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Just Say No to ATOH: How *HIC1* Methylation Might Predispose Medulloblastoma to Lineage Addiction

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Abstract

Hypermethylated in cancer-1 (HIC1) is a tumor suppressor frequently targeted for promoter hypermethylation in medulloblastoma, an embryonal tumor of the cerebellum. Recently, we showed that HIC1 is a direct transcriptional repressor of *ATOH1*, a proneural transcription factor required for normal cerebellar development, as well as for medulloblastoma cell viability. Because demethylating agents can induce reexpression of silenced tumor suppressors, restoring HIC1 function may present an attractive therapeutic avenue in medulloblastoma by exploiting an apparent addiction to *ATOH1*.

Introduction

During cerebellar development, neuronal progenitors known as granule cell precursors (GCP) proliferate in response to activation of the Hedgehog (Hh) pathway, driven by Sonic Hedgehog (Shh) ligand secreted by Purkinje cells (1). This GCP population is a potential substrate for the development of medulloblastoma, a primitive neuroectodermal tumor primarily associated with childhood, although it is also believed that a subset of medulloblastomas may arise from the deep-seated ventricular zone of the posterior medullary vellum (2). Although the Hh pathway is critical for the normal expansion of the GCP population, <25% of medulloblastoma cases are associated with activating mutations in the Hh pathway (3). The imperative to better understand the pathogenesis of this tumor is driven by two important issues. First, medulloblastoma and its treatment can cause devastating effects on the lives of patients and families. Second, and of broader significance, is the idea that medulloblastoma represents one of the best models for understanding how developmental processes can promote and sustain the growth of tumors, and how such pathways might be targeted for therapeutic benefit. Studying both a mouse model and human tumor cell lines, we have recently identified how epigenetic gene silencing of an important tumor suppressor can deregulate developmental pathways critical to the genesis of this tumor.

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No potential conflicts of interest were disclosed.

Chromosome 17p Is Frequently Deleted in Medulloblastoma

Loss of chromosomal arm 17p occurs commonly in sporadic human medulloblastoma. However, mutations in p53, the best-characterized tumor suppressor located on 17p, are rarely detected (3). Analysis of a commonly deleted region associated with poor prognosis in medulloblastoma, 17p12-13.3, suggested that novel tumor suppressor genes may be present at this locus (4,5). Two such genes have now been described as follows: *KCTD11* (also known as REN^{KCTD11}), located at 17p13.2, and *hypermethylated in cancer 1* (*HIC1*), located at 17p13.3. KCTD11 functions as a hedgehog pathway antagonist; and its overexpression causes a decrease in medulloblastoma cell viability, making it a putative tumor suppressor in medulloblastoma (6). HIC1 is a zinc finger transcriptional repressor hypermethylated in a variety of pediatric and human tumors. Until recently, HIC1 had only one known transcriptional target, *SIRT1*, a class III histone deacetylase that functions to protect cellular longevity by partially inactivating p53 by deacetylation (7). Recent work by our group has since shown Hic1 to be a specific transcriptional repressor of *Atoh1*, a proneural transcription factor necessary for both tumor cell viability and for the formation of GCPs (8). *Atoh1* up-regulation is commonly found in mouse models of medulloblastoma in which the tumors exhibit deregulated Shh signaling pathway expression (9–12). This relationship is maintained in a subset of human medulloblastomas in which tumors that express *ATOH1* also express *GLI1*, but it is uncertain whether this is an exclusive relationship (13). Because *Atoh1* is a likely target of Hh signaling, this finding provides a developmental framework for understanding how *Hic1* might function as a tumor suppressor in medulloblastoma.

Characterization of *HIC1* as a Tumor Suppressor Gene

HIC1 was first identified in a region of chromosome 17p frequently targeted for allelic loss and CpG island hypermethylation in cancer (14). The structure of the mouse and human loci consists of a large single coding exon, which translates an NH_2-terminal BTB/POZ domain, and 5 Kruppel-like zinc fingers. There are at least three first exons, each driven by separate promoters of variable CpG island density (15). Aberrant CpG island methylation in these promoter regions has been described in a wide variety of human tumors (16–22).

Analysis of HIC1 and the 17p13.3 locus reveals that they are commonly methylated in medulloblastoma and do not seem to be limited to any specific clinical or pathologic subgroup (5,23–25). Hypermethylation of the *HIC1* gene in medulloblastoma also correlates with a reduction or absence of gene expression. Methylation-dependent reexpression of *HIC1* in medulloblastoma cell lines after treatment with the DNA methyltransferase inhibitor, 5-aza-2′-deoxycytidine, provides further evidence for a causal role of hypermethylation in regulating expression (23,25). The frequency of *HIC1* epigenetic silencing in medulloblastoma makes it an attractive tumor suppressor candidate in medulloblastoma; and its potential role in regulating differentiation through transcriptional repression of *ATOH1* provide a mechanistic link to better understand how HIC1 might function in this context.

