

Updates on the WHO Classification of Pituitary Neuroendocrine Tumors

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The 4th edition of the WHO Classification of Endocrine Tumors recommends changes in the classification of tumors of the pituitary gland that will be discussed in this symposium. In regard to the adenohypophysis, changes include: 1. A novel approach for classifying pituitary neuroendocrine tumors according to adenohypophyseal cell lineages; 2. Changes on the histological grading of pituitary neuroendocrine tumors with the elimination of the term atypical adenoma; 3. Introduction of new entities like the pituitary blastomas, and re-definition of old entities like the null-cell adenomas. The new classification is mostly based on immunohistochemistry for pituitary hormones, pituitary specific transcription factors, and markers commonly used in pathology practice, not requiring routine ultrastructural analysis of the tumors. Evaluation of tumor proliferation potential, by mitotic count and Ki-67 index, and tumor invasion is strongly recommended on individual case basis for identification of clinically aggressive adenomas. In addition, the classification provides information on prognostic significance for the treating clinical team with the identification of specific variants of adenomas with elevated risk for recurrence. Changes in the classification of non-neuroendocrine tumors are also proposed, in particular those tumors arising in the posterior pituitary including pituicytoma, granular cell tumor of the posterior pituitary, and spindle cell oncocytoma. It is hoped that the 2017 WHO classification of pituitary tumors will establish more uniform biologically and clinically groups of tumors, will facilitate the practicing pathologist to better diagnose these tumors, and contribute to understanding of clinical outcomes for patients harboring pituitary tumors.

Non-functioning adenomas - a new approach for their classification

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To determine adeno-hypophysial cell lineages for the accurate subclassification of pituitary adenomas, the new WHO classification proposed the application of transcription factors immunohistochemistry. Approximately 30-40% of all surgically treated adenomas are clinically nonfunctioning. Morphologically they are classified into several histological subtypes with some different clinical behavior and prognosis. However, there are limitations in achieving accurate classification using hormone immunohistochemistry alone. An appropriate use of immunohistochemistry for the transcription factors has a complementary role in obtaining an accurate diagnosis for hormone-negative adenomas. Subclassification of nonfunctioning adenomas was revised accordingly in the new classification. (1) Gonadotroph adenomas: This subtype, the most common subtype, can be reliably detected by nuclear immunoreactivity for SF-1. Most of them are benign, slowly growing tumor presenting in middle-aged and elderly patients. (2) Silent corticotroph adenomas: ACTH-immunohistochemistry alone is insufficient to detect this subtype whereas assessment of Tpit expression is required for the accurate diagnosis. This subtype tends to show significant female preponderance and are more frequently large macroadenomas with marked cavernous sinus invasion. (3) Silent adenomas of pit-1 derivation: They are morphological heterogeneous including plurihormonal pit-1-positive adenomas (previously called subtype 3 adenomas), et al. They typically show preponderance in younger patients and are mostly invasive, large macroadenomas with a high Ki-67 proliferation index. (4) Null cell adenomas: The definition of this subtype was revised extensively in the new classification as follows: those composed of adeno-hypophysial cells that do not show any evidence of cell-type specific differentiation using pituitary hormones and transcription factors. Consequently, they became quite rare.

Updates on TTF-1 Expressing Posterior Pituitary Tumors

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TTF-1 expressing posterior pituitary tumors comprise pituicytoma, granular cell tumor of the sella region and spindle cell oncocytoma. This update will focus on the histopathology and current status of molecular understanding of these rare neoplasms.

Updates on Craniopharyngiomas

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Craniopharyngiomas are uncommon neoplasms of the central nervous system that arise above the sella turcica of the skull base. Although the tumors have benign histology, their proximity to critical neurological areas leads to extensive morbidity including pan-hypopituitarism, visual impairment, cognitive alterations, hyperphagia and morbid obesity. Craniopharyngioma comprise two variants: adamantinomatous craniopharyngioma (ACP) which arise in children and adults and papillary craniopharyngioma (PCP) which arise nearly exclusively in adults. These two variants can be defined based on integrated histologic and genetic analysis. In addition to distinct histologic features, ACP and PCP have different patterns of gene expression and DNA methylation. Moreover, most ACP have activating mutations in CTNNB1, the gene which encodes the beta-catenin protein, whereas nearly all PCP have BRAF V600E mutations. Several reports indicate that PCP can be highly sensitive to targeted therapy and a clinical trial is underway to evaluate the efficacy of this approach. Effective medical therapies for ACP are lacking. International research efforts continue to shed light on the importance of various signaling pathways in the development of ACP and studies are beginning to characterize the expression of immune checkpoint markers and the features of immune infiltrates in ACP and PCP. Murine models of ACP including new patient derived xenograft models are an important resource for exploring the biology of these challenging tumors and for testing therapeutic strategies.

Progressive Multifocal Leukoencephalopathy: Virology and Neuropathology

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Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease, caused by JC virus infection in immunosuppressed individuals. Pathologically, viral inclusions are detected in the enlarged nuclei of infected oligodendroglia-like cells, which is a diagnostic hallmark of this disease. Viral inclusions were initially noted with hematoxylin-and-eosin staining as amphophilic materials comprising the entire nucleus (full inclusions). Recent immunohistochemical analyses, however, revealed the presence of intranuclear viral inclusions in dots (dot-shaped inclusions), which reflect clustered progeny virions at punctuated subnuclear domains called promyelocytic leukemia nuclear bodies (PML-NBs). Normal oligodendroglia has compact small nuclei, but after virus infection, nuclear enlargement occurs with cell-cycle activation from S to G2. In the enlarging nuclei, PML-NBs also grow larger, where JC virus reproduces progenies to form dot-shaped inclusions. The progenies later fulfil the entire part of the nucleus and disrupt PML-NBs. Since PML-NBs are important for nuclear events, such as transcription, DNA replication, and cell-cycle regulation, PML-NBs disruption may induce death of host cells. Today, PML development in MS patients under disease modifying therapy (DMT) is a worldwide concern. Since early diagnosis of PML is essential for favourable outcome, understanding early pathological features of affected cells may help diagnosis in case of brain biopsy.

CADASIL and CARASIL: pathologic features and possible pathomechanisms

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) are hereditary cerebral small-vessel diseases (CSVD) leading to stroke and vascular dementia in adults. CADASIL is caused by mutations in the *NOTCH3* gene, which encodes the Notch3 receptor expressed in vascular smooth muscle cells (SMCs). The Notch3 extracellular domain (N3ECD) accumulates in arterial walls followed by SMC degeneration and subsequent fibrosis. Diagnosis is based on characteristic microscopic features such as granular osmiophilic material and N3ECD immunopositivity of SMCs, as well as genetic profiling. The pathogenesis of CADASIL is thought to be toxic gain of function related to a mutation-induced unpaired cysteine in N3ECD. Moreover, recent studies have provided new insight into the pathogenetic role of excess extracellular matrix proteins, including vitronectin and timp3. Despite having a similar clinical picture and white matter changes, CARASIL is much rarer than CADASIL. In addition, spondylosis deformans and early-onset alopecia are frequent features. High-temperature requirement serine peptidase A1 (*HTRA1*) gene mutations and a consequent decrease of HTRA1 protease activity cause CARASIL. Even though CARASIL is a recessive inherited disease, recent studies have identified patients with symptomatic CSVD with heterozygous mutations in *HTRA1*. We have observed that patients with heterozygous mutations and "CARASIL" share similar pathological findings including loss of SMCs and splitting of the internal elastic lamina with marked hyalinosis of cerebral arteries. We summarize the clinicopathologic features of CADASIL and CARASIL, and discuss their possible pathomechanisms.

Neuronal intranuclear inclusion disease (NIID)

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Neuronal intranuclear inclusion disease (NIID) is a slowly progressive neurodegenerative disease characterized by eosinophilic intranuclear inclusions in the central and peripheral nervous system, and in the visceral organs. We studied clinical and pathological features of NIID case, and made out the diagnostic flowchart of NIID. We studied 97 sporadic NIID cases and 23 familial NIID cases. 2 sporadic cases and 3 familial cases were also studied autopsy, and most of cases were performed skin biopsy. All histological samples were stained by hematoxylin & eosin, and immunostained with anti-ubiquitin and anti-p62 antibody. In the sporadic NIID cases, dementia was the most prominent initial symptom, followed by miosis, ataxia and unconsciousness. It was observed that, in familial NIID cases with onset age less than 40 years, muscle weakness was seen most frequently, followed by sensory disturbance, miosis, bladder dysfunction, and dementia. In familial cases with more than 40 years of onset age, dementia was most prominent. Elevated CSF protein and abnormal nerve conduction were frequently observed in both sporadic and familial NIID cases. Head MRI showed high intensity signal in corticomedullary junction in diffusion weighted image (DWI) in both sporadic and familial NIID cases. All of the dementia dominant cases presented with this type of leukoencephalopathy on head MRI, and with a decline in MMSE and FAB scores. The number of antemortem diagnosed NIID cases is increasing dramatically. We must consider NIID as a differential diagnosis of leukoencephalopathy and neuropathy, utilizing the NIID diagnostic flowchart.

Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP): pathologic features suggestive of "microgliopathy"

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Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is an autosomal-dominant progressive dementia, characterized pathologically by widespread loss of myelin sheaths and axons with axonal spheroids and macrophage infiltration. The causative gene of ALSP encodes the colony stimulating factor 1 receptor (CSF1R). CSF1R is a cell-surface receptor that regulates the survival, proliferation, differentiation and function of mononuclear phagocytes. In the CNS, the receptor is expressed predominantly in microglia, and therefore ALSP has recently been considered one of the primary microglial disorders known as microgliopathies. However, there has been little direct evidence to characterize ALSP as a microglial disorder. We have recently reported several unique histological features of microglia in the brains of ALSP patients. Activated microglia are spatially restricted rather than being distributed diffusely, despite the presence of diffuse white matter degeneration. The microglia show relatively uniform and delicate morphology with thin processes and many knot-like structures. The microglia in less affected regions are reduced in number relative to control brains, although Ki67-expressing proliferative microglia are frequently observed in areas of dense microglial distribution. Ultrastructurally, the microglial cytoplasm and processes show vesiculation of the rough endoplasmic reticulum and disaggregated polyribosomes, indicating impairment of protein synthesis. These findings suggest that the pathogenesis of ALSP is associated with microglial vulnerability and morphological alterations. Further investigations to clarify the underlying roles of microglia in this type of leukoencephalopathy are needed in order to develop suitable therapy.

S10-1

Neuropathological experience with 2244 LEAT from the European Epilepsy Brain Bank

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We have histopathologically described a series of 9523 patients with focal epilepsy submitted to epilepsy surgery in 36 centers across 12 European countries (the European Epilepsy Brain Bank consortium; Blumcke et al. 2017; NEJM, 377:1648-56). Low-grade epilepsy-associated brain tumours (LEAT) are the second most common structural lesions identified, i.e. ganglioglioma and dysembryoplastic neuroepithelial tumours, and comprise 69percent of brain tumours in our series. LEAT share the following catalogue of histopathological and clinical features: (1) > 70percent of tumors occur in temporal lobe; (2) they provoke seizure onset at mean age of 15y, were operated after disease duration of 16y, and > 70percent of patients were seizure free 1y after surgery; (3) > 90percent of LEAT behave clinically in a benign manner and correspond to WHO I. There is no definition of atypia (WHO II) for these tumours in the current WHO classification; (4) LEAT have a histologically variable appearance with oligodendroglial or astrocytic phenotypes with or without a prominent dysplastic neuronal component; (5) LEAT may be associated with cortical dysplasia (FCDIIIb); (6) LEAT lack IDH1/2 mutations or 1p/19q co-deletions. Instead, molecular alterations in RAS–RAF–MAPK and PI3K–AKT–mTOR signaling pathways prevail. Throughout all editions of the WHO classification, however, histopathological definitions of and descriptions for the broad spectrum of LEAT variants remain incomplete, thereby challenging the daily routine microscopic work-up. This symposium will tackle difficulties in classifying LEAT from various angles and discuss how to accomplish a reliable genotype-phenotype classification conform with WHO standards in the near future.

