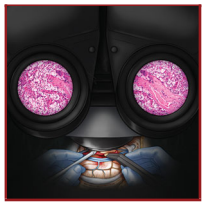

Ependymoma and Chordoma

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Ependymoma and chordoma are 2 tumors that occur throughout the craniospinal axis, and for which the extent of neurosurgical resection has a key prognostic role. Both tumors have distinctive pathologic features, yet can present significant diagnostic challenges to pathologists in cases without classical histology. The molecular understanding of ependymoma has had significant advances in the past decade, with the identification of 9 molecular groups with significant prognostic and clinical implications, while a comprehensive study of chordoma further emphasized the key role of brachyury overexpression in its pathogenesis. In this review, we discuss the pathogenesis, radiology and gross pathology, histology, and molecular features of these 2 tumors, as well as active research into targeted therapies, with an emphasis on practical diagnostic challenges, and the use of immunohistochemical and molecular tests in routine diagnostic practice.

KEY WORDS: Neuropathology, Molecular pathology, Brain tumors, Chordoma, Ependymoma

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Chordoma and ependymoma are tumors that are distributed throughout the cranio-spinal axis, with the former arising from the skull base and vertebral bodies, while the latter is located intra-axially in the brain and spinal cord. Both of these pathologies are relatively rare, and accordingly, optimal management paradigms remain to be elucidated. Currently, the first-line treatment and most consistent prognostic factor is gross total resection, when safely achievable. However, there is growing evidence about the efficacy of adjuvant treatments for both pathologies. The present manuscript reviews the pathological, radiological, and molecular features of these 2 tumors, as well as the status of targeted systemic therapies.

EPENDYMOMA

Ependymal tumors comprise 3 histologically distinct tumors – ependymoma, subependymoma, and myxopapillary ependymoma. They occur in all age groups and have widely varying outcomes, with some demonstrating aggressive behavior and high mortality, while others, particularly subependymoma, are indolent and slow growing.[1] Over the past decade, there has been significant progress in the molecular characterization of ependymomas, which has identified distinct molecular subgroups within tumors that are histologically indistinguishable.[2] This classification has demonstrated clinical utility in delineating high and low-risk groups of patients, and is recommended to be incorporated into future clinical trials.[3]

ABBREVIATIONS: BNCT, benign notochordal tumors; **CNS,** central nervous system; **CT,** computed tomography; **EMA,** epithelial membrane antigen; **GFAP,** glial fibrillary acidic protein; **MRI,** magnetic resonance imaging; **PNET,** primitive neuroectodermal tumor; **RTK,** receptor tyrosine kinase; **SWI,** susceptibility weighted imaging; **TKI,** tyrosine kinase inhibitor; **WHO,** World Health Organization

Development/Cell of Origin

Given their association with the ventricular system and similar immunohistochemical and ultrastructural features as normal ependymal cells, ependymomas are postulated to arise from cells of ependymal lineage, with both neuroepithelial cells and radial glial cells historically having been proposed as possible precursors. Recent evidence suggests that the latter view is correct, as ependymomas have a rare population of cells with an immunophenotype similar to those of radial glia that demonstrated stem cell properties of self-renewal and multipotency, and formed tumors when xenografted into mice.[4] Subsequently, compartment-specific mouse neural stem cells (supratentorial and spinal) with

transcriptomic similarity to human ependymomas were isolated, and when implanted into mice, developed tumors that were indistinguishable histologically from human ependymomas and had their classic ultrastructural features.[5]

Radiology and Gross Pathology

Intracranial ependymomas are more common in the infratentorial compartment (60%-70% of cases) than the supratentorial space (30%-40%). Classically, posterior fossa ependymomas arise within the fourth ventricle and can extend through the foramina (Lushka and Magendie) as finger-like extensions.[6] These lesions can encapsulate the cranial nerves or vasculature in the prepontine cistern and often lead to obstructive hydrocephalus. In contrast, supratentorial tumors are usually extraventricular and are thought to arise from the ependymal rests in the parenchyma. These are often quite large and commonly present with subfalcine herniation. Similar to other glial tumors, ependymomas are hypointense on T1 and hyperintense on T2. The soft tissue component of the tumor exhibits avid enhancement with intravenous gadolinium, although rare cases may show no enhancement. Diffusion weighted imaging often detects areas of increased cellularity and diffusion restriction, while susceptibility weighted imaging (SWI) highlights intratumoral calcifications or hemorrhage.[7] These tumors are iso- or hyperdense on computed tomography (CT) scan, with calcifications seen in 50% and hemorrhage in approximately 10%.[8]

Given their intra-axial location, ependymomas are typically received as fragmented microsurgical resection specimens, without distinguishing gross pathologic features. The one common exception is myxopapillary ependymoma, which may be received intact as a soft encapsulated mass. Cut sections demonstrate that the internal appearance is often lobulated, with cyst formation and gelatinous myxoid material.

Histology

Ependymoma

Where an interface with normal brain tissue is present, ependymomas are generally well-delineated and do not demonstrate the extensive infiltration of diffuse gliomas. Cytologically, the tumor is composed of small, fairly monomorphic cells with ovoid nuclei and speckled nuclear chromatin. The key histologic features include perivascular pseudo-rosettes, true ependymal rosettes, and ependymal canals (see Figure 1). In general, rosettes are a common histological architectural pattern that are present in a number of brain tumors, consisting of cells radiating outward from a central core or hub, in a halo or spoke wheel arrangement.[9] Perivascular pseudo-rosettes consist of cells with a tapered processes radiating around the wall of a central blood vessel and are highly sensitive for ependymoma, although nonspecific. In true ependymal rosettes, the cells radiate from an empty appearing lumen, rather than a blood vessel, forming a tubule-like structure. This pattern is highly specific for ependymoma, but is only present in a minority of cases, and mainly in the most well-differentiated tumors.

