Changing concept of microglia: microgliopathies

Atsushi Sasaki
Department of Pathology, Saitama Medical University

Our understanding of microglia function and potential has greatly expanded in recent years. This symposium is aimed to inform about the changing concept of microglia: Microglia influence synaptic density and connectivity; Gut-microbiota and microglia interact; Some leukodystrophies are considered microgliopathies; Senescent (sick) microglia may contribute to neurodegeneration; Microglial activation and true inflammation need to be distinguished, and neuroinflammation has no precise meaning. Nowadays, mounting evidence indicates that microglia are not in a resting state under healthy conditions, but instead play an essential role in maintaining homeostasis under physiological conditions. The term, microgliopathy, has been applied to disorders due to defects in microglia-specific gene products and/or in which microglia dysfunction could be at the center of the diseases process. These include roles for DAP12 or TREM2 in polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (Nasu-Hakola disease; NHD) and CSF1R in hereditary diffuse leukoencephalopathy with spheroids (HDLS). Interestingly, both DAP12 and TREM2 are implicated in the CSF-1R signaling cascade, and NHD shows the similar histological findings to those of the cerebral white matter in HDLS. Previous reports indicated that most NHD patients with DAP12 gene mutation had a decreased expression of DAP12 at mRNA and protein levels. The mechanisms by which DAP12/TREM2 or CSF1R dysfunction leads to impairment of white matter maintenance are still largely obscure. Future studies on the microglia-specific functions of the DAP12/TREM2/CSF1R signaling pathway in healthy and diseased brains are crucial for understanding the detailed pathogenesis of microgliopathies.
Physiological Implications of microglia-synapse interactions

Hiroaki Wake

Division of System Neuroscience, Kobe University Graduate School of Medicine

Microglia are the sole immune responding cells in the central nervous system. Their role as neuro-immune cells in the pathogenesis of various neurodegenerative and infectious diseases of the brain have been extensively studied. Upon brain disease and infection, the adopt an activated phenotype associated with the release of cytokines and neurotrophic factors and resulting in neuroprotective or neurotoxic outcomes. However, microglia are resident also in the healthy or physiological brain, but much less is known about their role(s) in the healthy brain, partly due to technical limitations involved with investigate these highly reactive cells in the intact brain. Recent developments in molecular probes and optical imaging in vivo has now helped to characterize microglia in the physiological or healthy brain. In vivo two-photon imaging of fluorescently labelled microglia have revealed they are highly motile cell in the healthy brain, extending and retracting their process that extend from a largely stationary cell soma. In this issue, we briefly summarize some of the physiological functions of microglia in the uninjured brain, with a focus on interactions they make with synapses.
Single-cell profiling of the myeloid cell compartment identifies new cell populations with distinct fates during neuroinflammation

Marco Prinz
Institute of Neuropathology, University of Freiburg

The innate immune cell compartment is highly diverse in the healthy central nervous system (CNS) including parenchymal and non-parenchymal macrophages. However, this complexity is increased in inflammatory settings by the recruitment of circulating myeloid cells. It is unclear which disease-specific myeloid subsets exist and what their transcriptional profiles and dynamics during CNS pathology are. By combining deep single-cell transcriptome analysis, fate mapping, in vivo imaging, clonal analysis, and transgenic lines we comprehensively characterized unappreciated myeloid subsets in several CNS compartments during neuroinflammation. During inflammation, CNS macrophage subsets undergo self-renewal, and random proliferation shifted towards clonal expansion. Finally, functional studies demonstrated that endogenous CNS tissue macrophages are redundant for antigen presentation. Our results highlight myeloid cell diversity and provide insights into the brain's innate immune system.
Microglia and neuronal degeneration in senescence-accelerated mice

