Medulloblastoma: From Molecular Pathology to Therapy

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Abstract

Medulloblastoma is the most common malignant tumor of central nervous system in children. Patients affected by medulloblastoma may be categorized as high-risk and standard-risk patients, based on the clinical criteria and histologic features of the disease. Currently, multimodality treatment, including surgery, radiotherapy, and chemotherapy is considered as the most effective strategy against these malignant cerebellar tumors of the childhood. Despite the potential poor outcomes of these lesions, the 5-year survival stands, at present, at 70% to 80% for standard-risk patients, whereas high-risk patients have a 5-year survival of 55% to 76%. Attempts to further reduce the morbidity and mortality associated with medulloblastoma have been restricted by the toxicity of conventional treatments and the infiltrative nature of the disease. Over the past decade, new discoveries in molecular biology have revealed new insights in signaling pathways regulating medulloblastoma tumor formation. Recent advances in the molecular biology of medulloblastoma indicate that the classification of these embryonal tumors, solely based on histology and clinical criteria, may not be adequate enough. Better understanding of the growth control mechanisms involved in the development and progression of medulloblastoma will allow a better classification, leading to the improvement of the existing therapies, as well as to the development of new therapeutic approaches.

Medulloblastomas are the most common cerebellar tumors of central in childhood (1). They are neuroepithelial tumors arising from the cerebellum and account for ~20% of all intracranial tumors in children, and for 40% of all childhood posterior fossa tumors. Although medulloblastoma peaks at 8 years of age, ~30% of medulloblastomas occur in adults (2).

Medulloblastoma arises from remnants of the primitive neuroectoderm in the roof of the fourth ventricle. It grows in cerebellar vermis and fills the ventricle, often invading through the ependyma in the floor of the ventricle to enter the brainstem. Less commonly, the tumor arises in the cerebellar hemisphere (3). The histogenesis of this tumor has been controversial for many years. Some authors support the idea that it arises primarily from primitive neuroectodermal cells in the germinal matrix surrounding the ventricle. According to another study, medulloblastomas have been shown to express the ZIC1 gene, which is normally expressed only in the external granular layer of the developing cerebellum, suggesting that this tumor arises from external granular layer precursor cells (4). It is also possible that cells from both of these locations give rise to medulloblastomas. In fact, there might be more than one cell type; the nodular variant originating from the external granular layer, and the classic histotype from cells of the subventricular matrix (1, 5). Historically, medulloblastomas and other central nervous system embryonal tumors are classified on the basis of their location within the central nervous system, and of their histologic features. Five histologic variants are recognized according to the WHO: (a) the classical variant medulloblastoma, in which the cells occasionally display features of neuroblastic differentiation; (b) desmoplastic medulloblastoma, in which tumor cells commonly show neurocytic differentiation, and are surrounded by a collagen-rich extracellular matrix; (c) large-cell anaplastic medulloblastoma, associated with poor prognosis and short survival; and finally (d) the melanotic and (e) medullosomyoblastoma variants, which are less common (1, 5).

Clinical outcome of patients with medulloblastoma varies according to age, postoperative tumor residuum, and metastatic stage (1). Therefore, this disease is also classified according to risk-adapted treatments. High-risk patients with medulloblastoma are distinguished from standard-risk patients, because they are either 3 years old or younger, have metastases, or show residual tumor postoperation, and should receive more intensive treatment (1).

Over the past decade, new discoveries in molecular biology have shown evidence that classification of embryonal tumors by histology and clinical criteria should also include the identification of specific gene mutations which could be necessary to differentiate medulloblastoma from other highly malignant tumors, otherwise not detectable by morphologic criteria (3). This review summarizes the signaling pathway alterations identified in medulloblastoma, with a particular attention on the
development of new molecular targets to improve the clinical management of this disease.

