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Genetics and Molecular Biology of Intramedullary Spinal Cord Tumors

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Intramedullary spinal cord tumors (IMSCTs) comprise 5% to 6%% of all central nervous system (CNS) tumors. Among intradural spinal tumors, intramedullary neoplasms account for only 8% to 10%, whereas extramedullary tumors, such as schwannoma and meningioma, predominate [1]. The most common intramedullary lesions are spinal ependymoma (60%) and spinal astrocytoma (10%–20%), but others include hemangioblastoma (3%–8%), cavernous malformation, metastases, and lipoma [1]. Although these tumor types exist in the brain, patients with spinal cord tumors tend to be younger and the spinal cord tumors do not seem to occur in connection with primary brain tumors. Spinal astrocytoma seems to occur in childhood or young adulthood, whereas ependymoma can occur throughout life but most commonly in the early to middle adult years. Much less attention seems to be paid to spinal cord tumors because of their overall rarity. Certain genetic syndromes are associated with IMSCTs and provide a special opportunity to study their genetics and molecular biology. Historically, ependymoma and astrocytoma of the spinal cord have been little distinguished from their cerebral counterparts. More and more evidence suggests that distinct differences in genetics and molecular biology exist between primary tumors in the brain and spinal cord with similar histology, however.

Genetic mutations associated with spinal ependymoma

Ependymomas are neuroectodermal tumors that occur in the brain and spinal cord. They are thought to arise from the ependymal lining of the ventricles and spinal canal. Most ependymomas are sporadic, but they are also associated with patients with neurofibromatosis. Intramedullary ependymoma is particularly amenable to surgical resection because of a defined tumor and cord interface, which is usually preserved and used to separate tumor from cord. A diagnosis of ependymoma on pathologic examination should prompt a full attempt at gross total resection without risking permanent spinal cord injury.

Three general types exist and include myxopapillary (World Health Organization [WHO] grade I), benign ependymoma (WHO grade II), and anaplastic ependymoma (WHO grade III). Myxopapillary ependymomas are a unique type of tumor because they are almost exclusively found at the conus and filum terminale and are therefore considered intradural extramedullary. Interestingly, myxopapillary ependymoma has been shown to exhibit a much higher propensity for aneuploidy or polyploidy, especially of chromosome 7, compared with other ependymomas based on chromogenic in situ hybridization (CISH) [2,3]. This unique genetic trait may explain its unique localization within the spinal cord. Anaplastic

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ependymomas are extremely rare, virtually only occur in the brain, and have the worst prognosis of any ependymoma. Little information is available on the genetics of grade III ependymomas.

Benign ependymomas comprise most ependymomas occurring in the CNS in the brain and spinal cord. Much of the earlier work in characterizing genetic lesions in sporadic intramedullary ependymoma derived from studies of patients with neurofibromatosis type 2 (NF2). Because of the propensity for patients with NF2 to develop CNS ependymoma, the NF2 gene, merlin, was an obvious target. Merlin is a tumor suppressor gene localized on chromosome 22q12, and mutations at this locus are the hallmark for NF2 [4].

A molecular distinction may exist between the events that lead up to a spinal ependymoma versus those that contribute to intracerebral tumor progression. In a recent study, Ebert and colleagues [5] analyzed 62 ependymal tumors, including myxopapillary ependymomas, subependymomas, ependymomas, and anaplastic ependymomas. They showed informative allelic loss of 10q (5 of 56 tumors) and 22q (12 of 54 tumors). Somatic mutations of NF2 were detected in 6 of the tumors examined, and in each case, the tumor was from a grade II spinal intramedullary ependymoma. These results were confirmed by another group that found NF2 mutations and loss of heterozygosity (LOH) of 22q in all spinal intramedullary ependymomas (n = 6) [6]. Allelic loss on 22q was also frequently observed and was significantly more common in intramedullary spinal ependymomas than in tumors in other locations [6]. Additionally, a low-penetrance ependymoma susceptibility locus has recently been mapped to chromosome 22q11 [7,8], suggesting the role of alternative predisposing genes apart from NF2/merlin. Overall, 75% of all ependymomas display chromosomal aberrations or rearrangements over several different chromosomes, with the most frequent LOHs being found on the long arms of chromosomes 6 (30.3%), 9 (27.3%), and 17 (50%) [9]. LOH was also detected on 3p14 (13.3%), 10q23 (10.3%), and 11q (18.2%). Monosomy of chromosome 22 is present in approximately 30% of ependymomas [10], with aberrations and/or alterations of 22q existing in up to 40% of all ependymomas. It is important to understand that although chromosome 22q abnormalities and NF2/merlin mutations are common in spinal ependymoma, they are not exclusive to ependymoma. Another genetic distinction between spinal and cranial ependymoma may lay in the methylation status of particular tumor-related genes. A recent study examining the methylation status of a putative tumor suppressor gene, HIC-1, on chromosome 17p13.3 showed a significant correlation between hypermethylation of HIC-1 and nonspinal localization ($P = .019$, n = 52) [11]. Losses in 1p and 16q, which correlate with other CNS tumors, have not been found in ependymomas [12]. A summary of genetic changes in ependymomas and hemangioblastomas is shown in Table 1. The apparent genetic differences and variations that segregate out between ependymomas in the brain and spine suggest that different molecular mechanisms exist that lead to their pathogenesis. Because primary brain and spine tumors are rarely, if ever, associated with each other, these distinctions indicate the need to reclassify spinal ependymoma separately from intracranial ependymoma.