Atsuyoshi Shimada
Kyorin University Faculty of Health Sciences

I here present our data on age-related changes in the morphology of microglia in Senescence-accelerated mouse prone 10 (SAMP10). In SAMP10 neuronal degeneration begins to appear earlier (around 8 months of age) and becomes progressively more remarkable with advancing age, compared to Senescence-accelerated mouse resistant 1 (SAMR1) as usual-aging controls. Parameters representing morphological features and pathological changes of microglia were quantified using an image analyzer. Microglia of SAMP10 mice at ages 3 and 8 months exhibit shortened combined projection length and smaller numbers of segments and tips than those in age-matched SAMR1 mice. Microglia of SAMP10 mice at all ages are characterized by having frequent pathological changes in processes. These morphological abnormalities in microglia of SAMP10 mice precede the onset of neuronal degeneration and may lead to making brain tissue less protective to neurons. I also present our data on hippocampal tissue responses to intraperitoneal injection of kainic acid (KA) in mice at age 3 months. On day 3 following KA challenge, microglia-derived interferon-gamma stimulate astrocytes via the receptors to induce the expression of CXCL10 and CCL3 in SAMR1 mice. Activated microglia produce GM-CSF and osteopontin. CD44, an osteopontin receptor, is strongly upregulated in neurons. This well-orchestrated glial reaction is strikingly reduced in SAMP10 mice. Thirty days after KA challenge, SAMP10 but not SAMR1 mice exhibit hippocampal atrophy. Since the osteopontin-CD44 system is essential for neuroprotection, these findings highlight the defects of SAMP10 mice in cytokine-mediated neuroprotective glial responses, which may be associated with early microglial abnormalities in SAMP10 mice.
Microglia were first described by Nissl in 1899 as rod cells in cerebral cortex where they are thought to engage in the activity that forms the focus of this Symposium: microglia-synapse interactions. Robertson also gave an account on microglia in 1900, and del Rio-Hortega finally named the cells in 1920. However, microglia were initially ignored by neuroimmunologists in the 1980s when astrocytes were incorrectly considered the main antigen presenting cells of the CNS. Over the last two decades microglial cells have been trivialised as macrophages although their presence in normal adult brain and spinal cord tissue is quite obviously not associated with macrophage activity. In addition, the question what exactly microglia do in classical non-inflammatory brain pathologies that light up on PET imaging has remained an open question. The tautological term "neuroinflammation" effectively denotes gliosis. We think it is likely that synaptic changes involving both astrocytes and microglia are the reality behind most claims of “brain inflammation” in classical non-inflammatory conditions such as autism, schizophrenia, depression, obesity and several forms of dementia to name a few. This lecture will focus on the engraftment of genetically modified bone marrow-derived microglia precursors, which we first reported in 1998 (Journal of Neuroimmunology 90:27/136). In our axotomy experiments, blood-derived cells assumed the typical morphology and even position of perineuronal activated microglia. Therefore, non-invasive access to the CNS via bone-marrow derived genetically modified microglia precursors should be a strong focus of research. We will discuss means to control these neo-microglia that may affect synapses.
Calcium channel protein aggregations and role of lysozymes in SCA6

Kinya Ishikawa

The Center for Personalized Medicine for Healthy Aging, Tokyo Medical and Dental University

Spinocerebellar ataxia type 6 (SCA6) is an autosomal dominant neurodegenerative disease caused by a polyglutamine (polyQ) expansion [control: 4 ~ 20Q; SCA6: 20 ~ 33Q] in the carboxyl (C)-terminal cytoplasmic domain of the α1A voltage-dependent calcium channel (Ca,2.1). The length of polyQ tract in SCA6 is the shortest among the nine polyQ diseases. Although the pathogenesis of SCA6 remains elusive, several fundamental observations suggest two plausible hypothesis. One is that a small polyQ expansion in a large membranous Ca,2.1 protein leads to aggregation within the cytoplasm of SCA6 Purkinje cells. The other is that a shorter carboxyl-terminus of the Ca,2.1 containing polyQ tract preferentially translocates into Purkinje cell nuclei, where it affects transcription of several genes involved in neurite outgrowth such as TAF1. On western blot, Ca,2.1 C-terminal fragment is detected in the nuclear fraction from human SCA6 cerebella. Importantly, Ca,2.1 protein aggregation is far more abundant in the cytoplasm compared to nucleus. This is convincingly recapitulated in SCA6 knock-in mice expressing Ca,2.1 with 118 polyQ. We found that antibodies against the lysosomal proteases cathepsin B (CaB) or cathepsin D (CaD) are both strongly immunoreactive in human SCA6 Purkinje cells. In accord with this, CaB- and CaD-immunoreactivities are intense in SCA6 knock-in mice. Furthermore, lack of CaB exacerbated Purkinje cell loss with acceleration of Ca,2.1 aggregation, suggesting that lysosomal enzyme(s) protect against polyQ-induced toxicity. Further studies investigating whether activating lysosomal catalytic enzymes is essential for mitigating polyQ-induced toxicity.
Autophagic function and dysfunction in Niemann-Pick type C neuropathology

Andrew Lieberman

Department of Pathology, University of Michigan

Niemann-Pick type C disease is a fatal, progressive neurodegenerative disorder caused by loss-of-function mutations in NPC1, a multipass transmembrane glycoprotein essential for intracellular lipid trafficking. This autosomal recessive lysosomal storage disorder is characterized by progressive neurodegeneration and early death, often in childhood. I will review data from cell culture and genetic mouse models defining impaired autophagic flux in models of disease. These findings will be extended to the brain of Npc1 deficient mice, where age-dependent Purkinje cell degeneration is a characteristic feature. Our analysis has demonstrated striking accumulation of autophagic substrates in the diseased brain, including both brainstem and cerebellum. Moreover, our studies establish that ER-targeted autophagy plays an essential role in degrading I1061T NPC1, the most common disease-causing missense mutant. Finally, I will discuss on-going work to develop novel strategies to alleviate neuronal lipid storage for therapeutic benefit.
The effect of impairment of autophagy versus lysosomal proteostasis on the survival of Purkinje cells

Masato Koike

Department of Cell Biology and Neuroscience, Juntendo University Graduate School of Medicine, Tokyo, Japan