Hic1-Mediated Suppression of *Atoh1* in Development and Medulloblastoma

As GCPs mature, they descend from the outer surface of the cerebellum known as the external granule cell layer (EGL), and ultimately take up residence as mature postmitotic mature neurons in the internal granule cell layer (IGL; ref. 1). This period of neuronal development provides a highly informative model of neuronal differentiation based on the topography of the developing cerebellum. Using immunohistochemical assays, we showed that as GCPs descend, they express Hic1 in a regulated fashion, corresponding precisely to the developmental phase in which *Atoh1* expression is lost (9). Because *Atoh1* is critically linked to the specification and proliferation of GCPs (8), we investigated the idea that Hic1 plays a key role in suppressing *Atoh1* expression in this progenitor pool.

The proneural bHLH transcription factor Atoh1 is the earliest marker of GCPs (8). Atoh1 is critical for proper cerebellar development as loss of the *Atoh1* murine ortholog blocks the development and proliferative ability of the EGL, as well as the production of mature granule cells in the IGL (8,26). Our data in cultured GCPs shows that Hic1-mediated repression of *Atoh1* expression is dominant to the induction of *Atoh1* by Shh. In other words, Hic1 can act downstream of Shh to repress *Atoh1* expression; thus, its expression may serve to render GCPs insensitive to Shh, and therefore permit terminal differentiation despite exposure to high levels of Shh during their migration (Fig. 1). This may explain a long-standing paradox in cerebellar development: although GCPs require Purkinje cell–derived Shh for their growth, postmitotic GCPs are insensitive to Shh, although they are exposed to the highest levels of Shh as they migrate past the Purkinje cell layer to take residence in the IGL (27).

A more rigorous test of this idea is a mouse model, in which we crossed $Hic1^{+/-}$ mice to animals heterozygous for a loss of function mutation in *Patched homolog 1* (*Ptch1*; ref. 9). In keeping with the role of *Ptch1* as a critical negative regulator of the Hh pathway, 10% to 15% of $Ptch1^{+/-}$ animals develop medulloblastoma (28). We showed that mice doubly heterozygous for mutations in *Hic1* and *Ptch1* have a marked increase in the incidence of medulloblastoma; and that in each case, the remaining wild-type *Hic1* allele was silenced by dense promoter hypermethylation (9). As predicted by our model, *Atoh1* expression is deregulated in these tumors. Additionally, we show that *Atoh1* expression is required for medulloblastoma cell viability (9), results that were independently confirmed by the Dr. Martine F. Roussel team, St. Jude Children's Research Hospital (29).

Available evidence suggests that *ATOH1* may not be a classic oncogene, but that its overexpression and functional requirement in medulloblastoma is more consistent with the concept of "lineage addiction." Here, cancer cells become addicted to the expression of transcription factors required for the development of an organ-specific progenitor (30). Typically, these genes cannot act as oncogenes but are required for the maintenance of a progenitor cell phenotype in cancer. This phenomenon is exemplified by *MITF*, a transcription factor expressed in, and required for, the maintenance of melanocytes and melanoma (31). *ATOH1* is similar in that it directs cerebellar granule cell lineage survival during development and protects the proliferative potential and survival of medulloblastoma; thus, *ATOH1* may function as a lineage-survival oncogene in medulloblastoma. Deregulation of *ATOH1* expression through loss of HIC1 function may well promote medulloblastoma formation by preventing terminal differentiation and, thus, expand the pool of transformation-competent GCPs during development.

Epigenetic Therapy in Medulloblastoma?

Regardless of whether *ATOH1* is a traditional oncogene or a lineage-survival oncogene, it is a potential therapeutic target due to its promotion of medulloblastoma viability. It is known that many pathways interact with *Atoh1* during cerebellar development, including the hedgehog-signaling pathway and the Notch pathway (1,32), suggesting that *Atoh1* is tightly regulated both spatially and temporally. Although transcription factors are notoriously difficult targets for small molecule drug development, recent interest in epigenetic therapies that remove silencing marks such as DNA hypermethylation can be effective in restoring expression of genes such as *HIC1* (23,25). *Atoh1* expression can be blocked by restoring Hic1 function, even when *Atoh1* expression is stimulated by pathways including Hh signaling. Because pharmaceutical approaches to reverse DNA promoter methylation are now used clinically in the treatment of myelodysplastic syndrome (33,34), it is possible that therapies targeting epigenetic events regulating HIC1 expression may be of use in medulloblastoma.

