation of amyloid β in vivo observed with multiphoton microscopy

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Background: Amyloid (Aβ), one of the causative proteins found in Alzheimer's Disease (AD), does not cross the blood-brain barrier besides transcytosis at the capillaries. Glymphatic pathway has been recently suggested as a possible lymphatic system in the brain and may be involved in the clearance of Aβ. To elucidate the pathomechanism of parenchymal Aβ accumulation, perivascular passage and accumulation of Aβ was analyzed in vivo.

Methods: Under isoflurane anesthesia, the skin covering left parietal skull of male mice was incised and a cranial window (3.5 mm in diameter) was opened with a dental drill. After removing the dura mater, HiLyte488-Amyloid 1-40 (Anaspec) or HiLyte488-Amyloid 1-42 (Anaspec) was topically applied on the cortical surface and the window was closed with a cover glass. Tie2-GFP mouse (Stock Tg[Tie2-GFP]287Sato/J) was introduced to visualize the vascular endothelial cells. Three dimensional imaging of the parietal cortex down to 400 μm was repeatedly conducted with multi-photon microscope (A1RMP+1080, Nikon).

Results: One-hour after the cortical application of Aβ, accumulated Aβ was observed along the penetrating vessels debranching from the pial vessels, as well as the small vessels in the deepest layer (400 μm). The Aβ accumulation was localized to the abluminal surface of endothelial cells. The penetrating arteries were involved more than veins.

Conclusion: Passage and accumulation of Aβ was repeatedly demonstrated along the penetrating arteries and capillaries in the deep cortical layer. Disturbance of the clearance system may contribute to the pathogenesis of AD.

Y-05 | The effectiveness of hydrogen inhalation in neonatal hypoxic-ischemic piglet model

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Background: Hypoxic-ischemic encephalopathy (HIE) of newborns results in unsatisfactory outcomes even when current standard treatment of mild hypothermia (HT) is given. Therefore, an adjunct

with HT might improve the clinical outcome. Among several therapeutic agents, we focused on hydrogen (H2) which is reported to have antioxidant, anti-inflammatory and anti-apoptotic properties.

Objective: To investigate the effectiveness of H2 inhalation with HT in neonatal hypoxic-ischemic (HI) piglets.

Methods: 23 piglets within 24 hours after birth are divided into; HI insult with normothermia (HI-NT, n = 8), HI with HT (HI-HT, n = 7) and HI with HT with H2 (HI-HT+H2, n = 6). HI insult was performed by reducing O2 to 3%–5% for approximately 40 mins and resuscitated with 100% O2. HT was performed for 24 hours after resuscitation. For HI-HT+H2 group, 3.8% H2 was used. Inhalation was continued for 24 hours. After weaning from mechanical ventilation, piglets were nursed in incubator. Neurological evaluation was assessed 4 times a day for 5 days. Motor function such as ability to walk, maintenance of posture and ability to control fore and hind limbs were observed. On day 5 post insult, piglets were euthanized under heavy anesthesia, brains perfused and fixed, stained with H&E and GFAP. Motor cortex was identified, cortical gray matter and subcortical white matter were studied and scored. For severity of HI insult, duration of low amplitude EEG (LAEEG) after insult was evaluated and compared among three groups.

Results: In evaluation of the severity of insult, LAEEG values showed no statistical significance. In neurological function, HI-HT+H2 group showed faster recovery. Also, more piglets in the same group were able to walk starting from day 3 post insult. In histopathology, score in motor cortex was the lowest in H2 inhalation group.

Conclusion: H2 inhalation with HT showed faster improvement and improved histopathological picture in our neonatal HI piglet.

FREE COMMUNICATIONS

F-01 | Blockade of lymphocyte entrance to Peyer's patches by inhibition of sphingosine-1-phosphate lyase ameliorates DSS-induced colitis

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Aim: Lymphocyte migration can be a therapeutic target for IBD by modulating gut immunity. 2-acetyl-4-tetrahydroxybutyl imidazole (THI) works as sphingosine-1-phosphate (S1P) lyase (SPL) inhibitor and has immunomodulating property, but the exact mechanism is still to be clarified. S1P receptor 1 (S1P1) expresses on lymphocytes and S1P agonist like FTY720 (FTY) has ameliorating effects on intestinal inflammation. Meanwhile S1P1 expresses on the endothelium of high endothelial venules (HEVs), suggesting that THI works on them also. We aimed to clarify the ameliorating effect and functional mechanism of THI for experimental colitis model from the aspect of lymphocyte migration.

