In terms of its molecular biology and molecular genetics, medulloblastoma is the most thoroughly studied of the pediatric brain tumors. Alterations in chromosome 17, usually an isochromosome 17q, are the most common cytogenetic abnormalities. Similarly, deletion of the short arm of one 17 chromosome, the result of formation of an iso17q, is the most common molecular biological abnormality found. The gene or genes important in the development of medulloblastoma found on chromosome 17 have not yet been identified. Both a tumor suppressor gene and an oncogene have been identified that may play a role in the development of this tumor type. The *Patched (PTC)* tumor suppressor gene is inactivated in approximately 15% of medulloblastomas; this alteration may be specific to the desmoplastic variant. Oncogenic mutations in the *beta-catenin* gene are found in a small subset of medulloblastomas. Both of these genes play central roles in developmental pathways. Prognosis in this tumor type has been related to the level of expression of the neurotrophin receptor trkC. In this review, these and other molecular biological and genetic findings are discussed with respect to the development of medulloblastoma.

**Key Words** * medulloblastoma * primitive neuroectodermal tumor * tumor suppressor gene * molecular genetics * molecular biology

Current theories of oncogenesis suggest that tumors develop as the result of sequential genetic alterations. These alterations may activate dominant-acting oncogenes or inactivate tumor suppressor genes involved in the regulation of the cell cycle. Oncogenes are altered or activated forms of normal cellular genes, which are termed protooncogenes. Protooncogenes encode growth factors, growth factor receptors, transcriptional regulators, or proteins which transmit signals that lead to cell division.[1,10,26,60] Mutations in these genes lead to increased cell division by a variety of mechanisms. Tumor suppression genes encode proteins that control entry of a cell into the cell cycle.[53] Inactivating mutations in these genes can lead to unregulated entry into the cycle and, thus, to increased cell proliferation. Oncogenes are often referred to as "dominant" because mutation in one allele of the gene serves to activate the transforming properties of the gene. Tumor suppressor genes are termed "recessive" because both copies of the gene need to be inactivated to allow unregulated entry into the cell cycle.

**MOLECULAR BIOLOGY OF MEDULLOBLASTOMA/PRIMITIVE NEUROECTODERMAL**
Whereas extensive research has been performed to identify the molecular events that lead to brain tumors in particular astrocytomas in adults, fewer studies have been performed on tumors that arise in the pediatric population. Medulloblastoma, the most common malignant brain tumor in children, is the pediatric brain tumor most extensively studied at the molecular level. Results of initial cytogenetic studies have revealed loss of genetic material on the short arm of chromosome 17 in approximately one third of tumors.[9,20,30] The loss of heterozygosity (LOH) on 17p has been confirmed by restriction fragment length polymorphism analysis. In the initial study, loss of all informative markers on 17p was shown in three of 13 tumors.[49] This work was extended to show 17p loss in between 25% and 50% of cases.[6,13,61] Because the p53 gene is located on 17p, a number of investigators have examined the status of this gene in medulloblastoma.[2,4,6,51,54] Taken together, the results of these studies clearly demonstrate that, despite the deletion of one p53 allele in many of the tumors, the remaining p53 gene is rarely mutated in medulloblastoma. Interestingly, a small subset of tumors have only a small area of 17p loss that does not include the p53 gene.[6,13,51] This finding suggests the possibility that another tumor suppressor gene is present on 17p. This putative suppressor might be important in the oncogenesis of medulloblastoma. The results of fine mapping studies of the region have suggested this putative suppressor is located near the abr gene on 17p13.3.[40]