S10-2

The differential diagnosis of ganglioglioma and DNT variants: avenues towards an integrated phenotype-genotype classification

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In the setting of long-term epilepsy, specific types of low-grade tumours can be grouped under the umbrella term 'LEAT' (low-grade epilepsy-associated tumours). LEATs are commonly located in the temporal lobe, typically present in late childhood, with epilepsy as the primary and often only neurological symptom. Many LEATs represent glioneuronal tumours with a mixed cell composition and are typically low grade (WHO grade I). Based on the WHO revised classification of brain tumours (2016) the two main tumour types encountered are ganglioglioma (GG) and dysembryoplastic neuroepithelial tumours (DNT). However many LEATs are difficult to classify using current WHO criteria: For example (i) many show diffuse growth patterns and lack the characteristic histology features of DNT or ganglioglioma, (ii) others can show mixed/composite features, (iii) in addition new LEAT tumour types continue to be described for example multinodular and vacuolated neuronal tumours and polymorphous low-grade neuroepithelial tumor of the young (PLNTY) that can histologically mimic DNT and GG. Agreement studies between pathologists, based on refined histological criteria alone, have shown to be insufficient. Advances in the molecular biology of LEATs with an integrated phenotype-genotype classification will be inevitable to improve their recognition and ultimately refine the classification of the LEAT and DNT-GG spectrum. There is evidence of involvement of the PI3K-AKT-mTOR and RAS-RAF-MAPK in the LEAT group and identification and study of common mutations to LEAT subtypes, as well providing diagnostic security, could indicate avenues for non-surgical treatments.

S10-3

Pleomorphic Xanthoastrocytoma: Pathology and Genetics

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Pleomorphic xanthoastrocytoma (PXA) frequently represented in epilepsy surgery series shares with long-term epilepsy associated neuroepithelial tumors (LEATs) the young onset age, the superficial cortical location, temporal lobe predilection and often slow growth rate. PXA has a less favorable prognosis than other LEATs with higher frequency of recurrence following surgical removal and a tendency to progression, prompting a WHO grade II designation. Histopathologically PXA is characterized by cellular pleomorphism with spindled and giant often multinucleated cells, xanthomatous change with cytoplasmic lipid droplets and frequent eosinophilic granular bodies, brightly eosinophilic and pale. Pericellular reticulin deposition is frequent. High mitotic activity (five or more mitoses per 10HPF) now defines anaplastic PXA (WHO grade III). Necrosis is frequent in anaplastic tumors, but its significance independently of mitotic activity remains indeterminate. Similar to other LEATs, PXA frequently shows BRAF V600E mutation (near 60%), which is more common in low-grade (WHO grade II) than high (WHO grade III) and associated with favorable outcomes, although it is unclear whether BRAF V600E is prognostically independent of tumor grade. CDKN2A/B homozygous deletion is present with equal frequency (near 90%) in classic (WHO grade II) and anaplastic (WHO grade III) tumors. Additional copy number alterations, including whole chromosomes or whole arm gains, losses and copy-neutral loss of heterozygosity, are frequent. These findings were confirmed in a recent study in which the authors found TERT to be the third most commonly altered gene in anaplastic PXA (alterations including gene amplification and mutations), and suggested its association with anaplastic transformation.

S10-4

Brain somatic mutations in MTOR and BRAF leading to intractable focal epilepsy

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Mutations occur during cell division in all somatic lineages due to the unavoidable DNA replication errors. Because neural stem cells continue to undergo cell division throughout human life, somatic mutations in human brain can arise during development and accumulate with the aging process. Although somatic diversity is an evident feature of the brain, the extent of somatic mutations affecting the neuronal structure and function and their contribution to neurological disorders remain largely unexplored. Recently, we have provided the molecular genetic evidence that brain somatic mutations indeed lead to intractable focal epilepsy. In this symposium, I will present our recent findings regarding brain somatic mutations as potential molecular lesions underlying intractable focal epilepsy, thereby providing a new insight into the molecular pathogenesis and therapeutics for the related disorders.

S10-5

New prospects for the classification of pediatric low-grade gliomas and glioneuronal tumors

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Neural tumors presenting in childhood often differ from their counterparts in adult patients. This is true of low-grade diffuse gliomas, IDH-wildtype / H3-wildtype tumors that have a distinct genetic profile and biologic behavior when presenting in children or adults. Attempts to delineate such tumors by molecular criteria need to account for the complex overlap between histologic and genetic features across the range of low-grade gliomas and glioneuronal tumors that present in children and young adults.

S11-1

Pathological and biochemical changes of tissue deposited amyloid protein by liver transplantation in hereditary ATTR amyloidosis patients

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Hereditary ATTR amyloidosis (familial amyloid polyneuropathy, FAP) is one of the most prevalent systemic amyloidoses caused by mutation of the transthyretin (TTR) gene. Several systemic organs are affected by amyloid accumulation, including the peripheral nerves, heart, eye, and GI tract. As the majority of TTR (over 95%) in the body is produced in the liver, therapeutic liver transplantation has been performed since 1990. While this increased survival paradoxical amyloid deposition occurred in other several organs, including the heart, eye, and central nervous systems (CNS), even after liver transplantation. Our previous study revealed that progression of amyloid cardiomyopathy was due to paradoxical post-transplant wild-type TTR deposition produced by the transplanted liver. Amyloid oculopathy and cerebral amyloid angiopathy are serious de novo complications in liver-transplant patients with FAP. However, the precise pathomechanism of post-transplantation ocular and CNS amyloid formation is not fully understood. Recently, we biochemically investigated amyloid fibril proteins using laser-microdissection and LC-MS/MS and evaluated the composition proportions of wild-type and variant TTR in amyloid fibrils. In this symposium, we describe the difference in biochemical amyloid proportions and the pathogenesis of amyloid formation between cardiac and ocular/CNS amyloids. Unfortunately, our results suggest that amyloid oculopathy and cerebral amyloid angiopathy cannot be prevented by liver transplantation and will therefore, require other therapeutic options.

S11-2

Diverse spectrum of hereditary ATTR amyloidosis: polyneuropathy, cardiomyopathy, and cerebral amyloid angiopathy

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Hereditary transthyretin (ATTRm) amyloidosis (familial amyloid polyneuropathy: FAP) is a life-threatening, systemic amyloidosis caused by mutant transthyretin (TTR). In addition to ATTR V30M in endemic and non-endemic areas, more than 140 non-V30M mutations occur worldwide. The clinical manifestations include length-dependent sensorimotor polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC), and familial oculoleptomeningeal amyloidosis (LMA)/hereditary cerebral amyloid angiopathy (CAA), with various degrees of amyloidogenesis and patterns of amyloid deposition. Patients with ATTR V30M in endemic areas shows autonomic-sensorimotor polyneuropathy at a relatively young age (<50 years old), and patients with ATTR V30M in non-endemic areas demonstrate large-fiber polyneuropathy relatively late. Patients with non-V30M ATTR shows FAP, FAC, or LMS/CAA. Since mutated TTR is mainly produced by the liver, liver transplantation has become an acceptable treatment for patients with ATTR V30M of early onset. Patients with ATTR Y114C develop LMA and CAA presenting fatal cerebral hemorrhage and rapidly progressive dementia. Postmortem examinations revealed that TTR amyloid deposition in the leptomeninges and the blood vessels of the cerebral parenchyma in the transplant patients were tend to be milder compared with those in the non-transplant patients. However, some patients with ATTR Y114C developed slowly progressive dementia even after liver transplantations. Liver transplantation is partially effective for CNS manifestations of CAA associated with ATTR Y114C reducing mutated TTR in the blood. However, continuing amyloid fibril formation from TTR produced by the choroid plexus and/or wild-type TTR produced by the transplanted liver might have caused the slowly progression of CAA after liver transplantations.

Pathology of familial amyloid polyneuropathy

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Hereditary (variant) transthyretin (ATTRv) amyloidosis, traditionally referred to as familial amyloid polyneuropathy (FAP), is a disease in which systemic deposition of amyloidogenic mutant TTR protein causes multi-organ failure. Although Val30Met is the most common mutation in FAP, over 130 other mutations have been reported thus far. The dissociation of TTR tetramers is a crucial step in the formation of amyloid fibrils. The process of amyloid formation in FAP has been well investigated *in vitro*, and a therapeutic strategy to stabilize TTR tetramers in the plasma is now available in clinical practice. However, the *in vivo* mechanisms of tissue damage resulting from mutant TTR have not been fully elucidated. Although some studies highlight the toxicity of pre-fibrillar TTR (i.e., the precursor of amyloid fibrils), the amyloid deposits are more widely believed to exert harmful effects on neighboring tissues. Previous studies have demonstrated differences in the morphology of amyloid fibrils in FAP patients with the Val30Met mutation depending on the age at onset. In Japanese FAP patients with the Val30Met mutation, long and thick amyloid fibrils are common in early-onset cases from endemic foci, whereas the fibrils are usually short and thin in late-onset cases from non-endemic areas. As the neuropathic features and modality of the nerve fiber loss in these two forms of FAP are distinct, the difference in the morphology of the amyloid fibrils may be strongly related to the mechanisms of its neuropathy.

S11-4

Transthyretin Amyloidosis (ATTR)

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Transthyretin amyloidosis (ATTR) was originally called Familial Amyloidotic Polyneuropathy (FAP) and subsequent studies have focused on the pathology of the peripheral nervous system. ATTR, however, is more than that. It is a systemic disease with life threatening manifestations throughout all organ systems including the peripheral nerves, autonomic nerves, CNS, and eye as well as the heart and gastrointestinal tract. Until recently there has been no specific therapy for treatment of ATTR except liver transplantation for patients with hereditary ATTR (FAP). Unfortunately ATTR can progress after liver transplantation due to synthesis of amyloid from wild type TTR. Now a phase 3 study testing a transthyretin specific antisense oligonucleotide for the treatment of patients with ATTR has shown very significant inhibition of disease progression. A study with siRNA to TTR, which is based on the same principle of inhibiting liver TTR synthesis, has also shown significant inhibition of ATTR progression. In addition to the studies on ATTR neuropathy we are now conducting an open label ASO treatment of ATTR cardiomyopathy patients, both hereditary and wild type, to determine safety and tolerability of the ASO in patients with advanced cardiac failure. To date, results have been encouraging with no serious safety issues and with efficacy parameters indicating significant inhibition of progression of cardiomyopathy over a time span from 1 to 4 years.

Overview of protein propagation in neurodegeneration

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Intracellular accumulation of abnormal proteins is the features of many neurodegenerative diseases, including Alzheimer's disease, Lewy body disease and frontotemporal lobar degeneration (FTLD). Recently, these diseases are pathologically classified by the major accumulated proteins such as tau, alpha-synuclein and TDP-43, which are collectively referred to as tauopathy, synucleinopathy and TDP-43 proteinopathy, respectively. Abnormal phosphorylation, ubiquitination, and proteolytic cleavage are the common pathologic signature of these proteins accumulated in diseased brains. Especially, the band patterns of C-terminal fragments of tau and TDP-43 on immunoblotting are associated with the clinicopathological features of FTLD-tau or FTLD-TDP. Recent research results of the biochemical analyses of the diseased brains and the cellular models suggest that different strains of these proteins with different conformations may determine the clinicopathological phenotypes of these proteinopathies, like prion disease. Detecting each protein strain in biological fluids may be useful for the differential diagnosis of proteinopathies. Furthermore, elucidating the mechanism of the conformational changes leading to the formation of multiple protein strains may be important for developing disease-modifying therapy for these diseases. For instance, therapeutic strategies targeting the cell to cell propagation of abnormal proteins using antibodies may be useful as novel disease-modifying therapy of proteinopathies.

