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Medulloblastoma Stem Cells

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Abstract

Medulloblastoma and other embryonal brain tumors are similar in appearance and differentiation potential to neural stem and progenitor cells. Expression studies performed using human tumor samples, as well as the analysis of murine transgenic models, suggest that both multipotent cerebellar stem cells and lineage-restricted progenitors of the external germinal layer can be transformed into medulloblastoma by genetic alterations. These molecular changes frequently involve constitutive activation of signaling pathways such as Wnt, Hedgehog, and Notch, which play a key role in non-neoplastic neural stem cells. Pharmacologic blockade of the Hedgehog and Notch pathways suppresses the growth of medulloblastoma in culture and in vivo and may prove effective in targeting the small cancer stem-cell subpopulation required for tumor initiation and long-term propagation.

INTRODUCTION

Medulloblastoma are tumors in which the majority of cells have an undifferentiated stem- or progenitor-like appearance. Signs of differentiation into neurons, glia, and other cell types can be detected, however, providing support for the concept that medulloblastoma have properties of multipotent stem cells. Indeed, their primitive appearance led long ago to the suggestion that medulloblastoma arise from CNS stem cells. Recent studies have sought to more precisely define stem-cell subtypes in the cerebellum and their relationship to medulloblastoma. Other types of embryonal brain tumors, most of which fall into the general category of CNS primitive neuroectodermal tumors (PNET) in the current WHO classification scheme,¹ also likely derive from stem-cell populations in the brain. In this review, we discuss our growing understanding of the stem-like phenotype of embryonal brain tumors such as medulloblastoma.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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We focus on three issues: first, the potential origin of medulloblastoma from cerebellar stem and progenitor cells; second, the signaling pathways required in both neural stem cells and medulloblastoma; and third, the role of so-called cancer stem cells in long-term propagation of medulloblastoma and the susceptibility of these rare cells to therapies targeting developmentally critical signaling pathways, such as Notch. Novel therapies are clearly needed, for although the 5-year survival rate for average-risk children with these aggressive neoplasms now approaches 90%, the outcome in patients with high-risk disease or in those too young for radiation therapy is much worse.² In addition, current therapies often result in serious long-term neurocognitive difficulties and other adverse effects.²

CEREBELLAR STEM CELLS AND MEDULLOBLASTOMA HISTOGENESIS

Brain tumor classification has historically been based on the morphologic or functional similarity of tumors to non-neoplastic cells types in the brain. Interest in the cellular origin of medulloblastoma therefore extends back to their initial description by the great neurosurgeon Harvey Cushing and his associate Percival Bailey. They named the tumor after the putative medulloblast, an undifferentiated cell on the cerebellar surface that was thought at that time to mature into both neurons and glia.³ Our understanding of how various stem and progenitor populations give rise to the cerebellum has evolved greatly since then, but it remains unclear what percentage of medulloblastoma and other embryonal brain tumors derive from true stem cells, lineage committed progenitors, or perhaps even fully differentiated cells (Fig. 1). By achieving a better understanding of the cellular origin of these tumors, we hope to also gain insight into what signals they require to survive and grow, permitting more effective targeted therapies to be developed.

The two primary germinal epithelia of the cerebellum are the deep-seated ventricular zone (VZ) of the posterior medullary vellum and the superficial external germinal layer (EGL), which covers the cerebellar surface (reviewed in Sotelo,⁴ Goldowitz and Hamre,⁵ and Chizhikov and Millen⁶). The midline VZ germinal matrix gives rise to many neuronal and glial cell types in the cerebellum. The EGL, in contrast, is thought to only produce cerebellar granule cells, the most numerous class of neurons in the entire brain. Peak cerebellar growth occurs relatively late compared to the rest of the brain, driven primarily by proliferation of EGL cells. In the mouse, the period of greatest growth occurs during the 2 weeks after birth, while in humans, the analogous proliferative peak occurs in utero during the third trimester, although EGL remnants can persist for up to a year after birth.⁷

In addition to these two relatively well characterized populations, other cerebellar stem cells have been described that could be a source of medulloblastoma. Lee et al⁸ recently isolated CD133-positive stem cells from the postnatal cerebellum, concentrated in the white matter, which have the ability to self-renew and display multilineage differentiation potential both in vitro and in vivo. Two other groups have identified novel stem/progenitor-cell populations in the rhombic lip that give rise to nuclei of the deep cerebellar white matter.^{9,10} Finally, Bergmann glia, like other radial glia, have some properties of stem cells.¹¹