We have defined the rearrangement in one tumor in which there is loss of one 17p arm and rearrangement in the other.[21] The rearrangement consists of a 9-kb deletion located near the minisatellite marker D17S34 rat Noc-2 gene.[35] We have named this homolog "(HAS)RPH3AL." High homology with rat Noc-2 was evident, with 77% identity on the amino acid level and 98% identity in the zinc finger regions of the protein. The intron/exon structure of the gene was determined; nine exons were identified. Our bank of 29 primitive neuroectodermal tumors (PNETs) with matched constitutional DNA was then screened for 17p LOH, and 10 tumors with LOH were identified. All exons of the Noc-2 gene were sequenced in all 29 tumors; no mutations were identified, which suggests that Noc-2 does not function as a tumor suppressor in PNETs.[59] Thus, the only PNET that has been described to have a homozygous deletion on 17p13.3 does not help identify a tumor suppressor gene in this region in PNETs. This result suggests the possibility that the actual rearrangement of chromosome 17 that occurs in a PNET, rather than the deletion of 17p, is the important genetic event.

The breakpoint on the 17p arm has been defined in two recent publications.[55,58] In the first paper the authors examined 24 tumors for LOH on 17p, which was demonstrated in nine cases. Careful analysis of the data presented in this paper reveals that the p arm breakpoint is between the loci D17S71 and D17S691. These markers are located within approximately 7 cM in band 17p11.2. We have mapped the breakpoint in 11 tumors with LOH by using 20 microsatellite markers in 17p11.2 and identified the breakpoint as lying between the markers D17S1843 and D17S122 in 10 of the 11 tumors. Because the i17q in PNET contains two centromeres, the rearrangement involves a break only in the p arm, and there is no q arm breakpoint.[7]

Although p53 appears not to be mutated in medulloblastoma, other means of inactivating the protein exist. For example, the mdm-2 gene product binds to p53 and inactivates it. Amplification of the mdm-2 gene has been described as a mechanism of p53 inactivation in sarcomas.[38,45] However, no evidence for mdm-2 gene amplification has been detected in medulloblastoma.[2]

In addition to loss of genetic material on 17p, medulloblastomas have been shown to lose portions of 6q
and 16q, but no candidate suppressor genes that may have a role in the development of PNETs have been identified in these regions.[61]

**INHERITED SYNDROMES AND MEDULLOBLASTOMA**

Medulloblastomas may occur in association with two different inherited cancer syndromes: Gorlin's syndrome and Turcot syndrome. Gorlin's syndrome, also called "nevoid basal cell carcinoma syndrome (NBCCS)," is an autosomal-dominant disorder.[19] Patients with Gorlin's syndrome develop multiple basal cell carcinomas, multiple odontogenic keratocysts of the jaws, palmar and plantar dyserkeratoses, and skeletal anomalies, especially rib malformations. In addition, at least 40 cases of medulloblastoma have been reported in patients with this syndrome, indicating that approximately 3% of patients with Gorlin's syndrome develop medulloblastoma.[15,37] The gene for Gorlin's syndrome has been mapped to chromosome 9q31.[16,17] However, in only five of 36 cases of medulloblastoma examined was 9q31 loss demonstrated, and in only one of these was loss of the 9q marker most closely linked to the Gorlin's syndrome gene.[3,56] Investigations have suggested that 9q deletions occur only in the desmoplastic subtype of medulloblastoma, which raises the possibility that a Gorlin's syndrome gene mutation is involved in the development of this subclass of tumor.[32]

The gene for Gorlin's syndrome has recently been identified as the **Patched (PTC)** gene, the human homolog of the Drosophila patched gene.[23,29] This Drosophila patched gene encodes a protein with 12 putative transmembrane domains, and it may function as a receptor or transporter.[25,43] The protein has an essential role in embryonic patterning in Drosophila; a similar role in humans may explain the congenital anomalies associated with Gorlin's syndrome.