There are 3 histological variants of ependymoma: papillary, clear cell, and tanycytic. Papillary ependymoma is characterized by well-formed papillae – finger-like projections in which a central vessel is lined by 1 or multiple layers of cuboidal tumor cells. The tumor cells form a smooth surface and are glial fibrillary acidic protein (GFAP)-immunopositive, in contrast to the bumpy hobnail surfaces that are present in other papillary tumors, such as choroid plexus papilloma and metastatic carcinoma. Clear cell ependymomas are composed of cells with cytoplasmic clearing that forms perinuclear halos. Finally, tanycytic ependymoma, a subtype that is most commonly found in the spinal cord, is composed of fascicles (ie, bundles) of varying width and cell density, containing elongated spindle-shaped cells.

Immunohistochemically, ependymomas demonstrate GFAP positivity, particularly in the perivascular tumor cells. Other common, and relatively nonspecific markers, include S100 and vimentin. More useful, is a characteristic cytoplasmic "dot-like" pattern of epithelial membrane antigen (EMA) positivity, which is thought to identify intracytoplasmic microrosettes. Importantly, this finding can be focal in some tumors and therefore its absence does not rule out an ependymoma. Recently, immunohistochemical markers have been identified for higher risk molecular group (see Prognostic Factors), allowing for rapid subtyping within the routine workflow of most pathology labs.

The grading of ependymomas is a challenging and unresolved issue.[1] Ependymomas are conventionally graded as World Health Organization (WHO) II and anaplastic ependymomas as WHO III; however, there is no consistent correlation between histologic grade and outcome.[10-12] This is in part due to differing grading criteria between studies and the inherent subjectivity in some aspects of grading. The diagnosis of anaplastic ependymoma can be readily made when a tumor shows widespread anaplastic features, including high cell density, high mitotic count, microvascular proliferation, and pseudopalisading necrosis (Figure 2). However, these tumors are often heterogeneous, and the importance of a focal area of anaplasia in an otherwise grade II tumor is unclear. Given the prognostic significance of the molecular subgroups, it is not recommended that treatment stratification be based solely on grade.[3]

Subependymoma

Subependymoma is a slow growing glial neoplasm that is often identified incidentally either on neuroimaging or at autopsy. The tumor is composed of small uniform cells, arranged in clusters within a dense fibrillary matrix (Figure 3). Tumor cell processes may orient around vessels, forming pseudorosettes; however, this is not a prominent feature of this tumor. Microcyst formation is common. Occasional cases of mixed ependymoma-subependymoma have been reported, in which the subependymoma usually forms the most superficial

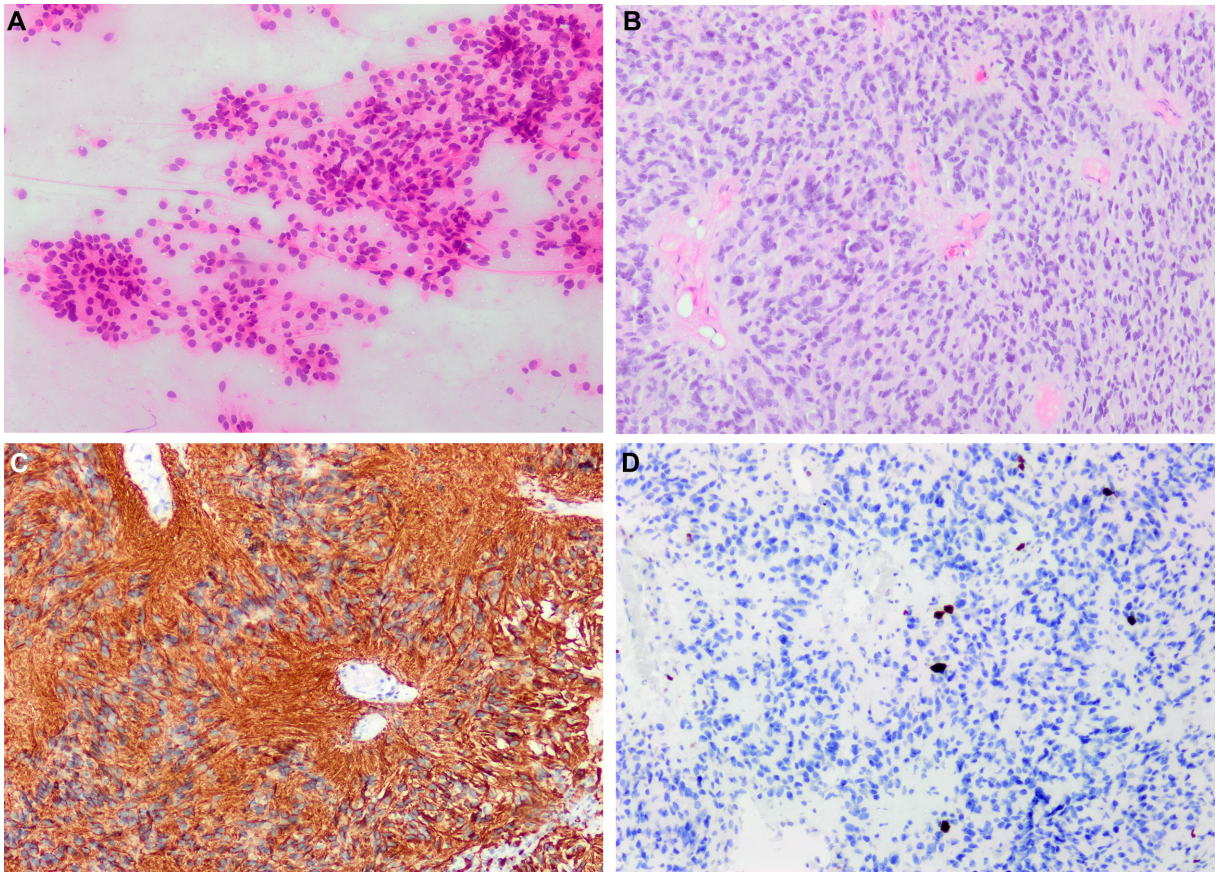

FIGURE 1. *Ependymoma histology showing* **A**, *monomorphic cells and glial processes on smear preparation (10x magnification),* **B**, *characteristic perivascular pseudorosette formation (10x),* **C**, *strong GFAP immunoreactivity with processes radiating out from central vessel (10x), and* **D**, *very low Ki67 proliferative index (10x)*

component.[13,14] Subependymomas are usually immunoreactive for GFAP, but unlike ependymoma, rarely express EMA.

