Anaplasia and Grading in Medulloblastomas

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The variable clinical outcomes of medulloblastoma patients have prompted a search for markers with which to tailor therapies to individuals. In this review, we discuss clinical, histological and molecular features that can be used in such treatment customization, focusing on how histopathological grading can impact both patient care and research on the molecular basis of CNS embryonal tumors. Medulloblastomas span a histological spectrum ending in overtly malignant large cell/anaplastic lesions characterized by increased nuclear size, marked cytological anaplasia, and increased mitotic and apoptotic rates. These "high-grade" lesions make up approximately one quarter of medulloblastomas, and recur and metastasize more frequently than tumors lacking anaplasia. We believe anaplastic change represents a type of malignant progression common to many medulloblastoma subtypes and to other CNS embryonal lesions as well. Correlation of these histological changes with the accumulation of genetic events suggests a model for the histological and molecular progression of medulloblastoma.

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Introduction

Because of their overall primitive appearance and aggressive biological behavior, medulloblastomas, as a group, are grade IV (of IV) in the current World Health Organization (WHO) system (41). However, patients with ostensibly similar neoplasms, and receiving identical therapies, can have widely disparate clinical outcomes. A significant percentage are cured, whereas the tumors in other patients recur quickly and disseminate widely. It would be advantageous to tailor specific therapies to individual lesions so that patients are not over or under treated. Clinical, histological, and molecular factors have been proposed for such medulloblastoma stratification. We review these three categories of prognostic variables, concentrating on histopathological criteria and the concept of "anaplasia" in these already small cell tumors. We also discuss the molecular and histological progression of medulloblastomas and other CNS embryonal tumors.

Clinical Prognostic Markers

Many clinical factors have been evaluated as possible predictors of outcome in patients with medulloblastomas (for review, see 12, 16, 23, 53). Significant variables include patient age, the presence of residual disease following surgery, and metastatic stage (59, 63). In most studies, patients less than 3 to 5 years of age have worse clinical outcomes, although this may be due to the need to delay radiation therapy rather than inherently more aggressive tumors. Significant residual local tumor, generally defined as more than 1.5 cm^3 postoperatively, is also associated with high rate of recurrence and shorter survival. Metastatic stage is perhaps the most powerful prognostic indicator. The widely used Chang system stages, in ascending order: *i)* tumor cells in the CSF, *ii)* intracranial leptomeningeal and ventricular spread, *iii)* spinal leptomeningeal spread, and *iv)* extra-CNS metastasis (10). Not surprisingly, metastasis generally presages a poor outcome. Male gender has also been associated with significantly shorter survival in some studies (54,61), but not in others (15,64).

Histopathological Factors Influencing Outcome in Medulloblastoma

Pathologists have been no less assiduous than clinicians, and molecular biologists, in identifying markers with which to tailor therapy. Multiple individual features have been identified, albeit with lack of agreement about some. The first was the presence of nodular foci, ie, "pale islands," containing cells with small, round, often bland nuclei and abundant cytoplasm (Figure 1A). Tumor cells in the internodular regions, in contrast, are more tightly packed, more proliferative, and have nuclei that are larger and cytologically more atypical than the often neurocytic cells within the nodules. Because of the connective tissue content in the internodular regions that is highlighted by stains for reticulin, these tumors are widely known as "desmoplastic medulloblastomas." However, the differentiation of cells within the nodules along neuronal lines is now thought to be the defining feature (18, 41). The designation "desmoplastic" is therefore, to us, rather a misnomer, since it is the nodular differentiation, not merely the presence of connective tissue, which appears the unique and critical feature of the nodular/desmoplastic subtype.

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Given their bland cytological appearance and low intranodular proliferation rate, it has long been suspected that these nodular/desmoplastic medulloblastomas might behave less aggressively than other variants. The difference in survival between nodular and non-nodular lesions, was, indeed, significant in some studies (11, 60), while only a trend towards better outcomes was seen in others (2, 40). One investigation of medulloblastomas in adults even found a negative correlation between desmoplasia and outcome (28). The discrepancies between these reports may to some extent reflect different definitions of desmoplasia, as some included cases containing desmoplastic regions but no nodules.