What evidence directly connects these stem- and progenitor-cell populations to medulloblastoma? In humans, the data are merely correlative. Expression of Calbindin-D, a

ventricular zone marker not found in the EGL, is present in less than half of medulloblastomas, mainly those of the classic (non-nodular) subtype.¹² In contrast, nodular/desmoplastic medulloblastoma were found to commonly express the EGL marker p75, whereas classic lesions did not, giving rise to a dual origin hypothesis, which proposes that tumors arise from either the VZ or EGL.¹³ Global gene expression profiling also suggests a kinship between medulloblastoma and cerebellar stem/precursor cells. Two groups have shown that at least a subset of human tumors display expression profiles similar to murine cerebellar EGL.^{14,15} In addition, as discussed below, the evidence for origin of medulloblastoma from the EGL is strong in murine models, with aberrant activation of Hedgehog (Hh) and other pathways.

One might ask how committed neuronal progenitors such as granule cell precursors of the EGL can give rise to multipotent medulloblastoma that also express glial markers. Interestingly, when unipotent EGL precursors are immortalized by SV40 Large T antigen, or exposed to sonic hedgehog and bone morphogenetic proteins in culture, they gain the ability to differentiate into multiple cell types, including glia, perhaps explaining how multipotent tumors can derive from EGL.^{16,17} As perhaps the most extreme example of multipotency in an EGL-derived cell, it has been reported that medulloblastoma cells from Patched (*PTCH*) heterozygous mice can direct development of mouse embryos after nuclear transfer.¹⁸ Such studies, however, call into question the strength of conclusions one can draw from expression of molecular markers when inferring histogenesis, as oncogenic transformation can clearly alter the molecular signature and cellular phenotype of restricted progenitors, causing them to take on a multipotent stem-cell phenotype.

A more radical concept is that fully differentiated cells give rise to medulloblastoma, acquiring a primitive, multipotent phenotype in the process. No data supporting this hypothesis has been shown for embryonal brain tumors. A recent report, however, suggests that embryonal tumors in the eye can form in just this fashion in a mouse model. Ajioka et al¹⁹ have shown that transgenic *Rb*(-/-);*p107*(±); *p130*(-/-) mice develop retinoblastoma, with the earliest lesions arising from differentiated horizontal interneurons that re-enter the cell cycle and clonally expand. It therefore seems premature to rule out the possibility that some medulloblastoma might be formed by fully differentiated cells.

SIGNALING PATHWAYS IMPORTANT IN BOTH STEM CELLS AND MEDULLOBLASTOMA

It is clear that many of the pathways required in neural stem cells are also aberrantly activated in medulloblastoma. Indeed, this tumor has more mutations and other genetic changes in developmental genes than in canonical regulators of the cell cycle such as p53.¹ Examination of the signaling pathways dysregulated in various medulloblastoma subtypes has also allowed further speculation about the relationship between tumor-initiating stem- or progenitor-cell populations and early molecular events in oncogenesis (Fig. 2). It is hoped that a better understanding of the molecular link between cerebellar development and tumor formation will lead to new therapies specifically targeting medulloblastoma cells. Listed on the next page are some of the better-understood signaling pathways active in both neural stem cells and in medulloblastoma.

Hedgehog

The Hh pathway has been shown to regulate neural stem-cell development in many species and plays a particularly important role in the cerebellar EGL. After their migration from the rhombic lip to the EGL, granule cell precursors are stimulated by Purkinje cell-secreted sonic hedgehog ligand to undergo a rapid burst of proliferation.^{20–22} Hh signaling also seems to be required for maintaining stem cells in the subventricular zone (SVZ) and hippocampus in the postnatal brain.^{23–25} Mutations in the PTCH receptor, which result in activation of the Hh pathway, have been identified in both sporadic medulloblastoma and in tumors arising in patients with Gorlin's syndrome, an autosomal dominant disorder in which patients present with various neoplasms including basal cell carcinoma and medulloblastoma.^{26,27} Approximately 15% of sporadic human medulloblastomas, particularly those of the nodular/desmoplastic subtype, harbor *PTCH* mutations.^{28,29} Global gene expression analyses have also associated Hh pathway activation with medulloblastoma-containing nodules.^{30,31}