Methods: Study 1: Mice were treated with 3% dextran sulfate sodium (DSS) and some mice were pretreated with THI (50 mg/L) or FTY (1 mg/kg). We investigated clinical score, histological damage and inflammatory gene expressions. Study 2: Rats were used to observe lymphocyte movement in Peyer's patches (PPs). THI was administered or FTY was gavaged before lymphocyte injection. Fluorescently labeled lymphocytes from intestinal lymph were injected into recipient rat. The microvasculature of PPs was continuously recorded in a manner of time-lapse photography to investigate the effect of THI on lymphocyte migration in PPs by a confocal laser scanning microscope.

Results: Study 1: THI induced peripheral lymphopenia. THI and FTY ameliorated the clinical score and histological damage significantly and reduced mucosa-infiltrating lymphocyte count. Although DSS increased expression of pathogenic mediators, THI and FTY suppressed them. Moreover, THI showed stronger ameliorating effects on colitis than FTY. Study 2: Lymphocytes moved from HEVs to stroma and then migrated to lymph capillaries under physiological condition. THI and FTY suppressed lymphocyte egression from HEVs, but only THI suppressed lymphocyte migration in stroma.

Conclusion: Intravital observation revealed that the main action site of S1P was HEVs and blockade of lymphocyte entrance to PPs might be one of the mechanisms how THI ameliorated DSS-induced colitis model.

F-02 | Intraoperative observation of the blood and lymphatic flow -First volume: Development and Introduction of the bright field color fluorescence cameras-

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In the past scientific meetings of the Japanese Society for Microcirculation, we reported on the in vivo observation of gastric microcirculation in rats. Our aim was intraoperative observation in the clinical practice. In the last 10 years, we were involved in the development of the bright field full color fluorescence camera (HyperEye Medical System: HEMS) for indocyanine green (ICG) and private importation of the bright field full color fluorescence laparoscope (PINPOINT) for ICG before the Japanese pharmaceutical approval.

HEMS is equipped with charge coupled device (CCD) image sensor covered with 4 filters i.e. red, blue, green and infrared and forms one image. PINPOINT has 2 images i.e. full color image and fluorescence monochrome image. Synchronised overlay technology enable to form a full color image with fluorescence.

This study was approved by the Ethics Committee for Biomedical Research of the International University of Health and Welfare (IUHW) Hospital and IUHW Mita Hospital. In the cases of rectal cancer, blood perfusion of the colorectal anastomosis could be observed after intra venous injection of 1 mg ICG. In the operation of the esophageal cancer, intra left gastric artery injection of 1 mg ICG may show the congestion area of the stomach before creating gastric tube after esophagectomy. Submucosal injection of 50 μg/mL ICG on the day before operation may be adequate administration for detecting the sentinel nodes in the early gastric cancer operation. Thirty-four cases with gastric cancer were enrolled for HEMS and 33 cases with gastric cancer were enrolled for PINPOINT. In total, metastasis was observed in 6 cases, and all of them had the metastasis positive sentinel lymph nodes.

Present study shows the potential of the ICG fluorescence guided surgery for the digestive organs.

F-03 | Intraoperative observation of the blood flow -Second volume: Evaluation in emergent surgery using the bright field/color fluorescence laparoscope system-

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Objective: In emergent operation, it is sometime difficult for surgeons to determine whether resection of the intestine should be performed or not. Especially in the incarcerated hernia operation, the resection of the intestine is a risk of mesh infection after the surgery. In the case of the resection, range of the resection is not always easy to be determined. We have investigated Indocyanine green (ICG) fluorescence for intestinal blood flow (IBF) in emergency abdominal surgery.

Method: We performed 4 cases of emergent abdominal surgery with ICG fluorescence using PINPOINT(Novadaq, Mississauga, ON, Canada), a brightfield full-color near-infrared fluorescence camera. ICG was injected to peripheral vein. The operative diagnoses of the 4 patients were incarcerated inguinal hernia, incarcerated femoral hernia, incarcerated umbilical hernia and superior mesenteric artery occlusion (SMAO).

Result: In incarcerated hernia patients, laparotomy was performed to reduce bowel incarceration. Bowel that had been incarcerated showed deep-red discoloration on gross evaluation. But intravenous injection of ICG revealed uniform fluorescence of the mesentery and bowel wall. This indicated an absence of irreversible ischemic changes to the bowel, so resection was not performed.