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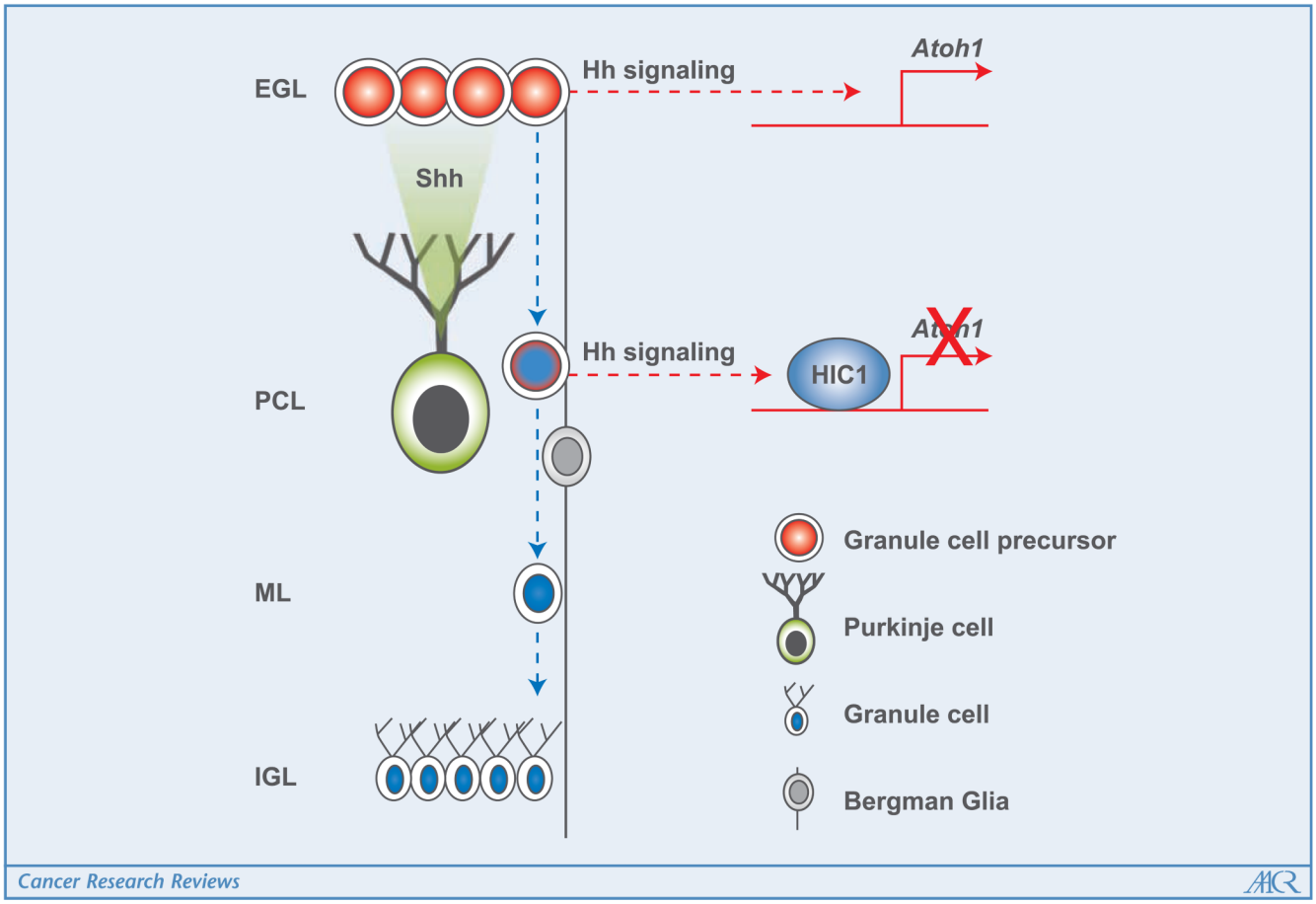

Figure 1.

Simplified representation of the developing cerebellum, showing the relationship between *Atoh1* and Hic1 expression in the differentiation of granule neurons. GCPs in the EGL proliferate in response to Shh ligand produced byPurkinje cells (*green*) and express high levels of *Atoh1* (*red*) driven byactivation of the Hh pathway. Hic1 (*blue*) is expressed bycells lining the inner EGL, and more strongly as the differentiating postmitotic granule cells migrate down the Bergmann glia through the molecular layer (*ML*) and Purkinje cell layer (*PCL*), to the IGL. Hic1 directlyrepresses transcription of *Atoh1* in maturing granule cells downstream of the Hh pathway, making them unable to respond to high concentrations of Shh in the PCL.