**Targeting Medulloblastoma: Small-Molecule Inhibitors of the Sonic Hedgehog Pathway as Potential Cancer Therapeutics**

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**Abstract**

Medulloblastoma is the most common malignant pediatric brain tumor for which no satisfactory treatments exist. The Sonic Hedgehog signaling pathway seems to play an important role in the pathology of this disease. Here we review our recent demonstration that a small-molecule inhibitor of this pathway can regress tumors that arise in a transgenic mouse model of medulloblastoma. These and other findings suggest that inhibitors of Sonic Hedgehog signaling may offer an effective way to target some malignancies. (Cancer Res 2005; 65(12): 4975-8)

**Introduction**

Current strategies to develop better cancer therapies rely to a great extent on targets identified from studying the genetics of human cancers. It is hoped that understanding the molecular pathways disrupted in cancer will yield a better understanding of molecular targets for therapy and thus lead to more specific and therefore less toxic treatments. Brain tumors offer a particularly difficult challenge not only because of their less accessible anatomic location but also because they are relatively rare, especially in the case of pediatric brain tumors.

Traditional drug development approaches for brain tumors were optimized for cytotoxic therapies involving radiation and chemotherapy. Although these treatments do show some efficacy, the drawback, especially for treatment of pediatric brain tumors, are the severe and life-long side effects (1). As yet, no specific "designer" drug has been made for brain tumors. One of the chief obstacles in the field is that testing new drugs relies on tissue culture and xenograft models, which recapitulate some but not all of the features of malignancy. In particular, medulloblastoma tumor cells are notoriously difficult to grow in cell culture and in murine allograft and xenograft (11). Therefore, developing and testing new targeted therapies. We propose here a three-step approach for preclinical development of such drugs: (a) Identify the control pathways driving tumor growth. (b) Create a genetically equivalent, high-incidence animal model, which recapitulates the specific subset of human disease with respect to developmental time frame and anatomy. (c) Design specific inhibitors of the growth control pathway impaired in tumors and test them in the animal model. Efforts to identify new cancer modalities have increasingly relied upon advances in realizing the three elements of this strategy.

Our laboratory studies the molecular causes underlying medulloblastoma, the most common malignant pediatric brain tumor. Whereas there are several genetic subtypes of medulloblastoma, the study described here is concerned specifically with tumors arising due to disruption of the Sonic Hedgehog (SHH) pathway. The first indication that mutations in the SHH pathway contribute to medulloblastoma was the finding that Gorlin syndrome patients, who inherit germ line mutations in the PTCH1 gene, are predisposed to medulloblastoma (2, 3). PTCH1 is a trans-membrane protein that functions as a receptor for Shh. In the absence of Shh, PTCH1 maintains the Shh pathway in the off state by acting on Smoothened (SMO). Upon Shh binding to PTCH1, this repression is lifted and a signal is transduced through Smo to the nucleus increasing expression of target genes including GLI1 and PTCH1 itself (Fig. 1). This pathway is crucial for normal development of the cerebellum, because it governs the proliferation of granule neuron precursor (GNP) cells, the most abundant neuronal population in the brain. GNP cells are believed to be the cells of origin for medulloblastoma, although definitive proof is still lacking. It has been suggested that medulloblastoma arises as an aberration of normal developmental processes; a GNP cell fails to exit the cell cycle at the appropriate time and remains in the EGL, eventually expanding to form a tumor (Fig. 2; ref. 4). The finding that over 30% of sporadic medulloblastomas show elevated expression of GLI1 supports this hypothesis (5).

The contribution of an inappropriately active SHH pathway to medulloblastoma formation makes it an ideal target to develop new therapies. In our recent study (6), we set out to investigate whether suppressing the overactive SHH signaling in medulloblastoma could cause tumor regression in vivo. To achieve this aim, we used an animal model of medulloblastoma (7), in which mice are heterozygous for the Ptc1 gene and null for the p53 gene. All Ptc1+/p53−/− mice spontaneously develop medulloblastoma as early as at 2 weeks of age and they die from the disease by week 16. The 100% incidence and short latency of tumor development makes this mouse strain well-suited to validate the therapeutic potential of Shh pathway inhibitors. The naturally occurring inhibitor of Shh signaling, cyclopamine (8, 9), which binds to Smo (10), has been shown to inhibit the growth of medulloblastoma cells in cell culture and in murine allografts and xenografts (11). However, although these studies are encouraging, extrapolation of
these results to appropriate tissue settings would be desired to address shortcomings of the artificial environments presented by tissue culture and xenograft studies. This is especially pertinent in the case of Shh pathway–dependent tumors, as we have shown that this pathway is down-regulated in cultured medulloblastoma cells, rendering them ineffective for testing therapies based on suppression of the Shh pathway (6).