Neurological phenotypes of cathepsin D (CD)-deficient mice, a murine model of neuronal ceroid lipofuscinoses (NCLs), indicate the importance of CD in the lysosomal proteostasis in CNS neurons. Furthermore, it is well known that impairment of autophagy leads to neurodegeneration. Because human patients with mutation in CD or autophagy related genes exhibit ataxia, we generated CD-deficient mice specifically in Purkinje cells (PCs), whose phenotypes were compared with those of PC-selective Atg7-deficient mice.
Radiation Induced Secondary Glioblastomas in patients with medulloblastomas showed alteration of the PDGFRA and TP53 in whole exome sequencing

Ji Hoon Phi\textsuperscript{1,2}, Sung-Hye Park\textsuperscript{3}, Seung Ah Choi\textsuperscript{1,2}, Ae Kyung Park\textsuperscript{5}, Ji Yeoun Lee\textsuperscript{1,2,4}, Kyu-Chang Wang\textsuperscript{1,2}, Seung-Ki Kim\textsuperscript{1,2}

\textsuperscript{1}Division of Pediatric Neurosurgery, Seoul National University Children's Hospital, Seoul, Republic of Korea, \textsuperscript{2}Department of Neurosurgery, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Republic of Korea, \textsuperscript{3}Department of Pathology, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Republic of Korea, \textsuperscript{4}Department of Anatomy, Seoul National University College of Medicine, Seoul, Republic of Korea, \textsuperscript{5}College of Pharmacy and Research Institute of Life and Pharmaceutical Sciences, Sunchon National University, Suncheon, Republic of Korea

Introduction: Currently, at least 1/4 of all patients with medulloblastoma suffer from tumor recurrence and the prognosis of recurrent MB (rMB) is dismal. Secondary malignancies such as glioblastoma (GBM) are rarely occurred in recurrent tumors after radiotherapy for MB patients. Methods: We performed whole exome sequencing (WES) in recurrent tumors of MBs and found 5 cases of RT induced secondary GBM (secondary GBM). The authors compared genomic and histopathological findings of secondary GBMs with rMBs. Results: The man age of initial tumor occurrence was younger in secondary GBMs than rMBs [5.58: 11.16 yrs], however, the mean age of recurrent tumors was similar [13.7: 14.2 yrs] because the time interval between primary and recurrence was longer in secondary GBMs than rMBs (8.1: 3.0 yrs). Three out of 5 cases of secondary GBMs were misdiagnosed as anaplastic variant of rMB. All secondary GBMs were positive for GFAP and Olig2, but not for rMBs. On WES, missense mutations or complex gene fusion in PDGFRA with augmented expression and genomic alterations of TP53, including loss or germline/somatic mutations of 17p, were found in secondary GBMs. On the other hand, rMBs revealed the loss of 17p region including TP53 and gain of 7q region containing EZH2 which already existed in primary MBs. Conclusion: The recognition of this secondary GBM is critical to finding secondary GBM. The PDGFRA and TP53 might play an important role in the development of a second malignancy and PDGFRA and EZH2 can be potential therapeutic targets in these fatal recurrent tumors.
**Astroblastoma is pathologically and genetically distinct from other mimics**

Takanori Hirose¹,², Sumihito Nobusawa³

¹ Department of Pathology for Regional Communication, Kobe University Graduate School of Medicine,
² Department of Diagnostic Pathology, Hyogo Cancer Center,
³ Department of Human Pathology, Gunma University Graduate School of Medicine

Astroblastoma is a rare, enigmatic tumor, which microscopically mimics ependymoma, and papillary meningioma, although some controversy remains regarding its distinctiveness. Recent molecular analyses of CNS primitive neuroectodermal tumors revealed some unique genetic aberrations in a subset that shared the clinicopathologic characteristics of astroblastoma. Astroblastoma predominantly affects children and young adults and shows a female preponderance. It usually arises in the cerebral hemisphere. Radiological examinations demonstrate a well-demarcated tumor, which is often associated with enhancement. Microscopic characteristics are well-developed perivascular pseudorosettes and perivascular hyalinization. The former are formed by the angiocentric arrangement of broad, short cell processes of tumor cells and are known as "astroblastic pseudorosettes". Astroblastoma is pathologically classified as low-grade or high-grade, based on anaplasia and proliferative activity, but diagnostic criteria and formal WHO grades have not been established. Astroblastoma usually shows immunoreactivity for GFAP and Olig2 in varying proportions. A dot-like reaction for EMA is also seen in some cases. Recently, astroblastoma was reported to possess MN1 gene rearrangement, which may form an aberrant fusion gene with BEND2 located on the X chromosome. Chromothripsis is suspected to be involved in the translocation of the X chromosome. Although high-grade astroblastoma is generally more aggressive, patients able to undergo total resection may follow an indolent clinical course. Considering the unique clinicopathologic features and distinct molecular profiles, astroblastoma is considered a distinct tumor entity despite several similarities with other glial tumors, especially ependymoma.
CNS Embryonal Tumors beyond the WHO 2016 Classification