One rare nodular variant that may have a more favorable outcome is that known as "medulloblastoma with extensive nodularity." Giangaspero and colleagues initially defined this lesion as a medulloblastoma so nodular, and so well differentiated, that there was little or no internodular component, (Figure 1B) (24). Their patients were all very young and generally had good clinical outcomes. Similar tumors had previously been designated "cerebellar neuroblastoma" (48). In our recent study of 330 similarly treated Pediatric Oncology Group (POG) patients, we found that limited nodularity was not significantly predictive of outcome, but the 14 cases that were mostly nodular had improved survival (19). The less aggressive biological behavior of the extensively nodular lesions is especially intriguing because they generally arise in young infants who are considered "high risk" using standard clinical stratification schemes.

Cell proliferation, independent of nodular or non-nodular architecture, is a second histological grading parameter, but its prognostic significance is not clear either. Ito and colleagues found that a bromodeoxyuridine labeling index of greater that 20% correlated with worse prognosis (37), but studies of adult and pediatric cases did not find a significant association between shorter survival and higher Ki67 labeling index (28, 57). Gilbertson and colleagues argued that a Mitotic Percentage Index (MPI) provides a more accurate measurement of proliferative activity, since that method takes tumor cellularity into account, and MPI was an independent prognostic factor in their analysis of 70 pediatric medulloblastomas (27). It has also been suggested that medulloblastomas in children have lower apoptotic indices and higher Ki67 labeling indices than those in adults (55).

Since it is the balance between cell proliferation and cell death that determines the rate of tumor growth, the impact of necrosis and apoptosis on outcome have also been considered. Using proliferation indexes measured in surgically removed tissue, Ito and colleagues calculated a theoretical tumor doubling time of 2 to 4 days for a group of 6 bromodeoxyuridine-labeled medulloblastomas. However, analysis of the 3 cases with preoperative serial imaging disclosed an actual doubling time of over 20 days, suggesting that significant cell loss was occurring (37).

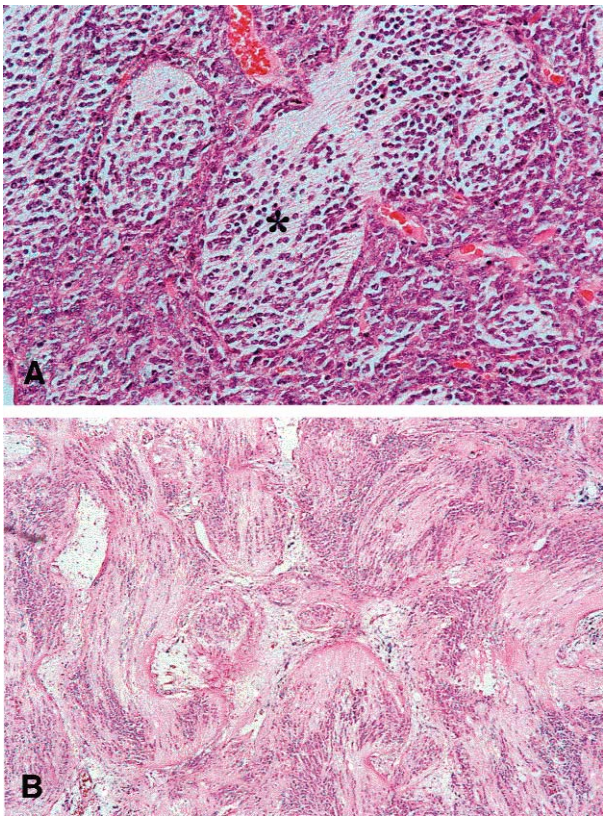

Figure 1. *Nodular medulloblastomas.* Well-circumscribed, pale nodules composed of differentiated cells characterize nodular/desmoplastic medulloblastomas (asterisk). There is a conspicuous disparity between the mitotic rate and degree of cytological atypia in inter- and intra-nodular regions (**A**). Rare tumors, known as "Medulloblastoma with Extensive Nodularity" are composed almost entirely of such nodules, with little if any internodular component. Neurocytic differentiation is pronounced, and cytological atypia is minimal (**B**).