Molecular Biology of Medulloblastoma

Genetic alterations and aberrant signaling pathways in medulloblastoma. Medulloblastoma is a heterogeneous cancer of unknown etiology. An important contribution to the understanding of medulloblastoma came from the study of the Gorlin’s syndrome and the Turcot’s syndrome, two genetic disorders which show an unusual predisposition to medulloblastoma formation (6, 7). Gorlin’s syndrome, or basal cell nevus syndrome, is a rare autosomal dominant disorder associated with skeletal anomalies, large body size, and a high incidence of basal cell carcinoma and medulloblastoma (3). It is characterized by germ line mutations of the PTCH gene, which encodes for a transmembrane protein capable of binding the Hedgehog (HH) family of signaling proteins (6, 8). How changes in PTCH and other pathway components predispose to medulloblastoma is currently under investigation. During the normal development of the cerebellum, PTCH is able to maintain the sonic hedgehog (SHH) pathway in the off state by interacting with smoothened protein (SMO). Upon binding of SHH, which is secreted by Purkinje cells, PTCH-mediated repression is alleviated and a signal is transduced to the nucleus to promote the proliferation of granule cells (9). Because most medulloblastomas seem to originate from this cell lineage, deletion of PTCH in granule-neuron precursor cells might result in malignant transformation. Mutations in the HH pathway may promote its constitutive activation and deregulated proliferation of granule cells to induce medulloblastomas (8). Mutations or deletions of the PTCH locus, and of the suppressor of fused (SUFU) locus, a downstream molecule of the HH signaling cascade, have recently been associated with sporadic medulloblastomas, indicating that PTCH and SUFU function as classic tumor suppressors in this subset of tumors (1, 6). Medulloblastomas that arise in children with Gorlin’s syndrome and ~10% of sporadic medulloblastomas have disregulated SHH/PTCH signaling (6) and have desmoplastic histology (10).

Turcot’s syndrome (glioma-colonic polyposis syndrome) is a rare heritable disorder, associated with colon cancer and malignant neuroepithelial brain tumor, usually glioblastoma multiforme or medulloblastoma (7). Mutations of the adenomatous polyposis coli (APC) gene have been linked to this syndrome (11). APC is a component of the wingless (Wnt) pathway, which coordinates a diverse array of developmental processes including the proliferation and the fate of neural progenitor cells (11). The binding of Wnt ligands to the receptor Frizzled activates the Wnt cascade, signaling to the nucleus through a molecular complex which includes, among other molecules, APC, glycogen synthase kinase 3β (GSK 3β), Axin, and β-catenin. Activation of the Wnt pathway inhibits the phosphorylation of β-catenin by GSK 3β, allowing unphosphorylated β-catenin to translocate to the nucleus and to promote the expression of a specific set of genes, such as c-MYC, CCND1, and NR5A1, which are involved in the proliferation, inhibition of apoptosis, and differentiation of cells in the developing central nervous system and in other organs of the body (8, 12). APC defines its role as a tumor suppressor by controlling levels of free β-catenin in the cytoplasm (7, 13).

Unlike the association between germ line APC gene mutations and medulloblastoma found in Turcot’s syndrome, mutations in this gene are quite rare, and may have no functional consequences in spontaneously occurring medulloblastoma (7). Although APC is the only gene of the Wnt pathway associated with inherited predisposition to medulloblastoma, to date, mutations of APC, axin and β-catenin have been associated in sporadic medulloblastoma (7, 13). From different studies on APC gene mutations, it has emerged that many medulloblastomas present alterations in the control of β-catenin levels, rather than APC inactivation (3, 7, 13). In particular, most of the Wnt pathway mutations reported in sporadic medulloblastomas target residues of serine 33 and 37 of β-catenin, which prevent phosphorylation-dependent degradation of β-catenin by GSK-3. As a consequence, β-catenin levels increase in an uncontrolled manner, leading to the development of a transformed phenotype (3, 13).

