A1

RARE TUMORS AND TUMOR-LIKE LESIONS IN THE REGION OF SELLA TURCICA AND IN ITS VICINITY

Adamek D

Department of Neuropathology, Institute of Neurology, Jagiellonian University, Medical College, Kraków

The sellar region, localized in the very center of the head contains different tissue structures (hypophysis with its bony encasement, sphenoid sinus, meninges, optic nerves, hypothalamus etc) in close approximation. Expansive lesions of this region represent an especially broad spectrum of both neoplastic and non-neoplastic pathologies. The neuropathological diagnosis of these lesions demands a meticulous and broad differentiation, and particularly cautious in the case where the material at the neuropathologist's disposal is very scanty (which is typical in the case of neurosurgical operations in this region). Apart from tumors "typical" of this localization like pituitary adenomas, craniopharyngeomas, optic gliomas, meningiomas there may occur (mostly rare) other tumors. Among them there are neoplasms like granular cell tumor, pituicytoma, chordoma, germinoma, dysontogenetic changes like cysts, hamartomas, and changes of inflammatory or of uncertain character like xanthogranuloma. Some of them make a real diagnostic and/or therapeutic problem. These rare changes will be reviewed and discussed based on own material.

A2

MENINGIOANGIOMATOSIS OF THE TEMPORAL LOBE IN AN 8-YEAR-OLD GIRL

Adamek D[1], Kwiatkowski S[2]

[1]Department of Neuropathology, Institute of Neurology; [2]Deartment of Pediatric Surgery, Division of Neurosurgery, Institute of Pediatrics, Jagiellonian University, Medical College, Kraków

The authors present a case of meningioangiomatosis (MA) of the temporal lobe in an 8-year-old girl presented with epilepsy which occurred 2 months before surgery. The neuroimaging (CT, MRI) showed well circumscribed, hyperdensive mass inside the brain parenchyma but attached to the meninges and apparently also to the petrous bone and suggested meningioma.

The tumor was totally resected through temporal craniotomy though it did not behave like a typical meningioma. The mass of hard consistence and rich in calcifications in its central and peripheral (juxtameningeal) part seemed, however, to immerse and permeate the brain parenchyma which suggested the infiltration like in a case of malignant neoplasm. Histologically, the central and peripheral part of the lesion showed features that might be suggestive of psammomatous meningioma. Toward the periphery, especially at the border zone with "normal" brain parenchyma a characteristic "wickerware" pattern of collagen-rich, fibro-vascular bundles encroaching the remnants of the brain tissue was visible. Both in the meningioma-like part and in the periphery the components of the lesion were totally EMA-negative. Rather unexpectedly, the CD34-positive endothelial network was not very dense. After almost 1.5 year no symptoms of recurrence occurred. The child has no epilepsy and her development is normal. The girl has no symptoms of neurofibromatosis.

One of unresolved questions about MA is whether it has any relation to meningioma. In the presented case the histological picture may suggest a coexistence of MA and psammomatous meningioma but it is at least just the same probable that in its periphery MA consolidates only into a meningioma-like structure and in fact it is a separate pathological entity rather of dysontogenetic character.

A3

DIAGNOSTIC POSSIBILITIES IN ANGIOTROPHIC LYMPHOMA. A CASE REPORT

Bertrand E[1], Wierzba-Bobrowicz T[1], Michalak E[2], Rycerski J[3], Szpak GM [1], Litwin T[4]

[1]Department of Neuropathology, Institute of Psychiatry and Neurology, Warszawa; [2]Department of Pathology, Institute of Mother and Child, Warszawa; [3]Department of Neuroradiology, Institute of Psychiatry and Neurology, Warszawa; [4]II Department of Neurology, Institute of Psychiatry and Neurology, Warszawa

A 57-year-old woman with rapidly progressing fluctuating dementia and headache followed by the gradual development of multifocal neurological symptoms. Routine blood samples revealed the elevated blood sedimentation rate. The level of cerebrospinal fluid (CSF) protein was increased, whereas the cell count was normal. Lymphocytes were mostly present. Cranial cerebral computed tomography (CCT) was negative. Cranial magnetic resonance imaging (MRI) in the FLAIR modality showed bilateral cortico-subcortical ischemic lesions initially interpreted as infarctions caused by

progressive occlusions of small blood vessels. The final diagnosis of systemic malignant angiotrophic lymphoma of the B cell type was immunohistochemically obtained postmortem from the brain and other organs. Intravital diagnosis was difficult because of negative CSF cytology or non-specific neuroimaging findings (MRI). However MRI scan, especially FLAIR imaging, may be uniquely useful in identifying the central nervous system (CNS) lesions not seen on CCT scans. The FlAIR MRI appears to be the modality of choice in characterizing CNN lesions especially before diagnostic surgical brain biopsy.

A4

MUTANT *EGFR* (*EGFRVIII*) EXPRESSION IN GLIOBLASTOMAS

Biernat W[1,2], Huang H[2], Yokoo H[2], Kleihues P[2], Ohgaki H[2]

[1]Department of Neuropathology and Molecular Pathology, Medical Academy, Gdansk; [2]International Agency for Research on Cancer, Lyon, France

EGFR amplification is a frequent genetic alteration in glioblastomas, and is often associated with structural alterations. The most commonly occurring variant III (EGFRvIII) results from a non-random 801 bp in-frame deletion of exons 2 to 7 of the *EGFR* gene. We assessed amplification and overexpression of *EGFRvIII* and wild-type *EGFR* in 30 glioblastoma biopsies. Immunohistochemically, EGFR overexpression was observed in 20 (67%) of 30 glioblastomas. Eight (27%) cases also showed immunoreactivity to an EGFRvIII antibody. In 6 of these cases, the pattern of EGFR and EGFRvIII overexpression was compared in serial sections. In 4 cases, areas with immunoreactivity to EGFRvIII largely coincided with the wild-type *EGFR* expression. In the other 2 cases, the areas immunoreactive to EGFRvIII were significantly less extensive than EGFR-positive areas. To assess whether *EGFRvIII* is predominantly amplified in tumors with concurrent wild-type *EGFR* amplification, we carried out real-time quantitative PCR using 2 sets of primers located in exon 2 and intron 15 of the *EGFR* gene. A >5-fold ratio of relative copy numbers between intron 15 (present both in wild-type *EGFR* and *EGFRvIII*) and exon 2 (present only in wild-type *EGFR*, but missing in *EGFRvIII*) suggested predominant amplification of *EGFRvIII* in only 3 (10%) of 30 glioblastomas. The observation that intratumoral wild-type *EGFR* overexpression is often more extensive and that predominant amplification of *EGFRvIII* is a rare event would limit the effectiveness of therapeutic approaches based on selective targeting of EGFRvIII.

A5

IMMUNOHISTOCHEMICAL ANALYSIS OF SELECTED PARAMETERS OF TISSUES IN THE IMMEDIATE SURROUNDINGS OF TUMOURS IN SURGICAL MATERIAL OF GLIOMAS AND METASTASES TO THE CENTRAL NERVOUS SYSTEM

Bierzyńska-Macyszyn G[1], Kwiek SJ[2], Właszczuk P[1], Gołka D[1], Bażowski P[2]

Departments of Pathomorphology[1] and Neurosurgery[2], Katowice, Medical University of Silesia, Katowice

Introduction: The continuously increasing number of primary and metastatic tumours in CNS, observed in the last years, induces an analysis of this phenomenon in various aspects. Our attention was drawn by the two fundamentally different patterns of the relationship between the neoplastic tissue and the surrounding glia: the infiltrating type of glia tumours' growth and distinct delimitation of the metastatic foci from the surrounding tissue. Spreading of the tumour in a tissue depends on many factors. In the glia setting, elements like anatomical structures of the white matter tracts, submeningeal spaces, cerebrospinal fluid and blood vessels gain more significance; they are connected with the extracellular matrix proteins. Neoplasmatic cells use, among others, ligands and receptors of cells and extracellular matrix – DCC, integrins, hialuronate receptors like CD44, SPARC matrix protein, tenascin.

Materials and methods: We performed an analysis of the surgical material obtained during resections of the following primary tumours: pilocytic, fibrillary, anaplastic astrocytoma, glioblastoma multiforme, anaplastic oligoastrocytoma and of the following metastatic tumours: bronchogenic squamous cell carcinoma, bronchogenic small cell carcinoma, bronchogenic adenocarcinoma, gastric adenocarcinoma, adenocarcinoma of the colon, serous adenocarcinoma of the ovary, endometroid adenocarcinoma, papillary carcinoma of the thyroid, clear cell carcinoma of the kidney, malignant melanoma. The immunochemical responses with antibodies against CD44 (hialuronate receptor) and osteopontin (integrines ligand) were carried out in the areas where tumours and the surrounding glia adjoined. 3 cases of each type of tumour were examined. The intensity of the reaction was assessed and compared in

Table I.

Tumour	Intensity of expression of reaction	CD 44			OPN		
		tumour	surrounding tissue	glia	tumour	surrounding tissue	glia
Gliomas	1	0.0%	100.0%	100.0%	0.0%	40.0%	100.0%
	2	6.7%	0.0%	0.0%	40.0%	60.0%	0.0%
	3	93.3%	0.0%	0.0%	60.0%	0.0%	0.0%
		P<0.05			p<0.05		
		p<0.05			p<0.05		
			NS			NS	
Metastatic tumours	1	20.0%	80.0%	80.0%	10.0%	10.0%	90.0%
	2	63.3%	20.0%	20.0%	20.0%	30.0%	10.0%
	3	16.7%	0.0%	0.0%	70.0%	60.0%	0.0%
		P<0.05			NS		
		p<0.05			p<0.05		
			NS			p<0.05	

a 3-grade scale and the expression of the antigens in the neoplastic and non-neoplasic tissues were compared.

Results and conclusions: In all the examined gliomas, the CD44 expression was high in the tumour tissue. However, this expression was not high, and of comparable intensity, in the immediate surrounding of the tumour and in the normal glia tissue of the vicinity area. As far as metastases were concerned, a similar high expression in the neoplastic tissue with lower expression in the surroundings was observed in cases of metastatic bronchogenic squamous cell carcinoma and clear cell carcinoma of the kidney. A very low CD44 expression was found in metastases of small cell bronchogenic carcinoma and malignant melanoma. In other tumours, the expression of CD44 was estimated as mediocre and differentiated in the surroundings of the tumour, nevertheless, it was always the same in the immediate surroundings and in the normal glia vicinity area. The OPN expression was distinct in the gliomas' tissues, its intensity increasing along with the malignancy grade of the neoplasm; in the surrounding tissues it was lower than in the tumour itself; it decreased with the distance from the tumour. In the small cell bronchogenic carcinoma, the OPN expression was vestigial, in the adenocarcinomas of the bronchus and of the digestive tract this expression was mediocre, in all others it was comparable to the one observed in highly malignant gliomas. Contrary to the situation observed in gliomas, the OPN expression in the tissues surrounding metastases was similar to the expression in the tumours' tissue. Like in the gliomas, this expression decreased along with the distance from the tumour, and these relationships were inverted in small cell bronchogenic carcinoma only.

It seems plausible that the statistically significant distinctions in the expression of the proteins analyzed can represent one of the elements responsible for the differences in the way tumors and metastases spread in the central nervous system.

A6

ASSESSMENT OF GLIOMAS' PROLIFERATIVE ACTIVITY IN STEREOTACTIC BIOPSY MATERIAL

Bierzyńska-Macyszyn G[1], Mazurek U[2], Lech A[3], Własczczuk P[1], Stępień T[3], Kapral M[2], Fijałkowski M[4], Blamek S[4], Białas B[4], Majchrzak H[3], Wilczok T[2]

Departments of [1]Pathomorphology in Katowice; [2]Molecular Biology and Medical Genetics in Sosnowiec; [3]Neurosurgery in Sosnowiec, Medical University of Silesia, Katowice; [4]Department of Brachytherapy, Center of Oncology, IMSC, Gliwice, Poland

Background and objective: The increasing number of gliomas diagnosed recently, their tendency to recurrence and to malignancy progression as well as their diversified, difficult to predict reaction to adjuvant or primary radiotherapy is a reason to develop new methods of precise determination of the tumor biology. The determination of the gliomas malignancy according to the WHO criteria, using immunohistochemical proliferative responses (PCNA,

Ki-67), being not always fully satisfactory, were supplemented with molecular analysis of gliomas: determination of transcriptional activity of *H3 histone* gene – a marker of proliferation in these neoplasms.

The objective of our study was to determine the transcriptional activity of the gene coding the H3 histone protein. This gene becomes active during DNA replication, and may be considered as an index of proliferative activity of the neoplastic cells taking part in the proliferation of the diffuse astrocytomas of the brain. We were also searching for a correlation between the concentration of the mRNA of this gene, the findings of the morphological examinations, the course of the disease and the response to the implemented treatment.

Materials and methods: The biopsy samples of 232 patients, who had undergone stereotactic biopsy in the last 6 years in the Department of Neurosurgery in Sosnowiec, Poland, were investigated. In these patients, radiotherapy was the only treatment or it was adjuvant to surgery. The morphological and molecular analyses were carried out on 85 gliomas (30 cases of G2 WHO diffuse astrocytomas of low malignancy and 55 cases of G3 WHO anaplastic astrocytomas), 62 of these patients underwent radiotherapy.

An analysis of full clinical data, cytological examination, routine histopathological examination, determination of malignancy grade according to the WHO criteria and immunohistochemical proliferative responses (Ki-67) were performed. A pattern of material processing for the molecular analysis was developed. In the molecular analysis, the concentration of *H3 histone* gene mRNA was determined by the RT-QPCR method (Taq-Man) with application of an ABI PRISM 7700 sequence detector. The response to the treatment was assessed with imaging techniques (CT, MRI).

Results and conclusion: We found that fixed in pure alcohol, dry and not-stained cytological smears are enough to obtain the amounts of mRNA sufficient to carry out molecular analyses to determine the number of mRNA molecules per cell.

Analyzing the clinical data, morphological findings, immunohistochemical reactions with antibodies against Ki-67 and number of the *H3 histone* mRNA molecules in the investigated material, we found that the transcription activity of the *H3 histone* gene is a more sensitive proliferation marker than the immunohistochemical response against Ki-67.

Our findings enable us to claim that assessment of the genes' expression may be considered a valuable additional marker in the diagnosis and prognosis of diffused astrocytomas of low and high malignancy.

A7

POLYMORPHISMS OF METHYLENETETRAHYDROFOLATE REDUCTASE GENE AND THIOL COMPOUNDS LEVELS IN PATIENTS WITH ALZHEIMER'S DISEASE

Chojnacka K[1], Kempisty B[1], Dorszewska J[2], Florczak J[3], Kozubski W[3], Trzeciak WH[1]

[1]Department of Biochemistry and Molecular Biology; [2]Laboratory of Neurobiology; [3]Chair and Department of Neurology, Poznan University of Medical Sciences, Poland

Increased oxidative stress, DNA damage and triggering of apoptotic and excitotoxic cell death pathways are involved in the pathogenesis of Alzheimer's disease. Disturbance in the prooxidant/antioxidant balance increases during aging and the production of free radicals are alleged to play a significant role in the neurodegenerative process. The epidemiological, genetic and experimental findings suggest that individuals with elevated homocysteine (Hcy) levels are at an increased risk of neurodegenerative disorders, such as Alzheimer's disease. Both homocysteine and amyloid β-peptide (Aβ) affect degeneration synergistically. Hcy can cause synaptic dysfunctions and neuronal death by promoting DNA damages or activation of apoptotic signaling pathways. Homocysteine is produced from the amino acid methionine (Met) by demethylation. Folate and vitamin B_{12} promote remethylation of Hcy to regenerate Met. Hcy can also be converted to cystathionine (a precursor of gluthatione) by the activity of the enzymes cystathionine-β-synthase (CBS) and cystathionase. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine.