To avoid these problems we relied on in vivo studies using a novel, small-molecule inhibitor of the Shh pathway, HhAntag-691 (12), that readily enters the brain and suppresses Shh signaling by binding to Smo (Fig. 3). This compound, a benzimidazole derivative, inhibits the Shh pathway at lower concentrations than cyclopamine. We treated Ptc1+/p53−/− mice with different doses of HhAntag-691, as well as with vehicle alone, and found that the treatment led to a dose-dependent down-regulation of several genes that are overexpressed in medulloblastoma (Gli1, Ptc2, Sfrp1, and Math1). In contrast, Reln, a gene highly expressed in medulloblastoma but not associated with the Shh pathway, was not inhibited. We also saw a reduction of Gli2 expression. This was important, because Gli2 can to some extent compensate for loss of Gli1 in medulloblastoma formation (13). Suppression of Gli1 expression indicated that HhAntag-691 is capable of reaching its target in vivo, in the cerebellum of tumor-bearing mice. Further analysis of tumors isolated from treated mice showed that HhAntag-691 leads to a drastic decrease in proliferation as well as a modest increase in tumor apoptosis. Additionally, we found that the treated brains became infiltrated with macrophages and reactive astrocytes, indicating that treatment incites the host immune response and tissue repair mechanisms. When mice were treated for a longer period, a dramatic loss of tumor volume was observed. In mice treated at intermediate doses, a 7-fold loss of tumor mass was noted, and no tumor mass remained after treatment with the highest dose. Finally, long-term treatment with HhAntag-691 prolonged tumor-free survival of Ptc1+/p53−/− mice, when compared with vehicle-treated mice. Importantly, daily monitoring of the treated mice did not reveal any weight loss or other obvious deleterious side effects. These results offer strong hope that targeting the Shh pathway in a subset of human medulloblastomas may provide a feasible, nontoxic therapy for this disease.

Traditional approaches to testing new therapies, which are based on tissue culture and murine xenograft studies, might actually hinder development of meaningful therapies, because they rely on artificial systems that do not fully recapitulate the disease in situ. Whereas it is also possible to fault some features

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1 Unpublished data.
of transgenic mouse models of cancer, the perspective provided by the study described above illustrates a new way to develop drugs for medulloblastoma and other solid tumors that are driven by deregulation of the Shh pathway. By directly assaying the molecular status of the pathway being targeted, the model offers an invaluable tool for preclinical pharmacodynamic and pharmacokinetic experiments. With molecular targeted therapeutics, the traditional strategy of testing drugs at the maximum tolerated dose is clearly not appropriate. Instead, the specific nature of such agents dictates that they should be used at doses that can effectively suppress the target; going beyond the biologically relevant level may lead to deleterious effects not associated with target inhibition. Caution needs to be exercised in applying results from mouse trials to human disease; the animal model system described here should be used in conjunction with studies done on human tumor material, if possible, before moving forward to clinical trials. However, in the case of medulloblastoma, human systems which reliably recapitulate the in situ disease are not yet available. If the mouse model recapitulates the genetic signature of the human disease, as well as its anatomic and temporal characteristics, targeting the same pathway in human disease may also be effective. One issue for medulloblastoma is that it is predominantly a pediatric tumor, and new treatments for cancer are usually tested first in adult patients. For this reason, the hope for clinical trials will likely start with tests on adult tumors that rely on Shh signaling, thereby paving the way for clinical trials for medulloblastoma in children. Basal cell carcinoma (BCC) is one example of a tumor in which the initial genetic lesion (PTCH1 mutation) is the same as in medulloblastoma, making BCC suitable for evaluating the efficacy of Shh suppression as a therapy (14). Additionally, recent reports point to other solid tumors that are dependent on Shh signaling for growth, although genetic lesion in the Shh pathway have yet to be identified in these cases (15–17). Such tumors offer additional candidates for initial clinical trials of Shh inhibitors. We hope that the efforts to produce animal models which recapitulate specific human diseases will offer an improved chance of developing nontoxic, targeted cancer therapies, each one designed for a specific subtype of tumor, based on its genetic profile. This would be especially beneficial in the area of pediatric brain tumors, where the currently available cytotoxic treatments leave patients to cope with sometimes very severe, neurologic and neuroendocrine consequences for the rest of their lives.

Acknowledgments


![Diagram showing the Shh pathway and its molecular targets.](image)

**Figure 2.** Medulloblastoma can arise as an aberration of cerebellar development, when a GNP cell fails to exit the cell cycle and remains proliferating in the EGL. Somatic mutations in different components of Shh pathway, including PTCH1 and SMOH may contribute to sporadic medulloblastoma (18–21) and germ line mutations of PTCH1 gene underlie tumors arising in patients with Gorlin syndrome (22, 23).
The link between the Shh pathway and medulloblastoma led us to investigate whether suppressing Shh signaling in these tumors in vivo could be therapeutic. We used a small molecule inhibitor of the Shh pathway (HhAntag-691, a Smo inhibitor) in a mouse model of medulloblastoma. We found that treating mice with the Smo inhibitor suppressed the Shh pathway and led to the elimination of tumors. This was accompanied by a decrease in tumor cell proliferation and an increase in apoptosis. Importantly, long-term treatment prolonged tumor-free survival of the mice. This experiment underlines the importance of developing relevant animal models in which to test new cancer therapies.
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