However important in concept, the prognostic role of cell death has been difficult to assess in practice, and is complicated by distinctions between necrosis and apoptosis. The former is often associated with rapid tumor proliferation, and in many CNS neoplasms is a negative prognostic indicator. In contrast, apoptosis can sometimes indicate sensitivity of tumors to radiation or chemotherapy, as seen in the response of some primary CNS lym-

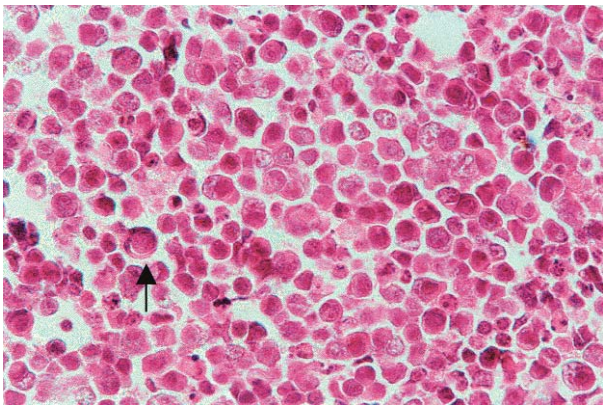

Figure 2. *Large cell medulloblastoma.* This medulloblastoma subtype is characterized by large, round cells with prominent nucleoli and numerous mitotic figures and apoptotic bodies. Cannibalistic wrapping of one cell around another is common (arrow).

phomas to corticosteroids. Caputy and colleagues found that 5-year survival was significantly worse in patients whose medulloblastomas contained necrotic areas (9). The role of apoptosis has been less clear. Korshunov and colleagues have calculated apoptotic indexes of over 1.5% to be associated with shorter survival (43), and a 4-fold higher mean apoptotic index in recurrent tumors and tumors from patients who died of their disease (44). Others have reported the opposite, with longer survival associated with high levels of apoptosis (34).

A final histopathological parameter is differentiation along glial or neuronal lines. Investigating 38 consecutively treated patients, Packer and colleagues divided tumors into 2 groups: undifferentiated tumors and those with glial or neuronal differentiation as appraised by the appearance of H&E stained sections (47). Patients with undifferentiated tumors had significantly longer survival. However, a study using similar criteria, but with more patients and longer follow-up, found the opposite, ie, improved clinical outcomes associated with differentiation (9). These differences can perhaps be explained by the subjective nature of assessing "differentiation" without, or even with, immunohistochemistry. Indeed, immunohistochemistry has not eliminated conflicting claims in this area. Some have found that glial differentiation, as evidenced by glial fibrillary acidic protein (GFAP) expression, is associated with worse clinical outcome (38) whereas others have reported the opposite (29), or found no association (13). As discussed above, extensive neurocytic differentiation in nodular medulloblastomas has been associated with longer survival.

- Increased cell size
- Increased mitotic rate
- Numerous isolated or confluent apoptotic bodies
- Cellular "wrapping"
- Either round discrete cells with prominent nucleoli (large cell subtype) or angular, pleomorphic, crowded cells with frequent molding (anaplastic subtype).

Table 1. Cardinal features of the large cell/anaplastic medulloblastoma.

A system for histological grading of medulloblastoma was proposed in 1983 by Kopelson and colleagues, who ranked increasing levels (0, 1, 2, 3) of necrosis, mitosis, and cytoplasmic processes, as well as decreasing degrees of desmoplasia, in 43 tumors (42). Those patients whose tumors' sums were 5 or greater had significantly shortened overall survival. While this system either included or anticipated many of the individual prognostic features discussed above, it was never widely adopted, and quantitation of histological features has not generally been used in medulloblastoma stratification.

Large Cell/Anaplastic Medulloblastoma

A recent and more subjective approach to medulloblastoma grading identifies "large cell" or "anaplastic" features. The former includes cells with large, round, vesicular nuclei containing prominent nucleoli, abundant mitotic figures, and numerous apoptotic bodies as first described in a series of 4 unusual medulloblastomas reported by Giangaspero and colleagues in 1992 (Figure 2) (25). While acknowledging that embryonal tumors such as medulloblastoma are expected to have a "primitive" appearance, the authors felt the nuclear features in these cases were distinctive, and proposed the name Large Cell (LC) medulloblastoma. In accord with the "malignant" histological features, all 4 of the patients died within one year of initial surgery. Subsequent reports of 2 individual cases by others described LC medulloblastomas with similar clinical, histopathological, and molecular features (39,52).