Genetic variants of genes encoding enzymes involved in homocysteine metabolism can influence development of the nervous system. The most common genetic abnormality of the homocysteine metabolism is a transition 677C>T in exon 4 of the gene encoding the enzyme MTHFR, rendering it about 50% less active and increasing Hcy levels.

The aim of our study was to determine the frequency of common *MTHFR* polymorphisms: 677C>T, 1298A>C, 1793G>A and the concentrations of thiols: homocysteine, methionine and cysteine in a group of patients with Alzheimer's disease.

DNA was isolated from peripheral blood leucocytes of 29 patients with Alzheimer's disease and 100 healthy

individuals. The *MTHFR* 677C>T, 1298A>C, 1793G>A polymorphisms were determined by PCR-RFLP analysis using specific primers and the following restrictases: *HinfI* (677C>T), *MboII* (1298A>C) and *MbiII* (1793G>A). The total Hcy, Met and Cys concentrations in serum were based on HPLC/EC/UV method.

The differences in the frequency of 677C>T, 1298A>C, 1793G>A polymorphisms of *MTHFR* in patients with Alzheimer's disease were statistically insignificant in comparison to the control group ($p>0.05$). The levels of homocysteine and cysteine in Alzheimer's disease were significantly higher than in the control group ($p=0.003$ for Hcy and $p=0.0046$ for Cys). There were not any statistically significant differences in the levels of methionine in both groups ($p=0.53$).

Our findings suggest that although the level of homocysteine in Alzheimer's disease is higher than in the control group, it is not associated with the appearance of common polymorphisms of *MTHFR* gene. The hyperhomocysteinemia in Alzheimer's disease may cause and increase irreversible neurodegenerative processes in the brain.

A8

CARCINOGENIC CEREBELLOPATHY IN CHILDREN

Dąmbska M

Department of Clinical and Experimental Neuropathology, M Mossakowski Medical Research Centre, Polish Academy of Sciences, Warszawa

Our investigations from before a few years concerning brain damage during infantile neoplastic diseases led to conclusions that this complex problem needs further investigations. In view to approach it we examined 20 cases with neoplasms, among others blastomas, histiocytomas, sarcomas all without metastases to the central nervous system. We divided the cases into four groups. Half of them was treated with chemotherapy and radiotherapy, others did not receive such treatment. Each group included two subgroups, younger aged from two months until three years and older aged seven or eight years. Among the changes in all cases cerebellopathy was seen even in 2 or 3 months old children. It suggests the diagnosis of carcinogenic cerebellopathy. In all cases Purkinje cells were damaged but the granular layer was also rarefied, particularly in older subjects and in chemotherapy-treated cases. All results showed that the most advanced changes were found in older cases and in those treated with chemotherapy.

A9

OXIDATIVE DAMAGE TO DNA AND THE LEVEL OF APOPTOTIC PROTEINS IN LYMPHOCYTES FROM ALZHEIMER'S DISEASE PATIENTS

Dorszewska J[1], Florczak J[2], Różycka A[3], Jaroszewska-Kolecka J[1], Trzeciak WH[3], Kozubski W[2]

[1]Laboratory of Neurobiology; [2]Chair and Department of Neurology; [3]Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poland

Oxidative stress is an important factor in the pathogenic cascade of Alzheimer's disease (AD) and in degenerative neuronal death (degeneration of neurons in the nucleus basalis of Meynert) and disease progression. In the brains of AD patients, the level of reactive forms of oxygen (RFO) increases. RFO induces DNA oxidation products of purines, such as 8-oxo-2'-deoxyguanosine (8-oxo2dG) is thought to represent a marker of oxidative DNA damage in cancerogenesis, in aging and in neurodegenerative diseases. One of the first responses of a cell to DNA damage involves posttranslational modification of poly-ADP-ribosylation by a poly (ADP-ribose) polymerase (PARP). PARP and p53 proteins are involved in DNA repair. P53 protein regulates the level of proteins of Bcl-2 family and their homologues in neuronal cell death.

The aim of the study was to estimate the extent of oxidative DNA damage (levels of 8-oxo2dG) and p53, Bax, Bcl-2, PARP (with 85 kDa fragment) proteins, and to analyze polymorphisms of the *CHRNA4* (the gene for nAChR alpha-4 subunit) in peripheral lymphocytes from AD and control individuals.

34 AD patients (36-83 years, 65.8±12.5) and 44 control subjects (22-73 years, 42.9±16.5) were studied. Oxidative damage in peripheral lymphocytes was carried out using HPLC/EC/UV system (high-pressure liquid chromatography with electrochemical and UV detection) and the level of p53, Bcl-2, Bax, PARP (with 85 kDa fragment) proteins with Western Blot method. The *CHRNA4* polymorphisms were detected by PCR. Two polymorphisms (*Cfo*1690 and *Fok*1651) situated within exon 5 of the *CHRNA4* and two in intron 5 (*Ssi1* 1770^{+192} and *Ssi1* 1770^{+11}) of the *CHRNA4* were analyzed.

The level of 8-oxo2dG in lymphocytes of AD patients was significantly higher ($p<0.05$) than in controls. The marker of oxidative damage showed a marked increase

(p<0.01) in comparison to the control group only in AD patients with polymorphisms of the *CHRNA4* situated within exon 5 (*Fok1*). Moreover, the level of p53 (p<0.01) and Bax (p<0.001) proteins was markedly higher than in controls but the content Bcl-2 protein was significantly lower than in controls (p<0.05). The increase of the content PARP protein was statistically insignificant but the 85 kDa fragment was statistically significant (p<0.001).

The findings indicate that polymorphisms of the *CHRNA4* might play a role in neurodegenerative disease and the presence of oxidative damage in lymphocytes is of interest in AD because it may reflect a condition of increased oxidative stress in this pathology.

A10

ESTIMATION OF PROLIFERATION ACTIVITY, APOPTOSIS AND MICROVESSELS' DENSITY IN EPENDYMOMAS

Duda-Szymańska J, Janczukowicz J, Lewy-Trenda I, Omulecka A, Nawrocka-Kunecka A, Papierz W

Chair and Department of Pathomorphology, Medical University of Łódź

The results of the estimation of a proliferation activity, apoptosis and vessels' density in 50 ependymomas (G2 WHO), including 5 re-operated tumors have been presented in the study. Immunohisotchemical reactions with the MIB-1 and PCNA antibodies (for the estimation of the proliferation index), Bcl-2 and Bax (for the estimation of the apoptosis) as well as CD31 and FVIII (for the estimation of microvessels density) were performed. The results of the reaction were estimated quantitatively and the data underwent statistical analysis.

The results indicate that histological forms of G2 WHO ependymomas do not differ significantly in the aspect of the estimated parameters. In the recurrent ependymomas a higher proliferation index than in the primary tumors has been revealed.

A11

DYNAMICS OF ULTRASTRUCTURAL CHANGES IN LUMBAR SPINAL CORDS IN TRANSGENIC SOD 1 RATS

Fidziańska A[1], R. Gadamski[2], J. Rafałowska[2], M. Modrzewska-Lewczuk[3], R. Szopiński[3], P. Grieb[4]

[1]Neuromuscular Unit, [2]Department of Experimental and Clinical Neuropathology, [3]Laboratory of Sciences Documentation, [4]Department of Experimental Pharmacology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

The purpose of this study was to determine what structural changes tigger the onset and progression of amyotrophic lateral sclerosis in rats expressing a human SOD1 transgene with mutation G93A. The lumbar spinal cord of affected rats at presymptomatic PM (60-93 day of age) and symptomatic S (120 days of age) stages were analyzed ultrastructurally. At 60 days the structure of the lumbar spinal cord as well as alpha motoneurons type 1 and 2 appeared normal, however a careful examination revealed that approximately 15% of axons were filled with mitochondria that were abnormal in number, size and morphology. Beside hypertrophied, giant, undergoing fission, mitochondria we could distinguish mitochondria with focal separation between the outer and inner mitochondrial membrane. In a few large axons aggregation of swelling and dilated mitochondria were observed.

Grossly swollen mitochondria with disrupted cristae were a prominent feature in all large axons at 93 days of age. Dilated mitochondria no longer carrying the remnants of cristae appeared as large vacuoles, which occupied almost the entire axonal caliber and blocked axonal transport. At this time swelling and dilated mitochondria were observed also in type 1 of alpha motoneurons while type 2 had small well preserved mitochondria.

At the symptomatic stage, the alpha motoneurons showed a moderate neuronal loss mainly type 1. There was still some degree of vacuolization of the large axon but the most interesting finding at this time was the occurrence of motoneurons with morphological signs of apoptotic degeneration. Nuclear and cytoplasmic condensation, chromatin compaction and formation of uniformly large clumps characterized such motoneurons. Numerous axons with very dark homogeneous and compact interiors as well as apoptotic bodies were irregularly scattered among the neuropile. In the smaller motoneurons apoptotic degeneration was not observed, however they showed numerous structural abnormalities. Their eccentrically located nuclei exhibited deep invaginations; the cytoplasm contained numerous autophagic vacuoles, fragmented Golgi complex and filamentous inclusion.

In some astrocytes filamentous aggregates were observed in the cytoplasm and in the nuclei. One ultrastructural study indicates that mitochondrial degeneration in axons and in type 1 of alpha motoneurons occurred long before the onset of motor impairment suggesting that it is the primary cause of disease.

Disrupted mitochondria could release the pro-cell death molecules normally residing in the inter membrane space and initiate motoneurons degeneration.

A12

POLYMORPHISMS OF METHYLENETETRAHYDROFOLATE REDUCTASE GENE *MTHFR* AND THIOL COMPOUNDS LEVELS IN DIFFERENT STAGES EVOLUTION OF PARKINSON'S DISEASE

Florczak J[1], Dorszewska J[2], Kempisty B3, Chojnacka K[3], Trzeciak WH[3], Kozubski W[1]

[1]Chair and Department of Neurology, [2]Laboratory of Neurobiology, [3]Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poland

Folate is a cofactor in one-carbon metabolism, during which it promotes the remethylation of homocysteine- a cytotoxic sulfur-containing an amino acid that can induce DNA strand breakage, oxidative stress and apoptosis. Folate is required for normal development of the nervous system, playing important roles regulating neurogenesis and programmed cell death. Recent epidemiological and experimental studies have linked folate deficiency and resultant increased homocysteine levels with several neurodegenerative conditions, including Parkinson's disease (PD). Homocysteine is produced from the amino acid metionine by demethylation. Methylenetetrahydrofolate reductase (*MTHFR*) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine.

The aim of the study was to determine the frequency of common *MTHFR* polymorphisms: 677C>T, 1298A>C, 1793>A and analyze the level of thiols: homocysteine, methionine and cysteine in PD and in control individuals.

92 PD patients (34-81 years) and 48 control subjects (22-76 years) were studied. The diagnosis of PD was based on Hoehn and Yahra criteria (five, I-V stage evolution of PD). The concentrations of homocysteine, metionine and cysteine in plasma of PD patients, and healthy controls were analyzed using HPLC/EC system (high-pressure liquid chromatography system with electrochemical detection). The *MTHFR* polymorphisms: 677C>T, 1298A>C, 1793>A were determined by PCR-RFLP analysis.

The differences in the frequency of 677C>T, 1298A>C, 1793>A polymorphisms of *MTHFR* in patients with PD were not statistically significant in comparison to the control group. The level of homocysteine isolated from plasma of PD patients was significantly higher ($p<0.05$) in II and III stage evolution of PD and ($p<0.01$) in IV stage evolution of this disease than in controls. Moreover, the level of methionine was markedly lower in all stages of PD (statistically significant in II, $p<0.05$ and in III, $p<0.01$) but the concentration of cysteine was higher in all stages of PD (statistically significant in II, $p<0.05$ and in III, $p<0.01$) in comparison to the control group. The content of methionine: homocysteine ratio ($p<0.01$ in II stage, $p<0.001$ in III stage, $p<0.05$ in IV stage) and cysteine: homocysteine ratio in PD patients was lower ($p<0.01$ in IV stage) than in controls (cysteine: homocysteine ratio, apart I stage of PD).

The findings indicate that homocysteine and different thiols might play a role in pathogenesis of neurodegenerative disorders, because the level of these amino acids changes in different stages of PD evolution.

A13

THE EFFECT OF CDP-CHOLINE (SODIUM SALT, CDPCH/NA) ON RAT BRAIN MEDULLA ULTRASTRUCTURE IN THE TRANSGENIC MODEL OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS (FALS)

Frontczak-Baniewicz M[1], Walski M[1], Chrapusta SJ[2], Herbik MA[2], Grieb P[2]

Departments of [1]Cell Ultrastructure and [2]Experimental Pharmacology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Background: Hemizygotic transgenic rodents that express, in addition to their own wild-type superoxide dismutase gene (*SOD1*), the mutated human *SOD1* gene whose product shows the G93A amino acid substitution, are an established model of fALS. Untreated Sprague-Dawley rats showing high expression of the *hSOD1* gene develop neurological symptoms of the disease at less than 4 months of age and die within 11 days on the average. We have found that daily treatment of the rats with CDPch/Na (1 mmole/kg, ip), beginning on day 61 of postnatal life, slightly but significantly delays the emergence of the symptoms and death.

Methods: The study was performed in rats subjected to euthanasia at postanatal day 94, i. e. in the late presymptomatic phase of the model disease. The tissue was processed for transmission EM using standard paraformaldehyde/glutaraldehyde fixation, osmium

tetroxide postfixation, and uranyl acetate/lead citrate staining.

Results: In physiological saline-treated (control) rats, there were many dying (necrotic) and dark neurons. A sizeable percentage of surviving neurons showed marked enlargement of the Golgi apparatus *trans* zone, and atypical lobate-looking nucleus with an increased pore number. Highly heterogenous, particularly in non-myelinized fibers, was the appearance of mitochondria, many of which showed oedema and characteristic 'lace-form' crest structure of varying arrangement; the inside of some mitochondria consisted of only few, or just one enlarged compartment. Fusion of the outer membranes was seen between some mitochondria, which was suggestive of the ongoing fusion or division. An axonal accumulation was also apparent of disordered neurofilament aggregates. A distinct pattern of aberrations was found in the CDPch/Na-treated rats. There were no necrotic neurons, and the dark neurons with electron-dense cytoplasm and condensed chromatin were relatively less numerous. Many of the remaining neurons showed the lobate nucleus structure with an increased pore number, but also apparent discontinuities in the cell membrane. The axonal accumulation of disordered neurofilament aggregates was less evident. No enlargement of the *trans* zone of the Golgi apparatus, mitochondrial aberrations, or forms suggestive of mitochodrial fusion/division were seen. In addition, some medullar capillary vessels showed newly formed vessel characteristics; no such vessels were seen in the control rats.

Acknowledgment: The study was supported in part by the Foundation for the Development of Diagnostics and Therapy, Warsaw, Poland.