In case 4, the small intestine showed a brown discoloration on gross evaluation. Decision of resection range was difficult. But intravenous injection of ICG revealed demarcation line of fluorescence. The part of bowel without ICG fluorescence was resected. The post-operative course was uneventful.

Conclusion: Fluorescence was seen in the arteries of the mesentery, followed by uniform fluorescence throughout the bowel wall. ICG fluorescence may enable to observe intestinal blood perfusion including microcirculation. And ICG fluorescence may be useful for evaluating IBF in emergency surgery.

F-04 | Nailfold capillary of rheumatoid arthritis in Tokachi area

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Numerization of nailfold capillary images has been advanced focusing on scleroderma and has been applied to evaluate the disease condition. However, there are not many reports on rheumatoid arthritis because there are many nonspecific findings of capillary images. Therefore, in this study, we searched 27 cases of rheumatoid arthritis in the Tokachi district of Hokkaido for the correlation between numerical values using scleroderma score and capillary image findings and clinical information including inflammatory findings.

Methods: We adopted six parameters proposed by Cutolo et al. and for 27 cases of rheumatoid arthritis were digitized in 4 stages. Six parameters are Capillary number, Irregularly enlarged capillaries, Giant capillaries, Microhemorrhages, Capillary ramifications, Capillary disorganisation. We examined the correlation between these numerical values and DAS28 score, CRP, ESR, disease duration, age, image findings often found in rheumatoid arthritis, such as capillary prolongation, high flow velocity and capillary depletion.

Results: There was no correlation between each score of scleroderma and DAS28 score. On the other hand, when there was a giant capillary of 50 μm in diameter, the number of bleeding was tripled, the CRP was 4 times, and the capillary prolongation group had CRP 3 times and ESR 2 times. The blood vessel of 50 μm was doubled when the capillary flow velocity was high. In cases where disease duration was 10 years or more, there were many giant vessels and bleeding tended to be more frequent. In addition, in the methotrexate(MTX) administration group, the blood vessel of 50 μm and the frequency of bleeding tended to decrease.

Conclusion: Observation of nailfold capillaries in patients with rheumatoid arthritis was considered useful for inflammatory conditions and treatment evaluation.

F-05 | Correlation between indices of facial skin blood flow measured using LSFG and skin properties

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Objective: The relationship between skin blood flow and skin properties was investigated using indices of blood flow waveform, which is the measured Mean Blur Rate (MBR), a blood flow index in Laser Speckle Flowgraphy (LSFG), normalized with one heartbeat.

Methods: The subjects were 40 healthy females in their 20s-30s (30.1 ± 2.0 years old). Under hygrothermal conditions at 24.0℃ and 50.0% RH, skin blood flow of the entire face with the eyes closed was measured by LSFG in a semi-supine position. Regarding the skin physical properties, skin viscoelasticity of the right cheek was measured using the suction chamber method and the stratum corneum hydration was measured using the capacitance method under hygrothermal conditions at 20.0℃ and 40.0% RH. The relationship between indices of blood flow waveform in LSFG, Falling rate (index of time variation of decreasing blood flow rate) and Fluctuation (index of blood flow variation) and the skin properties were investigated.

Results: A significant inverse correlation was noted between an index of skin elasticity, Ur/Uf, and the Falling rate. A significant inverse correlation was also noted between the stratum corneum hydration and the Fluctuation. These findings suggest that sufficient skin blood flow with less fluctuation may contribute to maintain skin elasticity and stratum corneum hydration.

Conclusions: It is concluded that LSFG is useful for measurement of facial skin blood flow and to clarify the relationship between the skin blood flow and skin properties. Its application in the medical and health care fields is strongly expected.

F-06 | Predictability of diabetic retinopathy progression by the assessment of foveal avascular zone using optical coherence tomography angiography

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Purpose: The foveal avascular zone (FAZ) has been used as an indicator of the perifoveal microcirculation, with some previous Fluorescein angiography (FA)-based studies showing that enlargement of the FAZ in diabetic retinopathy (DR) patients was associated with the progression of DR. Optical Coherence Tomography Angiography (OCTA) is a new method that enables to image retinal microcirculation without dye injection. The purpose of this study was to evaluate the long-term change of the FAZ area in DR eyes and the relation between enlargement of the FAZ and the progression of DR by means of OCTA.