S12-2

Histopathologic Subtypes of FTLD-TDP: Implications for propagation in neurodegeneration

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Most cases of FTLD-TDP can be subtyped into one of several histopathologic patterns based on the morphology and distribution of TDP-43 inclusions. The FTLD-TDP subtypes strongly correlate with several clinical and genetic features of disease. We recently described a series of FTLD-TDP cases which were difficult to classify based on existing criteria. TDP-43 aggregates were found in a wide neuroanatomic distribution consisting of ubiquitin-negative granulofilamentous neuronal inclusions and abundant neuropil grains. Clinically, these cases were associated with very rapid clinical courses with disease duration within three years of symptom onset. We propose that these cases represent a new subtype of FTLD-TDP (type E). Ongoing work reveals that human FTLD-TDP brain derived lysates induce TDP-43 pathology when microinjected into TDP-43 transgenic mice brains, supporting the protein propagation hypothesis for neurodegenerative diseases. The relationship between histopathologic FTLD-TDP subtypes and induction of TDP-43 pathology in experimental models will be discussed.

S12-3

Systemic propagation of alpha- synuclein in the human body

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Aggregated alpha-synuclein (aSyn) in characteristic Lewy bodies and Lewy neurites are the defining neuropathological characteristics of Parkinson's disease, Parkinson's disease with dementia, and dementia with Lewy bodies, so-called Lewy body disease. Recent studies revealed that Lewy related pathology appears not only in the central nervous system but also in the peripheral nervous system especially the autonomic nervous system and enteric nervous system. aSyn represents the primary pathology rather than a consequence on neurodegeneration. Therefore, in order to elucidate the spread of aSyn, it is quite important to investigate the whole body of a Parkinson's disease patient pathologically. We investigated the occurrence frequency and distribution of aggregated aSyn related pathology using the consecutive autopsy patients from the Brain Bank for Ageing Research. Based on the latest our findings and knowledge obtained from animal experiments and human studies about propagation of aSyn, we provide there are several progression pathways such as a pathway from the olfactory to the amygdala.

S12-4

Prion-like propagation of pathological tau in neurodegenerative diseases

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Abnormal tau pathologies are the defining features of many neurodegenerative diseases including Alzheimer's disease (AD), Pick's disease (PiD), corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP). Immunocytochemical and biochemical studies revealed widespread phosphorylated tau pathologies in these diseases, and the distributions and spread of these abnormal tau were closely correlated with clinical presentation and disease progression. In adult human brains, six tau isoforms are expressed, and they are classified to four-repeat (4R) tau and three-repeat (3R) tau. Tau is a highly soluble, natively unfolded protein, however, in these diseased brains, it is accumulated as filamentous inclusions in abnormally phosphorylated and partially ubiquitinated states. It is demonstrated that the conformations of the abnormal tau in these diseases are distinct between the diseases. Recent studies also have shown pathological tau proteins from brains of patients may have a prion-like activity that can convert normal tau into an abnormal form. Therefore, we prepared synthetic tau fibrils and sarkosyl-insoluble pellets of tauopathy brains and investigated the prion-like seeding activities using culture cell models and injection to wild-type mouse brains. Both synthetic tau fibrils and sarkosyl pellets of diseased brains showed prion-like seeding activities in these cellular and animal models. The results suggest that pathological fibrillar forms of tau have prion-like properties and propagate from cell to cell in brains of patients.

Epidemiology of clinically-diagnosed dementia in a Japanese community: the Hisayama study

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A population based prospective cohort study of dementia has been conducted in an elderly population of the town of Hisayama in the southern part of Japan since 1985.

To examine secular trends in the prevalence of clinically-diagnosed dementia and its subtypes, five cross-sectional surveys were conducted among Hisayama residents aged 65 years or older in 1985, 1992, 1998, 2005, and 2012. We conducted a two-stage screening survey of dementia at each examination, and the diagnosis of dementia was made clinically based on the DSM-III criteria in 1985 and the DSM-III-R in 1992, 1998, 2005, and 2012. The age- and sex-adjusted prevalence of all-cause dementia increased significantly from 1985 to 2012. With regard to subtypes of dementia, a similar significant trend was observed for Alzheimer's disease (AD), while the prevalence of vascular dementia (VaD) did not change with time.

In a prospective study of risk factors for clinically-diagnosed dementia conducted in Hisayama elder residents without dementia, diabetes and blood pressure variability in late-life were a significant risk factor for the development of all-cause dementia, AD, and VaD. In addition, smokers in both midlife and late-life had significantly greater risks of AD and VaD than non-smoker.

In addition to the rapid aging of the population, secular changes in risk factors may be partly responsible for these increasing trends in the prevalence of dementia, especially AD, in the Japanese elderly.

Trends in dementia prevalence over 31 years of the Hisayama study

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Introduction: In Japan, dementia has become a serious social problem. The Hisayama study is a prospective cohort study of lifestyle-related diseases including dementia that was started in 1961. In the population-based study, it has been reported that all-cause dementia and Alzheimer's disease (AD) significantly increased in recent years. *Methods:* The aim of this study is to clarify the trends in dementia using data from 1371 autopsies performed over the past 31 years (1986-2016). Throughout the whole period, the autopsy rate is about 75%. We divided the 1371 autopsy samples into five groups according to the year of death: I (1986-1991, 257 cases), II (1992-1997, 268 cases), III (1998-2004, 318 cases), IV (2005-2011, 296 cases), and V (2012-2016, 232 cases). In addition, we investigated the increases in brain pathology related to AD using automated morphometric analyses for quantifying tau pathology. *Results:* The prevalence of all-cause and AD significantly increased. A significant increasing trend was observed in both men and women. A rapid increase in senile dementia of the NFT type (SD-NFT) in recent years was notable. The morphometric analyses revealed a significant increasing of tau pathology in recent years. The significance was also observed regardless of the senile plaques. *Conclusion:* We revealed a recent trend of increased tauopathy such as AD and SD-NFT, which is partly independent of amyloid-beta pathology. Although aging is considered one of the important risk factors accelerating tau pathology, there could be other risk factors associated with lifestyle diseases.

S13-3

Independence and interdependence of neuritic amyloid plaques, neurofibrillary tangles, striatal A-beta, hippocampal abnormalities, and generalized brain atrophy as elements of Alzheimer's disease

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Concurrent neuritic amyloid plaques (NAP), neurofibrillary tangles (NFT), and generalized brain atrophy were recognized by Alzheimer himself in his reports of premature senility. While this triad remains central to diagnosis of Alzheimer's disease (AD) even today, the underlying mechanisms responsible for their mutual associations remain largely obscure. This uncertainty and the recognition of very high rates of multi-morbidity identifiable at autopsy represent challenges for correct diagnoses and recognition of pre-clinical disease during of life. Although an association of the ApoE epsilon 4 allele with the accumulation of neocortical neuritic plaques is well established, its independent associations with NFT, striatal amyloid, hippocampal and neocortical neuronal loss, and gliosis are less certain. Our analyses of data from the Honolulu-Asia Aging Study (Japanese-American men) and the Nun Study (Caucasian women) indicate that each of these elements appears to occur and influence cognitive impairment at least partially independently of the others. Nonetheless their full impact on cognition is apparent only when they are simultaneously present. Similar patterns of occurrence and associations with dementia are evident in both studies. These observations indicate a complex pathogenesis of AD and suggest the possible importance of interventions aimed simultaneously at the separate determinants which may exist for these three distinct neuropathologic elements.

S13-4

The Rush Community Studies of Aging with Autopsy - Highlights and Implications

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Rush Alzheimer's Disease Center / Neurology, Rush University Medical Center

BACKGROUND: The Rush Community Studies including the Religious Orders Study (ROS), Memory and Aging Project (MAP), and Minority Aging Research Study (MARS) are ongoing longitudinal clinicopathologic cohort studies of aging and Alzheimers disease (AD).

OBJECTIVES: To provide highlights and implications of the Rush community autopsy studies.

METHODS: Participants are older adults who enroll without dementia and agree to longitudinal clinical evaluations. Agreement to organ donation is required in ROS and MAP and is optional in MARS.

RESULTS: Rush clinicopathologic studies have shown that there is remarkable heterogeneity in brain pathologies of elders. AD pathology is rarely the sole pathology in elders with Alzheimers dementia while it is common in persons without cognitive impairment. Most persons with an Alzheimers dementia have AD pathology with additional neurodegenerative (lewy bodies, TDP43, hippocampal sclerosis) and, or vascular (infarcts, vessel diseases) pathologies. There are multiple individual combinations of these pathologies in any one person and overall these pathologies account for less than half of the variability of cognitive decline in aging. These and other data show it is imperative to study amyloid and tau but also to investigate multiple other pathologies. In addition to targeting individual pathologies for treatment, approaches should aim at upstream biologic factors, final common pathways, and mechanisms of resilience.

CONCLUSION: Longitudinal cliniconeuropathologic studies in community dwelling older persons have strongly contributed to a transformation in the current thinking of AD and related disorders, resulting in promising new research directions toward prevention, detection, and treatment.

S13-5

Overview of Japan semisupercentenarian study

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Center for supercentenarian medical research, Keio University School of Medicine

We are conducting centenarian study from 2000. At the initial phase, the target of our study is centenarian in Tokyo area (Tokyo Centenarian Study: TCS). Then we started longitudinal Japan semisupercentenarian study (JSS) from 2002 till now. The target of JSS was semisupercentenarian (105+; SSC) in whole area of Japan. Now target of JSS is shifting from SSC to supercentenarian (110+; SC). The object of centenarian study is, 1) to identify the factors associated with happy and healthy longevity, 2) to understand the phenotype of ultimate human aging. We use multidisciplinary approach for the study of centenarian, such as medical, genetic and psychological domain. The number of centenarian in Japan are increasing more than exponentially from 153 in 1963 to 67,824 in 2017. However, number of SC is only 146 in 2015. They are extremely rare (1 in 870,000 total population). ADL and cognitive function of SC at 100 years old is excellent compared with younger centenarians. And genetic make-up is also different from younger centenarians. Based on these findings, we think that SC is a real model of healthy longevity. In this presentation, we will briefly summarize the results of TCS and JSS. Then we will present characteristics of supercentenarian. Prof Takao will present the autopsy finding of SC. We think that SC study is fascinating and rewarding. We hope young scientists will actively participate SC study.

S13-6

Brain pathology of supercentenarians

Masaki Takao

International Medical Center, Saitama Medical University

Following the presentation by Professor Hirose, I would like to some information of brain pathology of supercentenarians. Since we had previously reported four autopsy cases of SC brains (*Acta Neuropathol Commun.* 2016; 97), we obtained three additional SC autopsy cases. All seven cases were of Japanese female and their mean age of death was 111.2 years old (111-114). The numbers of cases for each phase of Thal's A-beta were as follows: 1 in three, 2 in one, 3 in two and 4 in one cases; of neuritic plaque score (CERAD) were none in 2 and moderate in 5 cases and of Braak NFT stage were III in four and IV in three cases. There were four intermediate and three low level cases based on the NIA-AA criteria for AD. Three low level cases were consistent with PART possible (Braak NFT stage III or IV, A-beta Thal phase 1 or 2). Aging-related tau astroglipathy was consistently observed, particularly in the basal forebrain. Neither Lewy bodies nor glial cytoplasmic inclusion were observed. We believe that the neuropathologic changes of SC is not simple pre-pathologic condition of AD. Future studies of SC brains are needed to understand the process of aging.

S14-1

What's going on in the field of veterinary neuropathology

Hiroyuki Nakayama

Veterinary Pathology, The University of Tokyo

Rodent species such as mice and rats have been used as model animals to study human diseases. However, there are too much evolutionary distance between human and rodents. Ungulata such as cows, sheep, pigs and horses, and Carnivora such as dogs and cats, which are evolutionally located between human and rodents, can be used as model animals to fill in the gap of the missing link of revolution. We are studying neuropathology of nonhuman animals under the concept of evolutionary medicine. Some topics in the field of veterinary neuropathology like "Aging-related brain changes and neurodegenerative disorders in animals", "Possibly immune-mediated encephalomyelitis in dogs" and "Brain tumors in animals" will be introduced in the session.