A14

DYNAMICS OF MOTONEURONS LOSS WITH IN THE SPINAL CORD OF RAT EXPERIMENTAL FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

Gadamski R[1], Rafałowska J[1], Fidziańska A[2], Wojda R[1], Chrzanowska H[1], Grieb P[3]

[1]Department of Experimental and Clinical Neuropathology, [2]Neuromuscular Unit, [3]Department of Experimental Pharmacology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Examination of motoneurons loss in the spinal cord of transgenic rats in various periods of life was performed. The material was obtained from animals at 60-th day of life (2), 93-rd day of age (2) and 120-th day of life (2).

Density of alfa and gamma motoneurons was quantified on both anterior horns of the cervical and lumbar spinal cord. From the 93-rd day of life a progressive decrease of the motoneurons number was observed. At the 120-th day of age only single motor neuron cells were preserved.

A15

PROGRAMMED CELL DEATH IN CANCER CELLS

Gajkowska B, Wojewódzka U

Department of Cell Ultrastructure, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw

In each cell line, a balance of cell proliferation and cell death regulates the control of the cell number. There are many types of cell death defined by morphological or biochemical behavior of the cell.

- Severely injured cells may undergo necrosis.
- Physiological deaths include the best known form-apoptosis Type I cell death. The term apoptosis or programmed cell death (PCD) defines a genetically encoded cell death program, which is morphologically and biochemically distinct from necrosis or accidental cell death.

In the last decade, the molecular mechanism of apoptosis has been clarified. The caspase family of proteinases has been shown to play crucial roles in the execution of apoptosis, and also the involvement of mitochondria in apoptosis has been broadly documented. However, there are two variants of apoptosis:

1. caspase-dependent and
2. caspase-independent programmed cell death.

- Increasing lines of morphological and biochemical evidence indicate an alternative, caspase-independent form of PCD, named autophagic Type II cell death, which is associated with autophagosomes/autolysosomes execution pathways of cell death.

Autophagy occurs in many eukaryotic cell types, where organelles and other cell components are sequestrated into lysosomes and degraded. In some setting of autophagy and apoptosis, mitochondria may be central organelles integrating the two types of cell death. Permeabilization of mitochondria seems to be an event also shared by autophagy and necrosis.

- Our own studies and other laboratories ultrastructural data suggest also the existence of the Type III cell death, which is defined as non-lysosomal vesiculate degradation. This mode of death is subdivided into:

1. III A Type (non-lysosomal disintegration) and
2. III B Type (cytoplasmic type of degeneration).

The present paper review outlines the main types of cell death showing their morphological and immunocytochemical signs by electron microscopic techniques. Our own observations conducted on human cancer cell lines (COLO 205, MCF-7) stimulated to death by anti-cancer drugs or other substances evidenced that a variety of cell death programmes may be triggered in distinct circumstances.

A16

CEREBRAL BLOOD FLOW SPECT IMAGING IN POST-STROKE APHASIA

Gąsecki D[1], Jodzio K[2], Lass P[3], Nyka WM[1]

[1]Department of Neurology, [3]Nuclear Medicine, Medical University, Gdansk, Poland; [2]Institute of Psychology, University of Gdansk, Poland

Background: Aphasia is usually the result of cardiovascular diseases and occurs in 19-38% of stroke patients. Aphasic syndromes can be correlated to relatively specific brain lesions located in the left cerebral hemisphere.

Cerebral blood flow (CBF) was evaluated in patients with three distinct aphasic syndromes following stroke.

Material and method: The research involved 50 stroke patients with a single left-hemisphere lesion and residual aphasia. Language, assessed according to the Weisenburg and Mc Bride classification and by the Boston Diagnostic Aphasia Examination, was affected to various degrees by a wide range of pathologies. Single-photon emission computed tomography (SPECT) images of the brain were acquired with Tc-99m-labeled ECD on a triple-headed gamma camera. Comparisons of reduced cerebral perfusion between patients with different types of aphasia as well as between patients with good and with poor recovery from aphasia were analyzed.

Results: The most prominent perfusion abnormalities in expressive aphasia were found in the parietal lobe and to the lesser degree in the frontal lobe, whereas the most prominent deficits in receptive aphasia were found in the left temporal and parietal regions and the striatum. In mixed aphasia, SPECT images evidenced the most extensive damage, involving both cortical and deep structures of the left cerebral hemisphere, besides the occipital lobe.

Frontal CBF was significantly higher in patients with good language recovery compared to patients with poor recovery from aphasia/low degree of speech improvement.

Conclusions: Distinct aphasic features in the three syndromes appear to be due to specific changes in the cerebral blood flow in the left cerebral hemisphere. The present study highlights the integrative role of the subcortical structures in language and speech functions. Preserved CBF in the left frontal lobe appears to be crucial in recovery from aphasia.

The results support the usefulness of regional cerebral blood flow SPECT imaging as a diagnostic aid in the post-stroke aphasia.

A17

CEREBELLAR GLIOBLASTOMA IN A PATIENT WITH THE BRAIN STEM LOW GRADE ASTROCYTOMA – A CASE REPORT

Gąsecki D[1], Świerkocka-Miastkowska M[1], Iżycka-Świeszewska E[2], Kozera G[1], Jaśkiewicz K[2], Nyka WM[1]

[1]Department of Neurology, [2]Department of Pathology, Medical University of Gdansk, Poland

A 34-year old man with a 3-month long history of left hemiparesis was admitted to the Neurology Department in November 1993. MRI showed a brain stem tumor involving medulla oblongata from the fourth ventricle level to the occipital foramen (35x18 mm). Performed diagnostic stereotactic biopsy was unsuccessful. The patient was treated with ^{60}Co irradiation of the posterior fossa (5500 cGy/30 fraction). Six months later he developed discrete bulbar and right cerebellar signs without features of progression on MRI. During next 10 years his neurological signs were stable.

In September 2004 the patient was admitted to the Neurology Department with bilateral cerebellar signs and slowly progressing increased intracranial pressure syndrome. On MRI tumor progression was found in the form of areas of diffuse hyperintensity with focal enhancement involving pons and medulla oblongata, both cerebellar hemispheres and ring-like enhanced lesion with central necrosis in the left cerebellar medial peduncle. Radiologically malignant glioma or late postirradiation necrosis was suggested. Soon after, the patient died suddenly due to pulmonary embolism.

On the *post mortem* examination the low-grade fibrillary astrocytoma was diagnosed within the brain stem. The infiltration within the cerebellar peduncle

and hemisphere was of gliobastoma type. The nervous tissue around the cerebellar tumor showed prominent postirradiation changes.

Cerebellar glioblastomas are very rare. The origin of the presented case is debatable. This glioblastoma could develop on the way of malignant progression of infiltrating low-grade astrocytoma, or it was a primary tumor induced by radiotherapy.

A18

HSV-1 GENOME IN PATIENTS WITH DEMENTIA

Geppert A[1], Myga M[2], Przedpelska-Ober E[1], Goździcka-Józefiak A[2]

[1]Department of Neurology, University of Medical Sciences, Poznan; [2]Department of Molecular Virology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan

Herpes simplex virus type 1 (HSV-1) was revealed in a latent form in the brains of elderly people and has been proposed an environmental risk factor for Alzheimer's disease in carriers of the type-4 allele of the gene for Apolipoprotein E (APOEε4). The presence of HSV-1 was associated with β-amyloid depositions in the cerebral cortex. It was established that HSV-1 remains in a latent form in 50-80% of population. If a constant low level of HSV-1 production occurs asymptomatically in the brains of patients with AD, one key question concerning the molecular mechanisms of HSV-1 transport within the nervous system arises.

The purpose of our study was to examine patients with dementia for presence of HSV-1 genome in peripheral blood. 27 blood samples from 12 AD patients, 4 patients with other type of dementia and 11 control patients were collected and kept at -80°C until processing. To study HSV-1 DNA expression PCR was carried out and amplified reaction products were electrophoresed.

Recently, the theories of the hematogenous dissemination of latent HSV-1 or the transmission by promoting viral passage across the synapse has been discussed. In present, a preliminary study on HSV-1 in patients with dementia no evidence of HSV-1 DNA in the peripheral blood is reported. Confirmation that HSV-1 DNA is not present in the blood in the population of AD patients may focus our interest on other than hematogenous manner of viral transmission in the latency.

A19

PATHOLOGY AND PATHOGENESIS OF TUBEROUS SCLEROSIS SUBEPENDYMAL GIANT CELL ASTROCYTOMAS

Grajkowska W[1], Jóźwiak S[2], Roszkowski M[3], Chan J[4], Kwiatkowski D[5]

[1]Department of Pathology, [2]Department of Neurology, [3]Department of Neurosurgery, Children's Memorial Health Institute, Warsaw; [4]Division of Neuropathology, Brigham and Women's Hospital, Boston; [5]Experimental Medicine Division, Harvard Medical School, Boston

Introduction: Subependymal Giant Cell Astrocytomas (SEGAs) are rare, histologically benign brain tumors associated with tuberous sclerosis complex (TSC). TSC is a neurocutaneus syndrome resulting from mutations in two genes, TSC1 and TSC2, encoding hamartin and tuberin, respectively.

Recent studies show an important role for the TSC genes in a signaling pathway involving the mammalian target of rapamycin (mTOR) kinase.

The aim of the study was examination of microscopic features and an analysis of the mTOR-signaling pathway in TSC SEGAs.

Material and methods: We performed a histological examination, immunohistochemical and genetic analyses on 7 SEGAs from TSC patients. Immunohistochemical studies were performed using the following antibodies: GFAP, class III b-tubulin, phospho-p70 S6 kinase, S6 ribosomal protein, phospho-S6 ribosomal protein, STAT3, phospho-STAT3. TSC1 and TSC2 mutational analysis was performed on blood-derived DNA from these patients.

Results: All 7 cases of SEGAs showed classic histologic features: plump cells with abundant eosinophilic cytoplasm.

Five of 6 SEGAs showed of biallelic mutation of TSC1 or TSC2 genes.

SEGAs cells revealed high levels of phospho-S6K, phospho-S6, and phospho-Stat3, all proteins downstream of mTOR activation.

We concluded that TSC SEGAs likely arise through a two-hit mechanism of biallelic inactivation of TSC1 or TSC2, leading to activation of the mTOR kinase.

A20

LOSS OF HETEROZYGOSITY ON CHROMOSOME 1p AND 19q IN OLIGODENDROGLIAL TUMORS

Grešner SM, Rieske P, Woźniak K, Piaskowski S, Golańska E, Liberski PP

Department of Molecular Pathology and Neuropathology, Medical University, Łódź, Poland

Loss of heterozygosity (LOH) on chromosome 1p and/or 19q is common and early molecular alteration occurs in the majority of oligodendroglial tumors. In most of these tumors, all or almost all of 1p and/or 19q arms have been deleted. Nevertheless, a small fraction of tumors carries small terminal or interstitial deletions, which have been instrumental in identification of candidate regions for oligodendroglioma-associated tumor suppressor genes. Molecular alterations in the region of 1p and 19q are predictors of survival and chemosensitivity in oligodendroglial tumors. Noteworthy, 1p and 19q deletions may be associated not only with response to chemotherapy, but with tumor grade as well. Therefore, the tests for the loss of heterozygosity on chromosome 1p and/or 19q may be of particular justification especially in cases of ambiguous diagnosis of oligodendrogliomas.

The objective of our project was to explore the characteristic DNA fragment on chromosomes 1p and 19q which is deleted in oligodendroglial tumors. DNA fragments, isolated from 22 oligodendroglial tumors (WHO grade II and III), were amplified by PCR technique and subsequently analyzed for LOH using 5 polymorphic markers for 1p and 5 for 19q. Results of our experiments allowed us to determine the frequency of LOH within the chromosomes analyzed. Simultaneous LOH on 1p and 19q was observed in 8 cases (36.4%), only on chromosome 1p in 2 cases (9%) while 1 case showed LOH only on chromosome 19q (4.5%). The frequent coincidence of 1p and 19q deletions suggests a synergistic effect of both alternations in the development and progression of oligodendroglioma. Moreover, the loss of genetic material on 1p and/or 19q raises the possibility that these chromosomes may harbor important tumor suppressor genes involved in oligodendroglioma cancerogenesis. However, with respect to the large region of deletions, the precise location of these genes is as yet complicated and consecutive investigations remain necessary.

A21

ATTEMPTS FOR PHARMACOLOGICAL DEFERMENT OF DISEASE PROGRESS IN A RAT MODEL OF THE FAMILIAL FORM OF AMYOTROPHIC LATERAL SCLEROSIS (FALS)

Herbik MA[1], Chrapusta SJ[1], Woźniak M[2], Grieb P[1]

[1]Department of Experimental Pharmacology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw; [2]Department of Medicinal Chemistry, Medical University of Gdańsk, Gdańsk, Poland

Background: ALS is an incurable fatal neurodegenerative disease with a 2-5-year symptomatic survival prognosis. fALS represents nearly 10% of all ALS cases; about 20% of fALS cases are due to mutation (s) in the superoxide dismutase-1 gene. Transgenic rodents endowed with the mutated human gene (*hSOD1*) develop, despite the normal expression of their own wild-type *SOD1* gene, an illness that imitates ALS in most respects.

Methods: Hemizygotic transgenic rats carrying the G93A-mutated *hSOD1* (Howland et al, PNAS 2002; 99:1604) were randomized by both gender and litter between cytidine 5'-diphosphocholine sodium salt-(CDPch/Na, 500 mg/kg), 4-hydroxy-2,2,6,6-tetramethyl-piperidine 1-oxyl- (Tempol, an SOD1 mimetic and free radical scavenger, 100 mg/kg) and the respective physiological saline-treated (control) groups. Treatments commenced on postnatal day 61 and consisted of one daily ip injection of the drug or the

Table I.

Treatment	Symptom-free survival, days			Overall survival, days		
	Mean±SD (range, median)	P		Mean±SD (range, median)	P	
		Log-rank	Cox F		Log-rank	Cox F
Saline (15♀, 7♂)	115.0±7.5 (96-125, 116.0)	0.020	0.011	129.6±7.7 (117-146, 128.0)	0.045	0.025
CDPch/Na (14♀, 10♂)	118.3±8.1 (101-130, 122.5)			134.7±12.0 (111-159, 134.0)		
Saline (12♀, 13♂)	111.4±7.7 (98-137, 110.0)	0.80	0.42	134.6±16.5 (114-181, 125.0)	0.77	0.41
Tempol (13♀, 12♂)	109.9±8.4 (95-126, 111.0)			133.8±16.0 (112-180, 125.0)		

saline. The rats were weighed and checked daily for a slow-down in the reaction to touching the paw/foot underside, or the emergence of limb weakness or abnormal gait/posture. The treatment continued till the rats showed full paralysis of at least 3 limbs (surrogate "death point", at which they were euthanized).

Results: Disease onset site was hindleg in 51 rats, front paw in 29 rats, and both locations in 16 rats; there was no considerable difference between the treatment groups in this respect ($P>0.05$, χ^2 test). Both symptom-free and overall survival were slightly but significantly prolonged by CDPch/Na, but not Tempol (see Table); the length of the symptomatic phase of the disease was not significantly affected (Mann-Whitney U-test). The drugs did not appreciably modify body weight changes' profile (two-way ANOVA followed by Tukey test).

Acknowledgment: The study was supported in part by the Foundation for the Development of Diagnostics and Therapy, Warsaw, Poland.