Methods: Retrospective chart review of patients who had undergone OCT angiography fundus examinations with at least 12 months of follow-up. Eyes with previous laser photocoagulation and anti-vascular endothelial growth factor treatments were excluded. OCTA images were obtained by the Avanti RTVue XR with AngioVue (Optovue, Inc., Fremont, CA). ImageJ software was used to evaluate the FAZ area in the superficial capillary plexus (SCP) and deep capillary plexus (DCP).

Results: During the 22 months of follow-up, the FAZ area of 40 eyes with DR in the DCP enlarged from 0.64 ± 0.20 mm^2 to 0.70 ± 0.20 mm^2 (P = 0.021), which was a 10.1% increase from baseline (5.1% per year). No significant changes were observed during the study period for FAZ areas in the DCP of controls and diabetic patients without DR. Enlargement of FAZ in the DCP was significantly greater in eyes with DR progression versus those without progression (19.2% and 1.2%, respectively, P = 0.013).

Conclusions: Our data suggested that FAZ enlargement in DCP was correlated with DR progression. Assessment of FAZ by OCT angiography might be useful for predicting DR progression.

F-07 | Three-dimensional analysis of choroidal vessels in eyes with vogt-koyanagi-harada disease before and after treatment

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Purpose: To evaluate choroidal vessel densities by binarizing wide-field indocyanine green angiography (WFICGA) images and swept-source optical coherence tomography (SS-OCT) images before and after treatment in eyes with Vogt-Koyanagi-Harada (VKH) disease.

Design: Retrospective, observational, consecutive case series.

Methods: Seven eyes of 7 patients (2 men, 5 women; mean age, 48.3 years) with VKH disease and 20 control eyes of 20 patients (13 men, 7 women; mean age, 47.6 years) who visited Nagoya City University Hospital from August 1st, 2015 through July 31st, 2017 were enrolled. Simultaneous wide-field fluorescein angiography (WFFA) and WFICGA (Optos California, Optos plc, Dunfermline, Scotland, UK) were performed in all patients. Then, WFFA images were subtracted from WFICGA images in Image J software to evaluate the choroidal vessel densities. SS-OCT (Triton, Topcon, Tokyo, Japan) images at the acute phase and after the treatment also were binarized to evaluate the vertical analysis of the choroid.

Results: The mean choroidal vascular densities in both posterior and mid-peripheral areas in eyes with VKH disease were significantly (P < 0.01) lower than those in control eyes, and recovered after the treatment. The binarization of SS-OCT images showed both the

choroidal stroma and lumina increased at the acute phase and significantly ($P < 0.05$) decreased after the treatment.

Conclusions: Binarization of WFICGA and SS-OCT images enables three-dimensional analysis of choroidal vessels. These results suggest that high vascular resistance due to diffuse infiltration into the choroidal stroma might compress choroidal vessels and the change would resolve after the treatment.

F-08 | Effects of edaravone on NO production, OH− metabolism and nNOS activity during cerebral ischemia and reperfusion in mice

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Objective: We investigated the effects of edaravone on the nitric oxide (NO) production, hydroxyl radical (OH−) metabolism and the activity of nNOS during cerebral ischemia and reperfusion.

Methods: C57BL/6 mice (n = 13) were used. Edaravone 3 mg/kg was given by intravenous injection in 9 mice just before reperfusion, and others were control groups. Both NO production and OH− metabolism were continuously monitored by in vivo microdialysis. Microdialysis probes were inserted into the bilateral striatum. The in vivo salicylate trapping method was applied for monitoring OH− formation via 2,3 dihydroxybenzoic acid (DHBA), and 2,5-DHBA. A Laser doppler probe was placed on the skull surface. Forebrain cerebral ischemia was produced by occlusion of both common carotid arteries for 10 minutes. Levels of NO metabolites, nitrite (NO_2−) and nitrate (NO_3−), in the dialysate were determined using the Griess reaction. Brain sections were immunostained with an anti-nNOS antibody. To determine the fractional area density of nNOS-immunoreactive pixels to total pixels of the whole field, the captured images were analyzed. Mann-Whitney U test was used for group comparisons.

