Meeting Summary

68th Annual Meeting of the German Society of Neuropathology and Neuroanatomy (DGNN)

Meeting Abstracts

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Dear colleagues,

It is my pleasure and honor to host the 68th Annual Meeting of the German Society of Neuropathology and Neuroanatomy (DGNN) in Regensburg. Since the Magdeburg meeting in 2019 this is the first pure national meeting of our society that will be held in presence after five long years. While the meeting in 2020 was cancelled due to the Corona pandemic, the 2021 meeting (organized by the colleagues in Gießen) took place as a mere online meeting. In 2022 and 2023 our national society meetings were embedded in the "Neurowoche" and the International Congress of Neuropathology in Berlin. We are enthusiastic about this years' reunion of our society in Regensburg.

In our Regensburg meeting, we aim to provide a comprehensive update on the major and hot topics in neuropathology. Neuropathologists address some of the currently most relevant and discussed health care issues, such as for example cancer, neuroimmunological diseases like Multiple Sclerosis, neurodegenerative diseases including Alzheimer's and Parkinson's, and muscle/nerve diseases. As tissue specialists, neuropathologists directly study diseases in human materials. Neuropathologists use state of-the-art methods to uncover disease processes on the molecular level. During our congress, we will hear a lot on the methodical progress made in this regard. Neuropathology is also becoming increasingly clinical as many of our scientific and diagnostic findings influence and directly guide treatment decisions.

We were able to attract renowned national and international speakers and our meeting will allow for an intensive interchange both within our society and with our neighboring disciplines. Program highlights include a Pre-Congress hands-on Workshop on Next Generation Sequencing, a session on Molecular Tumorboards and a Mini-Symposium on Quality Assurance in Neuropathology.

We are delighted about the submission of 31 abstracts covering the research fields Neurooncology, Neuroimmunology, Muscle/Nerve, Neurodegeneration, and Methods/Free Topics. The abstracts are published below in this edition of Free Neuropathology. I want to thank the scientific committee of our congress for helping in evaluating the submissions and selecting the poster talks and poster spotlight presentations. Many of the abstracts were submitted by our young researchers. They deserve our special attention! Posters will be exhibited throughout the entire congress and we will have plenty of time for poster viewing and discussions on Thursday evening at the Welcome Reception and at the main poster session on Friday at noon.

So let me again welcome you all to our beautiful city of Regensburg. I am looking forward to inspiring talks, vivid discussions and enriching encounters with like-minded people.

Yours,

Prof. Dr. Markus J. Riemenschneider

Regensburg University Hospital, Department of Neuropathology
Congress President DGNN Annual Meeting 2024

I. Neurooncology

P01

Free Neuropathol 5:19:5

Meeting Abstract

Identification and description of a novel type of medulloblastoma

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Medulloblastoma (MB) is the most frequent malignant brain tumor of childhood and adolescence. Based on biology, histology, and its clinical course, MB is a heterogeneous disease with four different types (WNT, SHH, Group 3, and Group 4), which are most reliably distinguishable by their global DNA methylation pattern. As a result of integrating DNA methylation data of > 2,600 MBs and screening of > 140,000 data sets uploaded to the DKFZ brain tumor classifier (www.molecularneuropathology.org), we identified a small group of MB (n = 49) that displayed a homogeneous DNA methylation pattern, which was clearly distinct from previously known MB types. Tumors within this group have also been recognized as a separate methylation class by the latest version of the classifier (provisionally named as MB_MYO). Transcriptomic data (n = 14) were similarly distinct from other MB types. Comparison of these data to various cell types of the developing hindbrain revealed similarities to precursor cells of the rhombic lip with signatures of WNT signaling and myogenic differentiation. In line with the latter findings, 3/13 analyzed cases harbored hotspot *CTNNB1* mutations and 7/10 cases showed a myogenic differentiation based on histology. *MYC* amplifications were present in 12/49 (24.5 %). Median age at diagnosis was 16 years, and five-year overall survival was ~ 70 %. In summary, we describe a novel type of MB identified by DNA methylation profiling that likely needs to be addressed separately during the retrospective analyses of MB patient cohorts, for the design of future clinical trials, and when evaluating targeted therapies.

P02

Free Neuropathol 5:19:7

Meeting Abstract

Glutamatergic synaptic input to brain metastases drives metastatic colonization

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Brain metastases frequently occur in patients with cancer, primarily originating from lung carcinoma, breast carcinoma, and melanoma, resulting in high morbidity and mortality. The brain microenvironment significantly influences the progression of brain metastases. Recent studies have demonstrated that direct, glutamatergic synapses on glioma cells drive brain tumor progression. However, it is unclear if direct synaptic communication occurs between neurons and cancer cells from non-neural tumors, and if so, whether this can promote metastasis and tumor progression.

This study aims to identify and characterize direct glutamatergic synapses between neurons and brain metastatic cells in breast cancer and melanoma models. We hypothesize that direct glutamatergic synapses are formed on brain metastases, mediating signals via AMPA receptors (AMPARs). We employed ex-vivo and in-vitro electrophysiology. Patch-clamp recordings from melanoma and breast cancer cells revealed spontaneous excitatory postsynaptic currents (sEPSCs). The application of the AMPAR antagonist CNQX inhibited sEPSCs, confirming the involvement of AMPARs. In addition, genetic modification and pharmacological blockade of AMPARs using the approved antiepileptic drug perampanel in breast and melanoma cancer models led to a reduction in the number of brain metastases and overall brain metastatic burden.

This study demonstrates that brain metastases can integrate into the neuronal network by forming direct chemical synapses with neurons. AMPARs on cancer cells play a functional role for brain metastatic progression that can be pharmacologically targeted. This is the first evidence of direct synapses on brain metastases, highlighting the need for further characterization of these synapses in brain tumor biology to find novel therapeutic opportunities.

P03

Free Neuropathol 5:19:8

Meeting Abstract

Brain invasion in otherwise benign meningiomas: molecular characteristics and prognostic relevance

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Introduction: The prognostic value of brain invasion (BI) in meningiomas and thus the grading of these tumors has controversially been discussed for decades. The 2016 revised 4th edition of WHO classification of CNS tumors defined BI as a stand-alone criterion for grade 2. Still, the data published since then have been inconsistent. In the 2021 classification CNS5, brain-invasive otherwise benign meningiomas (BIOB) are still graded as CNS WHO grade 2, yet with emphasis on the lingering controversy.

Materials and methods: A multicentric series of 304 brain-invasive cases was analyzed. 673 meningiomas from previous studies were used for comparison. DNA-methylation classification, CNV-profiling (both EPIC, v12) and sequencing data (panel/whole-exome) were generated. Kaplan-Mayer analysis and multivariate cox regression were applied to analyze clinical outcomes.