Myxopapillary Ependymoma

Myxopapillary ependymoma is a well-circumscribed tumor that is almost exclusively found in the caudal spinal cord region. The tumor is composed of glial cells with elongated fibrillary processes radiating outward from hyalinized fibrovascular cores, forming papillae (Figure 4). There is frequent mucoid degeneration, in which Alcian-blue positive myxoid material accumulates between the tumor cells and blood vessels, as well as forming microcysts. However, some cases do not show prominent papillary architecture, and instead demonstrate sheets or fascicles of tumor cells. Given its location in the sacral spinal canal, the differential for myxopapillary ependymoma includes other tumors that are commonly present in this region, including paraganglioma, schwannoma, chordoma, and metastatic tumors. In classic appearing cases, the diagnosis is readily made histologically, while more challenging cases can be distinguished by strong GFAP immunopositivity. Notably, while this is typically a low grade and slowly growing neoplasm, dissemination throughout the neuraxis is not uncommon.[15,16]

Ultrastructure

Ultrastructural analysis using electron microscopy has been largely supplanted by immunohistochemistry and molecular genetics in the diagnosis of brain tumors; however, there are still occasional situations in which it is useful for determining the histogenesis of tumors.[17] Ependymal tumors characteristically retain the ultrastructural properties of normal ependymal cells, including cilia with a "9 + 2" microtubular pattern, microvilli, and tight junctions.[1]

Practical Diagnostic Considerations

In its most classical histologic appearance, characterized by both true rosettes and perivascular pseudo-rosettes, the diagnosis of ependymoma is straightforward. However, in small specimens in which the diagnostic features are not prominent, it can be

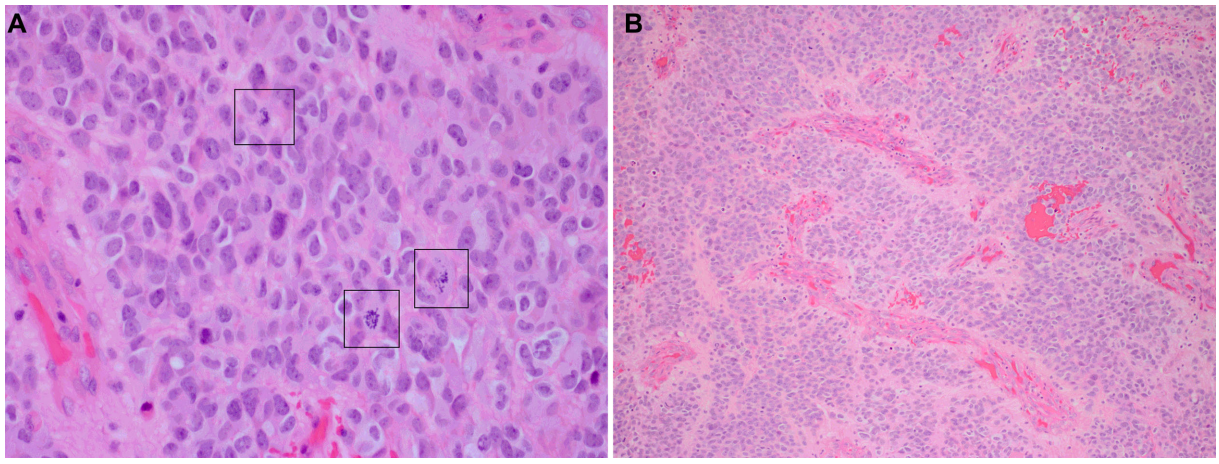

FIGURE 2. *Anaplastic ependymoma histology showing* **A**, *high cellularity, atypia, and multiple mitotic figures (40x magnification; mitotic figures in boxes), and* **B**, *a focus of microvascular proliferation (10x)*

challenging to differentiate ependymoma from other gliomas, particularly spinal cord astrocytomas. Similarly, anaplastic ependymomas, if poorly differentiated, can be difficult to distinguish from glioblastoma and other high-grade glial tumors. While perivascular pseudo-rosettes are highly characteristic of ependymomas, they can be seen in a number of other tumor types, including pituitary adenomas, medulloblastoma/primitive neuroectodermal tumor (PNET), central neurocytoma, and pilomyxoid astrocytoma.[9] Furthermore, the rosettes seen in ependymoma can be mistaken for different rosette types seen in other tumors, such as Homer Wright rosettes (seen in medulloblastoma and PNETs), Flexner-Wintersteiner rosettes (characteristic of retinoblastoma), and pineocytomatous and neurocytic rosettes.

The 3 histologic variants of ependymoma each have their own differential diagnosis. Papillary variant has a differential that includes other papillary tumors of the central nervous system (CNS), including choroid plexus papilloma, papillary tumor of the pineal region, and papillary meningioma. Clear cell ependymoma must be differentiated from other clear cell tumors of the CNS, including oligodendroglioma, central neurocytoma, hemangioblastoma, clear cell meningioma, and metastatic clear cell carcinomas (most often renal cell). This differential can be resolved immunohistochemically using a panel that includes IDH1 R132, neuronal markers (eg, synaptophysin), inhibin, and cytokeratins. Finally, tanycytic ependymoma can be confused with other gliomas with spindle cell morphology, particularly pilocytic astrocytoma, as well as other spindle cell tumors such as schwannoma and meningioma.

