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Specifics of spinal neuropathology in the molecular age

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Abstract

Tumors located in the spinal cord and its coverings can be diagnostically challenging and require special consideration regarding treatment options. During the last decade, important advances regarding the molecular characterization of central and peripheral nervous system tumors were achieved, resulting in improved diagnostic precision, and understanding of the tumor spectrum of this compartment. In particular, array-based global DNA methylation profiling has emerged as a valuable tool to delineate biologically and clinically relevant tumor subgroups and has been incorporated in the current WHO classification for central nervous system tumors of 2021. In addition, several genetic drivers have been described, which may also help to define distinct tumor types and subtypes. Importantly, the current molecular understanding not only sharpens diagnostic precision but also provides the opportunity to investigate both targeted therapies as well as risk-adapted changes in treatment intensity. Here, we discuss the current knowledge and the clinical relevance of molecular neuropathology in spinal tumor entities.

Keywords

classification | DNA methylation | histology | molecular pathology | spine

Intradural tumors of spinal cord and cauda equine only represent approximately 3% of primary central nervous system (CNS) tumors, but they may result in significant morbidity. They can affect different anatomical localizations within the spinal cord with many tumor entities showing typical predilection sites (Figure 1). Most common primary intradural tumor entities comprise meningiomas (35%), peripheral nerve sheath tumors (PNST) (30%), and ependymomas (17%).[1] Tumor frequency is different in pediatric cases, where ependymal tumors (17.7%) are most common.[1] Diagnostically, especially the differentiation of intramedullary tumors can be histomorphologically difficult.[2] In fact, countless reports during the last decades have proven that molecular alterations define clinically relevant tumor types that cannot always be recapitulated on a histological level alone. In addition to genetic precision diagnostics comprising (panel) DNA and RNA sequencing and FISH analysis, emergence of global DNA methylation profiling has driven new insights into the molecular characteristics of CNS tumors during the last decade and is today well established as a routine diagnostic tool in neuropathology.[3,4] Epigenetic analysis allows for robust molecular stratification of clinically and biologically relevant tumor groups and thereby enables subsequent characterization of additional molecular characteristics within these subgroups, including transcriptional and mutational profiles.[4]

These improved molecular insights during the recent decade allow the diagnosis of highly clinically relevant tumor subgroups—some of which have only recently been defined by the WHO (Figure 2). For example, *MYCN*-amplified ependymoma, which is significantly more aggressive than other spinal ependymoma is now defined as a distinct ependymoma type.[5] Also, identification of H3K28-altered diffuse midline glioma (DMG) in the spinal cord is of immense importance with respect to their exceedingly poor prognosis and the need for aggressive therapies.[6] Further, PNST including schwannomas and neurofibromas frequently occur spinally, and their molecular features