Three larger studies have extended the LC medulloblastoma concept, and established its negative prognostic import at the statistical level. The first, by Brown and colleagues, defined the incidence of the LC medulloblastoma phenotype and differentiated it from atypical teratoid/rhabdoid tumors (AT/RT) (7). They reviewed medulloblastomas from 474 Pediatric Oncology Group (POG) patients, and identified a group of "anaplastic" medulloblastomas, in addition to the LC group. These

anaplastic lesions shared the elevated cell size, mitotic rate, and apoptosis frequency with LC tumors, but also had cells that were pleomorphic, angular, and "anaplastic" rather than round as in LC medulloblastomas. Because LC and advanced anaplastic changes often comingled in individual tumors they were combined into a single subtype: large cell/anaplastic (LC/A) medulloblastoma. While 67 (14%) of the tumors fell into this category, statistical analysis focused on the 21 (4%) of cases with severe, diffuse LC/A features. Long-term survival of patients with LC/A tumors was approximately 10%, compared to over 50% for non-anaplastic cases.

A second study, by Leonard and colleagues, using similar criteria, identified LC/A features in 7 (9%) of 80 medulloblastomas (45). Three of these LC/A tumors arose focally in "classic" (ie, non-nodular) medulloblastoma, while 4 appeared at the time of recurrence or metastasis in nodular/desmoplastic tumors or medullomyoblastomas. Ellison and others have also highlighted the fact that both classic and nodular tumors can give rise to anaplasia (8, 21). Figure 3 demonstrates anaplasia in the internodular region of a nodular/desmoplastic medulloblastoma adjacent to a less atypical "pale island." These data suggest that anaplasia represents a malignant progression that can occur in many medulloblastoma subtypes, and potentially in other embryonal lesions as well (Figure 4).

The third large study of histological grading expanded the criteria for LC/A medulloblastoma, asking whether lesser degrees of anaplasia, or only focal anaplasia, would also affect outcome (19). We re-evaluated many of the POG patients reported by Brown et al, grading anaplasia using a 4-tiered scale: none, slight, moderate, severe. While slight anaplastic changes had no effect on outcome, moderate or severe anaplasia, identified in 14% and 10% of the tumors, respectively, were both significantly associated with shorter survival ($p<0.001$; Figure 5). The anaplasia was also classified as either focal or diffuse, and while tumors with widespread anaplasia were the most aggressive, even focal anaplasia had a significant negative effect on outcome. Based on this data, we proposed that medulloblastomas that were only moderately anaplastic, or in which the anaplasia was only focal, be included in the LC/A subtype. This binary approach thus treats tumors as either anaplastic or non-anaplastic. Interestingly, metastatic stage failed to predict outcome in patients for whom this data was available, while anaplasia remained prognostic, suggesting that histopathological grading is not

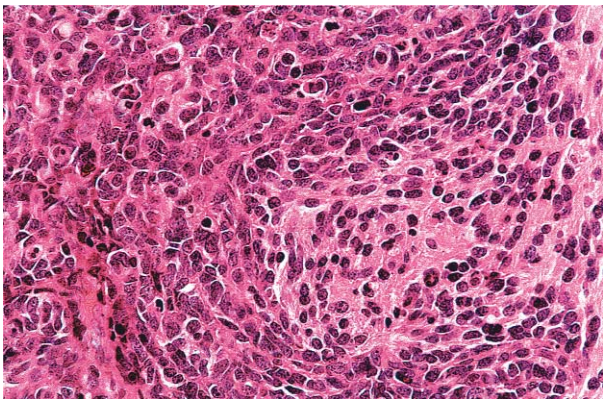

Figure 3. Anaplasia in a nodular medulloblastoma.