The PTC protein has an important regulatory role in the Hedgehog (Hh) signaling pathway, a complex series of inhibitory elements present in vertebrates as well as in Drosophila.[46] The regulatory element, Hh, in an undefined fashion upregulates the expression of the **wingless** and **cubitus interruptus** genes. Hedgehog also upregulates PTC, which in turn downregulates Hh, cubitus interruptus, wingless, and, interestingly, PTC itself. Cubitus interruptus plays an essential role between Ptc and Hh.[14] Hedgehog upregulates cubitus interruptus, which in turn upregulates other genes in the pathway. Conversely, PTC downregulates cubitus interruptus. Overexpression of cubitus interruptus is analogous to absence of PTC function. All of these proteins have human homologs. The **Hh** gene has three homologs: Sonic hedgehog (Shh), indian hedgehog, and desert hedgehog. The Shh in particular has a tissue distribution similar to PTC.[22] The human homolog of **cubitus interruptus** is gli, a transcription factor that may serve as an oncogene in rare glioblastoma multiforme.[33] The human homolog of **wingless** is Wnt, a gene whose overexpression causes mammary tumors in mice.

There have been descriptions of **PTC** mutations in patients with NBCCS and in spontaneous basal cell carcinomas. We have investigated PNET for alterations in **PTC**. In our initial study, medulloblastomas obtained in 24 patients were examined to determine the frequency of LOH in 9q22.3, the region of the **PTC** locus; two of these, M1 and M16, were of the desmoplastic subtype.[50] The microsatellite analysis revealed five tumors with allelic loss in the **PTC** region. Because all patients were heterozygous at one or more loci, the data suggest that no patient had germ-line loss of the **PTC** region.

Using a combination of single-strand confrontational polymorphism (SSCP) and direct sequencing analysis, all 23 exons were examined from the retained **PTC** allele in the five tumors with 9q22.3 LOH. The **PTC** gene mutations were identified in three cases, and interestingly, all of these were in exon 17. Two of the mutations were small duplications, and the third was a single base-pair insertion. None of
these alterations was observed in germ-line DNA. In using SSCP and sequence analysis of exon 17 in the remaining 19 tumors without LOH no additional mutations were revealed. We have extended these findings in an additional 37 tumors, of which six were desmoplastic.[69] Of those with matched constitutional DNA samples, one demonstrated LOH. The remaining PTC allele was sequenced in this tumor, but no mutation was identified. Of those without matched constitutional DNA, six were homozygous with all markers. Sequencing of the PTC gene in these samples revealed mutations in three tumors, M73, M93, and M111, of which two (M93 and M111) were desmoplastic. Sequence from M73 demonstrated that there was no LOH at the PTC locus in this tumor; however, a 7-bp insertion mutation (2465ins7) was identified in one allele. These results suggest that PTC mutations occur in approximately 10% of sporadic PNETs, a finding in agreement with later reports from other investigators.[47,63,66,67]

One possible reason for the few 9q deletions seen in medulloblastoma may be that other alterations in the Ptc/Hh pathway are the common alteration in this tumor. This hypothesis suggests that overexpression of any of the proteins normally downregulated by PTC could lead to medulloblastoma. In patients with Gorlin's syndrome, down regulation would fail because the PTC gene itself is mutated. In spontaneous tumors, alterations leading to overexpression of Shh, gli, or Wnt could be responsible. In addition, mutation of potential tumors suppressor genes in the pathway, other than PTC, might be occurring in PNETs. To address the frequency of genetic alterations affecting genes in this pathway, we used a combination of LOH analysis, SSCP analysis, and direct sequencing of DNA samples obtained from sporadic PNETs.[69] To identify alterations in the Shh and Smoothened genes, we analyzed all exons of both genes in 37 tumors by using SSCP, and we sequenced any exons that showed aberrant band patterns. No mutations were found in either Shh or Smoothened genes, or in any tumor. We also identified the following genes as candidate tumor suppressors based on their roles in controlling hh/ptc signaling in Drosophila: EN-1 and EN-2, the deletion of which results in a lack of cerebellar development in mice; SMAD family members 1 to 7; and protein kinase A subunits, RI alpha, RI beta, RII beta, C alpha, and C beta. Each of these genes was investigated in a panel of 24 matched constitutional and tumor DNA samples. Our analysis revealed no mutations in any of these genes. Thus, PTC is the only gene in this complex pathway that is mutated with notable frequency in PNETs.