Despite the fact that many medulloblastomas show identical histologic features, they respond differently to surgery, radiation, and chemotherapy. These differential responses may be ascribed to the involvement of other molecular pathways leading to a growth deregulation and to the promotion of invasion and metastasis. The molecular and biological analyses of medulloblastoma have identified new markers that could be potentially used for risk stratification and clinical trials. For example, the expression of several growth factor receptors has been linked to both good and poor prognosis: TrkC, the neurotrophin-3 receptor, for instance, has been found to be associated with favorable prognosis (1, 14). In fact, the biological actions of TrkC activation affect medulloblastoma outcome by inhibiting tumor growth through the promotion of apoptosis (14). Epidermal growth factor receptor 2 (ERBB-2), platelet-derived growth factor receptor, insulin-like growth factor receptor I, have also been associated with poor prognosis: ERBB-2 is a member of the ERBB family of tyrosine kinase I receptors, which regulate important cellular processes such as proliferation, apoptosis, migration, and differentiation (1, 8). ERBB-2 overexpression promotes the expression of proteins of the mitogen-activated protein kinase pathway, as well as the calcium-binding protein S100A4, which has already been linked to metastatic behavior in breast and bladder cancer (1, 8). Platelet-derived growth factor receptor-α was also found to enhance medulloblastoma migration in an in vitro assay and it has been correlated with metastatic behavior (8, 15). The expression of downstream effectors of growth factor receptors, such as Ras/mitogen-activated protein kinase and the transcription factor c-myc, has also been correlated with poor prognosis (8, 15).

Association with viral infections. Several lines of evidence suggest an association between the occurrence of medulloblastoma and the human neurotropic polyomavirus JC (JCV; ref. 16). More than 80% of neonatal hamsters inoculated intracerebrally, i.p., or s.c. with strains of JCV, isolated from progressive multifocal leukoencephalopathy lesions, develop a wide range of tumors including medulloblastomas, neuroblastomas, and pineocytomas (16, 17). Although the mechanism of JCV-induced neurtumorigenesis is not entirely clear, several studies point to the involvement of the viral early protein, T antigen, in this process. One of these studies showed that transgenic mice constitutively producing T antigen under the control of JCV early promoters/enhancers develop adrenal neuroblastomas, primitive-appearing mesenteric tumors and
Medulloblastoma and pRb family of tumor suppressor genes. The pRb family is a group of nuclear proteins including pRb/p105, p107, and pRb2/p130. These proteins are the major regulators of cell proliferation and cell differentiation through their ability to suppress cell cycle progression (17–19). Although pRb/p105 does not seem to be mutated in medulloblastomas, it has been identified as a tumor suppressor gene deleted or mutated in childhood retinoblastoma and in a variety of adult cancers (18). The role of p107 and pRb2/p130 in tumor suppression is less clear than that of pRb/p105, but there are several reports of pRb2/p130-inactivating mutations identified in human cancers (20–22). In humans, pRb/p105 mutations have been found in high-grade astrocytic tumors, and the loss of its function is considered to be involved in the progression from the benign form to the highly malignant astrocytoma. Therefore, the lack of an initiation step might be responsible for the lack of tumor formation of pRb/p105-null mutant astrocytes. Evidences suggest that loss of p53 function could be the initiator event in low-grade astrocytomas (23). Marino et al. (23) have studied the role of pRb/p105 in the neoplastic transformation of astrocytes. In their study, a mouse model for medulloblastoma was generated by Cre-LoxP-mediated inactivation of pRb and p53 in the external granular layer cells. They observed that pRb/p105 was not required for normal maturation of astrocytes, and that the independent inactivation of p53 or pRb/p105 was not sufficient to cause the neoplastic transformation of these cells "in vivo." These authors showed that pRb/p105 somatic inactivation, in combination with a somatic or a germ line p53 inactivation, leads to medulloblastomas in mice (23). Furthermore, it was evidenced that the biologically active NH2-terminal fragment of SHH acts to up-regulate and maintain the cyclin-retinoblastoma axis by regulating the cyclins CCND1, CCND2, and CCNE mRNA transcript and protein levels in a subset of primary cultures from neonatal murine cerebellum (24). SHH-induced CCND and CCNE expression clearly associated with cell cycle progression, as hyperphosphorylation of pRb/p105 and increased levels of DNA synthesis were observed (24). Therefore, activating mutations in the HH1 pathway, important for medulloblastoma formation, may cause alterations in the phosphorylation status of pRb/p105.