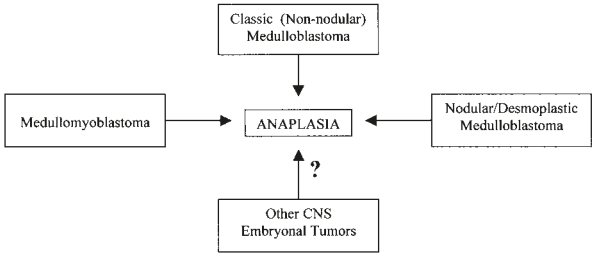

Figure 4. Anaplasia can develop in several embryonal tumor subtypes.

a surrogate for clinical stage, and may be a more powerful predictor of outcome than some clinical factors.

While grading of anaplasia in medulloblastomas is prognostically important, the criteria are subjective, and boundaries between anaplastic and non-anaplastic tumors are difficult to define numerically. However, the histological factors associated with anaplasia—mitosis, apoptosis, and cell size—are amenable to quantitation. Our analysis of mitotic index and cell size in 40 tumors of varying grade suggests that both increase in proportion to the degree of anaplasia, and that the establishment of quantitative standards is feasible (19). It will also be important to determine the relative prognostic power of LC/A changes compared to clinical and molecular predictors of outcome. This will be studied in imminent Children's Oncology Group protocols.

Differentiating LC/A Medulloblastoma from AT/RT

LC/A medulloblastoma must be differentiated from another aggressive pediatric brain tumor, atypical teratoid/rhabdoid tumor (AT/RT). AT/RTs are found in extremely young children, usually less than 2 years of age, and are characterized by a "jumbled" architecture and large but heterogeneous cells. Areas of densely cellular "small cell" tissues with an embryonal tumor appearance

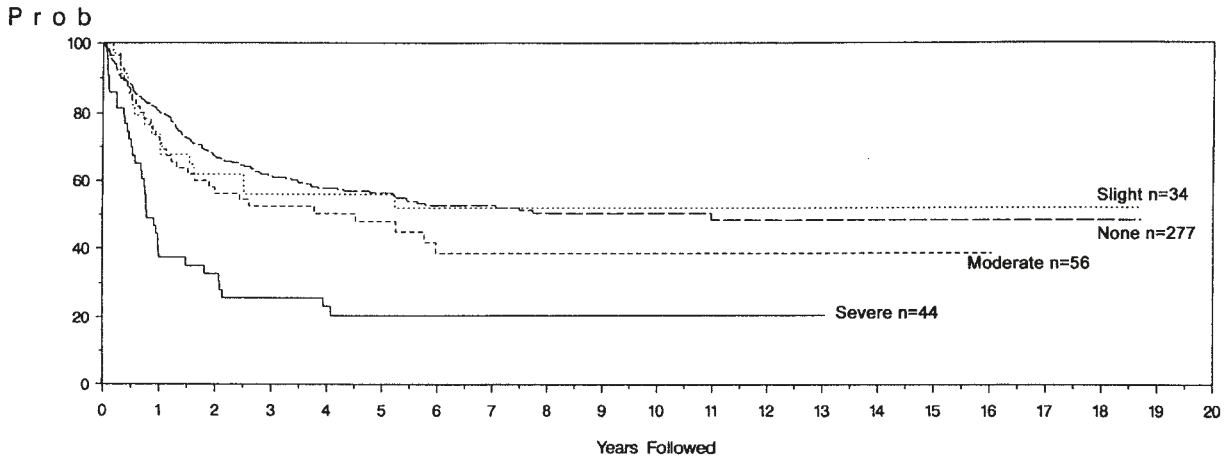

Figure 5. Effect of anaplasia grade on survival.

are common. The large, irregularly shaped cells of the AT/RT bear some resemblance to those found in LC/A medulloblastoma, and it was initially suggested that these entities might be the same. However, several lines of evidence indicate otherwise. Firstly, LC/A medulloblastoma foci often arise within a medulloblastoma of classic or nodular appearance, a phenomenon not seen with AT/RT. Second, as discussed below, the molecular changes in LC/A tumors are similar to those found in other medulloblastomas, but are distinct from the chromosome 22 loss and mutations in the *INI1* gene that characterize AT/RT. Third, unlike AT/RT, LC/A medulloblastoma are often seen in patients over 2 years of age. There is now general agreement that these are separate entities, and are so classified in the current WHO scheme.