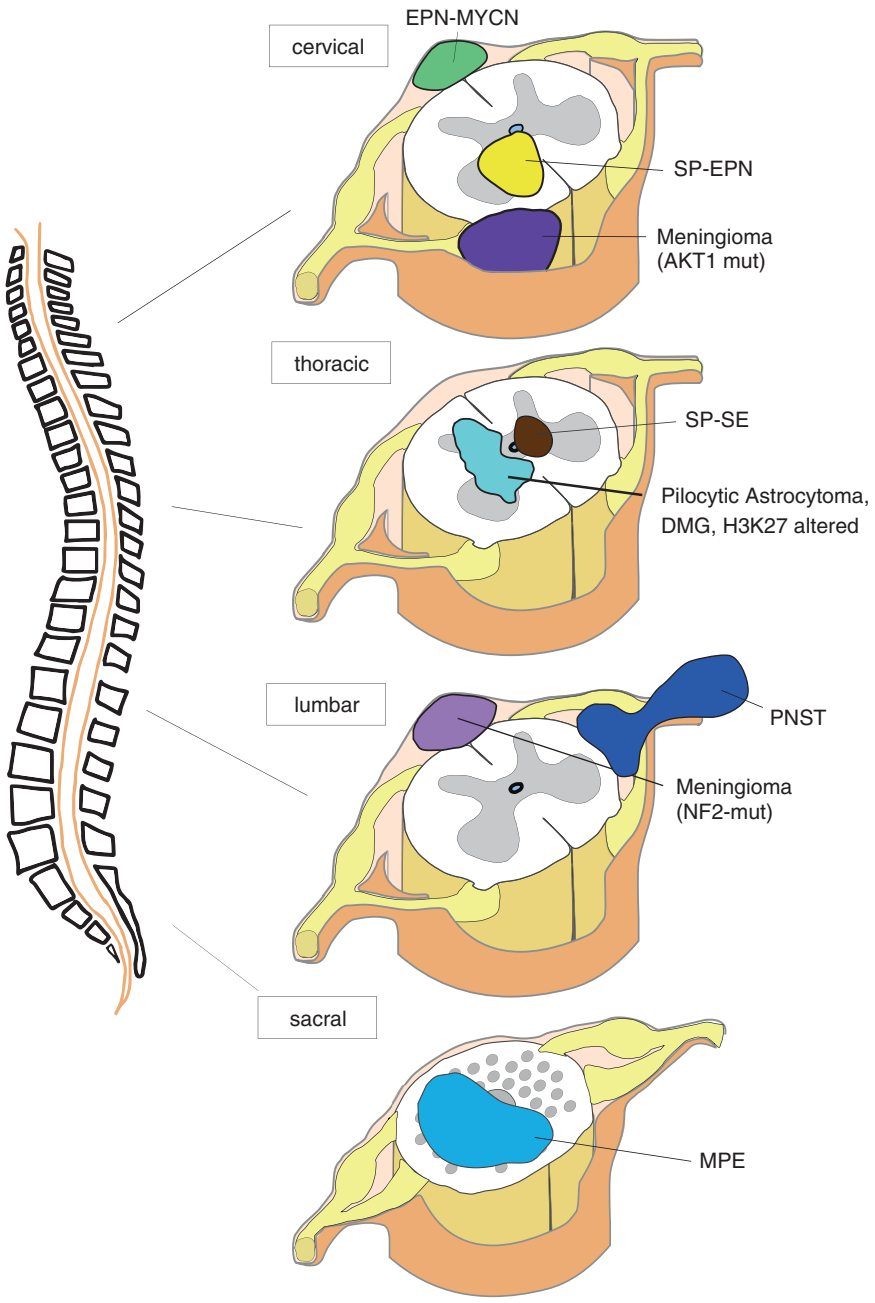


Figure 1. Most typical localization of common spinal tumors. Abbreviations: EPN-MYCN = spinal ependymoma, MYCN-amplified; Meningioma (AKT1 mut) = AKT1 mutated meningioma; DMG, H3K27-altered = diffuse midline glioma, H3K27-altered; Meningioma (NF2-mut) = :NF2-mutated meningioma; MPE = myxopapillary ependymoma; PNST = peripheral nerve sheath tumor; SP-EPN = spinal ependymoma; SP-SE = spinal subependymoma.

should be carefully considered, especially in the context of potentially underlying tumor predisposition syndromes. Here, we discuss the molecular features of the most common spinal tumors and match them with clinical characteristics.

Spinal Meningiomas

Only 1.2%–12% of meningiomas occur at the spinal cord, where they account for up to 25%–45% of the tumors within this compartment. Meningiomas have a broad morphologic spectrum, with meningotheliomatous, psammomatous, and transitional subtypes being most common in the spinal cord.[7] Meningiomas are classified as WHO grade 1, but may be graded 2 or 3 if there is brain invasion, specific criteria of anaplasia, *TERT* promoter mutation, or homozygous *CDKN2A/B* deletion.[2] Intracranial meningiomas have been extensively studied for their genetic (*SMO, AKT1, KLF4, TRAF* mutation) and epigenetic (benign-1, benign-2, intermediate A/B, malignant methylation classes) landscape, with the respective biomarkers