Vani Santosh

Department of Neuropathology, National Institute of Mental Health & Neuro Sciences (NIMHANS)

CNS embryonal tumors encompass a wide spectrum of histomolecular entities that are incorporated in the WHO 2016 classification. This includes the four new genetic subgroups of medulloblastoma (WNT activated; SHH activated, TP53mutant; SHH activated, TP53wildtype; nonWnt/nonSHH). Embryonal tumour with multilayered rosettes (ETMR) form their own group, sharing the common alterations in the oncogenic C19MC cluster. The diagnosis of Atypical Teratoid/Rhabdoid Tumors (AT/RT) mandatorily requires demonstration of mutation or loss of SMARCB1 or SMARCA4 genes. The CNS PNET entity is now obsolete, and the remaining tumours classified based on histology alone are termed other embryonal tumours. Following the WHO 2016 classification, high throughput data sets have identified newer molecular subgroups within embryonal tumors, such as; 7to12 molecular subgroups in medulloblastoma, each with its own clinical behavior. We have shown that mitochondrial DNA content based clustering identifies five subgroups of medulloblastoma. Three molecular subgroups of AT/RT which are genetically similar but epigenetically different are identified; AT/RT TYR, AT/RT SHH and AT/RT MYC. Newer embryonal tumor molecular entities that are not included in the WHO diagnostic lexicon include; CNS neuroblastoma FOXR2 activation, CNS Ewing sarcoma family tumor EFT&CIC alteration, CNS high grade neuroepithelial tumor with MN1 and BCOR alteration. Tumors that resemble CNS embryonal tumors histologically include pineoblastomas and the newly described pituitary blastoma. Germ line mutation in DICER1 is reported to be a key predisposing event in pituitary blastomas. The clinical significance of these newer molecular subtypes of CNS embryonal tumors would determine their practical incorporation into subsequent CNS tumor classifications.
Are IDH wt diffuse astrocytomas glioblastomas in disguise?

Ho-Keung Ng
Department of Anatomical and Cellular Pathology, Chinese University of Hong Kong

During the making of the WHO2016 classification, there was intense debate whether truly low grade diffuse IDH wt astrocytomas exist. The entity was finally retained reluctantly though it was noted that many of them would be glioblastoma in disguise. The TGCA paper (NEJM 2015) of lower grade gliomas more or less arrived at the same conclusion. We and some others propose that while many of IDHwt diffuse adult astrocytomas will indeed behave like high grade gliomas, a significant proportion will behave more indolently and have survivals worse than IDH mutant astrocytomas but much better than glioblastoma. The high risk and low risk IDH wt diffuse adult astrocytomas are separable by molecular biomarkers. We have set out our findings in Aibaidula, Ng. Neuro-oncology 2017. Around the same time, two other groups published findings corroborating our data: Aoki, Natsume. Neuro-oncology 2018 and Wijnenga, van den Bent. Acta Neuropathologica 2017. It seems that the most appropriate molecular biomarkers to delineate the low and high risk IDH wt astrocytomas will be EGFR, TERTp and 10q. At the time of writing this abstract, we understand the cIMPACT-now group will soon be publishing an update of the diagnosis of IDHwt astrocytomas largely based on the findings of these papers.
Muscle pathology in the era of NGS

Ichizo Nishino

Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP)

Next generation sequencing (NGS) is a powerful technique that allows us to screen theoretically all genes for mutations at one time. Nevertheless, the definitive diagnosis is made only around 30% of cases – it is sometimes called “30% issue”. The main reason for this relatively low diagnostic rate, in addition to technical limitations of NGS itself, is the fact that NGS does not simply tell the mutation(s) but provides a long list of variants – nucleotides different from the reference sequence. Among all those variants, mutation candidates need to be chosen based upon likely inheritance pattern, the features of the disease and often with the help of prediction software. Furthermore, even with a strong candidate, laborious functional assays are usually necessary to validate its pathogenicity.

In contrast, if the mutations whose pathogenicity has already been well established are included in the list of variants that NGS produces, theoretically, the diagnosis can be almost immediately. In fact, most of the “30%” whose diagnosis is successfully made have known mutations, clearly indicating the necessity of enriching genotype-phenotype correlation data for various genetic disorders.