Although there is no evidence regarding alterations of the pRb2/p130 pathway in medulloblastoma, it seems to be implicated in glioblastoma. In fact, one study shows that pRb2/p130 plays a role in radiation-induced cell death, indicating that the antitumoral activity of pRb2/p130 could regulate both the inhibition of cell cycle progression and induction of cell death (25).
in those with cerebrospinal fluid metastases at presentation (3). Several chemotherapeutic regimens have been investigated for the treatment of medulloblastoma, as shown in Table 1. Verlooy et al. (35) have analyzed preradiotherapy chemotherapy in high-risk patients in order to treat microscopic metastasis and to reduce the tumor burden prior to radiotherapy. In general, these studies have shown that the combination of postradiotherapy chemotherapy and surgery is more effective than radiation and surgery (26). It was also investigated if intensive postoperative chemotherapy alone could improve survival and cognitive function in young children. This treatment is based on three cycles of intravenous chemotherapy and intraventricular methotrexate instead of radiotherapy, and has been shown to be a promising treatment in young children without metastases (36).

The combination of chemotherapy and radiotherapy has currently improved the 5-year survival rate to 55% to 76% for high-risk patients and 70% to 80% for standard-risk patients (1). Despite these improvements in overall survival rate after the multimodality treatment including surgery, radiation, and chemotherapy, a small but substantial number of patients will have recurrent or progressive disease. Unfortunately, attempts to further reduce the morbidity and mortality associated with medulloblastoma have been restricted by the toxicity of conventional treatments and the infiltrative nature of the disease (1).

**Conclusions and Future Perspectives of Molecular Biology to Therapy**

Currently, many strides have been conducted in the field of medulloblastoma, and several molecular modifications have been identified, the elucidation of which might have direct effects on tumor management (1, 3). In fact, a better understanding of the genetic events underlying the pathology of these tumors may contribute to the development of new more effective and less harmful clinical strategies. Many lines of evidences suggest that response to the treatment is not determined by chance, but rather by the biology of the tumor (1, 3). Furthermore, drugs that target cell signaling pathways implicated in the formation of medulloblastomas might provide an alternative to conventional cytotoxic approaches of cancer treatment, decreasing treatment-related toxicity (1, 3).

In one study, gene expression profiling was able to distinguish classic and desmoplastic medulloblastomas and to separate a series of medulloblastomas from malignant gliomas and atypical teratoid/rhabdoid tumor (1, 3). Atypical teratoid/rhabdoid tumor was delineated as an embryonal tumor with poor prognosis and linked with mutations of the INI1/SNF5 gene (1, 3). They cannot be distinguished from medulloblastomas by histologic criteria alone; thus, mutation analysis of INI1/SNF5 is essential to establish diagnosis (1, 3). Combining molecular and histopathologic analyses of tumors, together with clinical staging, might identify patients who can be cured with very low-intensity treatment, patients requiring moderate therapy, or patients who require very intensive or experimental treatment. For instance, a multicenter study showed that combining tumor ERBB2 expression and analysis of the clinical features of the patient permits a better assessment of the disease risk than clinical factors alone, even if this study needs to be confirmed in large prospective clinical trials (37, 38). In this study, all of the children with standard-risk ERBB2-negative disease were alive at 5 years, compared with only 54% for children with standard-risk ERBB2-positive cancer. Numerous growth factor receptors have been considered molecular markers, and may be used for the stratification of tumor in clinical trials, such as TrkC, which has been associated with good prognosis, and ERBB2, platelet-derived growth factor receptor-α, insulin-like growth factor receptor 1 which have been associated with poor prognosis (14, 39).

In a variety of childhood malignancies, a risk-adapted therapy, according to both disease status and molecular profile,
is currently considered routine. Thus, accurate disease risk assessment for patients with medulloblastoma might also be possible considering clinical and molecular prognostic markers (1, 3). At present, clinical trials of medulloblastoma by the Children’s Oncology Group are associated with biological studies aimed at validating molecular markers as outcome predictors, which will be used for risk stratification (1, 3).