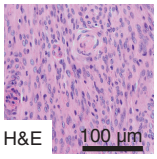
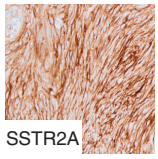
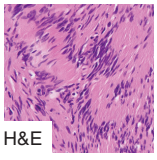
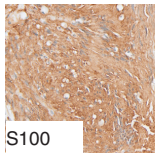
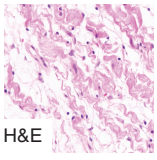
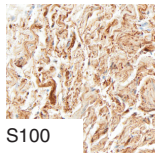
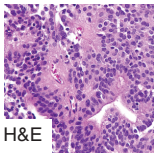
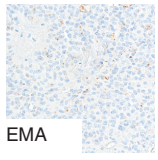
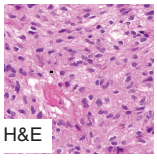
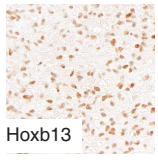
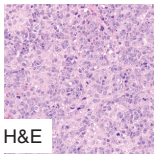
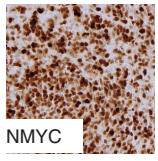
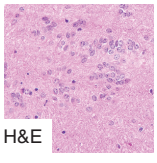
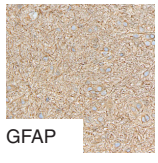
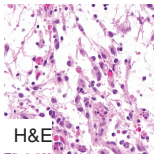
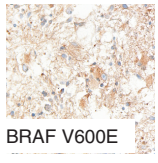
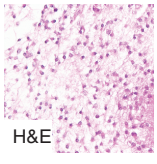
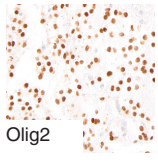
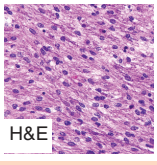
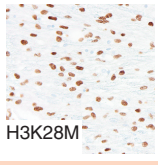
	Typical histological features			Typical molecular features
Meningioma			- Wide morphological spectrum, frequently meningotheliomatous, psammomatous, and transitional subtypes - CNS invasion and features of anaplasia lead to WHO grade 2-3	- *NF2* mutations - *AKT1* mutations - Malignant types: *TERT* promoter mutations or *CDKN2A/B* deletions - May occur due to a tumor predisposition syndrome
Schwannoma			- Spindle cell tumor - S100 positive - typically biphasic growth pattern nuclear pallisading (Verocay bodies)	- *NF2, SMARCB1*, or *LZTR1* mutations - Loss of chromosome 22q - May occur due to a tumor predisposition syndrome
Neurofibroma			- Spindle cell tumor - S100 positive - variably loose myxoid matrix - outlining perineurium	- Biallelic inactivation of *NF1* - May occur due to a tumor predisposition syndrome
Spinal Ependymoma			- Perivascular pseudorosettes - Absence of *NMYC* amplifications and of morphological criteria for MPE and SE	- Loss of chromosome 22q - Loss of *NF2* - May occur due to a tumor predisposition syndrome
Myxopapillary Ependymoma			- Papillary structures - Perivascular myxoid change or at least focal myxoid microcysts - Hoxb13 expression (IHC)	
MYCN-amplified Ependymoma			- Morphological und immunohistochemical features of ependymoma - Typically high-grade features - MYCN protein expression	- *MYCN* amplification
Subependymoma			- Clustering of tumor cell nuclei in a fibrillary matrix - Lack of anaplastic features	- Loss of chromosome 19 in a subset of tumors
Pilocytic Astrocytoma			- Biphasic growth pattern - Piloid cytology - Can show Rosenthal fibres	- MAPK pathway alterations
Diffuse lepto-meningeal glioneuronal tumor			- Oligodendroglia-like morphology - Low to moderate cellularity - Frequent desmoplastic and myxoid changes - Expression of OLIG2	- Loss of chromosome 1p, frequent co-deletion of 19q -Frequent MAPK/ERK alterations
Diffuse midline glioma, H3K27-altered			- Diffusely infiltrating glioma - Loss of H3K28me3 (IHC) - Mitotic figures, microvascular proliferations and necrosis are typical	- H3 p.K28M-mutations - Frequent mutations in *TP53, PPM1D* and *NF1*

Figure 2. Typical histological and molecular features of common spinal tumors.

having clear prognostic value. However, only 2 recent studies have investigated such molecular features in spinal meningiomas. In both studies, *AKT1-E17K* and *NF2* mutations emerged as the predominant molecular drivers of spinal meningiomas.[7,8] *AKT1*-mutated tumors originate in the cervical ventral spinal cord, whereas *NF2*-mutated tumors arise dorsally in the thoracic and lumbar spine (Figure 1). This was also recapitulated in 2 distinct methylation classes that are not yet integrated as such by the DKFZ methylation classifier. *NF2*-mutated tumors frequently exhibited loss of Chr.22q and were associated with female sex. Imaging revealed calcifications in thoracic-lumbar *NF2*-mutated tumors, whereas cervical *AKT1*-mutated tumors showed no evidence of calcification. Several reports have discussed calcification as a risk factor for postoperative neurological deficits and incomplete tumor removal.

Spinal Peripheral Nerve Sheath Tumors

The PNST neurofibroma and schwannoma both arise from Schwann cells, although they underlie different molecular mechanisms of tumorigenesis. Spinal PNST are predominantly located intradurally with a slight predilection of the lumbar spine. 10%–15% extend through the dural root sleeve as so-called dumbbell tumors (Figure 1).

Schwannomas

Schwannomas are histologically defined as S100-positive spindle cell tumors that typically display biphasic growth (Antoni A and B areas), nuclear palisading (Verocay bodies), and usually are encapsulated.[2] More than 90% of schwannomas arise solitary and sporadic.[2] Sporadic spinal schwannomas account for approximately 25% of spinal intradural tumors and are predominantly located in the cervical and lumbar spine. The genetic etiology of most sporadic schwannomas remains unclear. However, multiple schwannomas arise in the genetic context of the tumor predisposition syndrome schwannomatosis. Depending on the underlying molecular cause, 4 types of schwannomatosis have been defined: "NF2-related schwannomatosis," "SMARCB1-related schwannomatosis," "LZTR1-related schwannomatosis," and "22q-related schwannomatosis." Patients with clinical features of schwannomatosis but with missing or inconclusive molecular analysis are termed "schwannomatosis, not otherwise specified (NOS)" or "schwannomatosis, not elsewhere classified (NEC)," respectively.[9] Patients with *NF2*-related schwannomatosis have a heterozygous germline mutation of *NF2,* and schwannomas arise after the somatic inactivation of the second *NF2* allele in Schwann cells. *NF2*-related schwannomatosis typically comprises bilateral vestibular schwannomas as well as schwannomas in other sensory nerves and spinal dorsal roots. Patients with *SMARCB1/LZTR1/22q*-related schwannomatosis typically develop multiple schwannomas of peripheral nerves (90%) and spinal nerves (75%), with a predominance in the lumbar spine as well as in nonvestibular intracranial nerves (9%).[10] For *SMARCB1*- and *LZTR1*-related schwannomatosis, a 3-step/4-hit hypothesis has been proposed for tumorigenesis: a germline mutation of *SMARCB1* (or *LZTR1*) (hit 1) leads to loss of the other chromosome 22—thus loss of the wild-type copy of *SMARCB1* (or *LZTR1*) and one copy of *NF2* (hits 2 and 3)—and is followed by a somatic mutation of *NF2* (hit 4).[11]