In this line, precise phenotyping will be more important than before for successful NGS analysis. Without correct phenotype information, NGS analysis and result will be misinterpreted. Historically, most muscle diseases have been classified and/or defined by pathological features in addition to clinical features, but the disease definition will most likely be more gene- or mutation-based in the near future. In this transition period, we must establish detailed genotype-pathological phenotype correlation, for which muscle pathology should play a major role.
The road less traveled: the evolving role of morphological assessment in the era of precision medicine - through a window of mitochondrial myopathy

Kurenai Tanji
Department of Pathology & Cell Biology, Columbia University

This is a dynamic phase in the history of mitochondrial medicine. The increasing utility of genetic information in the diagnosis of myopathies, owing to the rapidly advancing next generation sequencing, is transforming the role of conventional tissue diagnosis. Morphological categorization remains essential for many myopathies, especially when genetic abnormalities of unknown significance might complicate a diagnostic odyssey. However, a morphological assessment must consider the specific genetic abnormality. Furthermore, morphology must take into account the clinical stage of the disease, since myopathies are increasingly degenerative at large, and the pathology can change dramatically as the disease progresses. Mitochondrial disease is one of the systemic or neurological conditions in which genetic-pathological correlation has long played a critical role in diagnosis and patient care. With the ever-increasing accessibility to high throughput genetic sequencing, the categories of this bioenergetic disease have been expanding at an unprecedented speed, and have led to a number of therapeutic approaches. For example, deoxynucleotide substrate enhancement therapy is being used for mitochondrial DNA (mtDNA) multiple deletions/depletion syndrome associated with thymidine kinase 2 gene (TK2) mutations. Against this background of rapid diagnostic changes, this presentation aims to highlight: 1. A brief history of muscle biopsy in mtDNA multiple deletions/depletion syndrome; 2. The evolving role of morphological assessment - from bedside to bench and back; 3. A novel imaging technology, cryo-electron tomography, and its potential value in the era of precision (accurate) mitochondrial medicine.
Muscle pathological changes of cancer associated myositis

Jun Shimizu

Department of Neurology, Graduate School of Medicine, The University of Tokyo

Idiopathic inflammatory myopathies (IIMs), also known as myositis, are a group of acquired, heterogeneous, autoimmune diseases that are classified into polymyositis (PM), dermatomyositis (DM), immune-mediated necrotizing myopathy (IMNM) and non-specific myositis based on clinical features and pathological findings. As a clinical phenotype, cancer association in myositis has been known from long ago. Since the first description of the cancer association in a DM patient in 1916, the association of cancers in DM patients has been extensively reported in the medical literature. Historically, cases of paraneoplastic necrotizing myopathies have been also reported from 1960s. Currently, more than 15 myositis-specific autoantibodies (MSAs) have been identified. Because a number of MSAs correlate with specific clinical features of patients with IIMs, and only one of MSAs is detected in individual patients, it is presumed that autoantibodies or their target molecules correlate with underlying pathomechanisms of IIMs. With regard to MSAs associated cancers, it is well-known that adult patients with DM who carry anti-transcriptional intermediary factor (TIF1) γ are more likely to develop malignancy than anti-TIF1γ negative patients. Besides anti-TIF1γ antibodies, a few studies have indicated the increased cancer risk in adult patients with anti-NXP2, anti-SAE, and anti-HMGCR antibodies. In this session, we will talk about our studied on the clinical and pathological features of cancer associated myositis (CAM), focusing on CAM with anti-TIF1γ and anti-HMGCR antibodies.
Interaction between cerebrovascular disease and Alzheimer pathology

Masafumi Ihara

Department of Neurology, National Cerebral and Cardiovascular Cetner

Epidemiological investigations have proposed strict control of vascular risk factors as a strategy to overcome dementia, because of the close interaction between cerebrovascular disease (CVD) and Alzheimer's disease. In light of recent advances in basic, translational, and clinical research in the area, I would like to focus on the significance of CVD in Alzheimer's disease pathogenesis. Alzheimer's disease and CVD share several risk factors, and the coexistence of both pathologies is frequently noted. CVD and subsequent cerebral blood flow reduction would increase amyloid β (Aβ) production by modulating β and γ-secretase. Furthermore, CVD impairs Aβ clearance, which is mainly driven by vascular mediated systems, including active transport across the blood-brain barrier, and perivascular lymphatic or paravascular glymphatic drainage systems. Thus, CVD may disturb homeostasis between Aβ production and clearance, thereby aggravating Alzheimer's disease. Recent translational researches in this field aim to facilitate Aβ clearance. Several candidate drugs are being tested in clinical trials such as our trial using cilostazol for MCI (COMCID study). Compared with Aβ pathology, little is known about the relationship between tau pathology and CVD, although some studies have shown that CVD has an influence on tau pathology. The close interrelationship between Alzheimer's disease and CVD suggests the necessity of the maintenance of cerebrovascular integrity, which may herald a new generation of dementia treatment strategies.
The Gliovascular Unit in White Matter Disease associated with Post-Stroke and Vascular Dementias