Currently, numerous anticancer drugs are designed to target a specific protein or a signaling pathway. The efficacy of several of these molecules is now being investigated in medulloblastomas. Among these, cyclopamine is a plant-derived teratogen, the inhibitory activity of which acts on the SHH pathway by binding to, and inactivating, smoothened protein (SMO; ref. 40). Cyclopamine seems to interfere with this pathway by modulating the SMO function (41). In particular, this inhibitory effect is mediated by a direct binding of cyclopamine to the heptahelical bundle of SMO, likely affecting the protein conformation. This drug is able to target the SHH pathway, inhibiting SHH-dependent gene expression in medulloblastoma in vitro, and is able to cause cell cycle arrest consistent with the initiation of neuronal differentiation and loss of neuronal stem cell—like character (41, 42). This compound also causes the regression of murine tumor allografts in vivo and induces rapid death of cells from freshly resected human medulloblastomas, but not from other brain tumors (41, 42). Other inhibitors of the SHH pathway, such as HhAntag, are in preclinical studies (43).

Medulloblastoma is a highly invasive tumor, which is generally disseminated at the time of diagnosis. Furthermore, conventional therapeutic approaches have not reduced the mortality associated with metastatic medulloblastoma (1, 3, 8, 28). Thus, it is very important to find agents that are able to reduce tumoral invasiveness. In fact, another class of potential molecular-targeted therapies for medulloblastoma is represented by small molecule inhibitors of receptor tyrosine kinases. These include dual-specific inhibitors of ERBB1 or ERBB2 activity, such as OSI-774 (erlotinib; ref. 44). This drug is able to inhibit ERBB2 signaling in medulloblastoma in vitro and in vivo. Treatment with this drug selectively blocks ERBB2-dependent prometastatic gene up-regulation and reduces the invasiveness of medulloblastoma cells (44). At present, the inhibitors of ERBB1 or ERBB2 activity are in phase I and phase II clinical trials in children with brain tumors in the Children’s Oncology Group and the U.S. Pediatric Brain Tumor Consortium (44).

Inhibitors of the SHH and ERBB2 pathways represent a great promise as a first-generation molecular-targeted therapy for medulloblastoma, even if the development of these agents for routine clinical use will not be easy (43, 44).

It is very important to develop agents that are able to block Wnt signaling, given its activation in medulloblastoma (12, 45). Lawinger et al. (12) have shown that neural silencer element NRSF/REST, which is transcriptionally regulated by the Wnt cascade, is highly expressed in medulloblastoma cell lines and that cell growth is inhibited by specific molecular blocking agents. Dkk-3 and FRPs are Wnt signaling antagonists that could be used as natural inhibitors of this pathway in cancer therapy. In fact, expression of Dkk-3 is down-regulated in human immortalized and tumor-derived cell lines and the expression of the exogenous Dkk-3 gene in small cell lung cancer caused the inhibition of cell proliferation (45). Furthermore, it was shown that transient expression of a recombinant transcription factor, REST/VP16, was able to compete with the endogenous REST/NRSF for DNA binding (12). It counteracts the NRSF/REST-mediated inhibition of neuronal promoters, causing stimulation of endogenous neuronal gene expression and induction of apoptosis. There are other inhibitors of this pathway, but they are still in preclinical studies and, hopefully, should reach clinical trials in the next few years (12).

Line-1 (L1) are abundant retrotransposons that comprise ~20% of mammalian genome (46). In particular, they are endowed with a reverse transcriptase—coding gene which enables them to retrotranspose autonomously (46). Engineered human L1 harboring a retrotransposition indicator cassette could retrotranspose in adult rat neural progenitor cells in vitro and in the brains of transgenic mice in vivo. It has been shown that these events could influence both neuronal gene expression and differentiation in neural progenitor cells in vitro (46). According to the study by Sciamanna et al. (47), two characterized reverse transcriptase inhibitors, nevirapine and efavirenz, seem to reduce proliferation, induce morphologic differentiation, and reprogram gene expression in melanoma and prostate cancer cells. Thus, the use of reverse transcriptase inhibitors might represent a novel approach to block cell growth and to induce differentiation in medulloblastoma, and in addition, might overcome the problem of the blood-brain barrier because most of these inhibitors are lipophilic.4

Elucidation of crosstalk of the pathways during tumor formation is required in order to study how these anticancer drugs could be combined. Moreover, a deeper understanding of the molecular mechanisms of the resistance to cytotoxic chemotherapy and radiotherapy might allow the combination of molecular and conventional therapies in the very near future.

References

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