A22

VASCULAR PATTERNS IN NEUROBLASTOMA

Iżycka-Świeszewska E[1], Drożyńska D[2], Rzepko R[1], Grajkowska W[3], Jaśkiewicz K[1]

[1]Department of Pathology, [2]Clinic of Pediatrics, Hematology, Oncology and Endocrinology, Medical University of Gdansk, [3]Department of Pathology, Children's Memorial Health Institute, Warsaw

Neuroblastic tumors are a heterogenous group of embryonal neoplasms of the adrenal medulla and sympathetic nervous system. Vascularization in neuroblastic tumors is an infrequent subject of investigation despite accepted classifications, where the quantity of tumor stroma (Schwannian type) is one of diagnostic criteria.

Seventy-nine cases of neuroblastic tumors (NB) were examined, including 60 cases of neuroblastoma Schwannian stroma-poor and 19 cases of ganglioneuroblastoma (GNB) Schwannian-rich. The type of vascular pattern and vascular changes were analyzed in HE and immunohistochemically with CD34 and αSMA.

Three types of vascular patterns were observed-reticular, trabecular and irregular. In reticular-fibrovascular stroma formed delicate meshwork surrounding small tumor nests (20 cases), in trabecular-fibrous stroma separated larger tumor sheets (22 cases). An irregular pattern was found in 37 tumors. In some tumors small delicate vessels dominated, in some-vascular changes (fibrosis, hyalinization, lumen widening) and microvascular proliferation (MVP) were present. MVP was observed in 27 cases. Fibrosis and hyalinization dominated in GNB and cases after chemotherapy. Evident co localization of developing Schwannian stroma and vascular framework was encountered. Immunostaining revealed a network of CD34 positive and αSMA positive extravascular cells in developing Schwannian stroma.

Vascular patterns in neuroblastoma are variegated. We observed remodeling of the vascular pattern parallel to NB maturation: irregular stroma-poor-reticular--trabecular-irregular stroma-rich. MVP is quite often a feature of NB vascularity, and is more frequent in differentiating subtype. Vascular and perivascular stromal elements contribute to Schwannian stroma formation.

A23

LOSS OF HETEROZYGOSITY ON CHROMOSOME 10 (LOH) AND SOME CLINICAL PATIENTS AND TUMOR CHARACTERISTICS IN GLIOBLASTOMA

Iżycka-Świeszewska E[1], Woźniak A[2], Limon J[2], Rzepko R[1], Jaśkiewicz K[1]

[1]Department of Pathology, [2]Department of Biology and Genetics, Medical University of Gdańsk

This study was performed to establish the frequency of loss of heterozygosity (LOH) on chromosome 10 in glioblastoma and to investigate its clinicopathological correlation. Sixty-two glioblastoma cases were examined (28 women, 34 men; aged: 25-75 years; 39 temporal lobe tumors). In HE dominant cellular population and type of microvascular proliferation (MVP) were assessed. Proliferative index and vascular density were counted on Ki-67 and CD34 stained slides, respectively. For LOH analysis the representative neoplastic tissue and normal brain tissue were cut out with lancet from paraffin blocks. Six polymorphic 10q markers localized close to *PTEN*, *LG11*, *DMBT1* and two 10p markers were used. DNA fragments were PCR-amplified and analyzed using automated sequencer ABI1310, with results under 0.6 scored as LOH.

44 glioblastomas presented with mixed cellularity, in 45 features of glomeruloid MVP were found. Vascular density ranged 66-312 vessel/mm2 and proliferative index ranged 9-39%. In 34 cases at least one marker from chromosome 10 was lost (54%). In seven tumors only *PTEN* locus was affected. In ten cases deletions were found in two or more loci on 10q, and in 17 losses involved loci on 10p and 10q. Statistical analysis revealed

a higher frequency of LOH 10 in tumors without glomeruloid MVP (p=0.05) and in men (p=0.008). Tumors with LOH 10 showed a tendency to higher proliferative index and localization of tumor in the temporal lobe.

Deletions on chromosome 10 were found in about 50% of glioblastoma. In the examined group LOH 10 shows some relations with tumor vascularisation and patients characteristics.

A24

OCCURRENCE OF DIFFERENT HISTOLOGICAL TYPES OF MENINGIOMAS AND THEIR RELATIONSHIP BETWEEN TOPOGRAPHY, SEX AND AGE OF PATIENTS-10-YEARS OBSERVATION

Jarosz B[1], Górniak J[2], Puzon K[2], Jarosz M[3], Szczepanek D[1], Trojanowski T[1]

[1]Chair and Clinic of Neurosurgery, Medical University of Lublin, [2]Medical Students' Research Association, Medical University of Lublin, [3]Institute of Agricultural Medicine in Lublin, Clinical Epidemiology Unit

Background: Meningiomas are a group of neoplasms growing from meningothelium cells. There are 15 histological subtypes of those tumors, according to the WHO classification of the CNS tumors issued in 2000. Histological malignancy of meningiomas is graded from I to III, and each histological type of the WHO classification has assigned the particular grade of malignancy.

Objectives: Epidemiological analysis of the occurrence of different histological types of meningiomas and their relationship between topography, sex and age of patients.

Material and methods: Surgical specimens from patients treated between 1994 and 2004 in the Department and Clinic of Neurosurgery and Child Neurosurgery, Medical University of Lublin, were studied. In statistical analysis the frequency and contingency tables were used. Statistical hypotheses were evaluated using Chi-square tests.

Results: There were 2716 intracranial neoplasm tumors surgically treated in the analyzed period. Among them, gliomas were more frequent – 46% (467), then meningiomas – 21.3% (579), metastases 16% (421) and other neoplasms – 17.2% (467). In the analyzed material, meningiomas were more frequent among women – 68.9% (399) than men 31.1% (180). That was statistically significant ($p<0.0001$). Age distributions among women and among men with meningiomas were similar and the observed differences were not statistically significant. Meningiomas were most frequent between the 5th and 7th decades of life, both among women and men. The most frequent histological type was meningothelial meningioma – 27.4% (158), then fibrous meningioma – 21.2% (122), transitional meningioma – 17.4% (100) and intracranially 90.3% (523), and only 9.7% (53) – in the spinal canal. There was an association between localization and histological types ($p<0.0001$). Meningiomas with grade II and grade III of malignancy were less frequent among women – 4.5% (18) of meningiomas than among men – 18.9% (34). We observed the association between sex and grade of malignancy ($p<0.001$). However, we did not observe the association between age and grade of malignancy.

Conclusion: The presented analysis provided evidence that meningiomas occurred more often among women and that a higher grade of malignancy is more often among men. Meningiomas were most frequent between the 5th and 7th decades of life, both among women and men. Finally, meningiomas are more often localized intracranially than in the spinal canal.

A25

COMPARISON OF THE Ki-67 EXPRESSION (USING MIB-1 ANTIBODY) AND PCNA EXPRESSION AMONG ASTROCYTIC GLIOMAS

Jarosz B[1], Puzon K[2], Górniak J[2], Mosiewicz A[1], Trojanowski T[1]

[1]Chair and Clinic of Neurosurgery, Medical University of Lublin; [2]Medical Students' Research Association, Medical University of Lublin; [3]Institute of Agricultural Medicine in Lublin, Clinical Epidemiology Unit

Background: Astrocytic gliomas are most frequent neoplasms of the central nervous system. According to the WHO classification, their histological malignancy is graded from grade I to grade IV. This type of neoplasm has an extreme tendency for progression. Proliferation activity of astrocytic gliomas has a direct influence on the clinical course of the disease. The quantitative assessment of proliferation activity is a very good extension of the WHO classification based on histogenesis.

Objectives: Comparison of the Ki-67 expression (using a MIB-1 antibody) and PCNA expression as a method of differentiation between grade II, III and IV of histological malignancy of astrocytic gliomas.

Material and methods: The study was carried out on the selected surgical material from 27 patients with astrocytic gliomas, treated between 1996 and 2000 in the

Department and Clinic of Neurosurgery and Child Neurosurgery, Medical University of Lublin. Among selected specimens we have found fibrillary astrocytoma GII (5 case), gemistocytic astrocytoma GII (5 cases), anaplastic astrocytoma GIII (6 cases), glioblastoma GIV (11 cases). Proliferation activity was studied using immunohistochemical reactions with monoclonal antibodies: Ki-67 (clone MIB-1, DAKO, 1:100) and PCNA (clone PC10, DAKO, 1:50) and EnVision system (DAKO). In each case the labeling index (LI) was calculated dividing the number of the positive cells by the number of all cells. Then the LI was expressed as percentage. The descriptive statistics, the Pearson correlation and the Kolmogorov-Smironov test were used in the statistical analysis.

Results: The average values of MIB-1 LI were as follows: grade II – 4% (SD=1.98 SE=0.63), grade III – 11.8% (SD=3.96, SE=1.62), and grade IV – 27.9% (SD=10.09, SE=3.04). The average values of PCNA LI were as follows: grade II – 54.5% (SD=11.63 SE=3.68), grade III – 61.5% (SD=7.65, SE=3.12), and grade IV – 71.5% (SD=7.59, SE=2.29). We have found a statistically significant difference of the MIB-1 LI values between grade II and grade III (K-S test, $p<0.001$). However, we have not found any statistically significant difference of the PCNA LI between grade II and grade III. Similarly, we have found a statistically significant difference of the MIB-1 LI values between grade III and grade IV (K-S test, $p<0.01$) and there was not any significant difference in PCNA LI.

Conclusion: Despite the positive correlation between MIB-1 LI and PCNA LI, their usefulness as markers of cell proliferation differs. Because the PCNA LI does not differentiate grade II and grade III, and because PCNA immunohistochemical reactions are vague, this antibody is not a relevant marker of the cell proliferation for astrocytic gliomas. On the contrary, the MIB-1 LI seems a relevant marker of the cell proliferation for this type of neoplasms.

A26

GENETIC BASIS OF 4R TAUOPATHY IN PROGRESSIVE SUPRANUCLEAR PALSY

Kowalska A

Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland

Progressive supranuclear palsy (PSP), called also Steele-Richardson-Olszewski syndrome, is a rare form of parkinsonism characterized by abundant pathology of microtubule associated protein tau. Neuropathologically, PSP is associated with a neuronal loss in the brain stem and basal ganglia. Within these brain regions there is a high density of fibrillary tau pathology, including neuropile threads and neurofibrillary tangles (NFTs), that are typically round or globular. The tau pathology in PSP seems to be characteristic and distinguished from that in other tauopathies. The following features are observed in a majority of PSP patients: 1/ tau lesions are exclusively composed of tau 4R isoforms with exon 10, 2/ tau aggregates have a specific biochemical pattern (Class II) with the upper tau doublet determined by hyperphosphorylation of 4R isoforms, which is found in the subcortical and cortical areas at the last stage of the disease, and 3/ ultrastructural analysis of the neurofibrillary lesions has revealed 15- to 18- nm straight filaments, and filaments with a long periodicity, in contrast to Alzheimer's disease with paired helical filaments (PHF).

The etiology of PSP is still unknown. Rare familial cases of PSP, which have been described, suggest the influence of genetic factors. Disturbances in tau protein metabolism seem to be crucial for etiology of PSP. However, the mutations in *Tau* gene have not been still found in patients with PSP. Although, several *Tau* mutations (e.g.: N279K, R5L, or E10 +16) have been associated with PSP-like symptoms in a clinical phenotype of some familial autosomal dominant cases of FTDP-17. Recent studies of patients with sporadic PSP suggest that PSP have a recessive pattern of inheritance. A strong association between the polymorphic 116507 (TG) n repeat in intron 9 of the *Tau* gene and PSP has been reported (Conrad et al., Ann Neurol 1997; 41: 277-281). Both the AO allele and AO/AO genotype were significantly overrepresented in the PSP patients, compared to controls. The association has been confirmed in many other studies. At least several hypotheses concerning possible effects of the DNA polymorphism on the *Tau* gene expression and its role in the pathogenesis of PSP will be discussed.

A27

IMMUNOHISTOCHEMICAL IDENTIFICATION OF NEURAL STEM CELLS FROM HUMAN UMBILICAL CORD BLOOD (HUCB-NSC) AFTER TRANSPLANTATION INTO THE NEONATAL RAT BRAIN

Kozlowska H, Markiewicz I, Habich A, Lukomska B, Domanska-Janik K

NeuroRepair Department, M. Mossakowski Medical Research Centre, Warsaw, Poland

The studies conducted by our research group enabled us to establish the neural stem-like cell line derived from human umbilical cord blood (HUCB-NSC). Recently, we have shown that HUCB-NSC have the capacity to proliferate and differentiate *in vitro* into the major phenotypes of the adult brain cells, i.e., neurons, astrocytes, and oligodendrocytes. The goal of this study was to evaluate the potential of HUCB-NSC to survive and differentiate after their transplantation into the neonatal rat brain. On postnatal day 0 (P0) Wistar rats were used as recipients. HUCB-NSC ($2x10^4$) labeled with CMFDA cell tracker was injected into the subventricular zone (SVZ) under stereotaxic surgery (coordinates: A-1.0, L-2.0, V-2.0). After various time brains were removed, frozen and cut into 20 µm coronary slices, then the immunocytochemical studies were performed to visualize HUCB-NSC presence in the brain. The phenotype of transplanted HUCB-NSC and their localization in the rat brain were identified using monoclonal antibodies anti-HuNu and HuMi as human cell markers. Results. Eighteen hours after transplantation most HUCB-NSC expressed neuronal markers (TUJ-1, NF-200) and few of them presented astrocytic markers (GFAP, S100). Seven days after transplantation the implanted HUCB-NSC started to migrate from the clump and 14 days after grafting a significant number of these cells were found in the white matters tracts. In conclusion, our study shows that transplanted HUCB-NSC survive in the neonatal rat brain, migrate and differentiate along the neuronal and glial lineages. These properties make them candidates for therapeutic transplantation in neurodegenerated disorders.

A28

REMOTE IN TIME AND LOCATION MALIGNANT GLIOMA METASTASES TRANSPORTED VIA CEREBROSPINAL FLUID PATHWAYS. A REPORT OF TWO CASES AND LITERATURE REVIEW

Kwiek SJ[1], Bierzynska-Macyszyn G[2], Ślusarczyk W[1], Kukier W[1], Bażowski P[1], Wlaszczuk P[2], Wójcikiewicz T[1]

[1]Department of Neurosurgery Medical University of Silesia, Katowice, Poland; [2]Department of Pathology Medical University of Silesia, Katowice, Poland

Introduction: Malignant glioma dissemination via the cerebrospinal fluid is known but relatively poorly documented in the literature. Recurrence of malignant glioma appeared usually at the site of removal, but cancer can reappear also from cells transported via fluid pathways in distant parts of the central nervous system. Cases of remote in time and location metastases of malignant glioma were described and radiologically documented in the literature quite rarely.

Materials and methods: Detailed clinical history and well-documented histology of two cases of glioma metastases are described. Case 1: a woman, 44 years old, treated for anaplastic astrocytoma of the left cerebral hemisphere appeared tumor of similar histology, located in the right cerebellar hemisphere 16 months after surgery. Case 2: a woman, 24 years old, was operated for astrocytoma of the lumbar segment of the spinal canal 6 years after treatment of the some histology glioma located within the cerebellum.