Genetic mutations associated with spinal astrocytomas

Spinal astrocytomas are glial neoplasms that are thought to arise from similar glial predecessors as primary brain gliomas. Astrocytoma of the spinal cord is usually always sporadic, and no associations with other genetic syndromes are currently recognized. By definition, intramedullary astrocytomas are infiltrative tumors, and

Table 1
Summary of common genetic aberrations for spinal ependymoma and hemangioblastoma

Tumor	Mutation	Sporadic	Familial
Ependymoma			
Myxopapillary	Chromosome 7 (ploidy)	> 50%	N/A
Low grade	LOH 22q12	25%–100%	N/A
	LOH 10q23	10%	N/A
	LOH 6p	30%	N/A
	LOH 9p	27%	N/A
	Monosomy 22	30%	N/A
	NF2 mutation	?	100%
Hemangioblastoma	Germline 3p25–26 mutation	23%	94%
	LOH 3p25–26 mutation	50%	62%
	LOH 22q13	60%	N/A

Abbreviations: LOH, loss of heterozygosity; N/A, not applicable; NF2, neurofibromatosis type 2.

most are considered low grade (WHO grade II). Anaplastic (grade III) and glioblastoma multiforme (grade IV) histopathologic findings have been reported in the spinal cord but are exceedingly rare (5% each). Surgery is less beneficial for intramedullary astrocytoma and is typically helpful only for biopsy followed by duraplasty. Juvenile pilocytic astrocytomas (WHO grade I) may also form in the spine but are rare (10%). These tumors should be the only spinal astrocytoma for which aggressive surgery and gross total resection are recommended.

There are few published studies specifically examining the genetic mutations in intramedullary astrocytomas. Extensive work has been done on the pathogenic events causing intracerebral glioma, however. It is likely that some, if not all, of the genetic alterations described in intracerebral astrocytoma play a role in the progression of spinal astrocytoma. Three general transitions have been studied as a paradigm for glioma progression: (1) astrocyte to astrocytoma, (2) astrocytoma to anaplastic astrocytoma, and (3) anaplastic astrocytoma to glioblastoma. In the first transition, mutations in p53 and losses of chromosome 17p and 22q have been implicated. Recently, Rubio and colleagues [13] have shown that the NF2 gene was not mutated in 30 astrocytomas examined, making it an unlikely candidate for the 22q locus lost during this transition. In the progression from astrocytoma to anaplastic astrocytoma, genetic defects include retinoblastoma gene mutations, chromosome 13q loss, p16 gene deletions, chromosome 9p loss, and chromosome 19q loss [14]. The transition from anaplastic astrocytoma to glioblastoma has been shown to involve chromosome 10 loss and epidermal growth factor (EGF) receptor gene amplification [15].

Several studies have identified the PTEN gene (also known as MMAC and TEP1) as one of the candidate chromosome 10 genes lost in glioblastoma [15,16]. The gene encodes a tyrosine phosphatase located at 10q23.3. The function of PTEN as a cellular phosphatase is consistent with a tumor suppressor phenotype. Phosphatases act by turning off signaling pathways dependent on phosphorylation. When phosphatase activity is lost as a result of genetic mutation, signaling pathways can become activated constitutively, resulting in aberrant proliferation.

More specific description of genetic lesions in intramedullary spinal astrocytomas requires analysis of large cohorts of patients. This may prove difficult because of the rarity of this disease process and the paucity of tumor that is resected once the diagnosis of astrocytoma is suspected. Unlike patients with intramedullary ependymoma, aggressive surgical resection of tumor from patients with intramedullary astrocytoma is currently of questionable long-term benefit.

von Hippel-Lindau disease and spinal hemangioblastoma

Hemangioblastomas are low-grade (WHO grade I) vascular tumors predominantly found in the cerebellum and spinal cord. First described by Arvid Lindau as cystic lesions in the cerebellum, CNS hemangioblastomas occur mostly as sporadic cases, but 20% to 30% occur in association with von Hippel-Lindau disease (VHL) [17]. VHL is an autosomal dominant disorder with 90% penetrance attributable to loss of a tumor suppressor gene on chromosome 3p25 through 3p26, named the VHL gene [18]. The VHL gene encodes for a protein required for oxygen-dependent degradation of hypoxia-inducible factor-1α (HIF-1α). Dysfunction or absence of the VHL gene leads to constitutive overexpression of HIF-1α, which then leads to increased levels of vascular endothelial growth factor (VEGF) and other angiogenic signals [19].