Histopathological Progression in Embryonal Tumors

A grading system for medulloblastomas is important prognostically, but can aid in deciphering the biological significance of molecular changes as well. As has been demonstrated for carcinomas of colon, pancreas, bladder, and breast, among others, the ability to place molecular alterations in the context of a stepwise progression of histological tumor grade has been invaluable in determining the hierarchy of genetic events. The evolving concept of histological progression in CNS embryonal tumors will provide a similar framework.

Histological progression over time, from non-anaplastic to anaplastic medulloblastoma, has been described in several studies. In the report by Leonard et al, 2 of the seven LC/A medulloblastomas showed such temporal evolution (45). One tumor that was initially nodular recurred as an anaplastic lesion; a second had anaplasia first detected in a lymph node metastasis. We have described a similar temporal progression in 5 cases. The first was a nodular/desmoplastic medulloblastoma removed from an 11-year-old boy that recurred 3 years later as a moderately anaplastic lesion (20). Additionally, 4 medulloblastomas metastatic outside the CNS showed histological progression (17). One advanced from moderate to severe anaplasia in a CNS recurrence, while 3 had advanced anaplasia only in extra-CNS metastases. Others have reported an increased incidence of LC/A features in 6 medulloblastomas metastatic to the suprasellar region (35).

Even more common is "progression" of medulloblastoma grade within a single lesion, as inferred from the presence of differing degrees of cytological atypia or anaplasia in one tumor. High-grade histological features were focal rather than diffuse in almost half of the LC/A medulloblastomas we identified in our recent POG study (19). An example of such intratumoral heterogeneity, with localized anaplastic change, is shown in Figure 6. We believe these foci of LC/A tumor represent more aggressive subclones emerging from a lower-grade lesion. Perry has advanced a similar hypothesis (49). Changes consistent with anaplastic progression have been reported in many medulloblastoma subtypes (classic medulloblastoma, nodular/desmoplastic medulloblastomas, and medullomyoblastomas). In addition, we have seen similar anaplastic transformation in recurrent medulloepithelioma and supratentorial small cell embryonal tumors ("sPNET"). Thus, the potential for anaplastic progression may be common to all CNS small cell embryonal tumors (Figure 4). This