Results: BIOB cases were more frequently of male sex and showed chr14q loss compared to non-invasive CNS WHO grade 1 tumors, ($p = 0.027$ and $p = 0.006$ respectively). BIOB had shorter PFS than CNS WHO grade 1 ($p = 0.003$), yet more favorable outcome than non-invasive CNS WHO grade 2 cases ($p = 0.006$). In multivariate analyses considering sex, WHO grade, methylation families (intermediate, malignant) and prognostically relevant CNVs, BI was an independent risk factor for shorter PFS (HR: 1.73; 95 %-CI: 1.34–2.22; $p < 0.001$).

Conclusion: In this study, BI confers an independently higher risk of reduced PFS even among otherwise CNS WHO grade 1 cases. However, the risk and outcome align neither with CNS WHO grade 1 nor 2 in our reference cohort. Further analyses are warranted for alternative risk stratification and data validation.

P04

Free Neuropathol 5:19:10

Meeting Abstract

Electrophysiological characterization of slow inward currents in glioblastoma

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Introduction: Glioblastomas are incurable primary brain tumors colonizing the entire brain. Long membrane protrusions called tumor microtubes (TMs) as well synapses on glioblastoma cells (GBCs) contribute to glioma progression. In a subset of GBCs neuronal input leads to excitatory postsynaptic currents (EPSCs) and slow inward currents (SICs). The exact molecular mechanisms of SICs and their biological relevance are incompletely understood.

Objective: This project addresses the question of the biological relevance of SICs for the growth of glioblastomas.

Materials & Methods: Patient derived xenograft models and in-vitro co-culture models of neurons and glioma cells are used. Whole cell patch-clamp recordings of glioma cells are performed to characterize SICs. Simultaneous patch-clamp recordings and calcium imaging are performed to understand the downstream effects.

Results: Acute stimulation with glutamate evokes SICs in GBCs from acute brain slices as well as from cocultures. A percentage of the evoked SICs are followed by intracellular calcium signals. The calcium signals are heterogeneous in size and subcellular localization. SIC-positive GBCs exhibit a total higher growth of TMs than SIC-negative GBCs. This is a new connection between electrical activity and the functional relevance for glioblastoma.

Conclusion: Neuronal input of glutamatergic synapses leads to SICs and heterogeneous, intracellular calcium events in a subset of GBCs. It expands upon these by showing a positive correlation between GBCs with SICs and the growth of TMs. Targeting SICs and subsequent calcium events in GBCs may be a novel therapeutic approach in this intractable disease warranting further investigation.

P05

Free Neuropathol 5:19:11

Meeting Abstract

Nanopore sequencing from formalin-fixed paraffin-embedded specimens for copy number profiling and methylation-based CNS tumor classification

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 # equal contribution

Microarray-based DNA methylation profiling has emerged as a powerful tool for central nervous system tumor classification and investigation of diagnostically relevant copy-number alterations such as 1p/19q-codeletion and the +7/-10 signature. Methylation arrays are well compatible with formalin-fixed and paraffin-embedded (FFPE) derived DNA but are time-consuming and requires batch processing. Nanopore sequencing has emerged as a rapid and scalable method, enabling measurement of DNA methylation and generation of copy-number profiles, but has been limited to high-quality DNA from native or cryopreserved samples so far. Here, we demonstrate the feasibility of nanopore sequencing from FFPE-derived DNA for methylation-based classification and generation of genome-wide copy-number profiles.

FFPE-derived DNA was isolated from 40 CNS tumors (average storage time: 19 months) including IDH-wildtype glioblastomas (n = 8), oligodendrogliomas (n = 6), posterior fossa ependymomas (PFA: n = 6, PFB: n = 6), medulloblastomas (WNT: n = 4, SHH: n = 5), pilocytic astrocytomas (n = 4), and one meningioma. All samples were analyzed with the Illumina EPIC methylation array and Nanopore sequencing on MinION devices.

On average, sequencing runs produced 205,000 reads and 201 Mb per sample. Methylation-based analysis using the random forest classifier nanoDx resulted in correct classification in 25/40 samples (63 %), whereas Sturgeon classified the vast majority of samples correctly (37/40, 93 %), including 14/16 samples with poor DNA quality (DIN < 5). All IDH-wildtype glioblastomas showed the +7/-10 signature, and all oligodendrogliomas harbored a 1p/19q-codeletion.

Taken together, our study demonstrates the feasibility of rapid methylation profiling and copy-number analysis of FFPE specimens using nanopore sequencing. Sturgeon, a neural network-based classifier, performed considerably better than the random forest-based classifier nanoDx.

Po6

Free Neuropathol 5:19:13

Meeting Abstract

Epigenetic profiling can improve diagnostics of MPNST with intratumoral histological heterogeneity

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Introduction: Approximately 10 % of NF1 patients develop malignant peripheral nerve sheath tumors (MPNST) with a poor prognosis, making early detection crucial. MPNST exhibit pronounced intratumoral heterogeneity, often containing areas resembling plexiform neurofibroma (PNF), atypical neurofibroma (ANNUBP), and MPNST. Consequently, CT-guided biopsies may miss critical tumor features. This study aims to delineate MPNST heterogeneity histologically and molecularly to improve diagnostic precision and to better understand the mechanisms of malignant progression.

Methods: Evaluation of tissue morphology on H&E-stained sections, immunohistochemistry, global methylation profiling (850k Illumina EPIC arrays), and gene sequencing panels.

Results: We chose one area with premalignant and one area with MPNST morphology per tumor for molecular analysis. Clustering analysis showed similar epigenetic characteristics in both areas in 5/10 cases. The remaining 5 cases displayed distinct epigenetic profiles, with premalignant areas clustering with ANNUBP and malignant areas clustering with MPNST. Copy number profiles showed marked alterations not only in the high-grade areas but also in the histologically benign areas in 8/10 cases. Gene sequencing identified identical mutations in SUZ12 and TP53 in premalignant and malignant areas in 2/10 cases, and MPNST-typical mutations in TP53 and EED observed exclusively in high-grade areas in 3/10 cases.

Conclusions: Our findings highlight that the histology of MPNST biopsies may often not fully represent the underlying tumor biology. We show that genetic and epigenetic changes occur before histological features of malignancy become apparent. This study underscores the diagnostic relevance of MPNST intratumoral heterogeneity and the need for comprehensive diagnostic approaches especially in needle biopsies.

P07

Free Neuropathol 5:19:15

Meeting Abstract

Anaplastic histology and distinct molecular features in a small series of spinal cord ependymomas

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Ependymomas (EPN) of the spinal cord encompass multiple types and subtypes with distinct histological, molecular and clinical features. Apart from subependymomas (CNS WHO 1), spinal EPN, and myxopapillary EPN (both CNS WHO 2), spinal EPN with *MYCN* amplification have been identified as the most aggressive type in the spinal cord. The latter include most of the spinal tumors that had been diagnosed as 'anaplastic ependymoma (CNS WHO 3)' in the pre-molecular era. While amplifications of *MYCN* therefore need to be investigated specifically, global DNA methylation profiling has emerged as a valuable tool to classify EPN.