Rarely, other chromosome 22 genes seem to play a role in schwannomatosis—for example, biallelic germline alterations of *DGCR8* in combination with somatic loss of chromosome 22 were recently described in a 3-generation family and an unrelated sporadic case with multiple schwannomas and multinodular goiter.[12] Patients with multiple schwannomas without germline pathogenic variants of *NF2*, *SMARCB1*, *LZTR1*, or *DGCR8* but with chromosome 22q loss in at least 2 schwannomas are subsumed under the term 22q-related schwannomatosis.[9] Recently, a new subtype of familial schwannomatosis was suggested, involving mutations of *SMARCA4*, a gene that lies on chromosome 19p and encodes for a key member of the SWI/SNF chromatin remodeling complex, just like *SMARCB1*.[13] Altogether, pathogenic *SMARCB1* or *LZTR1* variants can only be identified in approximately 70% of patients with familial and 30% of sporadic non-NF2-related schwannomatosis.[9] In addition, mosaicism can further impede diagnostics. In summary, according to current knowledge, diagnosis of spinal schwannoma in the context of multiple lesions or familiar history of schwannomas requires comprehensive molecular genetic testing regarding mutational status of *SMARCB1*, *SMARCA4*, *LZTR1*, and *NF2* in blood and/or tumor tissue. Further tumor-driving mutations are likely to be identified in the future and might help to define the individual patient's risk for extent and characteristics of expected tumor burden and prognosis and provide a basis for targeted therapies. For example, Brigatinib, an inhibitor of multiple tyrosine kinases including ALK has been suggested as a potential targeted therapy for schwannoma and meningioma with *NF2* inactivation and is currently investigated in the INTUITT-NF2 basket trial.[14]

Neurofibromas

A hallmark of the tumor predisposition syndrome NF1 is the presence of multiple cutaneous, diffuse, and plexiform neurofibromas (PN) composed of S100-positive spindle cells with thin, wavy nuclei in a variably loose myxoid stroma.[2] In contrast to *NF2*-associated schwannomatosis, where spinal tumors frequently lead to neurological deficits and represent a diagnostic hallmark, only 2% of NF1 patients present with neurological symptoms associated to a spinal tumor.[15] However, the prevalence of spinal neurofibromas in NF1 patients lies at around 40% as estimated based on imaging studies.[15,16] Spinal neurofibroma arise from the proximal part of spinal nerve roots and range from discrete masses to parts of large PN.[15,16] It has been suggested that a subgroup of NF1 patients with multiple spinal neurofibromas and few or none other additional NF1 symptoms might harbor specific *NF1* mutations in exons 46 and 47 associated with an overall milder course of the NF1 syndrome.[17,18] In contrast, *NF1* microdeletions are associated with an overall higher tumor burden, including cutaneous, spinal, and plexiform neurofibromas, as well as an elevated risk of malignant

progression to malignant peripheral nerve sheath tumors (MPNST).[19,20] Spinal MPNST in both NF1 and non-NF1 patients are rare and mostly described as case reports or in very small cohorts.[21,22] Identification of MPNST comprises clinical assessment (fast tumor growth, tumor-related pain), radiological signs of malignancy on MRI imaging, elevated SUV_{max} values on 18FDG PET scans and histological assessment.[23–25] En-bloc resection is the treatment of choice for MPNST but can be very difficult in spinal cases and was only possible for 1/18 cases in a published series.[21] Application of adjuvant irradiation is also very limited due to the surrounding vulnerable structures, such as spinal cord and nerves, and despite certain responsiveness to chemotherapy, complete surgical resection remains the only curative treatment for MPNST.[26] The more important is early identification of premalignant lesions—called atypical neurofibromas or atypical neurofibromatous neoplasms of unknown biological potential (ANNUBPs).[27] Atypical neurofibromas have been reported to progress to MPNST, although they usually do not tend to recur when resected. Histopathological diagnosis of atypical neurofibromas is very challenging, but recent reports suggest that atypical neurofibromas show a distinct methylation profile that can aid in the identification and better characterization of this tumor entity.[28] Several clinical trials have been conducted investigating molecular targeted therapy in patients with NF1 by targeting the RAF–MEK–ERK pathway.[29–32] The MEK inhibitor selumetinib was the first medical therapy approved by the US FDA for the treatment of pediatric NF1 patients with symptomatic inoperable PN in April 2020. Since, selumetinib has been shown to induce size reduction of spinal neurofibromas in an evaluation of 24 pediatric and adult NF1 patients.[33]