Murine medulloblastoma arising as a result of constitutive activation of Hh signaling represent the most common transgenic models of this tumor. Approximately 14% of mice heterozygous for *PTCH* develop medulloblastomas.^{32,33} Detailed flow cytometric and molecular analyses have confirmed that granule cell precursors from the EGL are transformed into medulloblastoma-like tumors in these animals by loss of *PTCH* function, which causes constitutive activation of the Hh pathway.³⁴ The Hh pathway genes *SMO* and *SUFU* are also occasionally mutated in human medulloblastoma, and have been used to develop murine tumor models.^{35,36} Preliminary reports from the groups of Wechsler-Reya and Rowitch, published as meeting abstracts, indicate that activation of Hh signaling in multipotent murine stem cells in vivo can also result in medulloblastoma formation. This suggests that not only granule cell precursors are susceptible to transformation by this signaling pathway.^{37,38}

Interestingly, it has been shown that medulloblastoma models in mice initiated by genetic changes to genes outside the Hh pathway also result in upregulation of Hh signaling.^{15,39,40} Indeed, in one such model based on *Cxcr6* inactivation, the tumors that resulted were susceptible to Hh pathway inhibitors in vivo.⁴⁰ Such results buttress the argument that by understanding the cellular origin and signaling pathways associated with medulloblastoma formation, new therapies may be discovered.

Wnt

A second canonical signaling pathway involved in both sporadic and syndromic medulloblastoma is Wingless/Wnt. The Wnt signaling pathway regulates proliferation of stem and progenitor cells in the fetal VZ, as well as the postnatal SVZ and hippocampus.^{41–44} Loss of *Wnt1* or the key pathway effector beta-catenin causes severe abnormalities in the development of the midbrain and cerebellum, but despite this important role in early cerebellar growth, Wnt does not seem to regulate EGL precursors.^{45–47} It is therefore interesting the Wnt has been found to be activated predominantly in medulloblastoma of the classic subtype, supporting the concept that these tumors derive from stem/progenitor-cell populations outside the EGL, such as those in the VZ (Fig. 2).

Consistent with this concept, constitutive activation of Wnt in VZ cells results in cortical overgrowth and some hyperproliferative masses histologically reminiscent of CNS PNET.⁴¹

The first Wnt pathway mutations to be identified in medulloblastoma were in patients with Turcot's syndrome, an autosomal recessive disease caused by loss of adenomatous polyposis coli function.⁴⁸ Mutations activating the Wnt pathway member beta-catenin are more common than adenomatous polyposis coli loss in sporadic medulloblastoma and are found in 5% to 10% of cases.^{49–51} Mutation or reduced expression of Axin2, a negative regulator of Wnt signaling, has also been detected in a small fraction of medulloblastoma.⁵² Nuclear translocation of beta-catenin, a sign of Wnt pathway activation, is found in up to 25% of medulloblastoma,^{51,53} and is associated with better clinical outcomes.⁵³ Activation of Wnt is seen predominantly in classic tumors not showing signs of Hh signaling or chromosome 17 aberrations, suggesting they represent a unique subset of medulloblastoma (Fig. 2).^{31,54} It is not clear, however, if Wnt activity is particularly important in cancer stem cells within medulloblastoma, and therapeutic applications of Wnt blockade in neuro-oncology await the development of drugs that can effectively target this pathway in patients.

Notch

Like the Hh pathway, Notch signaling is active in neural stem and progenitor cells in both the embryonic VZ and rhombic lip,^{55,56} as well as the postnatal SVZ⁵⁷ and EGL.⁵⁸ During CNS development, activation of Notch signaling generally promotes proliferation and/or survival of stem cells while inhibiting their differentiation. The presence in mammals of four Notch receptors, as well as multiple ligands and effectors, adds to the complexity of this pathway, which is notoriously cell and context dependent in its effects. For example, Notch2 seems to promote proliferation of cerebellar granule neuron progenitors, whereas Notch1 expression is associated with cell cycle exit and differentiation in these cells.⁵⁸ A similar paradigm may hold in medulloblastoma, as we found that Notch2 was overexpressed in human primary tumors, and promotes tumor proliferation, whereas Notch1 is largely undetectable and suppresses the growth of tumor cells in culture.⁵⁹ Some human medulloblastoma and CNS PNET showed chromosomal gains including the *Notch2* locus, and expression of the pathway target *Hes1* was correlated with poor clinical outcome.⁵⁹ Others have also found evidence of Notch pathway dysregulation in human and murine medulloblastoma, along with evidence that Notch activity can be driven by Hh signaling in these tumors.^{36,60} Finally, hypoxia has been shown to promote neural stem-cell proliferation via Notch, and a similar regulatory loop could be operational in cancer stem cells in medulloblastoma.^{61,62}