Prognostic Factors

As was previously discussed, the histological grading of ependymoma (as WHO grade II or III) has not demonstrated a reliable association with behavior. The recently described molecular classification into 9 subgroups (see Molecular Characterization section) has greater prognostic significance, with the supratentorial *RELA*-fusion and posterior fossa group A tumors reported to have worse outcomes than other tumors in the supratentorial and infratentorial compartments, respectively,[2,18,19] although these patients may not have uniformly poor survival when treated with postoperative radiation.[20] While most labs do not have access to methylation array profiling, as was used to define these subgroups, there are surrogate immunohistochemical markers for the high-risk groups. Specifically, supratentorial *RELA*-fusion ependymomas have high expression of cyclin-D1 and L1CAM,[19,21] while posterior fossa group A demonstrates loss of H3K27me3 expression.[22] Other factors that have independently been associated with poor outcome include high immunohistochemical expression of nestin,[23] and gains of chromosome 1q in posterior fossa tumors.[20,24,25]

Molecular Characterization

Ependymomas encompass a broad array of histologically similar but spatially and temporally distinct tumors, a fact that has previously confounded the search for molecular drivers. Analysis of genomic copy number also presented a paradoxical effect where the more aggressive tumors often harbored quiet, balanced genomes.[26] Using deoxyribonucleic acid methylation profiling, ependymoma has now been segregated into 9 distinct molecular subtypes in 3 compartments of the central nervous system – spinal (SP), posterior fossa (PF), and supratentorial (ST) – suggesting that these are a group of distinct spatially defined tumors with different cellular origins.[2]

In the spinal cord, the 3 subtypes are spinal ependymomas (SP-EPN), myxopapillary ependymoma (SP-MPE), and spinal subependymoma (SP-SE).[2] SP-EPN are most often associated

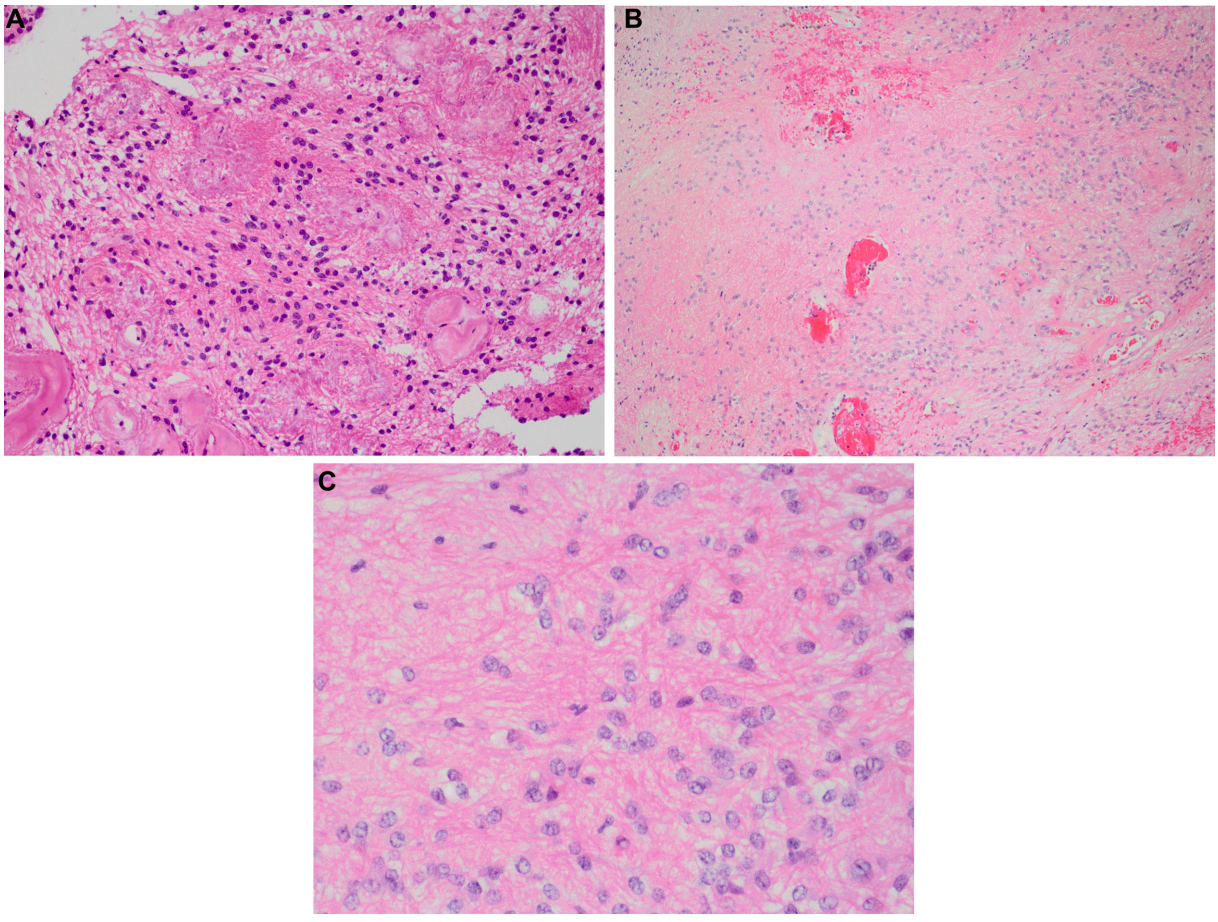

FIGURE 3. *Subependymoma histology on* **A**, *frozen section (20x magnification) and formalin-fixed tissue at* **B**, *medium (10x) and* **C**, *high (40x) magnification, showing characteristic features, including clusters of small uniform cells with dense fibrillary background and intervening paucicellular areas with microcysts.*