Ependymomas of the Spinal Cord

The classification of ependymal tumors has undergone major changes during the last decade. Today, the WHO classification of CNS tumors distinguishes 8 different ependymoma tumor types based on localization and molecular characteristics. Thereof, 4 entities commonly occur in the spinal cord: spinal ependymoma (SP-EPN), *MYCN*-amplified ependymoma (SP-EPN-MYCN), myxopapillary ependymoma (MPE), and subependymoma (SP-SE).

Spinal Ependymoma

Tumors of the molecular class SP-EPN arise primarily in the spinal cord, emerging at a medium age of 41 years. Children are affected as well as adults with an age range from 11 to 95 years.[34] SP-EPN are mostly benign tumors of WHO grade 2 and display morphological and immunohistochemical features of ependymoma (isomorphic glial cells, pseudorosettes, GFAP positivity, OLIG2 negativity) and absence of features of myxopapillary ependymoma or subependymoma.[2] However, with the presence of high mitotic activity and possible invasion of surrounding tissue, these tumors can also be graded as WHO grade 3.[2] The SP-EPN group was only recently defined as a distinct entity in the 5th edition of the WHO classification and was included in the groups of classic and anaplastic ependymoma before.[2,35,36] The current definition of this class is based on global methylation profiling, where SP-EPN form a distinct group compared to other ependymoma.[34] In the last decade, several studies have evaluated molecular properties of SP-EPN. The heterozygous loss of the chromosomal arm 22q is the most prevalent genetic alteration described for approximately 90% of spinal ependymoma.[34,35,37] Secondly, loss of tumor suppressor gene *NF2* was frequently reported in spinal ependymomas in older studies prior to the implementation of the molecular subgroup of SP-EPN.[34,35] While 22q loss occasionally also occurs in intracranial ependymomas, *NF2* mutations seem to be strongly associated with the spinal localization of ependymomas.[38,39] However, the newly defined molecular subgroup of SP-EPN has not yet been well characterized regarding the types and frequency of *NF2* mutations or other possible tumor-driving mutations.

While spinal ependymoma frequently arise sporadically, patients with the tumor predisposition syndrome NF2-associated schwannomatosis are at elevated risk of developing one or multiple spinal cord ependymomas throughout their lives.[40,41] This group especially comprises patients, who develop ependymoma relatively early in life.[40,41] Possible molecular and prognostic differences between tumors arising sporadically and tumors arising in the context of NF2-associated schwannomatosis remain to be elucidated.

Myxopapillary Ependymoma

Myxopapillary ependymoma are predominantly localized in the lumbosacral spinal cord. Diagnostic criteria currently comprise morphological features of a GFAP-positive glioma with papillary structures and perivascular myxoid change or at least focal myxoid changes. For unresolved lesions an additional DNA methylation profile aligned with MPE is advised.[2] Males are more frequently affected than females and tumors occur predominantly in adults, with 2 peaks of incidences at 25–29 and 45–49 years.[42,43] Despite a satisfying long-term survival rate of over 90%, the WHO grading of MPE was increased from 1 to 2 in the recent edition of the CNS tumor classification from 2021. This pays respect to a high rate of persistent disease due to locally advanced growth and/or cerebrospinal dissemination as well as frequent recurrences in around 20% of patients.[42,44,45]

Myxopapillary ependymoma show distinct transcriptomic and epigenetic features from all other spinal and extraspinal ependymoma subgroups.[3,34] However, the molecular data generated so far did not reveal any specific alterations on genetic or epigenetic level that drive the tumor formation of MPE. Copy number variation analysis revealed a variety of chromosomal gains and losses across the whole genome, whereas the most frequent alterations varied between different publications.[34,37,46]

Global methylation profiling recently revealed 2 distinct subtypes of MPE that differ in terms of clinical and molecular features.[46] The first subtype ("MPE-A") affects

predominantly young patients with a median age of 27 years and demonstrates relapses in 85% of patients within 10 years after resection. MPE-A cases are localized more caudally within the spinal cord and present a significantly worse progression-free survival than the other group ("MPE-B"). MPE-B cases exhibit a median age of 45 years at diagnosis, are most often localized in the conus medullaris, and gross total resection (GTR) can be achieved in almost all cases. This is consistent with the finding that a GTR predicts a more favorable disease outcome.[42,45] Zhang et al. observed similar clinical differences between MPE in the sacrococcygeal and other spinal regions.[43] However, methylation profiling was not conducted to verify the subtypes in this study, which would have been preferable to evaluate possible clinical implications for such subtypes.