Other Pathways

A number of other genes important in cerebellar stem and progenitor cells are dysregulated in medulloblastoma. Many of these have been linked to the three canonical developmental signaling pathways discussed above. *REN* promotes differentiation of granule cell precursors in the cerebellum at least in part by suppressing Hh signaling, and is located in a region of chromosome 17 frequently deleted in medulloblastoma.^{63,64} The oncogene *N-myc* plays an important role in the growth of the cerebellum, and is a key target of Hh signaling in cerebellar granule cells and in medulloblastoma.^{65–68} Amplification or overexpression of

myc oncogenes is associated with the large-cell/anaplastic medulloblastoma subtype and worse clinical outcomes^{69,70} and sometime occurs as tumors progress from less aggressive subtypes (Fig. 2). The development of drugs such as the quassinoid NBT-272 that target Myc proteins in medulloblastoma cells is therefore promising and could potentially affect stem-like cells in the tumors.⁷¹ The transcription factor RE-1 silencing transcription factor, a repressor of neuronal differentiation, is also highly expressed in both neural stem cells and in medulloblastoma and is able to transform *c-myc* immortalized granule cell precursors into medulloblastoma.^{72,73} OTX2 and BMI1 represent additional transcription factors that seem to regulate both neural stem/progenitor cells and medulloblastoma growth.^{74–77}

DO MEDULLOBLASTOMA CONTAIN CANCER STEM CELLS?

It seems that developmental stem-cell hierarchies similar to those in fetal brain are maintained in some brain tumors, including medulloblastoma. The cancer stem-cell hypothesis holds that not all cells within a neoplasm are equal in terms of their potential for self-renewal and long-term growth. Rather, as is the case in development of the brain, only a small fraction of stem-like cancer cells are capable of unlimited self-renewal, whereas the rest of the neoplasm is composed of progenitors of limited (albeit rapid) proliferative potential or fully differentiated cells (reviewed in Wang and Dick,⁷⁸ Tan et al,⁷⁹ and Reya et al⁸⁰). Although it seems likely that these cancer stem cells are the progeny of non-neoplastic stem or progenitor cells that are transformed by genetic and/or epigenetic alterations, it is also possible that better-differentiated cells give rise to tumors and acquire stem-like properties after transformation. The issues of histogenesis and hierarchy are therefore potentially, but not necessarily, linked.

Several pieces of evidence support the concept that medulloblastoma cells are functionally heterogeneous and that only a subset have a stem-like phenotype that drives tumor growth. First, nodular/desmoplastic medulloblastoma contain two karyotypically similar compartments,⁸¹ one comprised of rapidly proliferating, undifferentiated cells, and a second (the nodules) composed of largely nonproliferative cells that have differentiated into neurons. Extensively nodular tumors that lack the stem or progenitor-like component are known to be less aggressive clinically.¹ This suggests that by modulating the number of primitive or stem-like cells in a medulloblastoma, clinical outcome can be affected. Second, it has been shown that medulloblastoma contain a subset of stem-like multipotent cells that can be maintained as neurospheres under the same conditions used to grow non-neoplastic neural stem cells.^{82,83} Third, the stem-cell marker CD133 is expressed in only a fraction of medulloblastoma cells, and some have reported that these cells alone are able to form multipotent neurospheres in vitro or engraft in vivo.^{84,85} We have also found that CD133-positive cells from some established medulloblastoma cell lines have a greatly increased ability to form tumor xenografts.⁸⁶ Other groups, however, have recently reported that CD133 negative cells can propagate some glioblastoma lines,⁸⁷ suggesting that the CD133 marker may not identify all cancer stem cells in brain tumors. Indeed, in a recently published meeting abstract, Wechsler-Reya et al⁸⁸ reported that CD133 negative, CD15 positive cells are cancer-propagating cells in medulloblastoma derived from *PTCH* heterozygous mice.