S14-2

Encephalomyelitis in dogs

Kazuyuki Uchida

Veterinary Pathology, The University of Tokyo

In dogs, most typical encephalomyelitis is caused by canine distemper virus (CDV) infections characterized by subacute to chronic demyelinating encephalomyelitis. The pathologic features of CVD-associated encephalomyelitis are almost in conformity with those of human measles virus infections. Besides, necrotizing meningoencephalitis (NME) and granulomatous meningoencephalitis (GME) are idiopathic inflammatory diseases of the canine brains and are more commonly found in veterinary clinics in Japan. Typical NME occurs predominantly in Pug dogs. Recent molecular investigations have identified possible genetic risk factors for NME in Pug dogs. NME is characterized pathologically by necrotic lesions with lymphocytic infiltration in the meninges and perivascular spaces. On the basis of the distribution pattern of major necrotic foci, NME can be divided into cortex dominant and white matter dominant types; the latter is designated necrotizing leukoencephalitis (NLE) predominately found in Yorkshire terrier dogs. Lesions in GME are characterized by the accumulation of lymphocytes and macrophages with epithelioid morphology, forming granulomas around blood vessels. Some common genetic factors and/or some additional triggers, such as infection or vaccination, may play a role in the pathogenesis of NME, NLE and GME; however, the host immune responses may define the pathological phenotypes. Different cytokine and chemokine responses are seen in NME, NLE and GME, whilst autoantibodies against astrocytes are detected predominantly in NME. The pathological and immunological characteristics of these canine idiopathic inflammatory brain disorders are focused on the presentation.

S14-3

Aging-related brain changes and neurodegenerative disorders in animals

James K. Chambers

Veterinary Pathology, The University of Tokyo

Comparative neuropathology provides new insights on the pathogenesis of neurodegenerative diseases. As for age-related sporadic neurodegenerative diseases such as Alzheimer's disease, there are variations with the lesions that are observed in the brains of different animal species. Unlike other mammalian species, aged felids (domestic cat, leopard cat and cheetah) develop neurofibrillary tangles (NFT) comparable to that of human. Interestingly, these species do not form mature senile plaques, instead small granular amyloid-beta deposits are observed in individuals with NFT. Senile plaques and amyloid angiopathy are frequently observed in other mammalian species such as monkeys and dogs, although rodents do not show amyloid-beta deposits due to difference in amino acid sequence of amyloid-beta protein. Lesions associated with Parkinson's disease and amyotrophic lateral sclerosis (ALS) have not been widely examined in animal species. Mutation of the SOD1 gene has been identified in dogs with degenerative myelopathy that shows some similarity to ALS. As for inherited neurodegenerative diseases, counterparts of the human diseases have been found mostly in household pets. Mutation of the corresponding genes have been identified together with histological changes comparable to that of human disease. Here, we would like to share some of the pathological findings of animal cases of Lafora disease, Alexander disease, globoid cell leukodystrophy, neuroaxonal dystrophy and ceroid lipofuscinosis. For neurodegenerative diseases caused by alteration of a specific gene, non-rodent animal models may be valuable for future translational clinical trials.

S14-4

Brain tumors in animals

Takuya Evan Kishimoto, Kazuyuki Uchida, James K. Chambers, Hiroyuki Nakayama

Veterinary Pathology, The University of Tokyo

Primary brain tumors are relatively rare in domestic animal species with the exception of dogs and, to a lesser extent, of cats. The incidence of each tumor type differs among species, as well as among breeds or strains within a species. There is a relatively high incidence of meningiomas in cats, while gliomas are most prevalent in dogs. Within the canine species, short-nosed (brachycephalic) breeds such as Boxers and Bulldogs are predisposed toward gliomas, while long-nosed (dolichocephalic) breeds such as Collies and German shepherds tend to develop meningiomas. In addition, French bulldogs are the breed with a common onset of oligodendroglioma. In our study, over a half of glioma cases in dogs are observed in French bulldogs, and most of the French bulldog cases were anaplastic oligodendroglioma (over 90%). We have demonstrated histological and immunohistochemical characteristics of canine oligodendroglioma and have established a cell line from an original tumor. Canine oligodendroglioma bears striking similarities to its human counterpart. Moreover, as described in human, canine oligodendroglioma highly expresses oligodendrocyte precursor cell (OPC) markers such as nuclear transcription factors Olig2 and SOX10, platelet-derived growth factor receptor α (PDGFR α) and proteoglycan NG2. These results suggest that canine oligodendroglioma may be a suitable animal model for a comparative study on human oligodendroglioma.

Novel, improved grading system for IDH-mutant astrocytomas

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IDH-mutant astrocytomas comprise a wide range of malignancies, and patients' survival times vary from less than two years to over twenty years. The framework of grading was not updated in the WHO 2016 classification, and recent studies have demonstrated that the anticipated differences in survival between the IDH-mutant astrocytoma Grade2 and Grade3 have lost their significance. However, the idea of simply classifying such tumors exhibiting various survival times into two groups, such as glioblastoma and lower grade glioma, would be a clinically too rough classification. A new grading approach specialized in IDH-mutant astrocytoma is required. We undertook a comprehensive analysis assessing both morphological and molecular factors to establish a novel grading model associated with patients' outcome. A discovery cohort of 211 IDH-mutant astrocytomas was subjected to histological review, image analysis, and illumina 450k/850k array analysis. Most relevant for overall survival (OS) was CDKN2A/B homozygous deletion. Other parameters with major influence were necrosis and the total number of CNV. Importantly, tumors harboring CDKN2A/B homozygous deletion showed worse survival compared to the tumors harboring necrosis which is the hallmark pathological feature of glioblastoma. Employing CDKN2A/B homozygous deletion, necrosis and total number of CNV which were found to be most relevant for OS in our discovery set, we developed two grading models. The prognostic predictability of the models was validated in three independent cohorts of 108, 154 and 224 IDH-mutant astrocytomas. We propose that CDKN2A/B status, together with presence of necrosis, should be incorporated into a future grading framework for IDH-mutant astrocytomas.

A mathematical model for predicting the optimal timing of treatment to minimize the malignant transformation rate in WHO grade II diffuse glioma

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In WHO grade II diffuse gliomas (low-grade gliomas, hereafter called LGGs), chemotherapy and radiotherapy contribute to prolonged survival but could induce somatic mutations. The optimal timing of treatment in LGGs remain poorly understood. To delineate this, we propose a mathematical model for tumor growth and investigate the association among the treatment, the accumulation of somatic mutations, and malignant transformation (MT) in LGGs. Totally, 201 patients with LGGs between 1990 and 2014 were analyzed. We assessed the statuses of IDH mutation and 1p19q co-deletion in all tumors. Among all, 78 patients (39%) underwent MT during follow-up periods (mean: 78months). Tumor volume was evaluated with FLAIR and/or T2-weighted MR imaging. MT was evaluated with contrast-enhanced MRI and/or pathological diagnosis. Oligodendroglioma, IDH-mutant and 1p/19q-codeleted (OD) showed longer transformation-free survival compared to other subtypes. The growth rates and the risk of MT increased after surgery in OD and Diffuse astrocytoma, IDH-mutant (DA). They significantly decreased in the middle of chemotherapy but returned to the same as before when chemotherapy ended. Based on growth rate and the risk of MT, optimal timing of treatments was calculated for each genetic subtype. Early surgical resection could minimize the risk of MT only if the tumors were totally resected. Early radiotherapy and chemotherapy could reduce the risk of MT, if limited resection were performed in OD and DA, respectively. In other cases, adjuvant therapy could be waited. The mathematical model delineates the optimal timing of treatments in each subtype, which will help to decide the treatment for LGGs.

S15-3

Preoperative design of the treatment strategy for lower grade gliomas based on molecular diagnosis by imaging features

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In WHO2016, the lower grade gliomas (LrGGs) are classified based on IDH and 1p/19q status, and this classification correlates well with clinical outcomes. Since 2006, we have been treating the patients with LrGGs with 1p/19q codeletion or MGMT promoter methylation by upfront chemotherapy. The median volume change by chemotherapy in cases of initial incomplete resection (partial removal or biopsy) was -30-35% (SPD), and upfront chemotherapy enabled second-look resection to be performed following tumor volume decrease in many cases (J Neurooncol 124(1):127-35, 2015). However, molecular information relevant to treatment strategy is available only after tumor resection. If it is informed before initial surgery, such information could influence the initial surgical strategy as well as overall treatment strategy. To preoperatively predict tumor molecular status, we have established the scoring system to predict 1p/19q codeletion based on imaging features available on routine CT and MRI (Neurosurg Rev 2018 Apr 26). Quantitative texture analysis using commercially available software also showed high positive predictive value. Indeed, treatment strategy for some of the recent patients with 1p/19q-codeleted gliomas were preoperatively designed based on molecular diagnosis by imaging, including intentional staged operation and intentional placement of BCNU wafers. Molecular diagnosis based on imaging information would help to preoperatively design personalized treatment in patients with LrGGs.

PI3 kinase pathway activation to promote malignant progression in oligodendroglial tumor

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Oligodendroglioma (OD) is a subtype of diffuse astrocytic and oligodendroglial tumors. Although prognosis in OD tumors are initially favorable, majority of OD develop malignant transformation. Therefore, understanding of molecular mechanism is crucial to identify therapeutic target. However, there are few available patient-derived OD xenograft model, which limits preclinical investigations. Here, we present novel patient derived anaplastic oligodendroglioma (AOD) xenograft models. We have harvested two distinct cell samples with and without PIK3CA mutation. From PIK3CA mutant cells with rapid progression, we established xenograft model, while xenograft was not formed from PIK3CA wild-type cells, which was not developed malignant transformation. We confirmed AOD phenotype and the presence of IDH1 mutation and 1p/19q co-deletion in xenograft tissue, indicating recapitulating AOD model. We also tested to see if PI3K/AKT/mTOR gene mutation could induce patient-derived AOD xenograft formation. As expected, whereas no tertiary mutant tumor did not form xenograft, PI3K/AKT/mTOR mutant AOD cells formed xenograft. Also, we found such mutant tumor cells were vulnerable to alkylating agents and PIK/AKT/mTOR pathway inhibitors. These findings suggested the critical role of PI3K/AKT/mTOR pathway activation for xenograft formation in oligodendroglial tumors. Our xenograft models may allow figure out detailed mechanism for malignant transformation, which will contribute to identify optimal therapeutic strategy.

7-tesla MR susceptibility-weighted imaging can depict astrocytic and oligodendroglial pathology

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【Background】 There are very few reports of ultrahigh field 7-tesla magnetic resonance susceptibility weighted imaging (7T-SWI) used in the diagnosis of brain tumors. In the present study, we performed 7T-SWI on glioma patients to distinguish between astrocytic and oligodendroglial tumors. **【Methods】** Sixteen patients pathologically diagnosed as gliomas who underwent presurgical 7T-MR imaging were included. We used a 7T MR imager (General Electric) and obtained high resolution T2-weighted two-dimensional Fast Spin Echo (FSE) images and T2* weighted, two dimensional gradient echo (2D-GRE) images. SWI post-processing was performed using in-house software written in MATLAB (Math Works, Natick, MA, USA). The gliomas were pathologically diagnosed according to the 2016 WHO classification, and IDH mutations were detected by IDH1 R132H as well as Sanger sequencing and 1p/19 LOH was detected by Multiplex Ligation-dependent Probe Amplification (MLPA) analysis. SWI images were scored as astrocytic-like or oligodendroglial-like based on scoring system accounting for cortical thickening and shift of medullary vessels (minimum -2 to maximum +2). **【Results】** Ten cases were diagnosed as astrocytomas (WHO grade 2 to 4) and 6 cases as oligodendrogliomas (WHO grade 2 or 3). Cortical thickening and shift of medullary vessels were seen in oligodendrogliomas, whereas medullary vessels were not shifted in astrocytomas, suggesting an infiltrative nature. Scoring was significantly elevated in oligodendrogliomas ($p= 0.0057$; astrocytomas mean -0.9 vs. oligodendrogliomas 1.5). Microbleeding, necrosis, and thickening of medullary veins were seen in malignant cases. **【Conclusion】** 7T-SWI could reliably distinguish between astrocytomas and oligodendrogliomas and predict malignancy. SWI accurately reflected the vascular morphology of gliomas.