Discussion: In cases when part of malignant glioma is fluid, it is now assumed that some extra-cellular components of its matrix and also other soluble elements could have significance in the process of dissemination or recurrence. As some experimental research shows they can actively migrate or could be carried in a passive way by, for example, the tumour's liquids or by the cerebrospinal fluid.

A29

CURRENT METHODS OF DIAGNOSIS AND TREATMENT IN PRIMARY CEREBRAL LYMPHOMA CASES

Kwiek SJ[1], Bierzynska-Macyszyn G[2], Wolwender A[1], Wlaszczuk P[2], Bażowski P[1], Górowska E[1], Ślusarczyk W[1], Kukier W[1], Wójcikiewicz T[1]

1Department of Neurosurgery, 2Department of Pathology Medical University of Silesia, Katowice, Poland

Introduction: Modern standards of primary cerebral lymphoma histology diagnosis comprise stereotactic biopsy. Primary cerebral lymphoma is an extra-nodal lymphoma, developed within CNS without signs of the systemic disease. Radiation, chemotherapy and steroids supplementation seem to be the best schema of patients treatment even in large tumor cases, which are usually still qualified to open surgery.

Materials and methods: Diagnoses of 29 primary cerebral lymphoma were established from samples obtained from stereotactic biopsy (Brain-Lab system) or open surgery. The volume of the samples obtained from stereotactic biopsy ranges from 0.5 to 1 mm^3. Some of them were used for immediate diagnostics, others were

a reserve for immunocytochemical responses and evaluation of proliferation activity and apoptosis (Ki-67, p53, BCL2). Antigen expression was evaluated using semiautomatic microscopic analysis system VIDS IV.

Results: We diagnosed 23 B-cell lymphomas, 3 T-cell lymphomas and 3 cases without immunoblastic type definition. Radiation was conducted based on Varian Clinac 2300 C/D equipped with micro MLC together with dynamic treatment option LINAC radiosurgery. Chemotherapy and steroids supplementary supported patient's treatment. Patient's history is correlated to schema of treatment, subtypes of lymphoma and other factors.

Conclusions: Diagnosis of ultra-small samples obtained from stereotactic biopsy in cerebral lymphoma cases allows to correct patients' treatment.

A30

POSSIBILITIES OF CNS PATHOLOGIES DIAGNOSIS ON THE BASIS OF ULTRASMALL SAMPLES COLLECTED DURING STEREOTAXY

Kwiek SJ[1], Bierzynska-Macyszyn G[2], Tarnawski R[3], Maciejewski B[3], Ślusarczyk W[1], Kukier W[1], Bażowski P[1], Wlaszczuk P[2], Wójcikiewicz T[1]

[1]Department of Neurosurgery, [2]Department of Pathology Medical University of Silesia, Katowice, [3]M. Curie-Skłodowska Oncology Institute, Gliwice

Introduction: The key for decision made during stereotactic biopsy (SB), concerning the selection of treatment option (radiosurgery, brachytherapy, fractionated RT or surgery), is the histological diagnosis.

Material and methods: Between December 2000 and December 2003, 111 stereotactic procedures were performed, based on the system of stereotactic planning and treatment Brain-Lab. Samples obtained from SB ranged from 0.5 to 1 mm^3. Some of them were used for immediate diagnostics, others were a reserve for immunocytochemical responses and evaluation of proliferation activity and apoptosis (Ki-67, p53, BCL2). Antigen expression was evaluated using semiautomatic microscopic analysis system VIDS IV.

Results: Among stereotactic procedures were 73 SB, where after receiving histopathological diagnosis, appropriate treatment procedures were onsetted (HDR after loading brachytherapy – 35 patients, LINAC based radiosurgery – Varian Clinac 2300 C/D equipped with micro MLC together with dynamic treatment option – 8, fractionated irradiation – 15, and other methods – 15). In 3 cases, samples obtained from the biopsy were not diagnostic. 32 patients had the catheter implanted into the remnant tumor, or into its bed, in order to perform postoperative brachytherapy. Three patients underwent stereotactic evacuation of the intracerebral hematoma and in 3 cases stereotactic craniotomy have been performed.

Conclusions: SB permitted diagnosing deep located brain cancers. Small samples were sufficient for proper pathologic diagnosis. This allowed us to introduce correct treatment.

A31

DIAGNOSING CADASIL: THE ULTRASTRUCTURAL EXAMINATION OF SKIN AND MUSCLE BLOOD VESSELS

Lewandowska E[1], Wierzba-Bobrowicz T[1], Mazurkiewicz M[2], Barańska-Gieruszczak M[2], Bertrand E[1], Szpak GM[1], Leszczyńska A[3], Pasennik E1, Stępień T[1]

[1]Department of Neuropathology, [2]Second Department of Neurology, [3]Third Department of Psychiatry, Institute of Psychiatry and Neurology, Warsaw

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infracts and leukoencephalopathy) is a vascular disorder affecting mainly the central nervous system. CADASIL is a hereditary disease, with its onset in adulthood, caused by missense point mutation in a Notch 3 gene, mapped to chromosome 19. Most mutations have been identified within exons 3 and 4 of the Notch 3 gene.

Pathological alternations of the vascular wall are present in different organs, showing accumulation of granular osmophillic material (GOM) within the wall of small and medium-sized arteries. Diagnostic criteria for CADASIL involve a presence of mutation in the Notch 3 gene and/or deposits of granular osmophillic material in the vascular wall. So the final diagnosis can be confirmed either by ultrastructural examination of skin and muscle blood vessels or by a molecular analysis.

Fragments of skin and skeletal muscles were obtained from the left upper arm of a 51-year-old female patient suspected of having CADASIL. For ultrastructural studies, the small samples were fixed in 2.5% glutaraldehyde and post-fixed in osmium tetraoxide.

At the ultrastructural level, pathognomonic for CADASIL, accumulation of granular osmophilic material within the skin and muscle arterioles was found. Often within the wall of only one vessel numerous GOM deposits varied in size were present. GOM stored up in thickened basal lamina, that surrounded smooth muscle cells of the vessel wall and the deposits were often

found in infoldings of the vascular smooth muscle cells surface. The smooth muscle cells, coming in close contact with GOM were degenerated; commonly limited to only thin processes, where no nucleus was seen and contained a shrunken cytoplasm full of numerous vacuoles adjacent to a cell membrane or even in close contact with it. An irregular endothelial cell nuclei as well as a striking increase in the number of pinocytotic vesicles and microfilaments filling the cytoplasm were distinctive features in the affected vessels.

GOM phenomena, revealed at the ultrastructural level in the blood vessel wall, provided grounds for CADASIL to be diagnosed.

A32

IMMUNOHISTOCHEMICAL,ULTRASTRUCTURAL, AND NEUROIMAGING METHODS IN DIAGNOSTICS SNEDDON'S SYNDROME

Lewandowska E[1], Wierzba-Bobrowicz T[1], Wagner T[4], Bogusławska R[2], Rudnicka A[3], Pasennik E[1], Lechowicz W[1], Kuran W[3]

1Department of Neuropathology, 2 Department Neuroradiology, 3I Department of Neurology, Institute of Psychiatry and Neurology, Warszawa, 4Department of Pathomorphology, Institute of Rheumatology, Warszawa

Sneddon's syndrome is a rare progressive disorder affecting the blood vessels. It is characterized by typical skin lesions (livedo reticularis or livedo racemosa) and cerebrovascular lesions occurring at an early age. The typical pathological changes consist of non-inflammatory occlusive arteropathy of small and medium arteries of the skin and brain. The disease usually starts with vascular pathology in the epidermis. For this reason examination of skin biopsies and determination of arteriolar occlusion is of particular importance for early diagnosis.

We performed CT (computed tomography) and MRI (magnetic resonance imaging) of an 18-year-old female patient. TC showed two big ischemic focuses in the pons. MRI examination showed small disseminated ischemic focuses in the deep structure of both brains hemispheric – the biggest in the right thalamus. MRangiography showed no changes in the big extracranial and intracranial arteries. On the basis of these results, the skin and skeletal muscle biopsy was an examination to confirm the diagnosis of the Sneddon'syndrome.

The samples of skin and skeletal muscles for light microscopy were fixed in formalin. For ultrastructural studies, the small samples were fixed in 2.5% glutaraldehyde followed by osmium tetroxide.

The light microscopy revealed a significant reduction of the vascular lumen in the capillaries and small to medium-sized arteries of the skin and muscles. Proliferation of endothelial cells, frequently with multilayer arrangement and their nuclei placed perpendicularly to the vascular lumen was visible. Proliferating cells were CD31, CD34 and sometimes SMA positive

The electron microscopy showed in skin biopsies small and medium-sized arteries with a different range of lumen reduction, often resembling a narrow cleft. The cytoplasm of cells surrounding the lumen of the blood vessel showed abundant filaments (about 10 nm in diameter – a diameter of intermediate filaments), frequently filling a substantial part of cytoplasm. Both filaments and the present Weibel-Palade bodies are structures typical of endothelial cells. Numerous pinocytotic vesicles were seen under plasmalemma of those cells. Cellular nuclei, lobar or folded were arranged perpendicularly to the vessel lumen. These cells showed an ultrastructural picture typical of endothelium. It is generally accepted that, in addition to the Weibel-Palade bodies, the immunoreactivity for CD 31 constitutes one of the most specific ways to identify endothelial cells. Outside endothelia, were seen cells with a typical appearance of the smooth muscles cells. In numerous vessels with reduced lumen, the basement membrane was discontinuous and at its locations, cells of smooth muscles or their processes were in contact with the abluminal endothelial surface. Vessels with a different range of the lumen reduction were also observed in skeletal muscles. Cells surrounding the vessel lumen were characterized, contrary to cells in the skin vessels, by a very large number of pinocytotic vesicles under plasmalemma and in the all cytoplasm, whereas proliferation of intermediate filaments was not seen. Like in the skin vessels, a direct contact between smooth muscles and endothelia was seen.

The ultrastructural picture of blood vessels in both skin and skeletal muscles provided grounds for Sneddon's syndrome to be diagnosed.

A33

EXPRESSION OF MMP-9, MMP-2 AND THEIR TISSUE INHIBITORS TIMP-1, AND TIMP-2 IN PRIMARY AND RECURRENT VESTIBULAR SCHWANNOMAS

Marcol W[1], Kwiek SJ[2], Górka D[1], Korczyńska I[1], Ślusarczyk W[1,2], Bierzyńska-Macyszyn G[3], Larysz-Brysz M[1], Kukier W[1], Bażowski P[2], Lewin-Kowalik J[1]

[1]Physiology Department, Medical University of Silesia, Katowice, Poland; [2]Neurosurgery Department, Medical University of Silesia, Katowice, Poland; [3]Department of Pathomorphology, Medical University of Silesia, Katowice, Poland

Vestibular schwannomas do not form a homogeneous histopathological group. They are usually classified as benign tumors, however is some cases growth of the residual tumors has become malignant. Because of their changeable behavior as well as quite high rate of recurrences additional prognostic factors are still needed for their evaluation, and especially for planning of the strategy for their management.

Matrix metalloproteinases (MMPs – matrix metalloproteinases) are a family of endopeptidases that play an important role in development, tumor growth, angiogenesis or inflammatory reactions. Although many studies of different neoplasms have proven the role of MMPs in tumor growth and metastasis their prognostic role in patients with vestibular schwannomas has not been defined yet.

In this study, we analysed the expression pattern of MMP-9 and MMP-2 and their tissue inhibitors, TIMP-1 and TIMP-2, in vestibular neurinomas by means of zymography and immunoblotting. Primary as well as recurrent acoustic neurinomas were obtained from the same patients (n=5).

Zymography showed a significant increase in MMP-9 activity in recurrent tumors as compared to primary lesions. The activity of remaining enzymes (MMP-2, TIMP-1 TIMP-2) was similar in both groups. This indicates the important role of MMP-9 in generating the re-growth of subtotally removed neurinomas.

A34

EXPRESSION OF MMP-2, MMP-9 AND THEIR TISSUE INHIBITORS TIMP-1, AND TIMP-2 IN PAINFUL AND NON-PAINFUL PERIPHERAL NERVE NEUROMAS

Marcol W[1], Kwiek SJ[2], Ślusarczyk W[1,2], Górka D[1], Korczyńska I[1], Malinowska-Kołodziej I[1], Bierzyńska-Macyszyn G[3], Bażowski P[2], Lewin-Kowalik J[1]

[1]Physiology Department, Medical University of Silesia, Katowice, Poland; [2]Neurosurgery Department, Medical University of Silesia, Katowice, Poland; [3]Department of Pathomorphology, Medical University of Silesia, Katowice, Poland

The mechanisms of the origin of neuropathic pain are not fully understood. Most likely, they are complex and include both central as well as peripheral mechanisms. It is possible that this comes from an ectopic activity of sensitized C fibers, extra recruitment of nociceptors or abnormal spontaneous activity in re-growing nerve sprouts. Recently, much attention is paid to neuromas developing at the injury site as a probable neuropathic pain cause. After a nerve transection, in the lack of distal nerve stump, neuromas will be usually formed.

Matrix metalloproteinases (MMPs – matrix metalloproteinases) are a family of endopeptidases that play an important role in development, tumor growth, angiogenesis or inflammatory reactions.

We used zymography and immunoblotting to analyze the expression pattern of MMP-2, MMP-9 and their tissue inhibitors – TIMP-1 and TIMP-2 – in painful and non-painful neuromas of the peripheral nerves. Fresh neuroma samples were obtained after therapeutic removal of 6 painful and 3 non-painful neuromas. The non-painful neuromas were found in patients following nerve laceration injury, while the painful neuromas resulted from limb amputations.

We found that the level of activity of MMP-2 was significantly elevated in the painful peripheral nerve neuromas when compared to the non-painful neuromas. The level of activity of the remaining enzymes: MMP-9, TIMP-1 and TIMP-2 was comparable in both experimental groups. These results indicate that MMP-2 can participate in the generating of neuropathic pain.

A35

PLEOMORHIC XANTHOASTROCYTOMA COMBINED WITH ARTERIOVENOUS MALFORMATION. A CASE REPORT

Matyja E[1], Nagańska E[1], Ząbek M[2], Mossakowski Z[2]

[1]Department of Experimental and Clinical Neuropathology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; [2]Department of Neurosurgery, Medical Centre of Postgraduate Education, Bródnowski District Hospital, Warsaw, Poland

Pleomorphic xanthoastrocytoma (PXA) is a rare brain tumor with highly distinct histological and clinical features that occurs usually as a superficial discrete mass lesion in the cerebral hemispheres of young subjects. This usually benign tumor was characterized by extreme cellular and nuclear polymorphism and tumor cell lipidization. Its connection with other maldevelopmental abnormalities is unique.

We report a case of a 36-year-old woman with right occipital lobe PXA that was associated with vascular

malformation of the arterivenous type (AVM). The biopsy specimens from the surgery were composed of two fragments of tissue of quite different histology. The tumor mass demonstrated a typical PXA histology with marked cellular pleomorhism, cytoplasmic lipidization, presence of eosinophylic granular bodies, intercellular reticulin fibers, small lymphocytes infiltrates and positive immunoreactivity for GFAP. The prominent leptomeningeal involvement resulting in advanced desmoplasia of superficial parts of the neoplastic tissue. In some areas the tumor exhibited advanced vascular anomalies with abnormally enlarged, thick-walled blood vessels. The second fragment of the biopsy tissue was composed nearly entirely of cerebral AVM with tangled masses of arteries of various size, veins and thin-walled connecting channels. The abnormal blood vessels were separated by brain parenchyma with marked fibrillar gliosis with hemosiderin deposits.