Results: Blood pressure: There were no significant differences between the groups. Cerebral blood flow (CBF): There were no significant differences between the groups. NO_2− : Edaravone group (96.1 ± 37.3%; mean ± SD) showed significantly lower than that of the control group (127.1 ± 17.2) after reperfusion ($P < 0.05$). NO_3−: Edaravone group (173.8 ± 29.2) showed significantly higher than that of the control group (130.4 ± 16.0) after reperfusion ($P < 0.05$). 2,3-DHBA: Edaravone group (95.0 ± 4.8) showed significantly lower than that of the control group (108.7 ± 7.2) after reperfusion ($P < 0.01$). nNOS activity : Edaravone group (1.2 ± 0.2) showed significantly lower than that of the control group (1.7 ± 0.3) during ischemia ($P < 0.01$). Conclusion: These in vivo data suggest that edaravone effects not only on NO and OH− metabolites but also nNOS activity during cerebral ischemia and reperfusion.

F-09 | YangXue QingNao Wan, a compound Chinese medicine, attenuate cognitive impairment in aged LDLR (+/−) golden Syrian hamster via protection of blood cerebral barrier

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The purpose of the study was to explore the effect and the underlying mechanism of YangXue QingNao Wan (YXQNW), a compound Chinese medicine, on cognitive impairment in aged subjects with hyperlipidemia. Fourteen month-old female LDLR (+/−) golden Syrian hamsters were used with their wild type as control. YXQNW (0.5 g/kg) was administrated orally for a month. Y maze task, plasma TC, TG, LDL-C and HDL-C were measured both before and after administration. At the end of experiment, CBF was measured by laser Doppler perfusion image system. Albumin leakage in middle cerebral artery area was determined with an upright microscope. The number of opening capillaries was counted on the sections immunohistochemically stained by CD31. The ultrastructure of microvessels in hippocampus was examined by electron microscopy. The expression of the tight junction proteins claudin-5, JAM-1, occludin and ZO-1 in the hippocampus was assessed by immunofluorescence and western blot. YXQNW improved cognitive impairment of aged LDLR (+/−) golden Syrian hamsters without lowering plasma TC and LDL-C. YXQNW attenuated albumin leakage in middle cerebral artery area and neuron loss in hippocampus, concomitant with an increase in CBF, a decrease of perivascular edema and a preserved expression of claudin-5, JAM-1, occludin and ZO-1. In conclusion, YXQNW is able to protect cognition of aged LDLR (+/−) Golden Syrian hamsters from impairment, most probably via maintaining blood brain barrier integrity. These findings provide evidence suggesting YXQNW as a potential regime to counteract the cognitive impairment caused by familial hypercholesterolemia.

F-10 | Deterioration of Glycocalyx on cerebral and glomerular blood vessel in a rat model of pre-eclampsia. – " Et tu, Glycocalyx?"-

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Introduction: It is well recognized that pre-eclampsia (PE) is a major contributor to maternal and fetal mortality. Although the pathophysiology of cerebral edema in PE remains unclear, increased permeability of blood brain barrier might play a major role in this pathophysiology. Recently, it reported that the structure named

glycocalyx (GCX) on endothelial cells, which is involved in the maintenance of the vascular permeability barrier. In the present study, we investigated whether the degradation of GCX of cerebrum and glomerulus occurs in the PE rat model.

Methods: 20 rats were randomly divided into two groups: Control group (C-group, n = 10); PE group (n = 10). To establish experimental PE rats, a modified Sakawi's method using administration of L-NAME and LPS was employed in the PE-group. A catheter inserted for the perfusion fixation on the day 21. After the fixation using lanthanum, the both kidney and cerebrum were removed. The image of GCX was archived from 5 to 12 cites on each tissue and determined by histogram from the image of electro-microscope.

Results: Significant increases in the mean arterial blood pressure, urinary protein, VEGFR-1 values, and fetal death rate were observed in PE-group compared with C-group, while maternal body weight and kidney weight were substantially lower. While the degradation of GCX in PE-group was significantly higher than that of C in both tissue (Cerebrum; C: 117 ± 24, P: 48 ± 6, $P < 0.05$, glomerulus; C: 125 ± 30, P: 72 ± 10, $P < 0.01$, individually).

Conclusion: This is the first reports which the deterioration of GCX layer in endothelium occurs on the cerebrum and glomerulus as well as other organ already reported in other illness. Alterations of this structure may compromise endothelial permeability with associated interstitial fluid shift and generalized edema. The degradation of GCX might be involved in cerebral impairment and proteinuria in PE-patients.