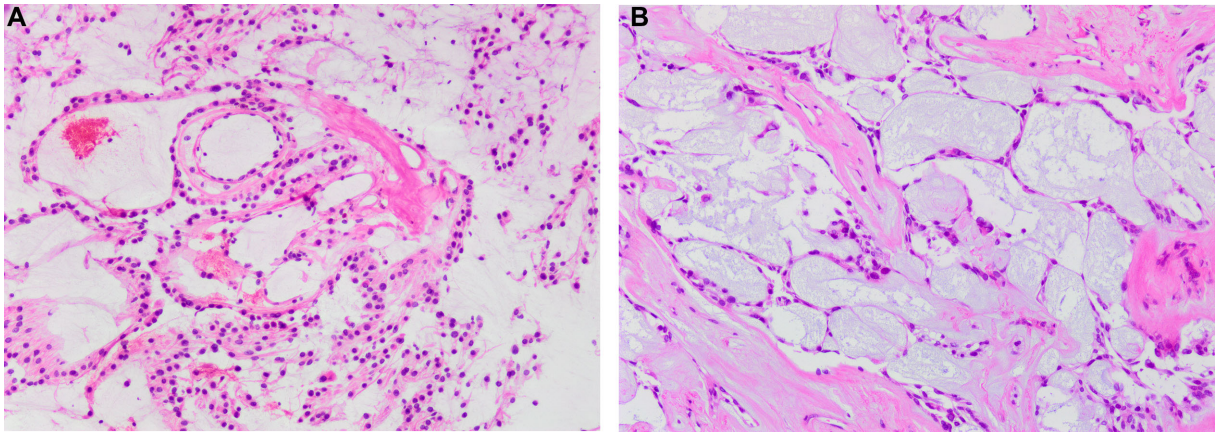

FIGURE 4. **A** *and* **B**, *Myxopapillary ependymoma histology showing hyalinized blood vessels with tumor cells radiating outward and myxoid degeneration (10x magnification).*

with somatic *NF2* mutation, the condition neurofibromatosis 2 (germline *NF2* mutation), or copy number loss of chromosome 22q (*NF2* locus).[27,28] Amplification of the oncogene *MYCN* is also observed in some spinal ependymomas.[29] SP-MPE are most often associated with aneuploidy or tetraploidy[30] and exhibit dysregulation of cellular metabolic processes such as upregulation of hypoxic pathways through *HIF1α* and increased reliance on glycolysis.[31] SP-SE tumors have been found to harbor losses of chromosome 6q, though no candidate tumor driver has been identified.[2]

Ependymomas of the posterior fossa can be separated into subependymoma (PF-SE) and 2 ependymoma groups – Group A (PF-EPN-A) and Group B (PF-EPN-B).[2] Clinically, PF-EPN-A tumors are most often diagnosed in pediatric patients, have a balanced genome with minimal aberrations, and are associated with bad prognosis, especially tumors with gains in chromosome 1q.[2,25] These tumors have uniformly low expression of the repressive histone marker H3K27me3[22,32] and small proportion have H3F3A K27M mutations, as are found in diffuse midline glioma.[33] In contrast, PF-EPN-B tumors are most often diagnosed in adult patients, generally have a good prognosis, and have multiple copy number abnormalities,[18] as well as *TERT* promoter mutations (C228T/C250T) in a small proportion.[34] Analyses of large cohorts of types A and B have demonstrated significant molecular heterogeneity within each group, with 9 distinct PF-EPN-A[35] and 5 PF-EPN-B[36] subtypes identified. Importantly, while PF-EPN-A generally has a poor prognosis, 1 subtype (2c) had relatively high survival (>90% at 5 yr). Similarly, while PF-EPN-B generally has a good prognosis, there were increased death rates in 2 of the subtypes.

Supratentorial ependymomas can be segregated into supratentorial subependymoma (ST-SE), *RELA* fusion ependymoma (ST-EPN-*RELA*), and *YAP* fusion ependymoma (ST-EPN-YAP). ST-EPN-*RELA* tumors typically occur in adolescents and young adults (but can occur in younger patients), are associated with poor prognosis, and are most often driven by chromothriptic events of chromosome 11 resulting in *C11orf95–RELA* fusions and oncogenic activation of NF-κB signaling.[19] ST-EPN-YAP tumors in contrast are most often diagnosed in young pediatric patients and confer good survival. These tumors are driven by fusion events involving *YAP1*, the most common being *YAP1-MAMLD1* and *YAP1-FAM11B*[2] which drive oncogenesis through activation of the Hippo pathway.[37]

Targeted Therapy

The historic lack of known molecular drivers and heterogeneous demographics of ependymoma patients has hindered exploration in the use of molecular targeted therapies. Studies have shown pediatric ependymoma to highly express members of the receptor tyrosine kinase (RTK) family (*ERBB1-4*, *EGFR*) which suggests RTK inhibitors may be a vulnerable target.[24] However, no effect was observed with erlotinib treatment in pediatric patients,[38] while treatment with lapatinib was able to achieve stable disease in under half of pediatric patients in two clinical trials.[39] Anecdotally, partial responses have been observed in 1 spinal ependymoma on imatinib[40] and 1 metastatic myxopapillary ependymoma on sorafenib.[41] There is currently one clinical trial that is also testing the efficacy of selumetinib, a MEK inhibitor, on *NF2* driven ependymoma (NCT03095248).

With mounting evidence of certain subtypes such as PF-EPN-A being driven by an aberrant epigenome, pre-clinical evidence in mouse models have shown efficacy of JQ1, a bromodomain inhibitor, and AZD4547, a WEE1 inhibitor.[42] Clinical trials are underway testing the efficacy of histone deacetylases inhibitors on ependymomas which lack H3K27me3, similar to those performed in pediatric diffuse pontine glioma (NCT02265770). Preclinical studies and ongoing clinical trials have also suggested that certain ependymoma subtypes may be candidates for immunotherapies – in particular, high expression of *PD-L1*, myeloid infiltrates, and high levels of CD4+/CD8 + tumor infiltrating T lymphocytes have been detected in ST-EPN-RELA.[43] The efficacy of nivolumab (a checkpoint inhibitor) for ependymoma and other rare CNS tumors is currently being evaluated (NCT03173950), and other active trials involving immunotherapy include tumor antigen vaccinations combined with imiquimod (NCT01795313) and *HER2* specific chimeric antigen receptor T-cell therapy (NCT03500991).