PXA is most likely derived from subpial astrocytes, which are normally invested by basal lamina and are associated with prominent reticulin network. Intracerebral arteriovenous malformation (AVM) is a congenital abnormality resulting from the lack of development of the local capillary bed. The rarity of combination of such different entities makes this case remarkable.

A36

DELAYED NEURONAL AND GLIAL CHANGES IN SLA MODEL *IN VITRO*

Matyja E, Taraszewska A, Nagańska E, Rafałowska J, Grzywaczewska E

Department of Experimental and Clinical Neuropathology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw

Chronic excitoxicity mediated through the defective glial and/or neuronal glutamate transport may contribute to several neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). To determine the detailed ultrastructural characteristics of excitotoxic motor neuron neurodegeneration, we used a model of slow excitotoxicity *in vitro* based on selective inhibition of glutamate uptake. The study was performed on organotypic cultures of the rat lumbar spinal cord subjected to glutamate uptake blockers: threohydroxyaspartate (THA) and L-trans-pyrrolidine-2, 4-dicarboxylate (PDC).

The chronic inhibition of glutamate transport by THA and PDC resulted in a dose-dependent slow degeneration of MNs in rat lumbar spinal cord cultures, which dealt with necrotic, apoptotic and autophagic mode of cell death observed up to 28 days of the experiment. Some MNs shared certain characteristics of a different type of cell injury including apoptotic-necrotic and apoptotic-autophagic changes. MNs degeneration was accompanied by distinct astroglial changes limited to protoplasmic type of astrocytes. The presence of irregular vacuoles and vesicles in the astroglial cell was observed. Occasionally, the astrocytes exhibited abnormal accumulation of the short profiles of smooth endoplasmic reticulum. There were no signs of increased production of glial filamentsin the protoplasmic astrocytes up to 3 weeks.

The results evidenced the coexistence of a different mode of MNs death in SLA model *in vitro* in association with abnormalities of the protoplasmic type of astroglia.

A37

INTRACRANIAL SOLITARY FIBROUS TUMOURS (SFTs) – PROBLEMS OF DIFFERENTIAL DIAGNOSIS AND TUMOUR CELL HISTOGENESIS

Matyja E[1], Taraszewska A[1], Ząbek M[2], Gębarowska J[1]

[1]Department of Experimental and Clinical Neuropathology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; [2]Department of Neurosurgery, Medical Centre of Postgraduate Education, Bródnowski District Hospital, Warsaw, Poland

Solitary fibrous tumours (SFTs) are rare neoplasms derived from mesenchymal cells that most often arise in the pleura. A number of extrapleural SFTs have been reported in different sites, including the cranial cavity and spinal canal. The intracranial SFTs are typically dural-based neoplasms of uncertain histogenesis. The majority of cases are benign, however some SFTs underwent malignant transformation with an unpredictable clinical course. The non-specific clinico-radiological characteristics of intracranial SFTs and the similarities of their morphological features with other spindle cell neoplasms resulted in difficulties in differential diagnosis. The meningeal SFTs should be distinguished from other frequent neoplasms of meninges, mainly fibrous meningioma, hemangiopericytoma, and sometimes neurofibroma or schwannoma. We discuss these diagnostic problems demonstrating the morphological, immunohistochemical and ultrastructural features of two intracranial cases of SFTs.

Histologically, typical SFT shows a patternless architecture consisting of spindle-shaped or ovoid cells that are arranged haphazardly, often in a fascicular or

whorled pattern and accompanied by hyalinized, dense bands of collagen deposition and branching, hemangiopericytoma-like pattern of vessels. The myxoid changes and mast cells are often seen. Some SFTs may contain adipocytes or giant multinucleated cells resembling variant of lipomatous hemangiopericytoma or giant-cell angiofibroma. The malignant SFTs usually exhibited hypercellular areas with marked cytological atypia, necrosis, numerous mitosis and/or infiltrative margins. Tumor cells show characteristically strong immunoreactivereactivity for CD34, CD99, vimentin and Bcl2, and they usually demonstrate negativity for EMA, GFAP, S-100 protein, NF, cytokeratin, actin and desmin. Ultrastructurally, the tumor cells exhibit some features of fibroblastic and endothelial cells.

Concluding, SFTs should be considered in the differential diagnosis of intracranial spindle-cell lesions. The characteristics of the immunophenotype of these tumours seem to be helpful in establishing the correct diagnosis. The histogenesis of tumor cells remain uncertain, nevertheless both, immunohistochemical and ultrastructural features indicate the endothelial differentiation of cells.

A38

LEUKOENCEPHALOPATHY WITH VANISHING WHITE MATTER IN THREE SISTERS

Mierzewska H[1], van der Knaap MS[2], Schrepes GC[2], Jurkiewicz E[3], Schmidt-Sidor B[5], Szymańska K[6], Kmieć T4, Pronicka E[1]

[1]Division of Metabolic Diseases, Department of Pediatrics, [2]Clinical and Human Genetics, Vrije Universiteit Medical Center, Amsterdam; [3]Department of Radiology, [4]Department of Neurology, The Children's Memorial Health Institute, Warsaw; [5]Department of Neuropathology, Institute Psychiatry and Neurology, Warsaw; [6]Department of Neurology, Institute of Mother and Child, Warsaw

Leukoencephalopathy with vanishing white matter (VWM) or childhood ataxia with the central nervous system hypomyelination (CACH) is the inherited autosomal recessive disease firstly described by van der Knaap et al. (1997), determined by mutations in the only one of five genes encoding subunits of the eucariotic translation initiation factor complex – *eIF2B*.

Neuropathological findings are severe cavitating orthochromatic leukodystrophy with the rarity of myelin breakdown products, predominating in hemispheric white matter. U-fibers, internal capsule, corpus callosum, anterior commissure and cerebellar white matter are relatively spared. In the white matter, an increased number of cells with the morphological features of oligodendrocytes, some of them with abundant cytoplasm like myelination glia is noted.

We present here the three sisters, 18, 11 and 8 years old, respectively, with the early-childhood and the late-childhood phenotypes. They were born to healthy, nonconsanguineous parents descending from small Highlander's neighbouring villages. The first signs of the disease were gait disturbances at 4, 2 and 6 years of age, respectively. The neurological examination showed the mild tremor of hands and head, truncal ataxia, dysarthria, and hypotonia followed by spasticity after several years. Increased tendon reflexes were also found. The course of the disease was slowly progressive. Both the oldest and the youngest sisters are able to walk up today without support, whereas the middle sister stopped walking at the age of 9 and is wheelchair-bound. Intellectual abilities are relatively spared.

The MRI showed diffusely abnormal white matter of the cerebral hemispheres, which after years had signals on T1 and T2 almost the same as CSF. The FLAIR images have revealed the rarefaction of white matter with some strip-like structures suggesting the presence of remaining tissue strands. The degree of the changes was most pronounced in the middle sister in whom the disease revealed the earliest.

Pathogenic homozygotic mutation in *eIF2B2* gene was found confirming the diagnosis of VWM; both the parents are carriers of this mutation. This disease in the Polish family is described for the first time.

A39

ACTIVATION OF C 1Q AND C 3B COMPLEMENT COMPONENTS IN THE CREUTZFELDT – JAKOB DISEASE

Nawrocka-Kunecka A[1], Papierz W[1], Liberski PP[2]

[1]Chair and Department of Pathomorphology, [2]Department of Molecular Pathology and Neuropathology, Medical University of Łódź, Poland

Neurons' loss in Creutzfeldt-Jakob disease (CJD) mainly occurs on the way of apoptosis and autophagy.

The activation of the process of apoptosis may be influenced by cytokines.

The role of a complement system in the induction of that process currently constitutes the point of the researchers' interest.

In our study we were investigating immunoreactivity of the cells with the use of the antibodies against C 1q and C 3b complement components (so called proteins of an early activation of a complement system) in the brains of the patients with CJD.

We have revealed a positive immunoreactivity for C 1q and C3 b in neurons. In the brains' hemispheres it mainly involves areas with an intense spongiosis. In the cerebellar cortex a positive reaction was demonstrated both in the molecular as well as granular and ganglionic layers' cells. PrPd plaques were also immunopositive.

The results of the studies suggest a significant role of a complement system in the process of neurodegeneration in Creutzlfeldt-Jakob disease.

A40

SPINAL CORD MYELIN COMPOSITION IN THE TRANSGENIC RATS MODEL OF AMYOTROPHIC LATERAL SCLEROSIS (ALS). A PRELIMINARY REPORT

Niebrój-Dobosz I[1], Rafałowska J[2], Fidziańska A[1], Gadamski R[2], Grieb P[3]

[1]Neuromuscular Unit, [2]Department of Neuropathology, [3]Department of Experimental Pharmacology, M. Mossakowski Medical Research Center, Polish Academy of Sciences, Warsaw, Poland

In the experimental models of ALS in mice and rats the biochemical studies were concerned on four main topics – the behavior of excitoxic amino acids and their transport, the function of mitochondria, the action of oxidative stress and participation of apoptosis in the disease process. Myelin in the central nervous system in the ALS experimental model has not been tested yet. We are presenting our results of electron-microscopic (ME) studies of spinal cord myelin and the biochemical examination of main myelin structural elements in transgenic rats in symptoms-free animals (30 and 90 days of life) and after the four legs paralysis occurred (120 days of life). In ME and the biochemical studies the myelin changes were starting already in the symptoms-free period and were more pronounced in the clinically affected animals. In ME complete myelin disorganization was observed. The biochemical examinations indicated a decrease of lipids, cholesterol and phospholipids concentration, connected with a shift of the phospholipids composition (increased proportion of phosphatidylathenolamine at the expense of phosphatidylcholine). Starting in the symptoms-free period a progressive decrease of the proteolipid protein (PLP), the DM-20, less so the basic myelin protein, was also present.

The mechanism (s), which are leading to the myelin structural changes, are hard to explain, yet. Abnormalities in the mitochondrial function responsible for decreased lipid synthesis, increased lipids peroxidation and the proteins oxidative damage may be, however, taken into account.

A41

CORRELATION OF ESTROGEN AND PROGESTERON RECEPTORS EXPRESSION WITH PROLIFERATION INDEX MIB-1 IN MENINGIOMAS OF CENTRAL NERVOUS SYSTEM

Omulecka A, Lewy-Trenda I. Nawrocka-Kunecka A, Janczukowicz J, Duda J, Papierz W

Pathology Department, Chair of Pathology Medical University of Lodz, Poland

Meningiomas are more frequent in women. Female to male ratio ranges from 3:2 to 2:1. These data suggest an influence of sex hormones in the genesis of meningiomas.

The aim of the study was to estimate the correlation between the expression of estrogen and progesterone receptors in some histologic types of meningiomas (G1 WHO) and atypical meningiomas (G2 WHO) with MIB-1 proliferation index and with sex of patients.

Material and methods: Tissue material was obtained from 68 surgically removed meningiomas (46 benign (meningothelial, fibrous and transitional), 21 atypical and 1 anaplastic). Expression of estrogen and progesterone receptors and expression of MIB-1 were examined in paraffin sections of tumors after immunohistochemical reactions with appropriate antibodies.

Results: A very weak immunoexpression of estrogen receptors was present in 33% atypical meningiomas, and in 35% benign tumors, and did not correlate with sex. Immunoexpression of progesterone receptor was present in the majority of benign meningiomas, and in 38% of atypical ones, and was more frequent in women.

Conclusion: Immunoexpression of progesterone receptors in meningiomas is more frequent in women than in men and it is more seldom in atypical than in benign meningiomas. The expression of estrogen receptors does not correlate with MIB-1 index, or with the histologic malignancy (G), and with the sex of patients, either.

A42

PATHOLOGICAL OPENING OF THE BLOOD-BRAIN BARRIER TO HORSERADISH PEROXIDASE, DIFFERENT FRAGMENTS OF AMYLOID PRECURSOR PROTEIN AND PLATELETS FOLLOWING ISCHEMIA-REPERFUSION BRAIN INJURY

Pluta R[1,2], Ułamek M[1], Pilis K[2], Januszewski S[1]

[1]Department of Neurodegenerative Disorders, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warszawa, [2]Department of Biological and Medical Sciences, Długosz's Academy, Częstochowa, Poland

Ongoing interest in brain ischemia-reperfusion research has provided data showing that ischemia may be involved in the pathogenesis of Alzheimer's disease. In ischemic disruption of the blood-brain barrier two pathological hallmarks deserve consideration. One relates to leakage of various molecules across the vessel wall and the other is important in view of the potential effect of various extravasated substances on the brain neurodegeneration. Finally, we wanted to document the vascular origin of β-amyloid peptide in Alzheimer's type of dementia. Using female Wistar rat's blood-brain barrier alterations, distribution of different fragments of amyloid precursor protein and β-amyloid peptide around microvessels and platelets behavior were examined following 10 min brain ischemia due to cardiac arrest with survival from 2 days to 1 year. The rats were perfusion fixed for light and electron microscopic analysis. In reacted vibratome sections scattered foci of extravasated horseradish peroxidase were observed throughout the brain and did not appear to be restricted to any specific area of the brain. However, they were restricted to branches of microvessels. The ultrastructural study of leaky sites frequently presented platelets adhering to the endothelium of microvessels. Some platelets were found in the perivascular space. Endothelial cells showed pathological changes with evidence of perivascular edema. At the same time, we noted C-terminal of amyloid precursor protein and β-amyloid peptide deposits around brain microvessels. Perivascular deposits of β-amyloid peptide took the same form as extravasated horseradish peroxidase. These deposits suggested diffusion of C-terminal of amyloid precursor protein and β-amyloid peptide out of the vascular compartment. We confirmed this phenomenon by i.v. injection of human β-amyloid peptide to ischemic rats. These data implicate delayed and chronic insufficiency of the blood-brain barrier after ischemia-reperfusion injury as a primary event in the pathological changes during neurodegeneration. Chronic blood-brain barrier dysfunction, abnormal behavior of platelets and different fragments of amyloid precursor protein accumulation in the perivascular space may be involved in the gradual maturation of the neuropathological process in the brain which causes a slowly progressing ischemic encephalopathy with dementia. Finally, we speculate about the possibility of a peripheral source of β-amyloid peptide that may, by crossing the blood-brain barrier contribute to the vascular and parenchymal deposits of β-amyloid peptide in the Alzheimer's disease brain.

A43

DYNAMICS OF MORPHOLOGICAL CHANGES WITHIN THE CENTRAL NERVOUS SYSTEM OF RAT IN A SUBCLINICAL STAGE OF EXPERIMENTAL FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

Rafałowska J[1], Fidziańska A[2], Gadamski R[1], Ogonowska W[1], Miodowska T[1], Grieb P[3]

[1]Department of Experimental and Clinical Neuropathology, [2]Neuromuscular Unit, [3]Department of Experimental Pharmacology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

Estimation of the dynamics of histological and immunocytochemical changes in CNS of transgenic rats in various periods of life was performed. Material was obtained from animals at 60-th day of age (4), 93-rd day of age (3) and 120-th day of age. Formalin fixed and paraffin embedded slices were stained with HE Kluver-Barrera method. Immunoreactions to GFAP, S-100, ferritin, neurofilament, ubiquitine, synaptophisine and tau protein were also performed.