According to the current WHO 2021, histological features remain the primary criteria for a diagnosis of MPE. For histomorphologically unresolved lesions, DNA methylation profiling is recommended.[2]

Immunoreactivity for the homeobox protein HOXB13 was proposed as a reliable marker for MPE, although this is not yet mentioned as a required criterion for diagnosis in the WHO classification.[16–18] The corresponding *HOXB13* gene is highly expressed in human MPE in comparison to other ependymoma.[37,47,48] Mouse experiments identified it as an important regulator of anterior–posterior axis patterning in the most caudal part of the spinal cord during embryogenesis.[49,50] First single-cell RNA sequencing experiments of 2 MPE detected the expression of ependymal-like and progenitor-like gene sets, leading the authors to the conclusion that MPE formation might recapitulate the caudal spinal cord development.[48] Apart from these first implications about MPE tumorigenesis, a large part of the pathology, including the exact cellular origin, remains unknown.

MYCN-Amplified Ependymoma

The novel molecular subtype of *MYCN*-amplified spinal ependymoma was first described in 2019.[51] It is a rare tumor entity that affects young adults and adult patients and is described to be located rather extramedullary on cervical or thoracic levels of the spinal cord.[51,52] Histologically, *MYCN*-amplified spinal ependymoma typically display pseudorosettes and papillary or pseudopapillary architecture. High-grade morphological features as elevated mitotic activity, microvascular proliferation, necrosis, and high nucleus-to-cytoplasm ratios occur frequently.[2] However, the cellular origin of MYCN-amplified spinal ependymoma remains unclear, especially as the extramedullary localization contradicts the ependymal morphology and expression of GFAP. From the 27 cases of SP-EPN-MYCN reported so far, it appears that this tumor entity is biologically very aggressive.[5,52] SP-EPN-MYCN typically show early metastases and leptomeningeal dissemination, rapid progression after relapse, and poor response to multimodal therapy.[51,52] In a study including 12 patients, relapse rate was 100% with a progression-free survival of 17 months and median overall survival of 87 months, revealing a dismal outcome compared to SP-SE, SP-EPN, and SP-MPE.[51]

SP-EPN-MYCN are molecularly defined by focal high-level *MYCN* amplifications and form a distinct global methylation cluster.[2,51,52] In addition, at least one spinal ependymoma was reported that was classified as SP-EPN-MYCN by methylation profiling but harbored a *MYC* instead of *MYCN* amplification.[53] Tumors typically show several additional chromosomal aberrations of varying frequencies, including loss of chromosome 10 (32% of cases) and focal losses on chromosome 11q (in 26% of cases).[51] Histologically, SP-EPN-MYCN typically show brisk mitotic activity, microvascular proliferation, and areas of necrosis, as well as strong immunohistochemical *MYCN* expression. Nuclear immunoreactivity for H3 p.K28me3 was retained in all tumors ($n = 12$) from one study.[51]

While available clinical data strongly suggest that SP-EPN-MYCN are an aggressive tumor entity with a poor prognosis, the recently updated 2021 WHO CNS tumor classification has not assigned this entity to a WHO grade, as no prospective clinical trials have been reported.

In conclusion, it is of great clinical importance to detect *MYCN* amplifications in spinal ependymoma by immunohistochemistry or FISH and to confirm unresolved cases by methylation profiling.

Subependymoma of the Spinal Cord

Subependymomas (SE) are often asymptomatic tumors with an excellent prognosis and rare recurrences after surgical resection. Most frequent sites of localization are the ventricles, and only a small subset of SE arise in the spinal cord, where they usually form eccentric masses in cervicothoracic segments.[54] Histologically, SE comprise circumscribed gliomas with clustering of tumor cell nuclei within an expansive, focally microcystic matrix.[2] Supratentorial, infratentorial, and spinal SE each have specific global DNA methylation profiles. However, some tumors with the morphological diagnosis of classic ependymoma may also cluster with SE.[35] The clinical significance of these discordant diagnoses remains to be clarified. A typical copy number aberration of SP-SE is loss of chromosome 19 in approximately 40% of cases.[35]

Other Glial and Glioneuronal Tumors of the Spinal Cord

In line with their cells of origin, nonependymal glial tumors of the spinal cord are predominantly located intramedullary. They are significantly more frequent in pediatric patients, where they comprise approximately 30% of all intramedullary tumors.[55] Molecular characteristics and biological aggressiveness show a wide variety, and molecular diagnostics is of great importance to stratify them.