Here, in depth analysis of the methylome and the proteome of spinal cord EPN revealed a novel distinct molecular EPN subtype. Unsupervised clustering of integrated methylation data of EPN revealed a distinct cluster of cases, that the established brain tumor classifier (Capper et al. (2018)) mainly defined as spinal subependymomas. Further liquid chromatography mass spectrometry-based proteomics measurements were conducted and an unsupervised analyses of matched proteome data revealed a similarity of the corresponding samples with spinal EPN, *MYCN* amplified. Reevaluation of histological features showed an anaplastic histology with high ki67 indices and positive nuclear staining for MYCN and OLIG2, which has not been described for subependymomas before. Although our cases had anaplastic features and showed expression of MYCN protein by immunohistochemistry, *MYCN* amplifications were neither detectable by FISH nor by whole genome copy number profiles. Limited follow up data indicated that respective cases may not relapse as commonly as *MYCN* amplified EPN.

We report a series of spinal EPN displaying distinct histomorphology, epigenetic and proteomic patterns and propose the provisional designation as MYCN-like spinal ependymoma (SP-EPN-MYCN-like). More in-depth investigations are warranted to uncover e.g. genetic drivers of the tumors and clinical outcomes of respective patients.

Po8

Free Neuropathol 5:19:17

Meeting Abstract

Integrated analyses reveal two molecularly and clinically distinct subtypes of H3 K27M-mutant diffuse midline gliomas with prognostic significance

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Histone H3 K27M-mutant diffuse midline gliomas (DMGs) are highly malignant tumours arising in the midline structures of the CNS. Recent studies suggested that epigenetic subgroups of DMGs can be distinguished based on alterations in the MAPK-signalling pathway, tumour localisation, mutant H3-gene, or overall survival. However, it is unclear how these parameters collectively influence survival. Hence, we analysed dependencies between different parameters, to define novel epigenetic, clinically meaningful subgroups of DMGs. We collected a cohort of 149 H3 K27M-mutant DMGs, that could clearly be allocated to the spinal cord (*n* = 31; one patient with an additional sellar tumour), medulla (*n* = 20), pons (*n* = 64) or thalamus (*n* = 33), including published data. We then performed DNA methylation profiling and, for a subset, DNA sequencing and survival analyses. Unsupervised hierarchical clustering of DNA methylation data indicated two clusters of DMGs, i.e. subtypes DMG-A and DMG-B. These subtypes differed in mutational spectrum, localisation, age at diagnosis and overall survival. DMG-A was enriched for MAPK-associated mutations, medullary localisation and adult age. 13 % had a methylated *MGMT* promoter. Contrarily, DMG-B was enriched for *TP53*-mutations, *PDGFRA*-amplifications, pontine localisation and paediatric patients. In univariate analyses, the features enriched in DMG-B were associated with a poorer survival. However, these parameters were dependent on the cluster attribution, which had the largest effect on survival: DMG-A had a significantly better survival compared to DMG-B. Hence, the subtype attribution based on two methylation clusters is best suited to predict survival as it integrates different molecular and clinical parameters.

P09

Free Neuropathol 5:19:19

Meeting Abstract

Spatial multiomics profiling reveals heterogeneity of B:T cell interactions and plasma cell formation in tertiary lymphoid structures in human gliomas

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Adult-type diffuse gliomas, the most common primary brain tumors, remain a clinical challenge in oncology with limited treatment options, restricted anti-tumor immune response and dismal patient prognosis. Despite their immunosuppressive microenvironment, the formation of lymphoid aggregates containing adaptive immune cells has been reported in gliomas. However, the cellular composition, immunological function and relevance of lymphoid aggregates for adaptive anti-glioma immunity is not well understood. Therefore, we performed a comprehensive, unbiased analysis of lymphoid aggregation in 642 adult-type diffuse gliomas using a multi-modal approach; combining DNA methylation, RNA sequencing with spatial transcriptome and proteome profiling. Overall, B cell aggregates and tertiary lymphoid structures (TLS) were observed in 15 % of tumors and associated with an improved overall survival. Gliomas containing TLS displayed a remodeled perivascular space, characterized by transcriptional upregulation and spatial redistribution of collagens associated with barrier functions. Spatial transcriptome and proteome profiling revealed heterogeneous B:T cell interactions that were associated with elevated CD8 T-cell numbers and differences in IgA and IgG plasma-cell forming capacity, suggestive of dynamic adaptive immune responses.

P10

Free Neuropathol 5:19:20

Meeting Abstract

Digital Biomarkers for an Improved Clinical Stratification of Meningioma Patients

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Introduction: Postsurgical therapy concepts in meningiomas are influenced by the World Health Organisation (WHO) three-tiered histomorphological grading, which is subjected to a high interobserver variability and low reproducibility. Risk-of-recurrence models, integrating histology and molecular tumor data, have been developed to improve patient stratification. However, the distinguishment between meningioma patients with a low risk of recurrence from those with an intermediate risk remains particularly challenging. Here, we propose image- and methylome-based biomarkers for a refined outcome prognostication.

Material and Methods: Whole slide images (WSI) of Ki67-stained meningioma samples were generated and DNA methylomes were collected (n = 173). A random-forest based pixel classifier for a machine-learning based tumor segmentation was generated on training images (n = 110). In a scripted batch processing, the Ki67 marker was spatially quantified within randomly distributed, segmented image tiles (10 tiles, tumor area 10 mm^2). Further, samples were epigenetically subcharacterized by reference-free tumor deconvolution.

Results: The WSI data is assignable to methylation subclasses within the Heidelberg v12.8 brain tumor classifier (median Ki67 grouped subclasses benign 1-3 and intermediate-A = 5.0 %; median Ki67 grouped subclasses intermediate-B and malignant = 7.0 %; p = 0.0096; Mann-Whitney U test; n = 135). Integrated risk-of-recurrence

scores (intS, Hielscher et al., 2022) are distinguishable based on proliferation (median Ki67 intS low = 4.1 %; median Ki67 intS intermediate/high = 6.5 %; $p < 0.0001$; Mann-Whitney U test; n = 118). Based on methylation-based tumor deconvolution, a meningioma clustering according to histological WHO grades is identifiable (n = 107).

Conclusion: Ki67-focused WSI and tumor deconvolution data translate into different patient outcome groups, representing promising novel biomarkers for future integrated models in disease course prognostication of meningioma patients.