Within the brain tissues patchy neuronal loss and dark or ischemic neurons were dispersed in cortical layers, CA1, CA3 CA4 hippocampal areas and depth structures of the hemispheres and brain stem.

In the spinal cord, numerous alfa motoneurons were dark or ischemic. Vacuoles or small pale spats were visible in their cytoplasm. Microspongiosis surrounded some motoneurons, particularly cells subjected to neuronophagy. Neuronophagy, sporadically observed at the age of 60-th day, was more extensive at the 93-rd day of age, and at age of 120-th day involved already all interneuron cells of the anterior and posterior horns. In immunereaction to neurofilament numerous fibers, often thick, fragmented or rosary – like were observed. They were located within the subcortical white matter,

external and internal capsules, anterior horns of the spinal cord. Changes become more intensive with age.

Astrocytic reactivity was week in animals at the 60-th and 93-rd day of life. Non-numerous cells were immunoreactive to GFAP and S-100, although an increase of astrocytic nuclei was observed. At the 120-th day of age astrocytic hypertrophy and proliferation were intensive.

But from the 60-th day of age ubiquitine and tau protein immunopositive material was accumulated in the perinuclear area of astroglial cytoplasm. Immunereactions of nerve cells to these proteins were negative.

Conclusions: 1) In the subclinical stage of the disease a pathological process within CNS take place already at the 60-th day of age and its intensity increased with age. 2) Morphological changes are not limited to motor neurons cells. Various structures of CNS are damaged. 3) A weak astroglial reaction is depended probably on pathologic accumulation of ubiquitine – and tau protein in the cytoplasm.

A44

INFANTILE MITOCHONDRIAL LEUKODYSTROPHY – A CASE REPORT

Schmidt-Sidor B[1], Szymańska K[2], Lewandowska E[1], Mierzewska H[3], Wierzba-Bobrowicz T[1], Pasennik E[1], Stępień T[1]

[1]Department of Neuropathology, Institute of Psychiatry and Neurology, Warszawa, Poland; [2]Neurological Clinic, Institute of Mother and child, Warszawa, Poland; [3]Department of Metabolic Diseases Pediatric Clinic, Center of Child Health, Warszawa, Poland

Myelin abnormalities are not particularly prominent in the mitochondrial diseases (Brown, Squier, 1996). We report an infantile leukodystrophy with some neuropathological features of mitochondrial diseases. Clinical data: A five-month-old boy was admitted to the neurological clinic because of the delay of psychomotor development. He was born after uneventful full term pregnancy (birth weight 4250 g, length 55 cm) in good state. At the time of admission his physical state was normal. Neurologically he was irritable, without any interest in the surroundings. There were slow pupillary light reflexes, bilateral horizontal nystagmus, spasticity with bilateral Babinski sign. There was bilateral optic nerve atrophy. Ventriculography showed widening of the lateral ventricles and cortical atrophy in the frontal lobes. EEG showed generalized changes with high voltage slow wave. The cerebrospinal fluid was normal. Metabolic test of urine was normal. At the end of life there were problems with swallowing. He died at seven months of age. There were three older children, none of them had neurological problems. The clinical diagnosis was *Leukodystrophia*. The general autopsy diagnosis was: *Pneumonia interstitialis*. *Hypertrophia cordis totium mediocri gradus*. *Induratio hepatis cum fibrosis*. Gross neuropathological evaluation showed a diffuse damage of central white matter of the cerebral hemisphere with cavitation in the parieto-occipital lobes. Also, the white matter of the cerebellar hemispheres was severely damaged. There was atrophy of the anterior part of the corpus callosum with widening of lateral ventricles. In the brain stem there was a similar in structure damage of cerebellar medium peduncles. Both pyramids in medulla oblongata were small. Microscopical evaluation showed normal for age development of the brain, cerebellum and brain stem with adequate to the age myelination. There was destruction of the white matter (with demyelination with moderate to severe hypertrophy and hyperplasia of capillaries and moderate glia reaction. In the better preserved white matter there were spongiform changes. The most severe white matter changes were seen in centrum semiovale of cerebral hemispheres, in cerebellar hemispheres and cerebellar medium peduncles. In both pyramids of medulla oblongata there was no myelin. There was a relatively good preservation of myelin in the axis of cerebral gyri basal ganglia and the brain stem (with the exception of cerebellar medium peduncles and pyramids). Electron microscope evaluation showed abnormal mitochondria, myelin and neurofibrils destruction. The neuropathological picture of the brain damage has showed orthochromatic leukodystrophy with some features characteristic of neuropathology of mitochondrial disease: capillary hyperplasia and hypertrophy, spongiosis and symmetrical, bilateral damage of the brain stem structures. The last one is characteristic of Leigh syndrome. The hypertrophy of the damage may be also connected with mitochondrial disease.

A45

ULTRASTRUCTURAL CHANGES IN THE HIPPOCAMPAL ASTROCYTES AFTER LONG-TERM VALPROATE ADMINISTRATION TO RATS

Sendrowski K[1], Sobaniec-Łotowska ME[2], Sobaniec W[1]

[1]Department of Child Neurology&Rehabilitation, [2]Department of Clinical Patomorphology, Medical University of Białystok, Poland

Abstract: Valproate (VPA) is one of the most often used wide-spectrum antiepileptic drugs. Prolonged

application of VPA even at the therapeutic doses can bring about severe neurological disturbances defined as "valproate encephalopathy".

The aim of the study was to analyze the astrocyte ultrastructure within the hippocampal gyre cortex in valproate encephalopathy induced by chronic administration of sodium valproate to rats for 1, 3, 6, 9 and 12 months, once daily intragastrically, in a dose of 200 mg/kg b.w. Prolonged applications of VPA caused damage to protoplasmic astrocytes of the examined hippocampal cortex, mainly in the pyramidal layer, which intensified in the later stages of the experiment, especially after 9 and 12 months. The most pronounced astroglial abnormalities, concerning about 2/3 of protoplasmic astrocytes after 9 and 12 months, were characterized by considerable swelling of cells, with the presence of empty vacuolar structures in the cytoplasm, a substantial decrease in the number of gliofilaments or even their complete loss, which indicated fibrillopoietic failure of the cell, and the appearance of astrocytes showing phagocytic activity. The astrocytic changes coexisted with distinct damage to pyramidal neurons and structural elements of the blood-brain barrier. In valproate encephalopathy, apart from any direct effect of VPA on astrocytes, the main cause of the protoplasmic astroglial damage in the examined hippocampal cortex could be associated with changes in microcirculation in the cortex (vasogenic factor), leading to its ischemia.

A46

CO-LOCALIZATION OF AUTOPHAGY MARKER MAP LC3 AND PRPD IN CREUTZFELDT JAKOB DISEASE

Sikorska B[1], Liberski PP[1], Preusser M[2], Budka H[2]

[1]Department of Molecular Pathology and Neuropathology, Medical University of Lodz, Lodz, Poland; [2]Institute of Neurology, Medical University of Vienna, and Austrian Reference Centre for Human Prion diseases, Vienna, Austria

Autophagy is a process by which subcellular constituents and organelles are targeted for degradation in lysosomes. In macroautophagy, proteins and organelles are sequestrated into a double membrane bound vacuole called autophagosome, formed by ER membranes, under the direction of various proteins including MAP-LC3, a microtubule associated protein – light chain 3. In addition to maintaining cellular homeostasis, autophagy may also contribute to cell damage. It is involved in autophagic programmed cell death, called programmed cell death type II. The role of autophagy in neurodegeneration is not only in removing protein aggregates but also in inducing the death of neurons. MAP LC3, a general marker for autophagic membranes has been used only in cell culture studies. We decided to use an anti MAP LC3 antibody on human Creutzfeldt Jakob disease brains to evaluate co-localization with the pathologic isoform of PrP (PrPd). Paraffin blocks of four autopsy cases of sporadic Creutzfeldt Jakob disease were evaluated for co-localization of PrPd and MAP LC3 immunoreactivities by confocal laser microscopy. The most prominent co-localization was observed with granular PrPd deposits in neuronal perikarya and along the outlines of axons. There was also some co-localization with synaptic PrPd deposits. The pattern of co-localization was variable, however. Sometimes PrPd deposits appeared surrounded by MAP LC3 immunoreactivity but occasionally more extensive aggregated deposits of PrPd overlapped LC3 immunoreactivity. Most of the observed LC3 reactivity could be interpreted as microtubules, but there were also ring-shaped and dot-like structures that could be interpreted as vacuoles. Their association with PrPd might indicate some pathogenetic relation.

A47

MICROGLIAL IMMUNE RESPONSE IN CEREBRAL AMYLOID ANGIOPATHY IN TRANSMISSIBLE AND NON-TRANSMISSIBLE CEREBRAL AMYLOIDOSES

Szpak GM[1], Lewandowska E[1], Bertrand E[1], Wierzba-Bobrowicz T[1], Sobczyk W[2], Kulczycki J[2], Łojkowska W[2], Mendel T[3], Pasennik E[1], Stępień T[1], Lechowicz W[1]

[1]Department of Neuropathology, [2]I Department of Neurology, [3]II Department of Neurology, Institute of Psychiatry and Neurology, Warszawa

Cerebral or congophilic amyloid angiopathy (CAA) is a degenerative disease of small and medium-size vessels in which the disruption of basement membrane, pericyte, and changes of endothelial cells, particularly progressive loss of smooth muscle cells, lead to acellular degeneration and necrosis of vessel walls, which can be the cause of progressive dementia, lobar or petechial hemorrhages, or ischemic strokes.

Accumulation of amyloid (different isoforms A, β, PrP, cystatin or other) affects sometimes only cerebral vessels, particularly in the elderly, or is associated with parenchymal amyloid deposition in other cerebral

amyloidoses and conformation diseases as Alzheimer disease (AD), dementia with Lewy body (DLB), and prion disease

Our recent immunohistochemical and ultrastructural investigations in both transmissible and non-transmissible conformation diseases have shown that in cerebral amyloidoses without CAA there occurs the immune response dominated by activated microglia. In the present investigations of 16 autopsy cases of cerebral amyloidoses (8 with CAA and AD, DLB or CJD and 8 without CAA) we used staining with Congo red, PAS, Thioflavin S, and antibodies to 4 isoforms of A, β (8-17, 17-24, 1-40, 1-42), and prion protein (3F4), cystatin, actin, α-synuclein, and also antibodies: CD31, HLA-DR and GFAP. In the cases of CAA, we have found multistep amyloid degeneration of the vessel walls with a loss of smooth muscle cells, increasing negative immunoreaction with actin and accumulation of amyloid fibrils in the vicinity of vascular basement membrane as well as the degenerative changes of smooth muscle cells in ultrastructural study.

We have shown that the cases, excluding the vascular type of CAA, were associated with scanty immune response of macrophages and perivascular microglia. Intensity and spatial pattern of the immune response in the cases CAA and AD, DLB or CJD, were characteristic of type parenchymal conformation disease and in all the cases was dominated by activated microglia.

A48

DEVELOPMENT AND EVALUATION OF VIRTUAL NEUROPATHOLOGY: NEW TOOLS AND METHODS

Szymaś J

Laboratory of Neurosurgical Pathology, Department of Pathology, University of Medical Sciences in Poznan

A standard microscope was reconfigured as a virtual slide generator. Robotized light microscope Axiovision2 was supplemented with scan stage, MCU 28 controller and AxioCam digital camera. Axiovision version 4.3 software controlled stage movement in the X-, Y-, and Z-axis and captured images at 1300x1300 pixels. Stage calibration, scanning algorithms, storage requirements, and viewing modes were standardized. Captured images were used to montage a large virtual slide image that was subsequently saved in TIFF format and converted in JPEG2000 file. Virtual slides were viewed at the dedicated workstation with the use of special software as well as using the viewer e.g. Adobe Photoshop and Kodak Imaging as well as Internet browser-based viewers. Dedicated server makes it possible to reach and to save virtual slides through the Internet. Software started by the user makes it possible not only to visualize virtual slide but also to use virtual microscope interface together with labeling and annotating tools. The images were served from a platform with 2 GB RAM, 500 GB of disk storage, and a 3.0 GHz P4 processor. To conserve disk space on the image server, TIF files were converted to the JEPG2000 file format using a compression ratio of 10:1. By using 1x, 5x, 10x, 20x, and 40x objectives, very large gigapixel images of tissue whole-mounts and tissue arrays with high quality and morphologic detail are now being generated for teaching, publication, research, and morphometric analysis. Technical details and a demonstration of our system can be found on the Web at http://www.telepath.poznan.pl/.

A49

EPIGENETIC CHANGES IN PILOCYTIC ASTROCYTOMAS

Szymaś J, Rembowska J, Szymańska K

Laboratory of Neurosurgical Pathology, Department of Pathology, University of Medical Sciences in Poznan

Differential methylation at CpG islands located in promoter regions is one of the most important mechanism of genes silencing and responsible for carcinogenesis of cells. Therefore methylation status of DNA could be an epigenetic marker of carcinogenesis useful for diagnosis as well as for prognostic purposes. Pilocytic astrocytoma belongs to the glioma group marked with the first grade of biological malignancy and connected with particular location and relatively good prognosis contrary to the other gliomas types. Recently, we have screened the profile of methylation of CpG island in a pilocytic astrocytoma group. We used the semiquantitative high throughput method MethyLight to analyze a gene panel comprising HICI, CALCA, MYODI, CTNNBI, PTGS2, TIMP-3, THBSI, TGFBR2, RB1, ARF, CDKN2B, APC and TP53 in 16 pilocytic astrocytoma. Only one of these loci, namely MYODI, has showed tumor specific hypomethylation changes. No hypermethylations were noted. Our results show that pilocytic astrocytoma subtype has characteristic methylation profiles and seems to be an exception in the glioma group which is mostly stratified by hypermethylation. The lower level of methylation in this glioma subtype could stimulate a process of tumor cell differentiation to stop the cell

cycle at S phase or trigger particular genes expression profile. Our results show that characteristic methylation profiles of pilocytic astrocytoma could be useful in differential diagnosis and could be helpful in the therapeutic decision.

A50

INTERACTION BETWEEN NEOPLASM AND ITS SURROUNDINGS IN METASTATIC LUNG CANCER TO THE CENTRAL NERVOUS SYSTEM

Tabaka J[1], Nowacki P[1], Pankowski J[2]

[1]Department of Neurology Pomeranian Medical University, Szczecin; [2]Department of Pathology Specialistic Hospital, Szczecin-Zdunowo

Primary lung tumors constitute 30-60% of all brain metastatic tumors. It is estimated that 18-65% of the patients with lung cancer will develop brain metastases. The number of metastatic cases of that tumor constantly increases, both in men and women. Firstly, it results from a longer time of exposure to the illness and, thus, a higher risk of the metastases to the brain. Secondly, it results from better diagnostic procedures. It is known that the frequency of appearance of the metastatic lung cancer to the brain depends on the type of the primary histologic tumor. Small cell carcinoma and adenocarcinoma give metastases twice as often as other types of lung cancer. This phenomenon, primarily, depends on the biological aggressivity of the tumor. It may be possible that the speed of spreading of metastasis in the brain is also affected by the tumor surrounding brain tissues. In connection with this we made attempts to answer the following questions:

1) How is the border between the tumor and its surrounding formed
2) Are there any differences in the gliosis and blood vessels around different types of brain metastatic lung cancer.