Raj Kalaria
Institute of Neuroscience, Newcastle University

Introduction: Diffuse white matter (WM) changes are described in cerebral small vessel diseases (SVD). We previously showed disruption of the gliovascular unit is associated with breach of the blood-brain barrier (BBB) in frontal WM disease. Besides astrocytes, the gliovascular unit also incorporates pericytes but their status is unclear in vascular dementias. Methods: We assessed post-mortem brains from prospectively assessed non-demented (PSND) and demented (PSD) stroke survivors, vascular dementia (VaD) subjects and normal ageing controls. Immunohistochemical and immunofluorescent staining methods were used to study the localisation, distribution and quantification of astrocytes and pericytes identified by glial acidic fibrillary protein (GFAP), collagen 4 (COL4) and platelet derived growth factor receptor-beta (PDGFR-beta) immunoreactive profiles of capillaries in the WM and grey matter. Results: COL4 and PDGFR-beta reactive pericytes adopted typical crescent morphology wrapped closely around capillary walls, readily evident in cross-sections. We estimated pericyte numbers per percent COL4 immunoreactive area and per mm vessel length in the frontal WM in ageing controls were 1.6 and 1.7, respectively. Whilst these numbers in controls were not altered in comparison to post-stroke non-demented subjects they were reduced by 30 percent in PSD and VaD subjects (P equal 0.001). Conclusions: Our results show decreased expression of COL4-positive pericytes in capillaries in the frontal WM of subjects with PSD and VaD. More profoundly, our findings suggest that down regulation of pericyte-like cells is associated with loss of control of the microcirculation within the deep WM in cerebral SVD and dementia associated with cerebrovascular disease.
Role of small vessel disease in the boundaries of large vessel disease and Alzheimer disease

Hidekazu Tomimoto
Department of Neurology, Mie University Graduate School of Medicine

Small vessel disease (SVD) encompasses vascular abnormalities in the vessels of various sizes ranging from small arteries to post capillary venules and small veins. The pathologic process in this size of vessels are mainly attributable to two etiologies; ie, hypertensive SVD and cerebral amyloid angiopathy (CAA). This presentation focuses on the boundary field between SVD and large vessel disease and those between SVD and Alzheimer dementia. First, large vessel disease may lead to small vascular lesions and may masquerade SVD such as granular cortical atrophy which is caused by microembolism and hypoperfusion in the border zone territory of the cerebral cortex. With advancement of MRI technologies, these small lesions are getting visible and should be paid attention not to be confused with SVD. Cortical microinfarctions are observed in high prevalence after transcatheter manipulation of heart and cerebral vessels and may remain visible or are transformed to embolic microbleeds after a long duration. Second, CAA may cause characteristic vascular lesions including subcortical hemorrhage, cortical superficial siderosis, strictly-lobar microbleeds and cortical microinfarctions. In the absence of Alzheimer pathology of the neuropil, CAA may be classified to a type of vascular dementia and postulated to be a link of overlapping pathology between vascular dementia and Alzheimer disease.
Exploring the pathologic targets in a white matter ischemic stroke model based on somatotopic mapping of the pyramidal tract

Min-Cheol Lee\textsuperscript{1,2}, Kyung-Wha Lee\textsuperscript{1}, Hyoung-Ihl Kim\textsuperscript{2}

\textsuperscript{1}Department of Pathology, Chonnam National University Medical School, \\
\textsuperscript{2}Department of Biomedical Science and Engineering, Gwangju Institute of Science and Technology

Background: Recent meta-analysis revealed that ischemic strokes are highly associated with white matter (WM) lesions, however, a few studies have investigated due to the difficulty in generating animal models of WM stroke. Method: We created a highly reproducible rat model of internal capsule (IC)-specific stroke using a photothrombotic technique based on anterograde somatotopic mapping of motor fibers, and studied neurologic behaviors, metabolic changes by PET analysis, comprehensive histopathology including ultrastructure, molecular pathogenesis and some therapeutic trials. Result: WM pathologies result in motor dysfunction. Motor function recovery was correlated to the extent of IC injury proper rather than the infarct volume. Pathologic changes indicate that WM is highly vulnerable to the effects of focal ischemia, among which myelin sheath is first damaged. Longitudinal PET results showed that capsular infarct resulted in a persistent decrease in brain metabolism in remote areas from ischemic lesion; diaschisis, and contributes to manifest the malfunctions of lesion-specific functional connectivity. The lesion of diaschisis revealed characteristic histopathology and electron microscopy, and molecular events. Conclusion: As the pathology of white matter stroke revolves around disrupted connectivity and injured axons and glial cells, the mechanisms of WM ischemic injury and the regenerative responses of glial cells and their signaling pathways are differ significantly from those in grey matter. Exploring the relationship between histopathology and metabolic changes by PET in the subcortical infarct model, the development of diaschisis, is a critical target to understand the underlying mechanisms leading to the progression of neurologic outcomes and the development of effective therapies.
Molecular heterogeneity in IDH-mutant gliomas

Michael E. Buckland¹²

¹ Neuropathology Department, Royal Prince Alfred (RPA) Hospital, Sydney, Australia,  
² Brain & Mind Centre, University of Sydney, Sydney, Australia