CHORDOMA

Chordomas are bone tumors that exhibit benign histopathologic features but follow a malignant clinical course. They are rare tumors, with a reported annual incidence of 1 per million individuals.[44] There is a slight male preponderance (males account for 60% of cases) and the median age at diagnosis is around 60. The major effect of the tumor is generally from local invasion and destruction, although 30%-40% of patients do have metastases, usually late in the disease course, to regions such as the lungs, liver, bone, and lymph nodes. In several large series, 5-yr survival rates of chordomas of the skull base or cervical spine have been reported to be between 55% and 86%, and for those of the sacrum and thoracolumbar spine are 54% to 84%.[44,45]

Development/Cell of Origin

Chordomas are thought to arise from undifferentiated notochordal remnants throughout the axial skeleton.[45] The notochord is a midline embryonic structure that typically disappears at about 8 wk of fetal development in humans.[46] It serves 2 key roles in development – as a source of secreted midline signals that provide position and fate information to surrounding tissue, and as a structural element for the embryo. In the latter role, the notochord is thought to be a primitive form of cartilage that eventually becomes ossified and forms the nucleus pulposus of intervertebral discs. In addition to the histopathological resemblance and similar distributions of notochordal remnants and chordoma, a key piece of evidence supporting their relationship is the role of the transcription

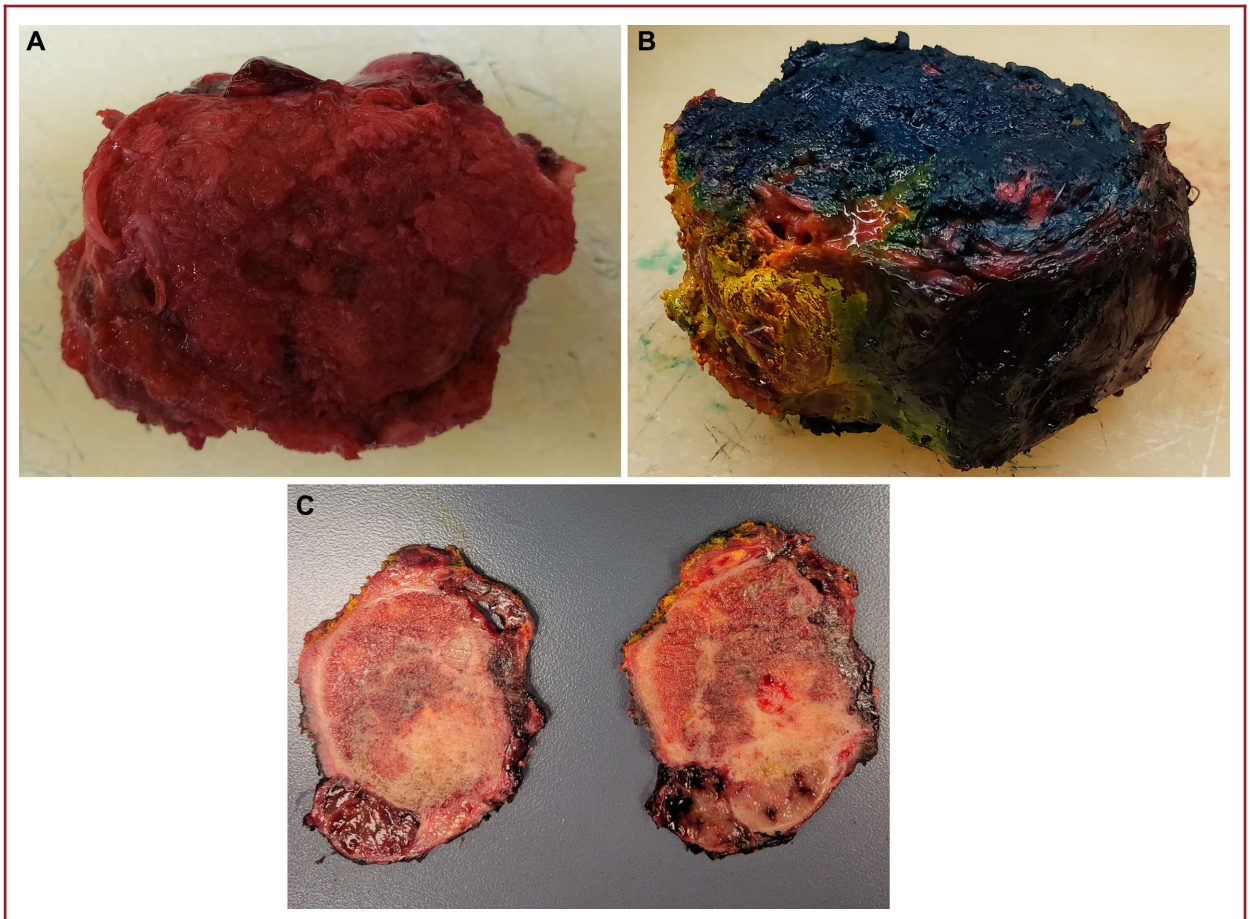

FIGURE 5. *Chordoma specimen gross pathology* **A**, *as received from operating room,* **B**, *with surfaces inked for orientation and to identify surgical margins microscopically, and* **C**, *transverse slabs following bandsawing but prior to formalin fixation*

factor brachyury, which is an important regulator of notochordal development and is ubiquitously and highly overexpressed in chordomas.[45,47-49]

The relationship between benign notochordal tumors (BNCT; also known as ecchordosis physaliphora) and chordoma development is still unclear. There are several case reports of a chordoma with adjacent BNCT that is the presumed precursor.[50,51] Microscopic BNCTs may be quite common in the general population, with one autopsy series of 100 randomly selected cases identifying 20 with BNCTs, although these are rarely large enough to be detected on imaging.[52] Given the rarity of chordoma, if this rate of BNCT is representative of the broader population, that suggests that even if BNCT can progress to chordoma, it is an exceptionally rare occurrence.