P11

Free Neuropathol 5:19:22

Meeting Abstract

Cellular senescence as a shared contributor to disease progression in Glioblastoma and Alzheimer's disease

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Introduction: Neurodegenerative disorders and cancer are increasingly prevalent in aging populations. Over 50 % of Glioblastoma (GB) patients show Alzheimer's Disease neuropathological changes (ADNC) in tumor-adjacent cortex, but the biological link remains unclear. Here, we investigate cellular senescence, one hallmark of aging, as a shared mechanism of disease progression.

Methods: We analyzed single cell/nucleus RNA sequencing datasets from 110 GB patients (GBmap core atlas) and 89 AD individuals (SEA-AD MTG atlas). Using established senescence gene lists, gene set enrichment analysis and machine learning we identified senescent cells. This allowed us to compare senescence in cell types, subclusters, and disease stages. Weighted gene co-expression network analysis (WGCNA) identified senescence-associated co-expression modules. Quantitative neuropathological analysis of published measurements supported the findings.

Results: Leveraging published datasets, we identified senescent cells in GB and AD. GB showed higher variance in senescence scores, indicating greater inter-patient variability. Among glial cells, microglia were enriched for senescence gene sets, with the highest scores in proinflammatory subtypes. Using WGCNA we found senescence genes co-expressed with known disease-associated genes like PTEN or SPP1 in AD or GB, respectively. In both diseases, microglia senescence correlated with disease progression. Accordingly, we identified higher ADNC and altered microglia morphology with signs of functional exhaustion in AD specimens with high senescence scores.

Conclusion: Our atlas-level transcriptomic analysis identifies cellular senescence as a shared mechanism in GB and AD pathology and disease progression, particularly enriched in microglia. Co-expression of senescence-associated and disease-specific genes in microglial modules highlights the complex role of senescence in these diseases.

P12

Free Neuropathol 5:19:23

Meeting Abstract

VOPP1::EGFR gene fusion as an oncogenic driver via NFκB pathway activation in a case of ganglioglioma

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Ganglioglioma is a well-differentiated glioneuronal tumor characterized by a combination of neoplastic ganglion and glial cells. Oncogenic driver mutations and gene fusions have been shown to be of prognostic significance in gangliogliomas and can offer potential therapeutic targets. Typical molecular alterations are MAPK pathway activations with BRAF p.V600E being the most frequent one. Here, we report for the first time a *VOPP1::EGFR* gene fusion as the single oncogenic driver in a case of ganglioglioma leading to activation of NFκB signaling. We show the respective histological and molecular evidence including gene fusion and mutational analysis, methylation profiling and clinical outcome. The case expands the known molecular spectrum of oncogenic drivers in ganglioglioma linking it with prognostic and potentially therapeutically relevant data.

P13

Free Neuropathol 5:19:24

Meeting Abstract

Insights from the additional immunohistochemical work-up of molecular tumor board patients from a regional brain tumor center

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Patients with CNS/PNS tumors are included into the molecular tumor board of our local brain tumor center to defined criteria and on a regular basis. Patients discussed in the period from 22.06.2021 to 30.06.2023 were selected for accompanying immunohistochemical (IHC) work-up in addition to comprehensive molecular analysis. Main objectives were to substantiate signaling pathway activation and to identify biomarkers harboring predictive value.

The project was approved by the local ethics committee, and informed consent was obtained from all patients. FFPE tumor tissues (same material as used for molecular analyses) from 84 patients were included on tissue-microarrays comprising the following entities: adult- /pediatric-type diffuse glioma, circumscribed glioma, ependymoma, meningioma, medulloblastoma, peripheral nerve sheath and pineal tumor. Tissue-microarrays were analyzed immunohistochemically using 14 different antibodies: p-AKT, p-mTOR, p-TSC1, p-TSC2, p-S6-RP, p-STAT3 (Ser727 and Tyr705), p-p42/44 MAPK(Erk1/2), p-MEK, p-Rb, MLH1, PMS2, MSH2 and MSH6. The H-score was used to classify results semi-quantitatively.

Immunohistochemical results were correlated to molecular alterations and revealed two significant associations: (1) Patients with at least one activating EGFR alteration exhibit significantly lower phosphorylation and activation of TSC1 than those without EGFR alterations. (2) Expression of the mismatch-repair proteins MSH2, MSH6, MLH1 and PMS2 was reduced in patients with a NF1 mutation.

In summary, additional IHC work-up of molecular tumor board patients generated two interesting scientific findings: (1) p-TSC1 might serve as a surrogate marker for EGFR alterations and (2) a potential interrelation between MMR-deficiency and mutational inactivation of NF1 exists. The exact mechanisms of this finding, however, need further investigation.

P14

Free Neuropathol 5:19:25

Meeting Abstract

Cytokine response in the cerebrospinal fluid after intraoperative radiation of primary and metastatic brain tumors

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Aim of our study is to characterize the immunological signature represented by the cytokine profile in the cerebrospinal fluid (CSF) after IORT of primary and secondary brain tumors.

For this, the cytokine patterns in 67 CSF samples obtained from 19 patients with brain tumor resection were analyzed. Samples were collected at four time points: 1. intraoperatively, before tumor resection and IORT, 2. intraoperatively, after tumor resection, before IORT, 3. intraoperatively, after tumor resection, after IORT and 4. approx. 24 hours postoperatively from drainage. Multiplex immunoassay CodePlex Secretome for the IsoSpark technology was used for level determination of 19 cytokines involved in the innate immunoresponse.

An increase of signal intensity was observed for 14 of the 19 analyzed cytokines (73 %) in the IORT group, including EGF, Granzyme B, IL-10, IL-1β, IL-6, IL-7, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-BB, sCD137 and VEGF. Seven of those cytokines (37 %) displayed a significant increase. When comparing the time points 1 and 4 between IORT and non-IORT group, the increase in signal intensity for IL-1β, IL-8, IP-10, MIP-1β, IL-10, and TNF-α in the IORT group is noticeably greater than in the non-IORT group, although not significantly.

Our results allow first conclusions about changes in the cytokine profile of CSF after IORT of primary and secondary brain tumors. The results indicate that IL-1β, IL-8, IP-10, MIP-1β, IL-10 and TNF-α are involved in the inflammatory response induced by exposure to ionizing radiation of the tumor bed during surgery.

II. Neuroimmunology

P15

Free Neuropathol 5:19:26

Meeting Abstract

In-Depth Analysis of Viral Distribution and Immunological Profiling in Human Bornavirus Encephalitis

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The Borna disease virus 1 (BoDV-1) has recently been shown to cause a usually fatal encephalitis in humans. To date, little is known about the pathogenesis and distribution pattern of the virus in human diseases. Aim of the present study is to characterize the virus distribution in the CNS and profile the immunological processes in human bornavirus encephalitis (BVE).

Complete sagittal and coronary sections of the brains from four individuals who died of BVE were embedded and stained immunohistochemically for the BoDV-1 nucleoprotein (antibody Bo18). The cross-sections were then digitally reconstructed, and the amount of BoDV-1-positive cells quantified using the software CellQuant. Furthermore, the viral loads were estimated for each block using qPCR. Immunological profiling was done using the nCounter technology (Nanostring).