The current WHO classification recommends that diffuse gliomas, including those with mixed (oligoastrocytoma) or ambiguous histological features, should be classified as either astrocytoma or oligodendroglioma based on key molecular alterations (e.g. IDH mutation status, ATRX immunohistochemistry and 1/19q codeletion status). However there have been several reports of diffuse gliomas showing evidence of genetically distinct oligodendroglial and astrocytic cell populations. While seemingly rare, the true incidence of these 'dual-genotype' gliomas is difficult to ascertain. We have examined over 70 IDH-mutant gliomas, originally reported to have either a mixture of two distinct neoplastic cell types (astrocytic, oligodendroglial), or morphological phenotypes ambiguous between these two lineages. All tumours underwent central histological review and IDH1(R132H)/ATRX dual staining. Further molecular analyses were then performed which included 1p/19q FISH and/or CHG-array, quantitative pyrosequencing and a targeted glioma deep next generation sequencing panel. Our findings confirm that dual-genotype 'oligoastrocytomas' are a rare but real phenomenon, and may provide insights into early stages of gliomagenesis.
CNS high-grade neuroepithelial tumor with BCOR internal tandem duplication

Sumihito Nobusawa
Department of Human Pathology, Gunma University Graduate School of Medicine

A recent study of tumors previously institutionally diagnosed as so-called CNS primitive neuroectodermal tumors revealed four new distinct entities defined by novel genetic alterations, including CNS high-grade neuroepithelial tumors with BCOR alteration (CNS HGNET-BCOR), characterized by somatic internal tandem duplication within exon 15 of BCOR (BCOR ITD). BCOR-ITD has also been found in clear cell sarcomas of the kidney (CCSK) and soft tissue undifferentiated round cell sarcomas/primitive myxoid mesenchymal tumors of infancy (URCS/PMMTI), and these BCOR ITD-positive tumors have been reported to share similar pathological features. Although these tumors had histologically similar structural patterns and characteristic monotonous nuclei with fine chromatin, CNS HGNET-BCOR exhibited glial cell morphology, ependymoma-like perivascular pseudorosettes, and palisading necrosis, whereas these features were not evident in CCSK or URCS/PMMTI. Immunohistochemically, diffuse staining of Olig2 with a mixture of varying degrees of intensity, and only focal staining of GFAP, S-100 protein, and synaptophysin were observed in CNS HGNET-BCOR, whereas these common neuroepithelial markers were negative in CCSK and URCS/PMMTI. Therefore, although CNS HGNET-BCOR, CCSK, and URCS/PMMTI may constitute a group of BCOR ITD-positive tumors, only CNS HGNET-BCOR has histological features suggestive of glial differentiation. CNS HGNET-BCOR are a certain type of neuroepithelial tumor relatively close to glioma, not CCSK or URCS/PMMTI occurring in the CNS.
Histological and molecular genetic features of epithelioid glioblastoma

Hideaki Yokoo
Department of Human Pathology, Gunma University

Epithelioid glioblastoma (E-GBM) is a rare aggressive variant of IDH-wildtype glioblastoma newly incorporated in the 2016 World Health Organization classification, composed predominantly of monotonous, patternless sheets of round cells with laterally positioned nuclei and plump eosinophilic cytoplasm, devoid of stellate cytoplasmic processes. Earlier studies uncovered that approximately 50% of E-GBM harbor BRAF V600E, which is much less frequently found in other types of glioblastomas. Most E-GBM are recognized as primary/de novo lesions; however, several E-GBM with co- or pre-existing lower-grade lesions have been reported. We collected 14 cases of E-GBM with (10) or without (4) lower-grade lesions, and molecular analyses demonstrated that the prevalence of BRAF V600E, TERT promoter mutations and CDKN2A/B homozygous deletions in E-GBM were 13/14 (93%), 10/14 (71%) and 11/14 (79%), respectively, and concurrent BRAF V600E, TERT promoter mutations and CDKN2A/B homozygous deletions were observed in 7/14 (50%) of E-GBM. These alterations were also frequently seen in the lower-grade lesions irrespective of the histology. Genetic analysis including array comparative genomic hybridization performed for 5 E-GBM with co- and pre-existing lower-grade components revealed that all molecular changes found in the lower-grade components were also observed in the E-GBM components, and additional changes were detected in the E-GBM components. The lower-grade components may be distinct infiltrative components of E-GBM, reflecting intratumoral heterogeneity, or a precursor of E-GBM.
Pilomyxoid Astrocytoma: What do we know about the tumor?

Tarik Tihan
Department of Pathology, University of California, San Francisco (UCSF)