S16-1

Arizona Study of Aging and Neurodegenerative Disorders and Brain and Body Donation Program 1988-2018

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The Arizona Study of Aging and Neurodegenerative Disorders (AZSAND) is a longitudinal clinicopathological study focused on normal aging, Alzheimer's disease and Parkinson's disease. Autopsies are performed as part of the Brain and Body Donation Program (BBDP) at Banner Sun Health Research Institute has been in operation for 30 years, with more than 1,900 autopsies performed (brainandbodydonationprogram.org). Autopsies and tissue banking were limited to the brain until 2005 but since then more than 630 whole-body donations have been received. Most Program subjects are enrolled as cognitively normal volunteers residing in the retirement communities of metropolitan Phoenix, Arizona. The median age at death is 83, with 376 that died over age 90 and 27 that were centenarians. Subjects receive standardized general medical, neurological, behavioral, neuropsychological and movement disorders assessments annually during life and more than 90% receive full pathological examinations by medically licensed pathologists after death. The Program has been funded through a combination of internal, federal and state of Arizona grants as well as user fees and pharmaceutical industry collaborations. Subsets of the Program are utilized by the US National Institute on Aging Arizona Alzheimer's Disease Core Center and substantial funding has also been received from the Michael J. Fox Foundation for Parkinson's Research. The Program has made rapid autopsy a priority, with a 3 hour median postmortem interval for the entire collection. The median RNA Integrity Number (RIN) for frozen brain and body tissue is 8.9 and 7.4, respectively. More than 3100 tissue requests have been served and currently about 200 are served annually. These requests have been made by more than 500 investigators located in 38 US states and 23 countries.

S16-2

The Netherlands Brain Bank

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The Netherlands Brain Bank (NBB) is a professional brain bank with extensive prospective donor programs that provides brain tissue from donors with neurological and psychiatric diseases and non-diseased controls on an open access basis for research worldwide. Annually, the NBB performs 120-160 autopsies with impressive short post mortem delays (mean 6.5 h) of donors that gave informed consent for the use of their brain and clinical files for research, in some cases supplemented with post mortem MRI. Since its start in 1985, the NBB has provided tissue from more than 4300 autopsies of donors with neurological and psychiatric diseases and controls to 700 research projects all over the world, resulting in more than 1700 scientific publications. Currently, the NBB has over 4800 registered donors. As partner of BrainNet Europe, a European consortium of brain banks, the NBB drafted an ethical Code of Conduct for Brain Banking. The sources for this Code of Conduct were laws, regulations and guidelines (Declarations, Conventions, Recommendations, Guidelines and Directives) issued by international key organizations, such as the United Nations, Council of Europe, European Commission, World Medical Association and World Health Organization. The Code of Conduct addresses fundamental topics, such as the rights of the persons donating their tissue, the obligations of the brain bank with regard to respect and observance of such rights, informed consent, confidentiality, protection of personal data, collections of human biological material and their management, and transparency and accountability within the organization of a brain bank. The Code of Conduct was ratified by all European brain banks in 2009.

S16-3

To establish all- Japan Brain Bank Network

Yuko Saito^{1,2,3}

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³The Japanese Brain Bank Network for Neuroscience Research (JBBNNR)

National Center of Neurology and Psychiatry (NCNP) is based on Ministry of Health, Labor and Welfare, Japan and its mission is for cure of intractable neurological and psychiatry disorders.

For this mission, NCNP has been trying to establish all- Japan brain bank network. NCNP established Research Resource Network of National Hospital Organization in 1997, followed by the brain bank network with brain donation program of Parkinson disease in 2006, expanding to intractable neurological disorders in 2014 and then, in- house psychiatric disorders in 2017.

In collaboration with NCNP, the Brain Bank for Aging Research (BBAR), Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology is playing a promoting role of brain banking in Japan and a core of the Japanese Brain Bank Network of Neuroscience Research funded by Ministry of Education, Culture, Sports, Science and Technology, Japan. The Psychiatric Bank of Fukushima Prefecture Hospital is recruiting brain donors of psychiatric disorders nationwide.

In 2016, Japan Agency for Medical Research and Development funded Japanese Brain Bank Net as NCNP Brain Bank as the core, focused on psychiatric disorders as well neurological disorders.

All brain bank representatives in Japan are members of the Brain Bank Committee, the Japanese Society of Neuropathology, which was established in 1986 and cultivate the consensus of detailed neuropathological studies.

We are now trying to develop our system to NPO- PO collaboration level as in U.S. and U.K. This symposium will be the starting point.

the Japanese Brain Bank Net: NCNP, Brain Research Institute, Niigata University; Aging Medical Institute, Aichi Medical University; Department of Psychiatry, Nagoya University; Department of Psychiatry, Okayama University; Tokyo Metropolitan Matsuzawa Hospital; Psychiatric Brain Bank, Fukushima Prefectural University (PBBF); BBAR
the Japanese Brain Bank Network of Neuroscience Research: BBAR, NCNP, Mihara Institute of Cardiovascular Disorders Brain Bank, Fukushima Brain Bank, PBBF

S16-4

Limitations of routine immunopathologic diagnoses in neurodegenerative diseases of the aging brain: implementing the added value of brain/biobanking (frozen-brain biochemistry, genetics, CSF, blood, other biofluids)

Colin Louis Masters

The Florey Institute, The University of Melbourne

The time is fast approaching when the standard morphological approach to neuropathologic diagnoses is no longer adequate. In unravelling the comorbidities of the aging brain, the diagnostic attributions of Alzheimer's disease (AD), small and large vessel vascular disease, diffuse Lewy body (DLB) disease, hippocampal sclerosis (HS) and the frontotemporal dementias (FTD) require a greater precision than can be achieved by morphologic examination alone.

The clinician who brings to the autopsy table the results of a full clinical history (including family history and neuropsychometrics on cognition and behavior), neuroimaging (3T MRI), molecular PET imaging ($A\beta$, tau, α -synuclein, and markers of synaptic integrity such as synaptophysin, neurogranin, SNAP-25, NfL etc.), plasma/serum biomarkers of neurodegeneration and vascular disease, and somatic and germline DNA sequences, must work hand-in-glove (so to speak) with the neuropathologist, who in addition to the routine morphometric approach to $A\beta$, tau, α -synuclein, TDP-43, and other determinants of neurodegenerative processes, will facilitate the quantitative estimation of these molecular entities from post-mortem frozen brain samples.

The technologies to achieve this holistic approach are now available. The challenge lies in implementation.

S17-1

Response assessment of bevacizumab for glioblastoma: Comparison between PET and pathological studies

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【Introduction】 MRI is the morphological imaging modality; however, it creates confusion about the efficacy of bevacizumab therapy. PET uses radiotracers to attain metabolic and molecular imaging; especially ¹¹C-methionine (MET) and ¹⁸F-fluoromisonidazole (FMISO). PET possibly provide precise evaluation of bevacizumab therapy. This study aims to assess MET and FMISO PET studies to evaluate pathological examinations induced by bevacizumab therapy for glioblastoma. **【Methods】** Between July 2013 and December 2017, seven patients with glioblastoma were treated by resection after bevacizumab therapy (biweekly with bevacizumab; dose, 10 mg/kg). Based on the MET and FMISO accumulation, tumor specimens were resected and assessed for VEGF and HIF-1 α expression levels. MET with a tumor-to-normal (T/N) ratio cut-off of >3 and the FMISO with a tumor-to-blood (T/B) ratio cut-off of >1.6 were associated with metabolically active tumor cells. Furthermore, we assessed sensitivities and positive predictive values (PPVs) for the evaluation of the presence of tumor and the accumulation of PET studies. **【Results】** MET accumulation correlated with the presence of a tumor (sensitivity, 88.5%; PPV, 50%; p =0.022). FMISO accumulation correlated with the presence of a tumor (sensitivity, 91.9%; PPV, 73.9%; p<0.001). Pathological studies revealed that high expression of VEGF and HIF-1 α were observed in tumors with high accumulation of MET and FMISO. Nonactive tumor was detected in regions with low FMISO T/B ratio. **【Conclusions】** Assumedly, high accumulation regions of MET and FMISO correlate with resistance to bevacizumab therapy, and MET and FMISO could be potential biomarkers for the assessment of pathological examination of glioblastoma during bevacizumab therapy.

S17-2

Definition of pathological total removal of glioblastoma multiforme

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【Background】 Glioblastoma multiforme (GBM) recurs within one year after microscopic total removal of the tumor. This suggests that microscopic total removal of GBM is totally different from pathological total removal. **【Methods】** 1. Expression of CD15 or CD133, which are stem cell markers, was investigated in cultured rat GBM C6 cells. 2. In GBM patients, expression of CD15 or CD133 was examined in the tumor tissue and in the normal brain tissue of 1cm and 2cm away from the pathological tumor border. **【Results】** No CD133 positive C6 cells were identified; however, a CD15-positive cell was confirmed 3 days after initiating cell culture. Ratio of CD15-positive cells to negative cells was constant during culturing, and this ratio was also constant even after temozolomide treatment. In 6 human GBM cases, CD15 or a CD133-positive cells were identified in 1cm away from the tumor border in one case, 1.5cm in two cases, and 2cm in three cases. **【Conclusions】** Based on the C6 cell culture study, it is obvious that some mature GBM cells can differentiate to precursor cells. This result also suggests that precursor cells have already existed when GBM is diagnosed on MRI. The precursor cells are identified not only in tumor tissue but also in the histologically normal brain tissue of 2cm away from a tumor border. Therefore, to reach to the pathological total removal of GBM, normal brain tissue of 2cm away from a tumor border should be resected.

S17-3

Immune checkpoint molecules in high-grade gliomas in adults

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Programmed cell death 1 (PD-1) and its ligand, PD ligand-1 (PD-L1) are representative molecules of various immune checkpoint molecules, which are attracting attention in high-grade gliomas (HGGs). Here we review recent literatures including our papers regarding expression of PD-1/PD-L1 and other immune checkpoint molecules in HGGs. Glioblastoma (GBM) showed significantly stronger expression of PD-L1 compared to lower-grade gliomas. Moreover, PD-L1 expression was different among GBM subgroups (for example, increased expression of PD-L1 in mesenchymal tumor samples). Association between PD-L1 expressions and outcome of GBM patients is debatable. In our study, which investigated PD-1 expression in GBM specimen pairs at the initial stage and recurrent stage after RT plus TMZ therapy at our institute, an increase in the number of PD-1-positive cells in the latter specimens was associated with worse outcome after recurrence. Theoretically, tumor vaccine therapies induce up-regulation of immune checkpoint molecules in tumor tissues, which may also be a mechanism of this vaccine failure especially in the recurrent phase. Thus, expression of the immune checkpoint molecules including PD-L1 depend on grading and subtypes of gliomas and affected by treatment for glioma patients.