While most cases show relatively low virus intensities in the cerebellum, the basal ganglia, brainstem and thalamus are frequently heavily affected as estimated by both, immunohistochemistry and qPCR. The immunological profile was examined for one BoDV-1 case based on low, medium and high viral loads. CD56dim natural killer

cells have the highest scores in areas with high viral loads. Interestingly, regions with medium viral loads show the highest signatures for immunological cells, like cytotoxic cells and macrophages.

BVE can be considered a model disease for neurotropic virus infections. Understanding the pathogenesis and viral spread mechanisms is crucial despite the rarity of the disease. A standardized, comprehensive analysis of autopsy cases, including the peripheral nervous system, is essential. This will significantly enhance our understanding and management of such infections.

P16

Free Neuropathol 5:19:28

Meeting Abstract

Histopathological Analysis of BoDV-1 Distribution in a Non-Human Primate Model: Comparing Intranasal and Subcutaneous Inoculation

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Borna disease virus 1 (BoDV-1) is known to cause fatal encephalitis in humans, yet the mechanisms and distribution of the virus within the brain remain poorly understood. This study investigates the histopathological impact of BoDV-1 in a non-human primate model. Twelve macaques were infected with BoDV-1, with six receiving intranasal inoculation which reflects the currently hypothesized portal of entry, and six receiving subcutaneous inoculation as setting for infection via the peripheral nervous system. The primary focus of this project is the histopathological examination of the brains to determine the differences of the viral distribution in the two settings of infection.

Complete sagittal sections of the brains were embedded and immunohistochemically stained targeting the BoDV-1 nucleoprotein. The stained sections were digitally reconstructed, allowing for a detailed analysis of the virus' distribution. Quantitative assessment was performed using the software CellQuant.

Preliminary results indicate differential patterns of viral spread and intensity between the two inoculation methods. Intranasally infected macaques showed a higher concentration of BoDV-1-positive cells in the olfactory bulb, spreading to the frontal cortex and deeper brain structures. Subcutaneously infected macaques demonstrated a more dispersed viral distribution with generally lower virus detection.

This study provides valuable insights into the pathogenesis of BoDV-1, highlighting the importance of the route of infection in determining viral spread within the CNS. The findings emphasize the need for comprehensive histopathological analysis to enhance our understanding of BoDV-1 and its implications for neurotropic virus infections.

P17

Free Neuropathol 5:19:29

Meeting Abstract

Systematic Analysis of Virus Spread to the Peripheral Nervous System in Fatal Borna Virus Encephalitis

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Borna disease virus 1 (BoDV-1) causes fatal encephalitis in endemic regions. Even if there are reports on spread of this highly neurotropic virus to the peripheral nervous system, there is no systematic analysis of the virus distribution aside from the central nervous system yet.

We analyzed autopsy material from four deceased with BoDV-1 infection regarding the presence of BoDV-1 nucleoprotein in visceral organs (including heart, lung, liver, thyroid gland, pancreas, stomach) with immunohistochemistry. Additionally, we evaluated the tissue morphology considering local inflammatory reaction, which is commonly observed in central nervous system and contributes to the fatal outcome.

BoDV-1 could be shown in small peripheral nerves in the thyroid gland, pancreas, adrenal glands, left side heart and in the stomach, without spread outside the nerve structures. One patient was a transplant recipient from earliest reported infection cluster and showed additional infiltration in peripheral skeletal nerves, esophagus, mediastinal and retroperitoneal adipose tissue, lungs, liver as well as in the implanted but not their own kidney. Neither inflammatory nor any other tissue reaction was observed. In one case, no peripheral spread could be shown (with also atypical brain distribution due to high-dose immunosuppression). No patient showed BoDV-1 positivity aside of the peripheral nerve tissue.

We confirm that BoDV-1 spreads to small peripheral nerves, pronouncing in organs innervated by the vagus nerve. Although neither specific reaction nor local damage was observed, consecutive autonomic nerve system dysregulation may occur in some patients. Investigations regarding alternative clinical presentations apart from the typical encephalitis should be considered.

P18

Free Neuropathol 5:19:30

Meeting Abstract

Global Cerebral Hypoxia-Ischemia: *Ex vivo* Ultrahigh Field MRI Signals Correlate with Differential Cortical Localization of Microglia and Gemistocytes Characterized by CHIT1 and CHI3L1 (YKL 40) Expression

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Previously, we have shown in survivors of global hypoxic-ischemic brain injury (HIBI) that the neuroinflammation- and ischemia-related glial markers chitotriosidase 1 (CHIT1) and chitinase-3-like protein 1 (CHI3L1, alias YKL-40) are expressed by microglia and astrocytic gemistocytes, respectively (Yilmazer-Hanke et al., 2022, Neuroscience 506:91–11). In the current study, we investigated the distribution of CHIT1-positive microglia and CHI3L1-positive gemistocytes in the cerebral cortex after HIBI. Tissue from HIBI cases was scanned using high-resolution ultrahigh field MRI (UHF-MRI) with ex vivo T1w, T2w and T2*w sequences. CHIT1 and CHI3L1 expression was compared to changes in microglial (e.g., IBA1, CD68) and astrocytic (e.g., GFAP) markers using immunohistochemistry and multiple-label immunofluorescence. Results indicated altered MRI signals in different cortical layers. These altered MRI signals correlated with severe neuronal cell loss, enhanced densities of rounded CHIT1-positive microglia in deep cortical layers, and the emergence of CHI3L1-positive gemistocytes, which populated superficial cortical layers and the juxtacortical boundary. Further analyses revealed that CHIT1-positive microglia aggregated around microvessels of deep cortical layers. In conclusion, altered cortical MRI signals that reflect the cortical damage as well as CHIT1- and CHI3L1-positive glial cell pathology with a differential cortical distribution could be a valuable *in vivo* biomarker for monitoring the outcome of global HIBI and for determining the prognosis of long-term HIBI survivors.

III. Muscle / Nerv

P19

Free Neuropathol 5:19:31

Meeting Abstract

Adipo-glial signaling mediates metabolic adaptation in peripheral nerve regeneration

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The peripheral nervous system exhibits an impressive potential to regenerate following acute nerve injury. However, complete functional recovery is uncommon and heavily relies on peripheral nerve Schwann cells, which orchestrate both the degradation and re-synthesis of myelin while also supporting axonal regrowth. These cellular processes elicit a substantial metabolic demand and the exact mechanisms by which Schwann cells cater to the high metabolic demands of nerve repair are still not well understood. In this study, we demonstrate that nerve injury triggers signaling from adipocytes to glial cells and we identify the adipokine, leptin, as a key regulator of glial metabolic adaptation during regeneration. Using conditional mutagenesis in mice, we demonstrate that Leptin Receptor ablation in Schwann cells or Leptin ablation in adipocytes renders a congruent phenotype characterized by abrogated injury-specific catabolic processes such as myelin autophagy and mitochondrial respiration in Schwann cells during nerve repair. Our research thus proposes a model wherein acute nerve injury initiates an adipo-glial communication that can be therapeutically targeted to modulate glial metabolism, ensuring sufficient energy for effective nerve repair.