Radiology and Gross Pathology

Chordomas occur along the length of the axial spine and can be classified based upon their anatomic location as sacrococcygeal, clival, or mobile spine. Useful imaging investigations include CT, magnetic resonance imaging (MRI) and positron emission tomography.[53,54] On CT scan, chordomas present as variably hypo- and hyper-dense lesions and are usually well circumscribed. This appearance is due to the lytic nature of the tumor which leads to areas of soft-tissue adjacent to calcified remnants of the destroyed bone. Chordomas appear hyperintense on T2 weighted MRI, while on T1 weighted MRI, they are heterogeneously hypointense. SWI may also demonstrate blooming artifact as a result of intralesional hemorrhage. Contrast enhancement on CT scan and MRI is usually heterogenous.

The recommended surgical treatment when feasible is en-bloc resection with negative surgical margins.[44,55] The resulting specimen received by the pathologist is complex (Figure 5) and typically includes 1 or more levels of vertebrae, as well as adjacent soft tissue, and potentially other involved structures, such as bowel. Margins are recommended to be classified as follows: R0 for negative margins of >1 mm of normal tissue surrounding the tumor, R1 for margins of <1 mm but no macroscopic tumor, and R2 for macroscopic tumor left or tumor spillage.[44]

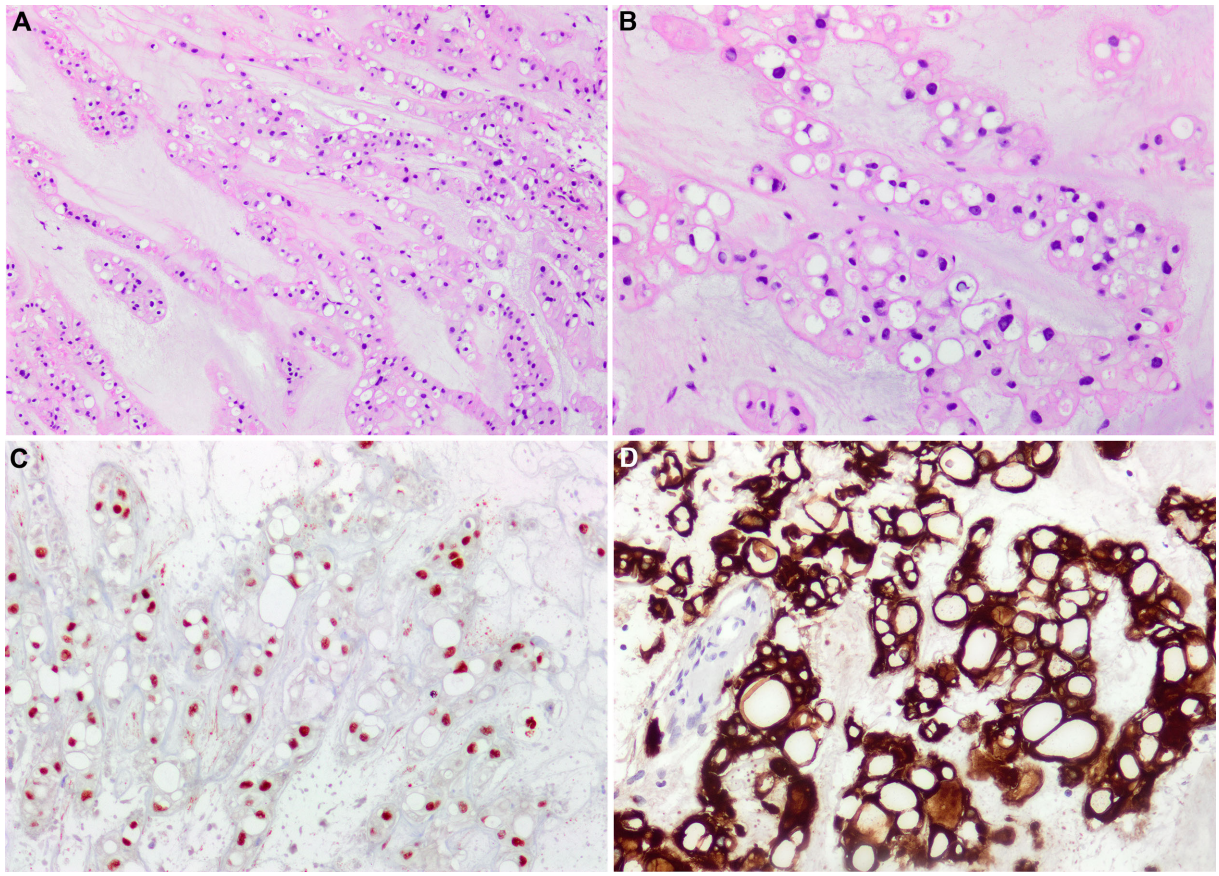

FIGURE 6. *Chordoma histology showing* **A** *and* **B**, *characteristic chordoid arrangement of physaliphorous cells (10x, 20x magnification), and immunopositivity for* **C**, *brachyury (20x) and* **D**, *keratin (20x).*

Given the challenge of processing these specimens and the importance of margin status in guiding adjuvant radiotherapy, they are best examined by pathologists with experience in bone and soft tissue pathology. In particular, the bony portions of the specimen usually require bandsawing into thinner sections (approximately 3-5 mm in thickness) prior to formalin fixation, as well as subsequent decalcification in acid before they can be processed for histology.[56] The orientation of the specimen can be challenging, and ideally is done with the surgeon present or using markers placed during the operation (eg, sutures), as well as using close correlation with preoperative radiographic imaging. Following thin sectioning on the bandsaw, the gross examination of the resulting slabs is a critical step that includes the measurement of tumor dimensions, gross assessment of margins, and identification of areas of necrosis. This guides the submission of sections for microscopy, which include representative areas of tumor and the closest margins to tumor.