S17-4

Persistent restoration of the immunosuppressive tumor microenvironment in glioblastoma by bevacizumab

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【Background】 Although vascular endothelial growth factor (VEGF) promotes the immunosuppressive microenvironment, the efficacy of bevacizumab (Bev) on tumor immunity has not been fully investigated. **【Methods】** The present study used 47 glioblastoma tissues obtained at 3 different settings: tumors of initial resection (naive Bev group), tumors resected following Bev therapy (effective Bev group), and recurrent tumors after Bev therapy (refractory Bev group). The paired samples of the initial and post-Bev recurrent tumors from 9 patients were included. The expression of PD-1/PD-L1, CD3, CD8, Foxp3, CD163, and CD11b was analyzed by immunohistochemistry. **【Results】** The PD-L1+ tumor cells significantly decreased in the effective or refractory Bev group compared with the naive Bev group. PD-1+ T cells significantly decreased in the effective or refractory Bev group compared with the naive Bev group. The number of CD3+ and CD8+ T cell infiltration increased in the refractory Bev group compared with the naive Bev group. Both Foxp3+ Tregs and CD163+ TAMs significantly decreased in the effective or refractory Bev group compared with the naive Bev group. CD11b+ myeloid cell infiltration was decreased in the effective Bev group compared with the naive Bev group. These findings were largely confirmed by comparing paired initial and post-Bev recurrent tumors. **【Conclusions】** Bev restores the immunosuppressive tumor microenvironment in glioblastomas, and this effect persists during long-term Bev therapy.

S17-5

Clinical experience of symptomatic epilepsy in patients with high grade gliomas

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【Background】 Brain tumor-related epilepsy (BTRE) has a tendency to appear in low-grade tumors more frequently than high-grade tumors in their clinical courses. Here, we report clinical cases of BTRE complicated with high-grade gliomas, and treated with combination therapy of antiepileptic drugs.

【Case 1】 A 68-yo male suffered status epilepticus initially, and a right frontal tumor was pointed out. Levetiracetam (1,000mg/day) was administered, and perampanel (4mg/day) was added after the second seizure. The tumor was resected surgically, and diagnosed as anaplastic oligodendroglioma, grade 3.

【Case 2】 A 39-yo female suffered a generalized epileptic seizure initially, resulting in diagnosis of anaplastic astrocytoma of her left frontal lobe. Valproic acid (600mg/day) and zonisamide (300mg/day) were administered. Eight years later, an epileptic seizure took place again, and recurrence of left frontal tumor was pointed out. Perampanel (4mg/day) was added. The tumor was diagnosed as secondary glioblastoma multiforme, grade 4, after the second surgery.

【Case 3】 A 68-yo female suffered status epilepticus initially, and treated with levetiracetam (2,000mg/day) for the diagnosis of symptomatic epilepsy caused by right frontal encephalitis. Ten months later, a right frontal tumor was pointed out, triggered by recurrent epileptic seizures. Perampanel (4mg/day) was added. The tumor was resected surgically, and diagnosed as glioblastoma multiforme, grade 4.

【Discussion】 BTRE in the present three cases of high grade gliomas were controlled successfully by the combination therapy with perampanel, which is a selective AMPA-type glutamate receptor antagonist, and other representative drugs of presynaptic sodium channel or calcium channel inhibitors and GABA receptor agonists.

S17-6

Vessel mimicry as a target of antiangiogenic therapy for glioblastoma

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【Purposes】 Vessel mimicry is an important target of resistance to anti-angiogenic therapy for solid tumors. In this study, the frequency and the treatment of vessel mimicry with glioblastoma were investigated. **【Methods】** 14 cases of glioblastoma sections were double-stained immunofluorescently with type IV collagen and CD34. The number of vessel mimicry (collagen+ / CD34- tubular structure) was counted and the frequency was calculated from total vessel number. Chetomin, a HIF-1 α inhibitor, was applied for U87 glioblastoma mimicry model in vitro and in vivo intracerebral model. **【Results】** Vessel mimicry was observed in all cases and 4.2% (range 2.0-5.7%) of tumor vessels. Chetomin (10nM) strongly inhibited U87 vessel mimicry in vitro (13.7% under normoxia and 33.3% under hypoxia). U87 intracerebral tumor size 28 days after implantation was similar with /without chetomin administration. Vessel density and vessel mimicry was under investigation. **【Conclusion】** Vessel mimicry was observed in 4.2% of glioblastoma vessels. Chetomin inhibited glioma vessel mimicry in vitro, but not inhibited tumor growth in vivo in the brain. Vessel mimicry is a treatment target of anti-angiogenic therapy of glioblastoma.

The ligand dependent EphB4 signaling is anchoring signaling in glioma

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【Introduction】 Despite the standard treatment of surgical resection of the glioblastoma followed by radiation and chemotherapy, patient survival remains a challenge. Extensive evidence implicates the Eph receptor family of tyrosine kinases and its ligand, ephrin, in glioma malignancy. The role of Eph-ephrin tyrosine kinase is gradually cleared. However, EphB4 of which ligand is ephrinB2 has not been investigated in glioma yet. We sought to reveal the role of EphB4 in glioma. **【Materials & methods】** The expression of EphB4 receptor was assessed in glioma cell lines by western blotting. The consequences of EphB4 activation by its ligand, ephrin-B2 were determined by migration and invasion assay. The alteration of signaling pathways induced by EphB4 activation was investigated by western blotting. Immunofluorescence staining was performed to detect the localization of EphB4 and ephrin-B2 expressing cells. **【Results】** EphB4 was highly expressed in U87 and SNB19 glioma cell lines and phosphorylated by ephrin-B2. EphB4 phosphorylation by ephrin-B2 suppressed migration and invasion in U87 and SNB19 and downregulation of EphB4 using small interfering RNA negated the suppression of migration and invasion induced by ephrin-B2. Stimulation of glioma cells with ephrin-B2 reduced the phosphorylated Akt levels dose dependently, which was abrogated by siRNA for EphB4. EphB4-positive cells existed only at the tumor core, whereas Ephrin-B2-positive cells existed both at invasive area and the tumor core. **【Conclusions】** Ligand dependent EphB4 signaling plays a role as stay there signaling. Extinction of its signaling promotes the release of tumor cell on its location.

ICOSLG-mediated IL-10 producing regulatory T cell expansion promotes progression of glioblastoma multiforme

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【Background】 Inducible T-cell co-stimulator ligand (ICOSLG) is a member of B7 family immune-regulatory ligands, expression of which in cancer is implicated in disease progression by regulating anti-tumor adaptive immunity. Although aberrant ICOSLG expression has been reported in glioma cells, underlying mechanisms promoting glioblastoma (GBM) progression remains elusive. **【Methods】** In the present study, we investigated a causal role of ICOSLG in GBM progression by analyzing ICOSLG expression in both human glioma tissues and the patient-derived GBM sphere cells (GSCs), and further dissecting its immune modulatory effects and the underlying molecular mechanisms. **【Results】** Bioinformatics analysis and GBM tissue microarray showed that upregulation of ICOSLG expression is associated with poor prognosis in patients with GBM. Results of our study further indicated that ICOSLG expression was upregulated preferentially in mesenchymal (MES) GSCs but not in proneural (PN) GSCs in a TNF- α /NF- κ B-dependent manner. Furthermore, ICOSLG expression of MES GSCs promoted IL-10 producing CD4⁺ Foxp3⁺ regulatory T cell expansion. Knockdown of ICOSLG gene markedly reduced GBM tumor growth in immune competent mice, with a concomitant downregulation of IL-10 levels in tumor microenvironment. Moreover, knock down of ICOSLG and ICOS-Fc treatment prolonged the survival of immunocompromised mice bearing GSC-derived mesenchymal GBM-like tumor. Knockdown of ICOSLG decreased CD44 and vimentin in tumor. **【Conclusion】** Collectively, our results indicated that inhibition of the ICOSLG-ICOS axis in GBM may provide a promising immunotherapeutic approach

S18-3**Olig2 positive Oligodendrocytes lineage cells induce chemo-radioresistant characteristics at the tumor border in glioblastomas**

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Glioblastoma (GBM) usually develops in adult brain white matter. Even after complete resection, GBM recurs around the tumor removal cavity, where GBM cells acquire chemo-radioresistance and survive. Characterization of the tumor border microenvironment is critical for improving prognosis in patients with GBM. Here, we compared microRNA (miRNA) expression in samples from the tumor, tumor border, and peripheral region far from tumor mass by miRNA microarray. The top three miRNAs showing higher expression in the tumor border were related to oligodendrocyte differentiation, and pathological oligodendrocyte lineage cells increased in the border. In 19 consecutive GBM samples that contained border tissues, Olig2⁺ cells were characteristically observed in the border region in all GBMs. From the results of Olig2 staining, GBMs were classified into two groups (high and low) based on the rate of Olig2⁺ cells in the tumor. Olig2^{high} cases (n = 10) showed many positive cells in the tumor and border, whereas Olig2^{low} cases (n = 9) showed few positive cells in the tumor, but many cells in the border. Cells positive for other oligodendrocyte lineage markers, such as O4, NG2, MBP, were also consistently found in the border region of Olig2^{high} and Olig2^{low} samples. These data suggest that most Olig2⁺ cells in the border region were OLCs. Medium cultured with oligodendrocyte progenitor cells (OPCs) induced stemness and chemo-radioresistance in GBM cells, similar to that produced by FGF1, EGF. Thus, OPCs may form a glioma stem cell niche at the tumor border.

S18-4**Detailed analysis of mutation change after treatment in glioblastoma**

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【Introduction】 Glioblastoma (GBM) is poor-prognosis cancer and tumor recurrence is inevitable despite intensive chemoradiotherapy. Thus, insights into the mechanism of tumor recurrence are critical for improved patient treatment. Here, we describe our integrated genomic analyses using next-generation sequencing of the paired samples from initial and recurrent tumors. **【Methods】** We collected paired GBM samples in patients who recurred after temozolomide (TMZ) treatment. Mutation analysis of the cancer-related genes was performed using Ion Ampliseq Cancer Hotspot Panel v2. In addition, *MGMT* promoter methylation and expression of mismatch repair (MMR) protein such as MLH1, MSH2, MSH6, and PMS2 were analyzed by pyrosequencing and Western blotting, respectively. **【Results】** Sixty tumor samples from 29 patients were included in this study. Mutation acquisition of cancer-related genes was observed only in 12 (41%) patients while remaining 17 (59%) patients were mutationally stable even after TMZ treatment. Mutations were gained in 5 *MGMT* methylated tumors and 7 unmethylated. Remarkably, 70% of acquired mutations in *MGMT* methylated tumor were G:C to A:T at non-CpG sites whereas only 8% in *MGMT* unmethylated tumors. In mutation gain group, MMR expression decreased significantly after treatment ($p=0.012-0.048$). Furthermore, in contrast to *MGMT* unmethylated tumors, *MGMT* methylated tumors showed marked MMR inactivation (40% vs. 7.5%, $p=0.078$). **【Conclusion】** We showed different types of the mutation acquisition after TMZ treatment according to *MGMT* status, providing further insights into the mechanism of TMZ resistance to improve treatment of the patients with GBM.

S19-1

Biological function and therapeutic potential of somatic mutations in meningiomas

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Meningiomas comprise the most frequent intracranial primary tumors, and with aging population the incidence of meningiomas is likely to increase. Meningioma therapy largely relies on surgical removal, while in some cases radiation therapy is encountered as primary or additional option. Recent genetic advances have identified somatic mutations in genes such as TRAF7/KLF4, AKT1, and SMO. Together with the well-known fundamental role of NF2 in meningioma development, the molecular characteristics of meningiomas are now on a solid basis. However, the true biological role of these somatic mutations for meningioma development and growth are not fully understood. Additionally, this holds true for their role in non-surgical therapies. The use of in vitro and especially in vivo mouse models to model meningioma biology is mandatory to explore the value of these mutations for the natural course, as well as potential treatment modalities. The current knowledge derived from model systems for AKT1, KLF4, SMO, and NF2 will be presented and discussed.

S19-2

Role of CD163 in meningioma progression

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【BACKGROUND】 CD163 is a 130-kDa transmembrane protein expressed in human monocytes and macrophages, and the aberrant expression of CD163 in breast and colorectal cancer associated with patients' poor prognosis was reported. Here, we analyzed the expression of CD163 in meningioma, a common intracranial tumor, and its molecular mechanism in association with meningioma progression.