F-11 | Spatiotemporal dynamic comparisons of cerebral blood flow responses evoked by optogenetic photostimulation to cortical neurons or astrocytes

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It is well known that cerebral blood flow (CBF) is regulated by multiple types of brain cells (e.g., neurons and astrocytes). However, physiological roles of the cell-type specific mechanisms of CBF regulation remain unclear. To dissect the cell-specific vasoactive mechanisms, we used genetically-modified animal models in which the cortical neurons (N = 30) or astrocytes (N = 30) specifically express photo-sensitive cation channels, channelrhodopsin-2 (ChR2), allowing cell-type specific stimulation with transcranial photostimulation (3-second irradiation of blue light followed by 3-second orange LED). Spatiotemporal dynamics of CBF responses to photosimulation was measured with laser speckle flowgraphy, and diameter response of cortical microvessels were captured with two-photon laser scanning fluorescent microscopy in a separate animal group. We observed reproducible robust changes in CBF following photostimulation to ChR2-neurons (1.8 ± 0.2 fold) and ChR2-astrocytes (1.2 ± 0.1 fold). The astrocytic stimulation evokes a rapid propagation of CBF changes compared to the neural stimulation. Pharmacological manipulation revealed that a separate mechanism of vasoactive signals participates in the CBF responses evoked by ChR2-neurons or astrocytes. Furthermore, vasodilatory responses of pial arteries differ between the photostimulation to ChR2-neurons or astrocytes. These results suggest that neural stimulation evokes concentrated responses of CBF, whereas the astrocytic stimulation produces wide-spread changes in CBF.

F-12 | Brain and brain tumor microcirculation evaluated by ultrasonography with Superb Microvascular Imaging technique and contrast agent

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Background: Ultrasonography (US) has been used as a useful tool for clinical investigation of many organs and as a reliable imaging during neurosurgical operations, providing real-time information. We combined the latest Superb Microvascular Imaging (SMI) technique with US contrast agent technique for detecting brain and tumor microcirculation during neurosurgery.

Methods: 14 patients diagnosed with brain tumor underwent neurosurgical operation with ultrasonography monitoring using a US system (Canon, Aplio) with the new SMI technique (imaging frequency: 10–12 MHz, frame rate: 28–31 Hz). Features of the SMI in the grayscale mode include (1) visualization of low-velocity flow with minimal motion artifact, (2) high resolution of images, and (3) high frame rates. We recorded SMI after injection of contrast agent (Sonazoid, 0.2 mL).

Results: The tumor vessels and shifted vessels were drastically enhanced by injection of contrast agent on the SMI in the grayscale mode, detailing the characteristics of normal brain tissue (vertically penetrating, fine, straight vessels), glioblastoma (rounding, dilating, and bending vessels), meningioma (many large and branching vessels). The microvascular flows of the normal brain and the brain tumors were clearly distinguished by contrast-enhanced SMI.

Conclusions: Combination of US SMI images and contrast agent enhances brain and tumor vessels and distinguishes tumor from surrounding normal tissue.

F-13 | Apparent dwell time of red blood cells in the cerebral capillaries

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To visualize and quantify the heterogeneity of capillary red blood cell (RBC) speed and pathways in the cerebral capillaries, we developed a new imaging approach using genetically-engineered fluorescent RBCs. Spatiotemporal dynamic behaviors of fluorescent RBCs and plasma labeled with sulforhodamine 101 were captured with two-photon microscopy in the anesthetized rat cortex at a rate of 12 ms per frame. The automatic segmentation for the RBCs and plasma areas was performed by means of custom-written Matlab software with machine learning methods as a post-processing. Then, apparent dwell time of RBCs was characterized on a pixel-by-pixel basis by calculating a ratio of presence for the RBCs and plasma over each 100 frames (i.e., per 0.12 seconds). We confirmed that the apparent dwell time measured was inversely correlated with a mean velocity of capillary RBCs ($R = -0.81$), and its temporal variation was independent of capillary diameter changes. This suggests that a local pressure gradient causes a temporal variation of capillary RBC speed. As expected, a variable speed of capillary RBCs was successfully visualized with mapping of the apparent dwell time within a single capillary and consecutive capillary networks. Furthermore, correlation analysis of the temporal variations in the maximum apparent dwell time provides a strength of connectivity across the multiple capillaries. In conclusion, the present unique approach allows for visualization of spatiotemporally-varying dynamic features of the RBC behaviors in a single capillary scale as well as across multiple capillary networks in the living animal tissue.