Pilocytic Astrocytoma

Pilocytic astrocytomas (PA) are the most common primary CNS neoplasm in children and are typically associated with an excellent prognosis.[55] However, tumors that are

located in regions that are not or only partly accessible for resection can cause progression. In order to avoid severely impaired quality of life, they might therefore require irradiation or chemotherapy.[56] Histologically, PA typically show a biphasic growth pattern, piloid cytology, a low proliferation rate, and sometimes Rosenthal fibers.[2] Most common localization especially in children is the cerebellum, but further typical localizations comprise the optic nerve, and spinal cord. Localization in the cerebral hemispheres is also typical in adult patients.[57] Pediatric PA typically harbor MAPK-pathway mutations, especially *KIAA1549-BRAF* fusions, but also (rarer) mutations in *FGFR1, PTPN11,* and *KRAS* as well as *NTRK2* fusions, alternative *BRAF* fusions, and *NF1* germline mutations.[58] Pilocytic astrocytomas may be associated with neurofibromatosis type 1 (NF1). Loss of NF1 activity leads to RAS activation, thereby contributing to tumor formation. In adults, PA occur less frequently and present a different molecular and clinical profile.[59] Survival rates significantly decrease with age and overall survival was only 52.9% in patients over the age of 60 years compared to 96.5% in patients between 5 and 19 years.[60] An inverse relation between the age of patient and the prevalence of *KIAA1549-BRAF* fusions has been suggested.[59] However, the molecular landscape of adult patients is not well characterized to date. Due to their rarity, only small cohorts and case reports of spinal PA have been investigated regarding their specific molecular profiles.[61] Recently, Métais et al. investigated 28 cases of pediatric spinal low-grade glioma (LGG) and diffuse leptomeningeal glioneuronal tumors (DLGNT) with the rational of addressing potential incorrect diagnosis of these histological differential diagnoses.[62] Of this cohort, 7 tumors that were initially diagnosed as PA had to be reclassified to DLGNT on histological and molecular basis. They discovered that spinal PA ($n = 15$) formed a unique methylation cluster distinct from other midline and posterior fossa PAs. *FGFR1* mutations occurred in 36% of spinal PA, which is higher than previous reports of PAs of different locations suggest. *FGFR1 TKD* alterations were exclusive to spinal PA, and, in contrast to DLGNT, spinal PA did not harbor chromosome 1p loss. Taken together, these results indicate that molecular data are of great importance in the differential diagnostics of spinal glioma.

Diffuse Leptomeningeal Glioneuronal Tumors

Diffuse leptomeningeal glioneuronal tumors are rare glioneuronal CNS neoplasms that mainly affect children but show a range between 5 months and 46 years.[63] To date, no genetic predisposition for DLGNT has been identified. DLGNT typically involve the spinal and intracranial leptomeninges; rare exceptions are located in the parenchyma.[64] The cell of origin remains unknown, but due to the molecular overlap with oligodendroglioma and pilocytic astrocytoma, a precursor cell upstream of this lineage segregation has been proposed.[65] Histologically, tumors appear as low-to-moderate-cell-dense neoplasms with oligodendrocyte-like morphology and only rarely histological features of anaplasia.[2] Main differential diagnoses are astrocytic or oligodendroglial gliomas with leptomeningeal dissemination. However, DLGNT have a distinct methylation profile, which allowed for further molecular characterization of this tumor entity: Deng et al. identified 30 DLGNT based on methylation profiling and further identified 2 stable subgroups that they called DLGNT methylation class (MC)-1 and DLGNT-MC-2. They found that all cases had a loss of chromosome 1p.[65] In addition, especially DLGNT-MC-1 tumors showed frequent codeletion of 19q—a typical feature of oligodendroglioma. Further, frequent MAPK/ERK alterations were identified in both subgroups, with *KIAA1549:BRAF* fusions in almost 70% of cases. DLGNT-MC-1 tumors additionally showed fusions resulting in aberrant MAPK/ERK pathway including *NTRK1/2/3*. Clinically, DLGNT-MC-1 comprised younger patients (median 5 years vs 14 years) and had a significantly better 5-year overall survival (100% vs 43%). In other studies, further less common MAPK alterations including *FGFR1* mutations, and *RAF* rearrangements were reported.[64–67] Together, these findings suggest that molecular diagnostics is of great relevance in differentiating glial spinal tumors and can help to stratify clinical prognosis and identify therapeutic targets like the MAP/ERK pathway in DLGNT. The WHO classification of 2021 recommends molecular workup including at least chromosome 1p status and *KIAA1549:BRAF* fusion testing for all cases, where some diagnostic uncertainty remains after radiologic and histologic evaluation.