Histology

Histologically, the 4 recognized chordoma subtypes are classical (or conventional), chondroid, sarcomatoid, and dedifferentiated.[44] Classical chordomas are distinguished by "physaliphorous" cells – large epithelial cells with prominent vacuoles and clear-to-eosinophilic cytoplasm (Figure 6). Architecturally, the cells are arranged in lobules with fibrous septae and small ribbons or cords. The extracellular matrix is often abundant and myxoid in nature. The nuclear features are typically bland, with a lack of atypia or pleomorphism, as well as low mitotic activity, belying the locally aggressive nature of this tumor. Necrosis is often present and can be extensive. Immunohistochemically, chordomas are typically positive for brachyury, cytokeratins (specifically CKs 8, 18, and 19), S100, and EMA, although these can be lost in dedifferentiated regions.

Chondroid chordoma has a hyaline matrix that mimics cartilaginous tumors. This appearance can be a focal component or extensive, but does not have different behavior from those

with exclusively conventional classical morphology. In sarcomatoid chordoma, the cells have a predominantly spindled morphology, rather than epithelioid, but do retain brachyury expression. Chordomas are not graded, but prognostically it is important to report areas of dedifferentiation, which typically have the appearance of a high-grade undifferentiated spindle cell tumor. Recently a subset of poorly differentiated chordomas with SMARCB1 (INI-1) loss, were reported to be particularly frequent in pediatric patients.[57] One important point for pathologists to be aware of is possible histologic differences in different anatomic regions, with 1 study reporting that skull base chordomas frequently have abundant chondroid matrix and diffuse growth pattern, while spinal chordomas have myxoid matrix and a more lobular growth pattern.[58]

Practical Diagnostic Considerations

The differential diagnosis for chordomas often includes chondrosarcoma, chordoid meningioma, metastatic carcinoma (particularly clear cell carcinomas, such as renal cell), and other bone and soft tissue tumors. In particular, the chondroid subtype of chordoma can be challenging to differentiate from chondrosarcoma. Brachyury expression is an important and highly specific marker, which reliably distinguishes chordoma (including chondroid subtype) from sarcomas and other bone and soft tissue tumors. However, immunoreactivity for brachyury and other markers can be lost due to decalcification in acid, as is often required for histological processing of bony tissue.[49] Furthermore, the brachyury immunohistochemical marker is a specialized test that many laboratories may not have on-site, and the need to send for external testing can cause delay in diagnosis.

It should be noted that brachyury does not differentiate chordoma from BNCT, as both entities are immunopositive.[59] While there are histologic differences between chordoma and BNCT, including a greater degree of nuclear atypia and an infiltrative growth pattern, they can be challenging to differentiate on small core needle biopsies, and careful radiographic correlation is often required.[60,61] Given the rarity of this tumor and crucial clinical implications, cases of chordoma that are diagnosed outside of a specialized referral center should be sent for pathology review to confirm the diagnosis.[44]

Molecular Characterization

Chordomas in general have an extremely low mutational burden.[48] Besides the ubiquitous overexpression of the transcription factor *Brachyury* (*T*),[59] the molecular drivers have remained enigmatic, with no detectable driver mutations in roughly half of sporadic cases.[48] Several genomic profiling studies have only identified a few genes, many implicated in epigenetic regulation, that are recurrently altered in a modest number of cases.[48,62] The most frequently identified alterations have been copy number losses of chromosomes 1p, 3 (*SETD2, BAP1, PBRM1*), 9p (*CDKN2A*), 10 (*PTEN*), 13, 14, and 22 (*SMARCB1*).[62,63] Loss of *SMARCB1* expression, due to 22q loss or epigenetic dysregulation, has been associated with poorly differentiated and dedifferentiated chordoma[64,65] and has a higher prevalence in pediatric cases.[66] Recurrent mutations occur in a small proportion of cases, with the most frequent being inactivating mutations in *LYST*, a lysosomal carrier, in 10%,[48] followed by *TERT* promoter (C228T/C250T) mutations in 8.7%.[67] The genomic origins of familial chordoma are better understood, with germline duplication of *T* on chromosome 6q27 identified in 4/7 affected families.[47] Minor allelic gains and amplifications of *T* also occur in 4.5% and 7% of sporadic chordoma, respectively,[68] and a germline polymorphism of *T* (rs2305089) has been associated with sporadic cases.[69]

Due to the ambiguous mutational landscape of chordoma, some studies have instead interrogated the proteomic landscape of known cancer drivers. High expression of at least 1 RTK (PDGFRα/β, c-Kit, c-Met, HER2, EGFR) and activation of downstream MAPK/AKT/mTOR pathways (pAKT, p-mTOR, pERK, p4E-BP1) has been observed in nearly all samples profiled.[70-72] Studies on the immune landscape have found that chordoma cells express the immune regulator PD-L1, and exhibit tumor infiltrating lymphocytes,[73] which is most pronounced in metastatic cases.[74] Patients with higher counts of CD4+ (T-helper), CD8+ (cytotoxic-T), and PD-L1 + T cells have also been correlated with better survival.[75]

Targeted Therapy and Current Research

The use of small molecule inhibitor therapies has been investigated in chordoma due to the high expression of several RTKs, but has been met with limited success.[44] The most widely tested drug is imatinib, a general tyrosine kinase inhibitor (TKI), which, in advanced patients, has resulted in predominantly stable disease responses,[76] as has treatment with other TKIs (sorafenib, erlotinib, sunitinib, temsirolimus, and lapatinib).[77,78] Imatinib has also been used in combination with mTOR inhibitors (sirolimus and everolimus), again with modest success.[79,80] In SMARCB1 deficient chordomas, an ongoing clinical trial (NCT02601950) has observed complete responses with tazemetostat, an EZH2 inhibitor.[81] Several on-going clinical trials are investigating the efficacy of the check-point inhibitor nivolumab in chordoma (NCT03173950, NCT02989636, and NCT03623854). Vaccines against the brachyury antigen elicit brachyury-specific T-cell responses, supporting the further investigation of immunotherapies.[82]

Disclosures

The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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