【METHODS】 First, we performed immunohistochemical analysis using 50 human meningioma specimens. Next, we established CD163-overexpressing human meningioma cell lines and investigated its roles in tumor progression in vitro and in vivo.

【RESULTS】 Immunohistochemically, 26 of 50 human meningioma specimens (52.0%) were positive for CD163 in tumor cells, including benign grade I (48.5%) and grade II (71.4%) cases. Furthermore, CD163 expression was correlated with histological atypical parameters that directly predict the prognosis of meningioma. CD163-overexpressing meningioma cells showed significant suppression of apoptosis and accelerated tumor growth in nude mice. In addition, unexpected splenomegaly affiliated with the xenograft predicted tumor-derived granulocyte colony-stimulating factor (G-CSF) production, which was confirmed by reverse-transcription polymerase chain reaction and enzyme-linked immunosorbent assay.

【CONCLUSIONS】 To our knowledge, this is the first report that demonstrates CD163 expression in meningioma not only by immunohistochemistry but also by reverse-transcription polymerase chain reaction, using primary culture cells, and provides the novel molecular function of CD163 to prevent apoptosis through the production of G-CSF in meningioma.

S19-3

Update of Meningioma Classification and Genetics

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Accounting for up to 37% of all primary CNS neoplasms, meningioma is one of the most common diagnoses encountered in neurosurgical practice. It has long been appreciated that the two most important prognostic variables for meningioma patients are extent of surgical resection and histopathologic grade. Grading criteria have varied greatly over time, although the current 2016 WHO scheme is largely derived from data reported in two large Mayo Clinic series published in the late 1990s. Since that time, the clinicopathologic associations have been independently validated, despite the concerns raised by some that the grade II category has become too commonplace. The derivation of this grading scheme and remaining controversies will be discussed, along with the identification of rare but biologically distinct variants, such as clear cell (including SMARCE1 alterations), chordoid, papillary, and rhabdoid (including BAP1 alterations) meningiomas. In contrast to diffuse gliomas and embryonal neoplasms, integrated diagnoses are not currently endorsed by the WHO 2016. Nevertheless, recent genetic advances beyond the long established role of NF2 will be discussed and now include driver mutations associated with skull base meningiomas utilizing TRAF7/KLF4 (including secretory meningioma), AKT, and SMO genes. The expanding list of biomarkers for tumor progression and aggressive biology now also includes Ki-67 LI, progesterone receptor expression, homozygous CDKN2A gene deletion, other copy number alterations, pTERT mutations, and epigenetic patterns, such as H3K27me3 loss and methylation profiling signatures.

S19-4

Radiation-induced meningiomas: experience at the Hiroshima University Hospital and review of the literature

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Secondary meningioma after cranial irradiation (i.e. radiation-induced meningioma) is considered as an uncommon late risk of cranial irradiation. We previously reported that the incidence of meningioma for atomic bomb radiation doses >0.1 sievert, or the exposed patients at distance of <2.0 km from epicenters was statistically higher than non-exposed group (P<0.005). The risk of secondary meningioma development was shown to increase with closer proximity to the atomic bombs' epicenters and in those exposed during childhood. It is now well-known that the increase incidence of meningioma is the delayed side effects of not only atomic bomb radiation exposure but also cranial/craniofacial irradiation for malignancy. In this report, we present our experience of secondary meningioma after cranial irradiation. Between 2000 to 2018, we radiologically diagnosed 8 patients with meningioma after cranial irradiation at the dose of 15 to 60Gy. Seven of 8 patients were surgically confirmed for the development of meningioma. Three patients had multiple meningiomas, and 4 patients had complicated cavernous angiomas. The mean age of the patients was 35 years (median 32) and the mean tumor latency was 18.4 years (median 18.3). Histopathology revealed the higher incidence of grade II & III meningiomas (3 atypical, 1 papillary subtype). We review the typical presentation of patients with secondary meningioma after cranial irradiation and discuss unique aspects of the clinical management of these tumors compared with sporadic meningioma, based on our clinical experience and literature review in treating these lesions.

Irradiated brain parenchyma provides favorable microenvironments for glioma stem cells to maintain their tumor-propagating ability

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Despite of aggressive multimodal treatment for patients with glioblastoma (GBM), the tumor recurrence is still a leading cause of mortality. In our previous studies using a rodent model of chronic cerebral radiation injury, whole transcriptome analysis revealed that, 3-6 month after X-ray irradiation, various gene pathways were significantly affected and became altered in the irradiated brains. Clinically, this would occur also in a patient's brain where surviving tumor cells are lurking. In the present study, therefore, we hypothesized that the pathophysiological changes raised in brain parenchyma by radiotherapy may involve in the tumor recurrence of GBM. We implanted TS cells, a B6 mouse glioma stem cell (GSC) line, into B6 mice brains which were locally irradiated by 60 Gy of X-ray, 3 months prior to the tumor implantation. Gliomas formed in the pre-irradiated and naive brains (control) were removed and minced for single cell isolation to recover alive tumor cells. In FACS analyses, the mean percentages of cells expressing CD133 and Nestin were significantly higher in the tumors from the pre-irradiated brains than those from naive brains; CD133: 78.6% vs 12.6% and Nestin: 25.8% vs. 8.7%, respectively ($p < 0.01$). Next, we transplanted these isolated tumor cells into naive mice brains. The animals implanted with the tumor cells from pre-irradiated brain showed significantly shorter survival than the animals with the control cells (Median survival: 15 vs. 20 days, $p < 0.0001$). Collectively, our data suggest that irradiated brain parenchyma might afford a favorable microenvironment to maintain the stemness in surviving GSCs after radiotherapy.

Mining-guided future prediction-The 20 hottest neuro-oncological fields in 2019

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【Background】 Grasping objective trends in today's rapidly changing academic fields is important. We analyzed the trends of brain tumor articles with our novel algorithm and predicted the 20 hottest neuro-oncological fields in 2019. **【Method】** Text-mining analyses were performed to whole MeSH data of all brain tumor articles published in 2017, and to particularly chosen text data of international conferences held in 2017. Sixty-nine frequently used keywords and 10 important brain tumor diagnoses comprised the subject fields of our survey. We chose the 15 highest impact factor (IF) journals among 8014 journals that have published neuro-oncological articles in 2017. In addition, we found 15 journals that had the largest number of published articles in 2017. These 30 journals were the subject journals of our survey. The annual impact (AI) of each year was calculated for each journal and each field (number of articles published in the journal \times IF of the journal). A field's AI index (AII) for the year was the sum total of AIs of each of the 30 journals. The AII trends of the 79 subject fields during 2008-2017 were analyzed with linear approximations. With the above algorithm, the 20 hottest neuro-oncological fields in 2019 were predicted. **【Results】** The 20 predicted fields comprise not only widely recognized fields such as immuno-oncology and epigenetics, but also emerging fields such as microenvironment. **【Conclusion】** This algorithm can be an effective tool for future prediction of medical field.

Tpr is an autophagy induced cell death suppressor in ependymoma

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【Background】 The nuclear pore complex (NPC) is composed of around 30 proteins and resides in the nuclear envelope. NPC regulates gene-expression, chromosomal division, and autophagy beyond nucleocytoplasmic transport, sustaining a life of the cell. Recent genomic analysis identified Tpr (translocated promoter region), one of NPC components, as an amplified gene in ependymoma. However, the significant role of Tpr in ependymoma is poorly understood. Previously, we reported that Tpr is highly expressed in ependymoma patients. Further, we verified that Tpr is required for malignant behavior by xenograft assay. We will present molecular mechanism regulated by Tpr in ependymoma. **【Method】** Samples from ependymoma patients and ependymoma cell line Vn19 were used. To measure autophagy activity, we employed qRT-PCR and Western Blot analysis. Further, immunofluorescent imaging was performed to analyze autophagosome formation processes. **【Results】** Ependymoma patients showed lower mRNA levels of autophagy molecules (BECN1, ATG3, ATG5, ATG7, ATG12). Further, protein levels of LC3B were reduced in ependymoma patients. The siRNA-mediated Tpr depletion resulted in the increase of autophagy molecules in Vn19. Finally, confocal-microscopy analysis using Vn19 silenced Tpr or expressing LC3-GFP determined autophagosomes were formed inside nuclear. **【Conclusion】** Tpr suppresses nucleophagy activation and mediates ependymoma malignancy.

S20-4

The correlation between 1p19q and TERT promoter mutation status in IDH-mutant gliomas

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【Background】 The revised WHO 2016 classification established the consensus that the diagnosis of oligodendrogliomas should be confirmed by IDH mutation and 1p19q codeletion. As these tumors are known to harbor TERT promoter mutation also, TERT status seems to be useful as a surrogate marker for 1p19q. Herein, we aim to verify this theory by analyzing the correlation between 1p19q and TERT status. **【Methods】** IDH and TERT status were determined by Sanger sequencing. 1p19q codeletion was confirmed by LOH on total arms using multiple microsatellite markers. We reviewed our case series of 65 gliomas with IDH mutation whose 1p19q and TERT status were examined. **【Results】** Whereas 60 out of 65 showed the consistency of 1p19q and TERT status, the remaining 5 were 1p19q non-codel/TERT mutant. In these 5 cases, 3 showed consistent clinical, radiographic and pathological features of oligodendroglioma. In contrast, the pathological diagnoses of the remaining 2 were anaplastic astrocytoma and glioblastoma, both of which also showed astrocytic molecular findings. **【Conclusion】** This study seems to support the theory that, in general, TERT mutation can be used as an effective surrogate marker of 1p19q for IDH mutant gliomas. Although, there were a few exceptional cases, thereby, we need further efforts to elucidate the correlation between 1p19q and TERT status.

Clinico-pathological findings of 1p19q LOH by using FISH method in high grade glioma: With findings of MLPA

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【Introduction】 Integrated diagnosis for G2 or G3 oligodendroglial tumor is needed to proof both IDH mutation and 1p/19q-codeletion. However, we sometimes experienced 1p/19q-codeletion, 1p or 19q LOH in high grade glioma by using FISH. We have some questions why high grade gliomas sometimes have 1p or 19q LOH. Then, we compared in clionico-pathological findings. **【Material and method】** We pick up consecutive operation cases in 2016~2018. Median age was 55yo, 18 cases of GBM IDH-wildtype, 1case of GBM IDH-mutant, 9 cases of AA IDH-mutant, and 6 cases of AO IDH-mutant and 1p/19q-codeleted were eliminated. After ordinal pathological findings, we examined sequences, FISH, and MLPA. Probe of FISH was LSI1p36/LSI1q25,LSI19q13/19p13, and one of MLPA was SALSA MLPA P088-C2 Oligodendroglioma 1p-19q probemix. **【Result】** We defined the cutoff point 20% of 1p19qLOH, 13 cases of high grade gliomas without AO IDH-mutant and 1p/19q-codeleted. In these cases, 5 cases were codeleted 8 cases were 1p or 19q LOH. There was no close relations between these cases and GBM IDH-wildtype in pathological findings of calcification, perinuclear halo, chicken-wire like vessels, microvascular proliferation, pseudoparisading, MGMT methylation status, and MIB-1. We tested MLPA method for these high grade glioma, and 1p or 19q point or hole deletion were seen acutually. Prognosis depended on integrated diagnosis. **【Conclusion】** Even though 1p/19q-codeleted was proofed by FISH, MLPA would showed a point or hole deletion, and biological behavior depended on the integrated diagnosis clinic-pathologically.