IV. Neurodegeneration

P20

Free Neuropathol 5:19:32

Meeting Abstract

Alpha-synuclein co-pathology in Alzheimer's disease drives tau accumulation

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Background: The molecular basis for accelerated cognitive decline seen in Alzheimer's Disease (AD) cases presenting with cortical alpha-Synuclein co-pathology is not well understood. Mouse experiments have shown adverse interactions between tau and alpha-synuclein, but how this finding translates to humans from a genome-centered point of view remains unknown.

Materials and Methods: Whole genome sequencing was performed on 137 neuropathologically defined AD cases, 36 of which presented with neocortical alpha-Synuclein co-pathology (Braak stage 6). Polygenic risk scores were calculated. Single-nucleus RNA sequencing and Western Blot data were collected from post-mortem tissue. Bioinformatic analysis of a large external dataset (n > 300) served as external validation. Transcriptomic and proteomic experiments were carried out in cell lines and cortical organoids derived from iPSCs.

Results: AD brains with alpha-Synuclein co-pathology had significantly higher polygenic risk scores for Parkinson's Disease, which could be partially explained by variants associated with higher expression of SNCA (the gene symbol for alpha-Synuclein). Single-nucleus RNA sequencing revealed a higher expression of MAPT, the gene encoding microtubule-associated protein tau, in co-pathology cases. Protein and mRNA expression of MAPT and SNCA were positively correlated in an external cohort. Ultimately, cell culture experiments demonstrated that overexpression of SNCA was sufficient to drive accumulation of soluble tau.

Conclusion: We show that alpha-Synuclein co-pathology brains are characterized by higher tau levels and that increasing alpha-Synuclein expression is sufficient to drive tau accumulation. Our results reveal that tau and alpha-synuclein can synergistically drive dementia-related pathology.

P21

Free Neuropathol 5:19:33

Meeting Abstract

Frontotemporal dementia patient neurons with the *MAPT-N279K* mutation are responsive to tau filaments and contribute to neuroinflammation *in vivo*

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Frontotemporal dementia (FTD) is a heterogeneous group of early-onset dementias leading to impairment of cognition, language and behavior. FTD can be caused by deposition of hyperphosphorylated tau (p-tau) in neurons and glial cells in various brain regions throughout the patient brains. However, the mechanisms leading to neurodegeneration are still largely unknown and a curative therapy does not exist. Here, we applied a stem cell-based approach combined with single-cell analyses of FTD patient brains and differentiated FTD patient-derived induced pluripotent stem cells (iPSCs) carrying the *MAPT-N279K* mutation and healthy control iPSCs into neurons. We found disease-relevant changes in FTD neurons associated with mis-splicing of tau, p-tau pathology, neurite outgrowth deficits, and increased oxidative stress and neuroinflammation with an upregulation of pro-inflammatory genes, several of which were also upregulated in neurons in FTD patient brains carrying the same *MAPT-N279K* mutation. Tau filaments isolated from FTD patient brains with *MAPT-N279K* further upregulated expression of the pro-inflammatory marker osteopontin in FTD neurons and altered pathways related to the unfolded protein response and proteasomal function. When injected into the mouse brains, FTD neurons showed decreased survival and induced an increased microglial response. Decreased survival of FTD neurons was also noted when cells were co-injected with FTD tau filaments into mouse brains. Interestingly, alterations of inflammatory gene expression in engrafted neurons resulted in altered engraftment and microglial infiltration. These findings point towards an immune-modulatory role of neurons in FTD and indicate that its alteration may represent a potential therapeutic target in FTD.

P22

Free Neuropathol 5:19:34

Meeting Abstract

Alterations of ER-co-chaperone SIL1 in Amyotrophic lateral sclerosis (ALS)

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** equal contribution*

Amyotrophic lateral sclerosis (ALS) is a devastating disease characterized by progressive loss of upper and lower motor neurons (MNs). Recent studies showed that selectively viable neurons are often equipped with protective factors, while vulnerable neurons lack them. Among others, the ER co-chaperone SIL1, mutated in Marinesco-Sjögren syndrome (MSS), supports such selective MN viability not only in MSS pathology but also in ALS pathology. However, the precise molecular mechanism(s) of such neuroprotection as well as its role in maintaining

neuronal proteostasis is largely unclear. We aimed to validate the correlation between SIL1 and ALS-associated protein aggregates in human ALS together with investigating the neuroprotective role of SIL1 associated with the protein quality control (PQC) mechanism. We used ALS postmortem autopsy materials and compared them with ALS cell models expressing ALS-causing mutant proteins. Our results confirmed that in the ALS MNs, SIL1 protein levels were specifically elevated in slow fatigue-resistant MNs (sMNs), and reduced SIL1 levels were linked to ALS-associated protein aggregates. Besides, SIL1 was found to be sequestered with various forms of pTDP-43 and other aggregates in ALS spinal MNs as well as ALS cell culture models. Overexpression of SIL1 facilitated the clearance of the above-mentioned pathogenic aggregates in these models. Furthermore, SIL1 could activate autophagy pathways by promoting fusion of autophagosomes with lysosomes. In summary, our results suggest that SIL1 is promoting neuronal PQC and survival. Conversely, disturbed SIL1 function could be detrimental to neurons.

P23

Free Neuropathol 5:19:36

Meeting Abstract

Characterizing granular Tau aggregates in astrocytes in Multiple System Atrophy

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Introduction: Multiple system atrophy (MSA) is a rare adult-onset fatal neurodegenerative disorder characterized by alpha-Synuclein aggregation in oligodendrocytes. Moreover, small granular Tau inclusions in the white matter were occasionally, but not extensively described in the literature and have been controversially discussed. Based on the finding from our snRNA-Seq dataset that *MAPT* is significantly upregulated in astrocytes in the frontal white matter of MSA patients, we sought to better and more comprehensively characterize the observation of granular Tau inclusions in MSA.

Materials and Methods: Immunohistochemistry and immunofluorescence as well as RNAScope were applied.

Results: AT8-positive granules were a common finding - although to a highly variable extent - in the white matter of MSA patients, whereas no such staining was observed in a series of non-neurodegenerative controls or patients with LBD. The granules partially colocalized with pTau-thr231 and pTau-ser396 as well as with RD3 or RD4. Co-staining of AT8 with GFAP revealed an association with astrocytes without any obvious association with oligodendrocytes or microglia. While there was no noticeable colocalization of pTau and alpha-Synuclein inclusions, we find a positive relationship between the load of alpha-Synuclein pathology and granular Tau inclusions.