Another tumor suppressor gene has been identified on chromosome 9, the MTS-1 gene. This gene encodes an inhibitor of cyclin D-cyclin-dependent kinase 4 complex and has been mutated or deleted in a large proportion of adult astrocytomas. In two studies of medulloblastomas, however, no mutations of this gene were found in any of the 25 medulloblastomas that were investigated, suggesting that this gene is not involved in medulloblastoma oncogenesis.[28,52]

Turcot syndrome is a hereditary disorder in which the affected individuals have multiple colonic polyps and a brain tumor.[62] In one study, mutations in the APC gene were identified in a group of patients with Turcot syndrome in whom the type of brain tumor was a medulloblastoma.[24] The relative risk for developing a medulloblastoma in the patients with Turcot syndrome and an APC gene mutation was 92 fold that in the general population. Surprisingly, in light of this association, APC gene mutations have not been identified in spontaneously occurring medulloblastomas. In one study, DNA samples obtained from 47 medulloblastomas were examined for mutations in the portion of the APC gene that contains at least two thirds of the mutations seen.[41] In no tumor was an APC mutation observed. Interestingly, in the two medulloblastomas that were removed from patients with Turcot syndrome, the germ-line mutation was identified, but no mutation was seen in the other APC allele. In a second study, DNA obtained from 23 medulloblastomas was examined for deletions in the region of the APC gene.[68] No LOH was found in this region in any tumor. Analysis of these results suggests that the APC gene is not
important in the development of sporadic medulloblastoma and even suggests that the gene may not be important in the development of medulloblastoma in patients with Turcot syndrome.

Although we were surprised at the lack of \textit{APC} mutations in sporadic PNETs given the association with Turcot syndrome, we believed that PNETs should also be evaluated for oncogenic mutations in \textit{beta-catenin}, because these mutations result in the same phenotype as \textit{APC} inactivation. In one study exon 3 of \textit{beta-catenin} (the location of all glycogen synthase kinase-3 beta phosphorylation sites), obtained from 67 sporadic medulloblastomas was investigated by direct sequencing.[70] Two tumors were found to have point mutations in codon 33 (tumors M4 and M43S), and one tumor (M18S) had a point mutation in codon 37. In each case, the alteration results in the substitution of a cysteine for a serine at a glycogen synthase kinase-3 beta phosphorylation site. Sequencing of exon 3 in the constitutional DNA obtained in these patients with medulloblastoma failed to show a mutation, indicating that the identified mutation is tumor specific. Two additional mutations were found, both of which altered an amino acid in the ubiquitin-binding region, also in exon 3. These results suggest that oncogenic mutations in \textit{beta-catenin} do occur in PNETs. Whether alterations in other genes in the \textit{APC/beta-catenin} pathway also occur is a question worthy of further study.

That close to 20\% of medulloblastomas contain double minute chromosomes suggests the possibility of gene amplification.[9] Gene amplification of 11 oncogenes has been investigated.[65] Of 20 primary medulloblastomas examined in a study conducted by Wasson, et al.,[65] only one tumor was found to have amplification of the \textit{c-erbB1} protooncogene; no other tumor had amplification of any of the oncogenes examined.[65] Interestingly, of the four established medulloblastoma cell lines that were also tested, three were shown to have protooncogene amplification, one had amplified \textit{c-myc}, one \textit{n-myc}, and one \textit{c-erbB1}. These investigators suggested that in vitro culture may select for a small subpopulation of cells within the tumor that have preexisting amplification or that culture is more successful with tumors that have an amplified protooncogene.[8]

Amplification of the \textit{myc} family of protooncogenes in medulloblastoma has revealed \textit{c-myc} amplification in five of 43 tumors in reports other than the aforementioned study.[5,39,49] In addition to the amplification of \textit{n-myc, n-ras} mutations, \textit{epidermal growth factor receptor (EGFR) c-erbB-2} expression, and \textit{bcl-2} expression have all been investigated in medulloblastoma.[5,18,27,44,65] No consistent pattern of expression, mutation, or amplification has been identified. Because \textit{EGFR} is frequently amplified and/or rearranged in glial tumors, seven medulloblastomas were analyzed for \textit{EGFR} copy number and gene rearrangements;[65] neither was found in any tumor. \textit{Homeobox} genes have also been examined in medulloblastoma. Interestingly, the \textit{PAX5} gene, which is not expressed during cerebellar development, is expressed in medulloblastoma.[36] The significance of this finding is unclear at this time.