A post-mortem neuropathological examination was done on 68 patients with lung metastatic tumors to the brain (40 men and 28 women). In all the cases the primary focus of the tumor was known and complete pulmonary diagnosis was made. The material was divided into 2 groups: I- metastatic tumors of small cell carcinoma, II- metastatic tumors of non small cell carcinoma. Group II was divided in two subgroups: IIa-squamous cell carcinoma (18 cases), IIb-adenocarcinoma (36 cases). The material was fixed in buffered formalin and embedded in paraffin and then stained with hematoxylin and eosin, van Gieson and Heidenhain's methods. Immunohistochemical evaluation was done with GFAP, cytoceratin, vimentin and factor VIII-related antigen. The computerized morphometric analysis was done by means of KONTRON imaging system KS-100 v. 2.0. Three general types of borders between the neoplasm and its surrounding were observed: 1 – distinct, 2 – penetration of the cluster of neoplastic cells or infiltration along the vessels, 3 – gradual cellular infiltration into the surrounding.

The first type very often concerned metastasis of small cell carcinoma (64% cases from group I), the second type more often appeared in adenocarcinoma metastases (69% cases from group IIb), the third type concerned more often of squamous cell carcinoma metastases (55% cases from group IIa). In the surrounding of metastatic lung cancer the evident reaction from blood vessels was observed. Regardless of the subtype of the tumor, vessels in diameter bigger and smaller than 25 μm and of different wall thickness (from 5 to 8-12 μm) were observed. In the surroundings of adenocarcinoma metastases prevailed vessels in diameter not bigger than 25 μm. Astrogliosis was the most evident around metastases of small cell carcinoma – in 86% cases evident astrogliosis was observed.

Conclusions: 1) From among different types of metastatic lung tumors to the brain, it is squamous cell carcinoma metastases that most infiltrate into the surrounding. 2) The most evident astrogliosis occurs around small cell carcinoma. It may result from the highest aggressivity of that tumor among all histologic lung tumor subtypes.

A51

IMMUNOHISTOCHEMISTRY OF LECTIN RECEPTORS IN THE SELECTED TYPES OF INTRACRANIAL MENINGIOMAS

Taraszewska A, Matyja E, Zielińska M

Department of Experimental and Clinical Neuropathology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw

Meningioma cells are particularly rich in glycoproteins of cell membranes, in particular involved in the formation of basement membranes and intercellular adhesion structures. The use of lectins as cytochemical markers for specific glycoprotein-bound sugar residues may contribute to further characterization of meningiomas in regard to the diversity of the neoplastic cells

transformation. In this study the immunohistochemical pattern of binding sites for six lectins was evaluated in 28 cases of the benign meningiomas of various histological types, including secretory, microcystic and angiomatous type and in 3 cases of anaplastic meningiomas. The biotinylated lectins used for the study were: Cocanavalin A (Con A), Wheat germ agglutinin (WGA), Peanut agglutinin (PNA), Ulex europaeus (UEA I), Soybean agglutinin (SBA) and Dolichos biflorus agglutinin (DBA).

The gycoconjugates reacting with PNA, WGA and SBA were distributed to a variable extent on the cell surface, interstitially and intracellularly within all meningiomas. Differences in their expression between some meningioma subtypes were mostly related to the specific cytoarchitectural patterns such as whorled formation, center of lobules or trabecular arrangements. Pseudopsammoma bodies exhibited strong staining with PNA, SBA, Con A and occasionally with WGA and DBA. In contrast, the psammoma bodies were labeled only with SBA. The selective reactivity with UEA-1 was limited to blood vessels endothelia, mostly in the capillaries. It resulted in a distinct visualization of the vascular network in various histological subtypes of meningiomas. In anaplastic meningiomas increased staining with PNA and loss of the DBA labeling predominated.

A52

IDENTIFICATION OF THE T-ANTIGEN SV40 IN THE BIOPSY OF CEREBRAL CORTEX IN A PML CASE

Taraszewska A[13], Mróz A[23], Baraniecka J[1], Bardadin K[23]

[1]Department of Experimental and Clinical Neuropathology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences; [2]Department of Pathomorphology, Medical Center of Postgraduate Education; [3]Department of Pathomorphology, Bielanski Hospital, Warsaw

Progressive multifocal leuconcephalopathy (PML) is caused by a lytic infection of oligodendrocytes with human polyomavirus JC (JCV) and replication of the virus in these cells. The presence of virus has been also demonstrated in astroglial cells and rarely in neurons. This report presents the study of the cerebral cortex biopsy taken from the region adjacent to subcortical PML lesion in a 25-year-old woman with HIV-infection, AIDS symptoms and suspicion of the brain lymphoma. Histological examination revealed small fragments of the cerebral cortex with the margin of focal necrosis. In the surrounding of the necrosis there were shrunken degenerated neurons and glial cells, hypertrophied astrocytes with bizarre or numerous nuclei and few oligodendroglial-like cells with enlarged basophilic nuclei. These findings suggested PML diagnosis. To prove the diagnosis the immunohistochemical study was performed using monoclonal antibody (PAb416) specific for SV40 large T antigen, recognizing also the homologous determinants of T antigen of the human polyomaviruses, JCV and BKV. By immunohistochemistry the presence of viral antigen was demonstrated in numerous nuclei of the abnormal neuronal and glial cells exhibiting shrunken cytoplasm. Expression of the antigen was also occasionally present in nuclei and/or cytoplasm of hypertrophied astrocytes. Immunostaining for p53 showed strong immunoreactivity in the numerous cellular nuclei. These results suggest that in the surrounding of subcortical PML lesions the JCV infection of neurons may occur resulting in apoptosis of these cells in the early phase of viral infection.

A53

THE ROLE OF PRNP AND PRND GENES PRODUCTS IN NEURONAL DIFFERENTIATION OF PC12 CELLS

Witusik M, Wójcik I, Hułas-Bigoszewska K, Szybka M, Piaskowski S, Golańska E, Liberski PP, Rieske P

Department of Molecular Pathology and Neuropathology, Medical University of Lodz,

Prnp and *Prnd* are the genes encoding PrP^c and Doppel proteins. The role of these proteins has not been established yet, however, according to many reports they may participate in the process of neuronal differentiation.

The present study was performed on a culture of neuron-like cells in order to investigate the regulation of several genes expression in relation to differentiation. The main aim of our study is to determine the expression of *Prnp* and *Prnd* genes and to compare it with specific expression pattern of neuronal differentiation markers. This strategy may contribute to understanding of PrP^c and Doppel proteins role in the proces of neuronal differentiation.

As a model system we used the nerve growth factor (NGF) -responsive line of PC12 rat pheochromocytoma cells, which undergo neuronal differentiation and develop into cells with morphological, electrophysiological, and neurochemical properties of sympathetic neurons, despite a very low level of PrP^c protein.

We used reverse transcription-coupled PCR method to examine the expression of the following genes:

Prnp, *Prnd*; neuronal differentiation markers: *Map-2*, *nestin*, *Nurr-1*, *Msi-1*, *Gfap*.

Our preliminary results indicated the presence of several markers of neuronal differentiation: *Map-2*, *nestin*, *Nurr-1*, *Msi-1*, *Gfap*, which seems to support the primary assumption that PC12 cells undergo neuronal differentiation, despite a low level of PrP^c. Treatment of PC12 cells with NGF resulted in a slight increase of PrP^c mRNA level and significant increase of Dpl mRNA level, which may suggest the participation of these proteins in the neuronal differentiation process.

To establish a definite expression pattern of the tested genes, further research is needed, including examination of potential expression changes on the protein level.

(The study was partially supported by KBN grant No. 3P05A11423)

A54

ANALYSIS OF THE EXPRESSION OF THE *REN*, *BMI1* AND *OCT-4* GENES IN MEDULLOBLASTOMAS

Wójcik I[1], Zakrzewska M[1], Witusik M[1], Szybka M[1], Zakrzewski K[2], Polis L[2], Liberski PP[1], Rieske P[1]

[1]Department of Molecular Pathology and Neuropathology, Chair of oncology, Medical University of Lodz, [2]Department of Neurosurgery Polish Mother Memorial Hospital Research Institute, Lodz

Medulloblastomas (MBs) constitute 20-25% of all brain tumors in pediatric population and are the most common malignant brain tumors in children. Molecular biology of this tumor is still unclear. Loss of heterozygosity (LOH) of the 17p13.2 region, that contains new identified RENKCTD11 gene, is a frequent genetic change in MB. Recently, a significant reduction of expression of the REN gene in medulloblastoma that might lead to deregulation of Sonic Hedgehog (SHh) pathway has been reported. BMI1 gene, which is essential for cerebellar development, has an important potential role in the pathogenesis of Mbs. Overexpression of Bim1 oncogene in medulloblastomas and deregulated proliferation of cerebellar precursor cells by activation of the sonic hedgehog (Shh) has been reported recently. OCT-4 gene is a marker of cell differentiation and has not been analyzed in medulloblastoma yet.

In this study, analysis of the expression of the *REN* and *BMI1* genes was performed in 14 children with medulloblastomas using real-time RT-PCR (SYBER® Green) and $2^{-\Delta\Delta Ct}$ methods, and *OCTt-4* gene was analyzed using multiplex RT-PCR method. Expressions of analyzed genes were assessed in comparison with level of these genes in three normal cerebellum samples. Expression of the *REN* gene was reduced in 13 cases, expressions of the *Bim1* and *Oct-4* were increased in 8 cases; expressions of both of these genes of 5 cases were simultaneously increased. Our results suggested that abnormalities of the analyzed genes may be important in the pathogenesis of medulloblastomas.

A55

CAN THE LEVEL OF SULFANE SULFUR BE RELATED TO THE GRADES OF GLIOMA MALIGNANCY?

Wróbel M[1], Czubak J[1], Adamek D[2], Bronowicka P[1], Czepko R[3]

[1]Institute of Medical Biochemistry, [2]Institute of Neuropathology, [3]Departament of Neurosurgery, Jagiellonian University Medical College, Cracow, Poland

In most mammalian tissues, cysteine, a sulfur-containing amino acid, is metabolized to cysteine sulfinic acid, which is either decarboxylated to hypotaurine and then oxidized to taurine, or transaminated, leading to the formation of inorganic sulfate. Alternatively, cysteine undergoes desulfuration by several enzymes present in animal tissues; these enzymes include γ-cystathionase (cystathionine γ-lyase, EC 4.4.1.1) and cysteine aminotransferase (EC 2.6.1.3) in conjunction with 3-mercaptopyruvate sulfurtransferase (MPST, EC 2.8.1.2). Sulfane sulfur atoms created by the action of these enzymes are involved in the detoxication of cyanide and inorganic sulfide, the incorporation of sulfur in iron-sulfur centers of redox proteins, enzyme activity regulation through a mechanism that involves the incorporation of sulfur, sulfuration of tRNA; these atoms have an antioxidant potential and may affect the toxic function of exogenous xenobiotics or drugs. Cysteine is also a rate-limiting amino acid in the synthesis of glutathione (GSH). The characteristic feature of neoplastic tissues is the absence or a decreased level of principal enzymes involved in L-cysteine desulfuration and, consequently, the absence or a low level of sulfane sulfurs compounds. Malignant cell proliferation may be related to a deficiency of sulfane sulfur and the uncontrolled operation of a set of enzymes normally inactivated by sulfane sulfur. Since biochemical changes may be related to the growth rate of cancer cells, they can be thought of as markers of tumor cell proliferation.

We have undertaken the investigations of the level of GSH and sulfane sulfur and the activity of the enzymes involved in sulfane sulfur metabolism (MPST, γ-cystathionase, rhodenase) in homogenates obtained from human gliomas collected intraoperatively at the Department of Neurosurgery, Collegium Medicum, Jagiellonian University. According to the WHO classification, the tumors included 1 astrocytoma fibrillare (WHO I), 1 oligodendroglioma malignum (WHO III), 4 astrocytoma malignum in glioblastoma multiforme vertens (WHO III/IV), and glioblastoma multiforme (WHO IV).

The level of GSH was found to be significantly increased in gliomas classified as WHO III/IV and IV (6 and 10 nmol x mg^{-1}, respectively) in comparison to those classified as WHO I and WHO III (1.6 and 2.1 nmol x mg^{-1}, respectively). The level of sulfane sulfur also increased significantly with the increasing grades of malignancy. Low-grade astrocytomas (grade I) showed about two times lower level of sulfane sulfur in comparison to that determined for grade III or about 3 times lower in comparison to grade III/IV or IV. MPST and rhodanese activities were also lower in low-grade astrocytomas in comparison to grade III, III/IV and IV. In all the gliomas, the γ-cystathionase activity was either absent, or trace activity was found, which might have been a result of contamination of neoplasms with the surrounding tissues.

Based on the above results we may conclude that: in human gliomas, cysteine desulfuration via the MPST reaction is the main pathway of sulfane sulfur generation because they lack the activity of γ-cystathionase. Sulfane sulfur production occurs in all the investigated tissues and its level is elevated in high-grade gliomas in comparison to low-grade neoplasms. As the increased sulfane sulfur level is accompanied by an increased activity of the enzymes participating in its formation and using, an explanation should be provided whether malignant cells require sulfane sulfur for proliferation or if they accumulate sulfane sulfur as a waste product.

A56

MOLECULAR ABNORMALITIES OF HSNF5/INI1 GENE IN ATYPICAL TERATOID/RHABDOID TUMORS

Zakrzewska M[1], Wójcik I[1], Zakrzewski K[2], Polis L[2], Fiks T[3], Liberski PP[1,2]

[1]Department of Molecular Pathology and Neuropathology, Chair of Oncology, Medical University of Lodz, Lodz, Poland; [2]Department of Neurosurgery Polish Mother Memorial Hospital Research Institute, Lodz, Poland; [3]Department of Pathology Polish Mother Memorial Hospital Research Institute, Lodz, Poland

Atypical teratoid/rhabdoid tumor (AT/RT) represents about 2% of pediatric central nervous system tumors, and the majority of them is observed in children under 5 years of age. Aggressive clinical course and poor outcome are connected with this type of tumor.

Up to 60-90% of AT/RT cases are connected with *hSNF/INI1* (*Integrase interactor 1*) suppressor gene alterations. Germ-line gene mutations are responsible for rhabdoid predisposition syndrome (RPS) characterized by the presence of brain tumors (AT/RT, choroid plexus carcinoma, medulloblastoma, sPNET) and peripheral malignant rhabdoid tumors. The most frequent abnormalities of *hSNF/INI1* are homozygous deletions and point mutations, in brain tumors exons 4, 5 and 9 are predicted to be changed.

We present five cases of AT/RT operated on at the Department of Neurosurgery Polish Mother Memorial Hospital Research Institute in Lodz. Among them, there were four girls and one boy. At the time of diagnosis, all of them were under 2 years of age. Molecular analysis was based on direct sequencing of exons 4, 5 and 9 of *hSNF5/INI1* and expression analysis of mRNA level by using multiplex-PCR method.

Sequencing analysis revealed a frame shift deletion in one case (delG: exon 9, codon 372) and a missence mutations in the two other cases (Val→Leu: exon 5, codon 185; Ala→Pro: exon 9: codon 384). There was one germ-line mutation. Multiplex-PCR analysis revealed a decreased mRNA level in three out of four analyzed cases, in two of them there was no mutation in the sequenced regions.

Abstracts
Index of authors