Conclusion: Granular Tau accumulations are a common feature in MSA. The inclusions, however, seem to be different from other known Tau aggregates. While it is generally assumed that glial cells take up misfolded Tau from neurons, the upregulation of *MAPT* in astrocytes may indicate that in this case Tau accumulation may be a consequence of an increased expression of endogenous Tau.

V. Methods and Free Topics

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Meeting Abstract

Ultra-rapid bacterial detection from neuropathology specimens using next-generation PCR and nanopore sequencing

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Bacterial infections of the central nervous system (CNS) are severe and potentially fatal diseases. Early detection of causative pathogens is crucial for the rapid administration of a tailored antibiotic regime. Routine neuropathology diagnostics is typically limited to histological staining and microbiological culture, which are time consuming and result in false-negative results in one-third of cases. Metagenomic sequencing of the bacterial 16S rRNA gene offers a rapid and unbiased alternative. In this study, we present a method for detecting bacterial pathogens in neuropathology specimens using ultra-rapid PCR combined with nanopore sequencing of 16S rDNA amplicons.

For protocol optimization, a microbial community standard and varying concentrations (5 pg to 5 ng) of gDNA from *E. coli* and *S. aureus* were used. Ten FFPE samples with known bacterial CNS infections were retrieved from the Institute of Neuropathology Münster. After DNA isolation, library preparation was performed using classic PCR and NextGenPCR with four primer pairs covering the variable regions V3–V7 of the 16S rRNA gene. Amplicons were sequenced on a MinION device and data was analysed with EPI2ME, MABA16S and EMU. NextGenPCR followed by nanopore sequencing identified bacterial taxa more accurately (64 % vs. 26 % correct hits, $p < 0.001$, t-test). Compared to classic PCR, the optimized NextGenPCR protocol significantly reduced the amplification time from 165 to 35 minutes.

In conclusion, NextGenPCR of 16S rDNA followed by nanopore sequencing offers an ultra-rapid method for detecting bacteria in neuropathology specimens. Further investigation of a larger cohort including cerebrospinal fluid samples is warranted.

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Meeting Abstract

CCNV: R package for enhanced cumulative copy number variation analysis

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Copy number variation (CNV) analysis is the study of genomic alterations resulting in an abnormal copy number of genes, chromosomal segments or whole chromosomes. The identification of chromosomal aberrations can help identify targetable proteins or markers for specific tumour entities (e.g. the MYCN amplification in spinal Ependymomas, MYCN-amplified or the loss of SMARCB1 in atypical teratoid/rhabdoid tumours). CNV information can be derived from global DNA methylation data, which has become an integral part of tumor diagnostics. CNVs can help in or even guide tumor classification and the number of available data constantly increases. In order to characterize CNVs in large datasets comprising tumor subgroups or types we developed CCNV, a tool for fast, accurate and efficient cumulative CNV analyses.

CCNV is an R package that integrates two main algorithms for the segmentation of inferred copy number data from methylation data; the circular binary segmentation integrated in conumee/conumee2.0 (Hovestadt, Zapatka (2015), Daekanas (2024)) and the piecewise curve fit algorithm (Nilsen (2012)). The package is compatible with all major available DNA methylation chips (450K, EPIC; EPICv2, Mouse Chip) and can automatically read array data from .idat files. Each segmentation algorithm produces one intensity plot, representing how strong aberrations are across the cohort, and one frequency plot, representing how often an aberration occurs. While the circular binary segmentation is applied consecutively to each sample, the piecewise curve fit algorithm can be applied to all samples simultaneously, achieving a significant decrease in runtime. The latter further extracts the main aberrations of the investigated samples, making it particularly suitable for large datasets.

With different selectable segmentation algorithms and parameters the CCNV package is a flexible and efficient tool to generate cumulative CNV plots from big datasets.

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Meeting Abstract

Rapid slice-free intraoperative histology in neurooncology using multiphoton microscopy – first study results

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Multiphoton microscopy (MPM) enables histological examination of tissue samples without the prior production of thin sections using paraffin or frozen preparation. It can be applied in the operating room (OR) with less effort and in just a few minutes. In a proof of concept study, we evaluated MPM on neurosurgical tumor samples and compared the diagnostic results to the routine histology.

MPM uses a laser to create non-linear optical effects that allow tissue blocks to be scanned at adjustable depths. After a 2:30 mins long staining process the MPM scanned the tissue at a speed of 4 min/cm², with the potential for further acceleration to 30 s/cm². The results are digital images with high similarity to standard H&E-stained slides. 12 tissue samples were collected at the Clinic for Neurosurgery at UKSH Lübeck. The digital images were evaluated in a blinded manner by a board-certified neuropathologist with no prior training in MPM image diagnosis. Only minimal clinical input data was provided (patient's age, sex, and sample localization). In 11 of the 12 cases the diagnosis was consistent with the result of the routine analysis. Tissue histomorphology assessment revealed a good assessability of the cell morphology and tissue texture compared to standard slides.

The initial results show that the MPM is a valuable tool for accelerating and simplifying intraoperative histological examinations in neurosurgery without the need for device-specific training of the neuropathologist. In ongoing research, we will further optimize the technical settings and increase the cohort size to validate a broad applicability in neurooncology.

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Free Neuropathol 5:19:40

Meeting Abstract

Predicting epigenetic ependymoma types from histological whole-slide images using neural networks

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Ependymomas are neuroepithelial neoplasms of the central nervous system and comprise (at least) 10 main clinically distinct types based on epigenetic (DNA methylation) profiles. For their diagnosis, the current standard practice is to integrate time consuming epigenetic analyses with histological assessment. We asked whether neural networks can predict the DNA methylation class of ependymoma types from hematoxylin and eosin stained whole-slide images. Using explainable AI, we further aimed to prospectively improve the consistency of histology-based diagnoses with DNA methylation profiling by identifying and quantifying distinct morphological patterns of these molecular ependymoma types.

We collected sample-matched epigenetic profiles and whole-slide images (Hematoxylin- and Eosin stain) of > 500 ependymomas from different anatomical compartments. Attention-based classification models (CLAM) or

Multi-Grid Vision Transformers (ViTs) were trained to predict the epigenetic ependymoma types from the slide images and the models were compared the results from histological annotations by neuropathologists for a fraction of cases.

Our approach yielded reliable predictions of the epigenetic types based on histomorphological data. Self-supervised encoder training was crucial for classification performance. The classifiers improved over board-certified neuropathologists and its attentions scores were leveraged to correlate epigenetic and morphological ependymoma characteristics. Image normalization and augmentation facilitated domain adaptation towards whole-slide images from other medical facilities and brightfield microscopy images.