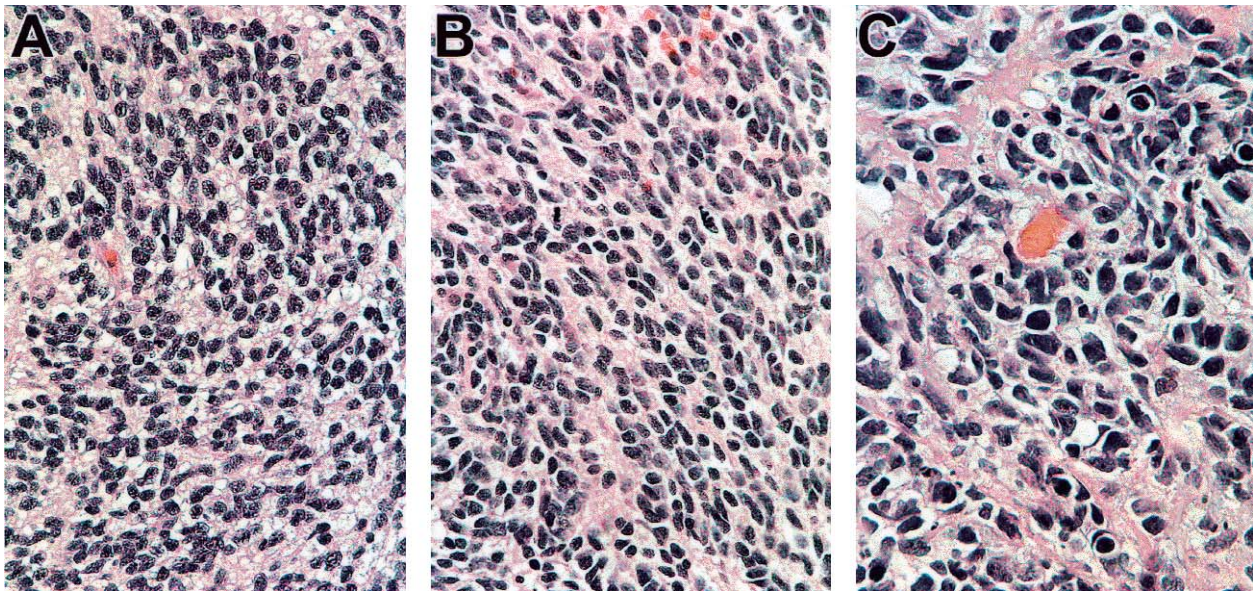

Figure 6. *Intratumoral variation in anaplasia.* Varying degrees of cytological atypia often occur within the same medulloblastoma. In this case, many regions were highly cellular but uniform (**A**), while others had slightly more nuclear atypia (**B**). Still others were overtly anaplastic with nuclear pleomorphism and cell "wrapping" (**C**). Original magnification for all images ×400.

should not be surprising, given similar anaplastic changes in systemic embryonal lesions such as Wilms' tumor (5).

Molecular Prognostic Variables

Molecular studies have unearthed numerous gene or chromosome alterations in medulloblastoma. Predictably, some of these are associated with better or worse clinical outcomes, but the relationships between molecular features and clinical behavior are not fully understood. Some researchers have found loss of chromosome 17p to predict poor clinical outcome (3, 14, 26), but others have failed to find this negative association (6, 22). Amplification of the *myc* oncogenes has been associated with malignant tumor behavior in a number of studies (1, 7, 20, 56), and increased c-myc mRNA expression has been proposed as a marker of aggressive lesions (31, 36). Overexpression of ErbB2 protein may also mark those tumors more likely to recur (26). On the positive side, increased TrkC expression is associated with better clinical outcomes, and the simultaneous evaluation of both TrkC and c-myc expression appears to have increased prognostic power (32,58). Finally, 2 recent large-scale studies of gene expression profiles have identified panels of genes whose differential expression correlated with tumor metastasis (46) or patient survival (51).

Molecular and Cytogenetic Alterations in Large Cell/Anaplastic Medulloblastoma

The molecular alteration most strongly associated with the LC/A medulloblastoma subtype is *myc* oncogene amplification. Prompted by the presence of numerous double minute chromosomes in one LC medulloblastoma, Giangaspero and colleagues identified amplification of *c-myc* in this case in their initial report (25). Myc is known to promote cellular proliferation, increased cell size and apoptosis – all features of LC/A medulloblastomas. In our comparative genomic hybridization (CGH) analysis of 33 medulloblastomas, tumors amplified at either the *c-myc* (4 cases) or *N-myc* (5 cases) loci were all anaplastic (20). *N-myc* or *c-myc* amplification has been detected in the majority of LC/A medulloblastomas examined (4, 7, 20, 25, 39, 45). It has recently been reported that increased c-myc mRNA levels also predict worse clinical outcomes (31, 36). Using in situ hybridization, we have shown that c-myc mRNA level is increased in LC/A medulloblastomas (CGE and PCB, unpublished data).

Loss of chromosome 17p, generally seen in conjunction with isochromosome 17q formation, is the most common cytogenetic abnormality in medulloblastomas. Interestingly, the 7 tumors with 17p loss in our CGH study were all anaplastic, and most had amplified *myc* oncogenes (20). In one case, 17p was intact in the primary lesion but lost in the recurrent tumor, suggesting it could be involved in medulloblastoma progression.

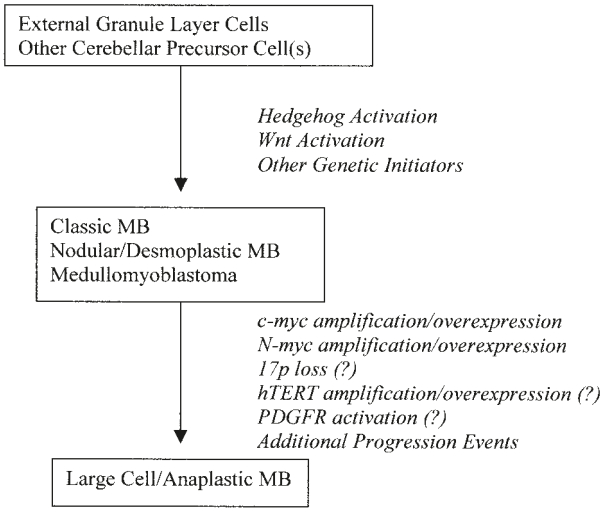

Figure 7. Possible molecular progression of medulloblastoma.