S20-6

Detection of 1p19q codeletion by targeted sequencing for glioma genotyping

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Detailed genotyping for glioma detecting the mutation in IDH1/2, ATRX, p53, TERT promoter region in addition to 1p19q codeletion is mandatory for final diagnosis especially for selecting the treatment after surgery. Here we established rapid and valuable targeted sequence pipeline for glioma genotyping for not only SNV detection but also 1p19q codeletion. Capture sequencing panel targeted 16 genes including ATRX, IDH1, IDH2, TP53, TERT were designed by SeqCap EZ target enrichment system (Roche). Genomic DNA was extracted from surgically resected glioma tissue (FFPE; formalin fixed paraffin embedded, or PFPE; PAXgene fixed paraffin embedded) and the libraries were sequenced by the MiSeq (Illumina). The raw read data obtained from amplicon sequencing were processed by originally designed, dedicated analysis pipeline by Genome Jack (Mitsubishi Space Software Inc.) We detected IDH1 R132H mutation in 3 out of 9 grade II-IV gliomas by our sequence pipeline, while IHC for IDH1 R132H was positive in 5 cases including these 3 cases. Our sequence pipeline and also FISH identified 1p19q codeletion only in these 3 cases with IDH1 R132H mutation positive, thus we concluded that the other two IHC-positive cases were false positive. Moreover, we successfully detected LOH of PTEN and amplification of EGFR and MET. Our workflow can be finished within 7 working days with reasonable cost, offering a practical laboratory developed test for glioma genotyping.

Intracranial remote recurrence in IDH mutant gliomas is associated with TP53 mutations and 8q gain

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Most IDH mutant gliomas harbor either 1p/19q co-deletions or TP53 mutation, and 1p/19q co-deleted tumors (oligodendrogliomas) demonstrate significantly better prognoses than tumors harboring TP53 mutations (astrocytomas). To investigate clinical factors which contribute to the difference in tumor progression of the IDH mutant groups, we classified the tumor recurrent patterns based on MRI, and correlated this with genomic characterization. Accordingly, in IDH mutant gliomas (N = 66), almost all 1p/19 co-deleted gliomas showed local recurrence, whereas TP53 mutant gliomas showed intracranial remote recurrence, as well as local recurrence. In addition, diffuse tensor imaging suggested that intracranial remote recurrence in the astrocytomas, IDH-mutant with TP53 mutations may occur along major fiber bundles. The remotely recurrent tumors showed a higher mortality and significantly harbored an 8q gain; astrocytomas with the 8q gain revealed a significantly shorter overall survival than those without the 8q gain. Next-generation sequencing indicated that the specific regions of 8q (i.e., between 8q22 and 8q24) show a high copy number. In conclusion, only tumors with TP53 mutations showed a distant recurrent pattern in IDH mutant gliomas. Furthermore, 8q gain was significantly associated with intracranial remote recurrence and can be considered as a poor prognostic factor in astrocytomas, IDH-mutant.

A phase I/IIa clinical trial of Ad-SGE-REIC for malignant glioma

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Expression of the gene encoding reduced expression in immortalized cells/Dickkopf-3 (REIC/Dkk-3) was shown to be reduced in a variety of human cancer cells. We investigated the anti-glioma effect of an adenoviral vector carrying REIC/Dkk-3 gene (Ad-CAG-REIC). We also recently developed a novel adenoviral vector (Ad-SGE-REIC), which could express more REIC/Dkk-3 protein than Ad-CAG-REIC. We evaluated the anti-glioma effect of Ad-SGE-REIC against malignant glioma in vitro and in vivo. In the cytotoxicity assay of malignant glioma cell lines (U87dEGFR and GL261), after treatment with Ad-SGE-REIC, the number of malignant glioma cells attached to the bottom of culture wells was significantly reduced in a time-dependent manner. Mice treated with Ad-SGE-REIC survived significantly longer than mice treated with Ad-LacZ or Ad-CAG-REIC, demonstrating promising anti-glioma activity for Ad-SGE-REIC. We conducted toxicology tests using intracranial injection of higher doses of Ad-SGE-REIC for Shinnihon Kagaku Mfg., Ltd. We aim to submit notification of a plan for a phase I/II clinical trial of Ad-SGE-REIC for the treatment of malignant brain tumor this year. This will be an open-label, single-armed, phase I/II study involving three cohorts of three or six subjects each in the dose-escalation phase. This Ad-SGE-REIC may help to improve the prognosis for patients with malignant gliomas.

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IDH gene status is associated with pattern of relapse in malignant gliomas

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【Introduction】 Since the local recurrence was found in majority of malignant glioma, the patients received local irradiation around resection cavity. However, the distant recurrence sometimes occurred without local recurrence. In this study, we analyzed the association of the pattern of recurrence and molecular alterations. **【Methods】** We retrospectively analyzed 245 cases of malignant gliomas (WHO grade III: 86 cases, Grade IV: 159 cases). Data regarding patient characteristics, recurrence pattern, and prognosis were obtained from medical records. We examined molecular alterations such as IDH mutation, 1p19q loss, TP53 gene mutation. **【Results】** Of the 86 patients with anaplastic gliomas, 58 carried IDH mutation, and 40 experienced recurrence. The first recurrence was local in 25 patients and distant in 15. Patients without IDH mutation showed more frequent distant recurrence than those with IDH mutation. $P=0.022$). No distant recurrence was found in the tumor with IDH1 mutation and 1p/19q co-deletion. On the other hand, of the 159 patients with glioblastomas, 4 carried IDH mutation, and 127 experienced recurrence. The first recurrence was local in 99 patients and distant in 28. No Patients with IDH mutation showed distant recurrence. **【Conclusion】** IDH gene status is the strong predictor for recurrence pattern in malignant gliomas.

An important role of histopathology and immunohistochemistry in immunotherapy against high grade gliomas

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We have conducted WT1 peptide immunotherapy against high grade gliomas in some investigator-initiated clinical trials. Safety and efficacy of the therapy were reported and nowadays sponsor-initiated trials are on-going. In this paper, a role of histopathology and immunohistochemistry was focused and reviewed. To enroll the clinical trials, the patient must have matched HLA type and tumor cells must have positive WT1 expression. After the basic research revealed the correlation between mRNA level and WT1 expression in immunohistochemistry, we have used the WT1 immunohistochemistry with WT1 6F-H2 antibody to judge the enrollment of each patient to the clinical trials. Accordingly, we created WT1 score for positivity evaluation and revealed that the patients with WT1 score 3, 4 (strong expression) survive longer than those with WT1 score 1, 2 (weak), concluding that WT1 score by immunohistochemistry can be a predictive factor. Gathering the data on the WT1 immunohistochemistry, a working group in the international conference on WT1 in human neoplasia advocated an international harmonization on immunohistochemical evaluation of WT1 positivity in solid cancers in 2012. Further, we have conducted immunohistochemistry using antibody of WT1 / HLA / various cytokines, and subset markers of tumor infiltrating lymphocytes (TIL). By comparing those expressions on pre- and post- immunotherapy surgical samples, we found some mechanisms of escape phenomenon of tumor cells from immunotherapy. An important role of histopathology and immunohistochemistry will be summarized in the presentation.

MGMT promoter methylation in patients with glioblastoma multiforme: Is methylation-sensitive high-resolution melting superior to methylation-sensitive polymerase chain reaction assay?

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The methylation status of O6-methylguanine-DNA methyltransferase (MGMT) gene promoter is not only the predictive marker in adult glioblastoma (GBM) but also acts as an information useful for clinical decision-making in elderly patients with GBM. Although pyrosequencing analysis has been introduced as the gold standard for evaluating the methylation status of MGMT, many institutions and studies still employ methylation-specific polymerase chain reaction (MSP) since this method is cost-effective and does not require special analyzers. Although MSP shows good correlation with the prognosis of GBM patients treated with Stupp protocol, it has some limitations, such as ambiguity in judgment and non-applicability for evaluating heterogeneously methylated samples. In this study, we focused on the methylation-sensitive high-resolution melting (MS-HRM) for an alternative to MSP. The methylation levels of 6 glioma cell lines were estimated by MS-HRM, and the data was validated by the bisulfite sequencing of cloned alleles. Strong correlation was observed between MS-HRM and bisulfite sequencing, indicating that MS-HRM could be a reliable method for estimating methylation levels (Correlation coefficient for #1 primer: 0.959 and #2 primer: 0.960). Methylation levels of 75 samples from patients with GBM were estimated using MS-HRM and compared to that of the MSP data. MS-HRM is superior to MSP in discriminating between responders and non-responders to temozolomide (TMZ) (p value of log-rank test, PFS; MS-HRM: 0.00023, MSP: 0.0035, OS; MS-HRM: 0.00019, MSP: 0.00028). Taken together, our study suggests that MS-HRM is a superior method for detecting MGMT promoter methylation status as compared to MSP.

A subgroup of IDH-mutated astrocytomas with 19q-loss presents oligodendroglioma-like morphology and better prognosis

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IDH-mutant gliomas are classified into astrocytic or oligodendroglial tumors by 1p/19q status in WHO 2016 classification, with the latter presenting with characteristic morphology and show better prognosis in general. However, the morphological and genetic features within 1p/19q intact astrocytomas are varied, and there may be distinguishable subtype. We analyzed gliomas with only 19q-loss in 170 WHO grade II to IV gliomas. 1p/19q status was analyzed by microsatellite analysis, and genetic mutations were analyzed by next-generation sequencing and Sanger sequencing. For validation, the Brain Lower Grade Glioma dataset of the TCGA was analyzed. Of the 42 grade III IDH-mutated gliomas, 12 were 1p-intact/19q-intact (anaplastic astrocytomas: AA), 7 were 1p-intact/19q-loss (AA), and 23 showed 1p/19q-codeletion (anaplastic oligodendrogliomas: AO). All of the seven 1p-intact/19q-loss AAs harbored TP53 mutation, but no TERT promotor mutation. All 19q-loss AAs had regions presenting oligodendroglioma-like morphology, and were associated with significantly longer overall survival (OS) compared to 19q-intact AAs ($p=0.001$). This tendency was observed in the TCGA Lower Grade Glioma dataset. In contrast, there was no difference in OS between the 19q-loss GBM ($n=14$) and 19q-intact GBM ($n=74$) ($p=0.4$). In a case of 19q-loss AA, both oligodendroglial morphology and 19q-loss disappeared after recurrence, possibly indicating correlation between 19q-loss and oligodendroglial morphology. We showed that there was a subgroup, although small, of IDH-mutated astrocytomas harboring 19q-loss that present oligodendroglial morphology, and also were associated with significantly better prognosis compared to other 19q-intact astrocytomas.

Usefulness and pitfalls of 1p/ 19q-codeletion analysis by FISH method in glioblastoma

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【Objective】 According to the revision of WHO classification, diagnosis of glioblastoma is accompanied by the status of IDH-mutation. However, when it is necessary to differentiate from anaplastic oligodendroglioma, information of 1p/ 19q-codeletion is also required. We examined the usefulness and problems of fluorescence in situ hybridization (FISH). **【Methods】** Of the 242 diffuse glioma cases in which 1p/ 19q-codeletion was searched by FISH since 2009 to 2016, 141 cases of primary glioblastoma were analyzed. Vysis LSI DNA probe 1p36/ 1q25 and 19p13/ 19q13 were used as probes, and the cutoff value was set at 20%. **【Results】** When judging that there was deletion when the signals of 1p or 19q were less than the signals of 1q or 19p respectively, 33 cases in 1p-deletion and 18 cases in 19q-, codeletion was 6 cases. On the other hand, when judging that deletion was present only when single signal of 1p or 19q and two signals of 1q or 19p were detected, 12 cases in 1p-deletion and 6 cases in 19q-, codeletion was one case. **【Discussion】** Although in glioblastoma there are many atypical deletion patterns compared to oligodendroglial tumor, they should not be judged as deletion. Since the case with codeletion had a histological feature of glioblastoma, FISH might detect partial deletion of 1p36, then it is necessary to interpret combined with the status of IDH-mutation. **【Conclusion】** In differentiating between glioblastoma and anaplastic oligodendroglioma, 1p/ 19q-codeletion analysis by FISH can be useful with consideration of its pitfalls.