Diffuse Midline Glioma, H3K27-Altered

Diffuse midline glioma, H3K27 altered, are highly aggressive, infiltrative glioma that occur in the midline of the CNS. The term DMG was introduced by the WHO classification of CNS tumors in 2016, subsuming diffuse intrinsic pontine gliomas and gliomas from thalamus, spinal cord, and other rarer CNS midline localizations. DMGs are predominantly located in the brainstem and pons as well as bithalamic in pediatric patients and unilaterally thalamic or spinally in adult patients. Irrespective of localization, DMGs are associated with a dismal prognosis.[68] Spinal DMGs represent approximately 40% of spinal astrocytomas,[6,69,70] present at a median age of 35 years, and show a slightly better prognosis than H3K27M-altered brain tumors.[6,71] Histologically, DMGs present as diffusely infiltrating neoplastic cells that are mostly small and monomorphic but can also be polymorph with astrocytic, piloid, oligodendroglial, giant cell, epitheloid, or undifferentiated cytology. Mitotic figures, microvascular proliferations, and necrosis may occur but are not required for diagnosis.[2] Molecularly, DMGs are characterized by genetic alterations leading to inhibitory effects on PRC2, ultimately resulting in widespread loss of H3 p.K28me3 (formerly K27) trimethylation. Four main molecular subgroups of DMG have been suggested: H3.3 p.K28M-mutant, H3.1 or H3.2 p.K28M-mutant, H3-wild-type with EZHIP overexpression, and EGFR mutant.[72,73] However, EZHIP overexpression has so far not been reported for primary spinal DMG, and *EGFR* amplification has only been reported in one spinal case.[71,72,74] A recent publication specifically characterized 77 cases of H3K28M-mutant spinal cord glioma.[71] Of 34 sequenced spinal DMG, 58.8% revealed mutations in *TP53*, that were mostly mutually exclusive with *PPM1D* mutations (26%)

and *NF1* mutations (44%).[71] Also, further alterations in genes of the *RTK/RAS/PI3K/MAPK* pathway and *RB1* pathway were frequently detected and *MYC* and *MYCN* amplifications were reported in 9/34 cases. The group further reported that several specific molecular alterations were significantly associated with worse survival and the same was true for a Ki67 index ≥10% and histological features of glioblastoma. Neuropathological diagnostics of DMG should comprise a minimum of molecular workup including the following investigations: as trimethylation is lost in all DMG and H3 p.K28M mutations are frequent, a combination of H3p.K28M and H3K p.K28me3 antibodies is extremely effective at detecting the vast majority of DMGs and even detecting single cells in the infiltration zone of the tumor.[2,75] Immunohistochemical loss of H3K p.K28me3 is required for WHO conform diagnosis of DMG as well as detection of one of the molecular criteria of H3 pK28M/p.K28I mutation, *EGFR* amplification, EZHIP overexpression, or a matching DNA methylation profile.[2]

Conclusions

Discrimination of primary spinal tumors can be challenging on a histological level. However, as prognosis and treatment of the different entities differ significantly, an exact diagnosis is of utmost importance. The molecular advances during the last decade made significant contributions to a better understanding of the underlying biology but also to improved patient care. For example, DMG are highly aggressive glial tumors with dismal prognosis and require irradiation and chemotherapy, whereas pilocytic astrocytoma have a favorable overall survival and rarely reoccur after complete resection. Also, discriminating the various ependymal neoplasms occurring in the spinal cord is extremely challenging if relying on histomorphological features alone. However, this can be straightforward upon application of DNA methylation profiling, FISH (for *MYCN*), and specific antibodies (eg, HOXB13 for MPE) and thereby allow the identification of patients with a considerably worse prognosis and a higher need of adjuvant and targeted therapy.

Current developments suggest that molecular profiling will play an increasing role and be further refined in the future. Besides methylation profiling, which might be further improved by steadily growing available reference data and by optimization of current classification algorithms, other diagnostic modalities are becoming more and more available. For example, large-scale sequencing of DNA and RNA will likely contribute to improved diagnostics and has the potential to detect targetable genetic changes. Further, large-scale proteome analysis is becoming increasingly relevant in tumor research and will likely improve brain tumor diagnostics. Importantly, besides diagnostics based on resected tumor tissue, liquid biopsy-based molecular analyses are rapidly developing and already today allow diagnosis of brain tumors from peripheral blood and cerebrospinal fluid (CSF).[76,77] CSF-based diagnostics is especially promising in CNS tumors with a localization close to the CSF and might enhance upfront diagnostics of spinal tumors.

While molecular diagnostical approaches are gaining more and more relevance, they still need to be seen in the context of histopathological features. Artificial intelligence can help to extract quantitative information from histological images and thereby improve the objectivity and consistency of diagnostics.[78] To date, AI-based histopathology is mostly performed in a research setting, due to the need of extensive upfront annotation of scanned samples in most approaches, and a lacking infrastructure and expertise to make annotated data sets available and usable. However, with bioinformatical techniques improving, AI-based histopathology is likely to develop to a point where it might significantly enhance neuropathological diagnostics.

Apart from the need to identify better therapies for aggressive tumors, future efforts will also have to unravel cellular origins and growth patterns as well as molecular driving events, which are unclear in many instances.

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