Others have reported that 17p loss in conjunction with *myc* oncogene amplification is associated with poor outcome (56). These 2 genetic alterations may therefore interact to promote tumor anaplasia.

Several groups have detected increased aneuploidy in LC/A medulloblastomas, suggesting that additional genetic changes associated with anaplasia are yet to be discovered (4,20). We identified, on average, more than twice as many chromosomal copy number alterations in tumors with anaplasia than in their non-anaplastic counterparts. Examination of additional LC/A medulloblastomas may ultimately reveal specific chromosomal loci commonly altered in these tumors. Expression profiling, which has already been used to discriminate between classic and nodular lesions (51), will also prove useful in identifying genes contributing to the LC/A medulloblastoma phenotype.

Molecular Progression in Embryonal Tumors

While evidence of molecular progression in medulloblastomas and other CNS embryonal tumors is only beginning to emerge, it seems clear that sequential genetic alterations of the type seen in most other solid tumors can occur in these small cell lesions as well. The strongest support comes from cases in which material from more than one operation is available for molecular analysis. We recently reported the CGH analysis of material from both a primary nodular medulloblastoma and an anaplastic recurrence removed 3 years later (20). Genetic alterations in the recurrent tumor affected both the same 4 chromosomes as in the initial case and 6 additional chromosomes as well. A similar progression of chromosomal abnormalities was identified when comparing a primary and recurrent medulloepithelioma, with 12 additional chromosomal alterations in the recurrent tumor, including amplification of the *hTERT* gene (62).

MacDonald and colleagues recently compared gene expression profiles of 23 metastatic and non-metastatic medulloblastomas using oligonucleotide arrays (46). They identified increased signaling through the MAP kinase pathway via upregulation of platelet-derived growth factor receptor alpha (PDGFα) in tumors that spread from the cerebellum, suggesting this pathway may also be involved in malignant progression and metastasis.

While many details of molecular and histological progression in medulloblastoma and other CNS embryonal tumors are still unclear, a rough framework can be suggested (Figure 7). The increased incidence of medulloblastomas in patients inheriting mutations that lead to activation of Wnt and Hedgehog signaling indicates that these pathways act early in medulloblastoma formation, with the latter pathway especially important in the pathogenesis of nodular lesions (50, 51). Animal models also support this concept (30, 33). Additional genetic hits, including "initiation" events involving pathways other than Hedgehog or Wnt, almost certainly occur as well. All medulloblastoma subtypes, along with other embryonal tumors such as medulloepithelioma, can then progress histologically and molecularly via mutation or amplification of oncogenes (*c-myc*, *N-myc*, *hTERT*, etc), upregulation of various signaling pathways (PDGF) or loss of as yet uncharacterized tumor suppressor loci (17p).

Future studies will map the genetic and histological course of medulloblastoma progression more fully. Primary and recurrent lesions should be compared both histologically and molecularly to determine what molecular abnormalities and gene expression changes are associated with recurrence and progression. Similar analyses of microdissected anaplastic and non-anaplastic regions will yield additional information. Finally, examination of genetically engineered mice developing medulloblastoma with anaplastic progression will enable more rigorous testing of proposed genetic interactions. A better understanding of how molecular and microscopic events interrelate will hopefully facilitate greater precision and efficacy in the treatment of patients with medulloblastoma.

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Note Added in Proof.

McManamy and colleagues recently identified large cell/anaplastic changes in 52 (19%) of 273 of non-desmoplastic medulloblastomas from children entered into the pan-European PNET3 trial. They found tumor anaplasia to be a significant prognostic indicator in both univariate and multivariate survival analyses.

McManamy CS, Lamont JM, Taylor RE, Cole M, Pearson AD, Clifford SC, Ellison DW (2003) Morphophenotypic variation predicts clinical behavior in childhood non-desmoplastic medulloblastomas. *J Neuropathol Exp Neurol* 62:627-632.