Members of the neurotrophin family (nerve growth factor [NGF], brain-derived neurotrophic factor, NT-3, and NT-4/5) and their family of high-affinity receptors (trkA, trkB, and trkC) are important in the proliferation, differentiation, and survival of neuroepithelial cells. In addition to the trk receptors, a low-affinity receptor for neurotrophins (p75LNGFR) has also been described. Because medulloblastoma is thought to arise from primitive neuroepithelial stem cells, neurotrophins and their receptors may be important in the development and growth of these tumors.

Three studies have been performed in which the status of the low-affinity NGF receptor in medulloblastoma was examined.[31,34,48] Approximately one third of tumors had messenger RNA for
this receptor. Expression of p75LNGFR may be restricted to the subset of PNETs in which neuronal differentiation is demonstrated. The addition of NGF had no effect on the growth characteristics or morphology of a PNET cell line that expressed the receptor.

Expression of trk receptors and two neurotrophins has been analyzed in medulloblastoma specimens by immunohistological investigation.[64] Variable expression of trk receptors was found in the 29 tumors examined. Most tumors expressed at least one functional receptor, some tumors expressed more than one receptor, and often one of these receptors was in a truncated form. Brain-derived neurotrophic factor and neurotrophin-3 were seen in 22% and 9% of tumors, respectively. Interestingly, in one study of a small number of tumors good clinical outcome was correlated with high expression of full-length trkC messenger RNA.[57] Transfection of trk family members into PNETs has yielded contradictory results. In one study, transfection of trkC into the Daoy and D283 Med cell lines followed by treatment with NT-3 had no effect, whereas transfection of trkA into the same cell lines followed by treatment with NGF resulted in massive apoptosis.[42] In a second study, transfection of trkC into Daoy followed by NT-3 treatment resulted in apoptosis.[32] In our laboratory, treatment of PNET cell lines, including Daoy and D283med, with NT-3 resulted in increased cell growth.[11] The reasons for the differences in results obtained in different laboratories need further investigation.

Just as the neurotrophins and their receptors have a role in the growth and differentiation of neuroepithelial cells, the insulin-like growth factors (IGFs), IGF-1 and IGF-2, and the type 1 IGF receptor, IGF receptor-1, have a role in the growth of many tissues including those of the central nervous system. In one study of medulloblastoma cell lines, all were found to express IGF receptor-1.[12] Blocking of ligand binding with a monoclonal antibody to the extracellular portion of the receptor inhibited growth of the cells in vitro. Further work is needed to extend these findings to the tumors themselves.

CONCLUSIONS

A large body of information has been generated concerning the genetic alterations that occur in adult brain tumors. Although less is known about pediatric tumors, considerable progress has been made in PNETs. Despite this work, knowledge of the many and complex pathways that lead from a normal cell to tumor initiation and progression is far from complete. As new techniques are developed and new genes with potential roles in tumorigenesis are isolated, these pathways will become better delineated.

With current and future information, the diagnostic, prognostic, and therapeutic implications of the genetic alterations in tumor cells will be explored. Indeed, molecular tumor typing may eventually supplant histological examination as a way of identifying tumors. Studies are needed in which the presence or absence of specific genetic alterations is correlated with patient prognosis. Genetic alterations in tumors may also become important in treatment. Specific genetic deficiencies in tumors could be replaced by gene-transfer techniques, whereas overexpression of a gene may lead to use of drugs which specifically inhibit that gene's protein product.

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