Pilomyxoid Astrocytoma (PMA) was reported originally in 1999 as a piloid neoplasm with distinct features including monomorphic bipolar cells, myxoid matrix and an angiocentric pattern. The tumors occurred almost exclusively in the hypothalamic region and in young children, and the prognosis was worse than pilocytic astrocytoma (PA) in the same location and age group. PMA often had higher rate of recurrence and cerebrospinal seeding compared to PA, but still better than diffuse gliomas. Subsequently, examples were reported in the spinal cord and cerebrum, and some case reports were published suggesting that pilomyxoid astrocytomas occurred in adults as well as in odd locations. However, many such cases fail to demonstrate the diagnostic criteria, and probably qualify as tumors other than PMA. In 2008, a tandem duplication producing an oncogenic BRAF fusion was discovered in PA and some PMAs. The MAPK pathway alterations are now considered typical of both PA and PMA, and tumors that do not harbor genetic alterations in this pathway often belong to other entities. In summary, PMA is a tumor in the pilocytic category and typically occurs in young children in the hypothalamic/chiasmatic region and show monomorphous histological characteristics. There is clear evidence in the literature that PMAs have a higher rate of recurrence and cerebrospinal spread compared to PMA even after matching for age and location. It is important to be judicious in using diagnostic criteria, and remember that tumors with only focal pilomyxoid features probably do not belong to this group.
CNS tuberculosis, Cryptococcal infection and cerebral toxoplasmosis are common AIDS associated opportunistic infections in developing countries. There is paucity of information on astrogial and microglial alterations in human brain following these infections as most studies focus on clinical, imaging, and laboratory diagnosis. In an autopsy study, we evaluated the pathomorphologic alterations with quantitative assessment of astroglia and microglia, with in prefrontal cortex and hippocampus in cases of tuberculous meningitis (TBM) and cryptococcal meningitis (CM) and cerebral toxoplasmosis to determine its possible contribution to long term neurological sequelae in survivors. Quantitative proteomics using iTRAQ labeling and mass spectrometric studies was performed to determine alterations in host at proteome level. Studies on neuronal-astrogial interactions will provide insights into the neuropathogenetic mechanisms in the cellular and pathomorphological evolution to modulate the progress of these common opportunistic infections.
Neuroinvasion via peripheral nerves: Increasing evidence for its importance in viral encephalitis

Kum Thong Wong¹, Tzeh Long Fu¹, Kien Chai Ong², Soon Hao Tan²

¹Department of Pathology, Faculty of Medicine, University of Malaya,
²Department of Biomedical Science, Faculty of Medicine, University of Malaya

Neuroinvasion by viruses is either via a haematogenous route or by neural pathways. The best known examples of neuroinvasion by neural pathways are the rabies virus via the peripheral nervous system and herpes simplex virus via the olfactory nerve. Among the enteroviruses, although poliovirus has been thought to enter the CNS via a haematogenous route following a viraemic phase, more recent evidence in transgenic mouse models strongly suggests retrograde axonal viral transmission in peripheral motor nerves. Among the other non-polio neurotropic enteroviruses, Enterovirus A71, Coxsackievirus A16 and Enterovirus D68, there is also increasing clinical, brain MRI and autopsy evidence that strongly suggest the same retrograde axonal transmission is important for CNS invasion. Flaviviral encephalitides caused by Japanese encephalitis (JE) virus, Tick borne encephalitis virus and Murray valley encephalitis often involve bilateral thalami in particular, apart from other CNS regions. We hypothesise that skin peripheral sensory nerve may be a portal for viral entry following an infected mosquito bite, and viruses could enter the CNS via established sensory pathways. In a footpad-inoculation JE mouse model we found that the peripheral nerves, dorsal root ganglia (DRG) and thalami were preferentially infected early. Organotypic cultures of DRG also showed the cells supported viral replication. These findings support the involvement of sensory neural pathways in JE, and possibly other flaviviruses as well. If confirmed, neuroinvasion via the peripheral nervous system could be very important for further investigation to advance understanding of viral encephalitides.
Neuropathology and neuropathogenesis of congenital Zika syndrome

Leila Chimelli\textsuperscript{1,2}

\textsuperscript{1} State Institute of Brain Paulo Niemeyer, \textsuperscript{2} Federal University of Rio de Janeiro

Zika virus, a flavivirus transmitted by Aedes aegypti, arrived and in Brazil in 2015. Infection varies from mild fever, arthralgia, rash, headache, myalgia, but may be asymptomatic. A major concern was the increased incidence of microcephaly with frequent calcifications in neonates born from mothers infected in the beginning of pregnancy. Intrauterine ultrasound/CT showed that cerebral maturation and growth were drastically affected. Many were born with arthrogryposis. Neuropathological lesions in neonates infected during pregnancy included three patterns of lesions, with a mixture of destruction, calcification, hypoplasia and migration disturbances. Hydrocephalus was severe in the first, due to midbrain damage and aqueduct obstruction. Small brains with mild/moderate (ex-vacuum) ventriculomegaly characterized the second pattern, both when infection occurred early. The third, with well-formed brain and mild calcification, coincided with infection late in gestation. Absence of descending fibers resulted in hypoplastic basis pons, pyramids and cortico-spinal tracts. Spinal motor cell loss explained the intrauterine akinesia, arthrogryposis and neurogenic muscle atrophy. The sensory system was normal. Viral detection in the germinal matrix and hemispheres confirmed infection and death of neural progenitors and glial cells, interfering with proliferation and migration. Topography and severity of lesions and timing of infection during gestation indicated a developmental vulnerability of the immature brain and explained the manifestations of the disease. Vascular occlusions in meninges and placenta do not discard a secondary ischemic process in the pathogenesis of destructive lesions. It is also possible that viral persistence, may be responsible for progression of lesions and symptoms observed after birth.