Lesions associated with VHL include CNS hemangioblastoma, retinal angioma, renal cysts, renal cell carcinoma, pancreatic cysts, pheochromocytoma, and epididymal cystadenoma [20]. VHL families can be grouped according to the presence or absence of pheochromocytomas [21]. Nearly all families with pheochromocytomas have missense mutations of the VHL gene. Hemangioblastomas are predominantly made up of endothelial cells and pericytes in a dense network of vascular channels intermixed with lipid-laden stromal cells. Using tissue microdissection, Vortmeyer and colleagues [22] have demonstrated consistent loss of heterozygosity at the VHL gene locus in the stromal cells, implicating these cells in the pathogenesis of hemangioblastoma.

CNS hemangioblastoma occurs in type I (without pheochromocytoma) and type II (with pheochromocytoma) VHL families. Sites of predilection are the posterior fossa (80%) and the cervical or lumbar regions of the spinal cord (20%). Specific point mutations and deletions in the VHL gene have been characterized in sporadic and VHL-related spinal hemangioblastoma. VHL-related hemangioblastomas have been

reported to harbor 94% germline mutations and 62% LOH at the VHL gene [17,23,24]. More than 150 different germline mutations have been identified and include deletion, missense, and nonsense frameshift mutations. The resultant biallelic inactivation of the VHL gene suggests a ''two-hit'' model of tumor genesis in patients with VHL. In other words, patients with VHL are usually heterozygous for the germline VHL mutant, and a ''second hit'' at the remaining wild-type VHL gene then causes tumor formation. Similar findings have been reported in VHL-related renal cell carcinoma and pheochromocytoma. In contrast, sporadic hemangioblastoma contained only 50% LOH and 23% germline mutations at the VHL gene, suggesting alternate pathways to biallelic inactivation and tumor genesis in sporadic cases [17]. Other mutations and sites of LOH have been implicated in the development of sporadic hemangioblastomas as well. LOH of chromosome 22q13 was found in five of eight patients with hemangioblastoma not related to VHL, with only three of eight patients harboring LOH at chromosome 3p [25]. Hypermethylation of the VHL gene or its promotor is an unlikely mechanism for VHL inactivation. Recent studies have failed to show hypermethylation in hemangioblastomas of the VHL gene from VHL and sporadic cases [17,26]. Differences in the molecular and genetic origins of hemangioblastoma may explain why patients with VHL and CNS hemangioblastoma fare much worse compared with those with sporadic CNS hemangioblastoma.

Neurofibromatosis and spinal intramedullary tumors

There are two distinct types of neurofibromatosis, each affecting cells embryologically derived from the neural crest. Neurofibromatosis type 1 (NF1) is a disease characterized by autosomal dominant inheritance with almost complete penetrance and variable expressivity [27]. Approximately 50% of cases are new mutations, with a 1 in 3000 prevalence. The NF1 gene is located on the long arm of chromosome 17 and codes for a guanosine triphosphatase–activating protein called neurofibromin that influences cell proliferation and differentiation. Tumors associated with the NF1 syndrome include neurofibromas, malignant nerve sheath tumors, optic nerve gliomas, rhabdomyosarcomas, pheochromocytomas, and carcinoid tumors.

NF2 also segregates by autosomal dominant inheritance with high penetrance. The NF2 gene is located on chromosome 22q12, with approximately 50% of reported cases representing new mutations. It is much less prevalent than NF1, with a rate of 1 in 40,000. The NF2 gene product encodes the protein merlin, which is a member of the ezrin-radixin-moesin protein family that links the cytoskeleton to the plasma membrane [28,29]. Neoplasms associated with NF2 include bilateral acoustic schwannomas, neurofibromas, ependymomas, gliomas, and meningiomas. There are two subtypes of NF2. The severe or ''Wishart'' form is characterized by early onset, rapid clinical progression, and multiple tumors. The mild or ''Garner'' form has a later onset, slower clinical progression, and fewer tumors.

In 1996, Lee and colleagues [30] published one of the largest series of IMSCTs in patients with NF. Nine patients were described, including 3 with NF1, 5 with NF2, and 1 with ''type uncertain.'' The predominant pathologic finding associated with NF1 was astrocytoma (two low-grade cases and one anaplastic case), whereas ependymoma was most closely associated with NF2 (4 of 5 patients). The reported incidence of IMSCTs in the NF population was approximately 19% (9 of 48 patients). This incidence may reflect referral patterns associated with highly specialized neurosurgical services. In 1997, Yagi and colleagues [31] described a series of 44 patients presenting with IMSCTs, 2 of whom had NF1. In both cases, the pathologic finding of the lesion was astrocytoma (anaplastic astrocytoma and glioblastoma). Taken together, along with selected case reports in the literature, these studies support the presumption that solitary IMSCTs in patients with NF1 are most likely to be astrocytoma. Similarly, it is reasonable to assume that a patient with NF2 presenting with an intramedullary tumor is most likely to have an ependymoma [32].

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