We established an interpretable method to reliably predict epigenetic ependymoma types from histological whole-slide images. Our approach provides a fast and inexpensive way for first assessment of molecular ependymoma classification, provides morphological interpretability and may prospectively enable rapid decisions on patient-specific treatment in the upcoming era of digital pathology.

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Free Neuropathol 5:19:42

Meeting Abstract

Established in 1902: a brief history of the Institute of Neurology (Edinger Institute) in Frankfurt, Germany

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The Institute of Neurology was established by the Jewish neuroscientist Ludwig Edinger on the premises of the Dr. Senckenberg Institute of Pathology in Frankfurt, headed by Carl Weigert. Edinger was considered to be the leader in comparative neuroscience of its time by the Spanish Nobel prize winner Ramon y Cajal. Edinger was among the founders of the "Königliche Universität zu Frankfurt" (later renamed as Goethe University) in 1914 and was appointed as the first Professor of Neurology in Germany.

During the "Third Reich", Jewish scientists, including Edinger´s successor Kurt Goldstein, were forced to leave the Institute. As a consequence, scientific activities in the Institute of Neurology ceased almost completely.

After WWII, Wilhelm Krücke, an expert on peripheral neuropathies, became the director of the Institute and founder of the German Society of Neuropathology in 1950. In 1960, the Edinger Institute served as founder for the Max-Planck Institute for Brain Research, the legal successor of the Kaiser-Wilhelm Institute for Brain Research (KWIH, established 1914). As a consequence of the merger of Edinger´s Institute with the Department of Histopathology of the former KWIH, previously unrecognized specimens of victims of the Nazi-regime were detected.

Wolfgang Schlote became the director of the Institute in 1984 until his retirement in 1999. This position was carried on by Karl H. Plate in 2001. The year 2002 marked the 100th anniversary of the Edinger Institute. Several members of the Edinger family returned to Germany for the first time after WWII on this occasion. 122 years after its foundation, the Institute of Neurology continues to serve as the department of neuropathology of Frankfurt University Hospital, hosting three professors and six research groups, consisting of neuropathologists, molecular biologists, developmental biologists, bioinformaticians and research staff to provide a unique, lively environment for translational neuropathology, coming close to how it was initially intended by its founder Ludwig Edinger.

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Free Neuropathol 5:19:43

Meeting Abstract

Paving the path: the powerful effect of substrate topography on axon-repulsive Schwann cell-astrocyte barrier formation for spinal cord injury repair

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Spinal cord injury is a devastating condition that leads to permanent impairment of sensory, motor and autonomous functions. The severely limited regenerative capacity of the lesioned central nervous system (CNS) is mainly attributed to the formation of a glial scar, a process that is mediated by reactive astrocytes (ACs). Transplantation of Schwann cells (SCs) into the severed spinal cord represents a promising therapeutic approach to support tissue regeneration but is limited by mutually repulsive interactions with resident ACs, resulting in the formation of well-defined interfaces. Such interfaces act as effective barriers for regenerating axons, allowing the transition from the AC-dominated CNS environment into the SC compartment but not *vice versa*, leaving regenerated axons trapped within the SC graft.

For this investigation, the well-established confrontation assay model was adapted to study the effect of topographical cues derived from substrate-bound, highly oriented poly(ε-caprolactone) nanofibers on such devastating SC-AC interactions as well as axon regeneration *in vitro*. Immunocytochemistry was performed to quantify the extent of SC-AC intermingling and the success of axon regeneration across the modified SC-AC interface.

Our study demonstrates the powerful effect of oriented substrate topography on SC-AC interactions, preventing the formation of barrier-forming SC-AC interfaces and enabling substantial directed and long-distance axon outgrowth across the SC-AC interface and into the AC compartment.

This is the first demonstration of non-functionalized nanofibers providing a substrate that can override such mutually repulsive cell-cell interactions. It is anticipated that these findings will profoundly influence the design of biomaterial-based scaffolds for spinal cord injury repair.

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Meeting Abstract

Using Eye-Tracking to find Differences in the Analysis of Whole-Slide Images Between Physicians and Machine Learning Models – A Study Design

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Neuropathologists are experts in their field, quickly detecting and classifying tumors in Whole-Slide Images (WSIs). However, it is often unclear what features they focus on and which aspects they rely on for their decision. Machine Learning (ML) is also increasingly used in pathology, but its decision-making process is similarly opaque. To better understand expert analysis and evaluate ML reasoning, an eye-tracking study will compare detection accuracy and Region of Interest (RoI) selection among neuropathologists, pathologists in general, medical students, and ML models.

The study involves experts (neuropathologists and pathologists with high experience in brain tumor diagnostics), intermediates (pathologists without any particular expertise in brain tumor diagnosis), and beginners (students, physicians from other domains) analyzing hematoxylin-and-eosin (HE) stained sections of 50 Glioblastoma (GBM) WSIs. Participants must determine whether neoplastic tissue is present in the section and, if so, mark the most relevant regions for their analysis. The EyeLogic LogicOne eye-tracking device records their gaze during this process.

Post-study, gaze data from different participant groups will be compared with ML probability masks to identify differences and similarities between human and artificial analysts. The study will also correlate selected RoIs with ML probabilities and gaze duration to see if the most critical areas are those most viewed by humans and deemed most important by ML.

An eye-tracking study is planned for October 2024, comparing the analysis of HE-stained WSIs of GBMs between human analysts and ML models. The study is currently in the recruitment phase, with results expected by end of the year.

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Meeting Abstract

Why We Don't Save Whole-Slide Images as Lego Mosaics – Putting the Scale of Whole-Slide Images into Perspective

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Why do we save Whole-Slide images (WSIs) as DICOM-WSI, TIFF, SVS, … and not as Lego mosaics? While the answer to this question might be pretty obvious (working with digital data is much easier, and the staining is closer to the original), we want to use the latter to put the scale of WSIs into perspective.

WSIs typically scale up to 80,000 x 60,000 pixels (larger sizes are possible). When transforming such images into Lego, we need 4.8 billion 1 x 1 Lego plates and 4,687,500 32 x 32 Lego base plates. This would accumulate to a total cost of 330 million Euros for a single WSI using original Lego blocks. The final mosaic would result in a size of 625 m by 468.75 m, a total area of 292,968.75 m^2 (41.03 soccer fields or approx. 2.09 times Legoland Germany). Since Lego currently offers only 40 different colors for its plates – of which only about 10 to 20 are relevant for pathology (depending on the staining method) – the conversion to a Lego mosaic would result in a significant loss of information.

While these numbers may seem absurdly large, processing a digital WSI with all its information at once using a machine learning model would result in 14.4 billion input parameters.

While converting a WSI to Lego does not make sense from a practical view point, it illustrates the enormous amount of data in such images. These numbers further demonstrate the immense value of digital pathology, allowing computers to process such data quantities for diagnostic uses.