TARGETING CANCER STEM CELLS IN MEDULLOBLASTOMA

If the cancer stem-cell hypothesis is true, stem-like cells within medulloblastoma must be removed if a patient is to be cured. However, it is thought that most cancer stem cells are resistant to standard therapies.⁸⁹ The mechanisms for such resistance are beginning to be elucidated in some CNS tumors, although relatively little is known in medulloblastoma. It has been suggested that cancer stem cells in malignant gliomas are radiation resistant because of an intrinsic ability to induce the DNA damage response.⁹⁰ At least one group also documented increased radiation resistance in CD133-positive stem-like cells in medulloblastoma.⁹¹ Regarding chemotherapy, it is thought that cancer stem cells have an increased ability to efflux harmful chemicals because of their expression of the adenosine triphosphate-binding cassette-type transporters.⁹² Although no direct evidence supporting this has been generated in medulloblastoma, it has been reported that stem-like side population cells are resistant to mitoxantrone in a similar tumor, the neuroblastoma.⁹³

Hh inhibitors represent one class of therapies that may deplete cancer stem cells. Hh signaling seems to be elevated in the more primitive-appearing, progenitor-like regions of nodular medulloblastoma.⁹⁴ Cyclopamine, an inhibitor of Hh signaling, has been shown to reduce medulloblastoma growth in vitro and prevent tumor propagation in vivo as a result of reduced proliferation and induced apoptosis.^{95,96} Other Hh inhibitors also showed encouraging results in vivo in transgenic models.⁹⁷ In neither case, however, was it clear whether the stem-like tumor fraction was effectively targeted, as was recently shown using cyclopamine to treat malignant glioma.^{25,98} It is also not clear whether Hh inhibitors will only prove effective in tumors with extremely high levels of pathway activity, or if even medulloblastoma with lower levels of signaling will be susceptible. In one experiment, freshly resected cells from all seven medulloblastoma tested proved sensitive to Hh blockade, suggesting that even tumors without high-level Hh activity might need this pathway.⁹⁶

The Notch pathway represents a second signaling cascade for which cancer stem cells might maintain a requirement present in their non-neoplastic cognate cells. When we separated cells from established medulloblastoma lines into CD133 positive and negative fractions, the level of Notch signaling was several-fold greater in the former than in the latter.⁹⁹ After Notch signaling blockade in medulloblastoma lines using pharmacologic inhibitors of gamma-secretase, the cancer stem-cell population marked by CD133, side population, and Nestin was markedly reduced or abolished. Moreover, the viable cells remaining after Notch blockade were unable to efficiently form colonies in soft agar or xenografts in nude mice.⁹⁹ Although the mechanism by which these stem-like cancer cells were depleted was not entirely clear, cell cycle exit and neuronal differentiation were observed in the cultures treated with gamma-secretase inhibitors. In addition, double immunolabeling for Nestin and apoptotic markers revealed that the primitive cells were more than 10 times more sensitive to apoptotic induction after Notch blockade than better-differentiated ones (Fig. 3). Hallahan et al³⁶ have also shown that gamma-secretase inhibition caused reduced proliferation and increased apoptosis in medulloblastoma cells. Consistent with the idea that Notch is required in tumor-propagating cancer stem cells, we found that after gamma-secretase treatment, the remaining viable cells could proliferate for a time in culture but were no longer able to form

xenografts in immunocompromised mice.⁹⁹ This experimental evidence supports the hypothesis that reagents that target cancer stem cells, most likely in combination with standard therapies, may eventually help to completely treat medulloblastoma.

Another avenue by which cancer stem cells in medulloblastoma and other tumors might be depleted is through ablation of the so-called niche required for their survival. The stem-cell niche is a micro-environment composed of supportive cells, extracellular matrix, and other factors required for stem-cell self-renewal.¹⁰⁰ The nature of the niche likely varies during various phases of neural development and in different regions of the brain. In the SVZ, for example, astrocytes, neuroblasts, and endothelial cells all help form the niche by providing factors required for neural stem-cell self-renewal.^{101,102} In the developing cerebellum, the niche required for the various stem- and progenitor-cell fractions is largely undefined. It is not yet clear whether cancer stem cells require a niche similar to that present in their non-neoplastic counterparts, but a recent report by Calabrese et al¹⁰³ supports this notion. They showed that CD133-positive putative cancer stem cells in medulloblastoma are more commonly found near endothelial cells and small vessels, suggesting the latter may function as a niche and provide critical growth factors and stromal support required for self-renewal. This raises the possibility that antiangiogenic therapy could have the added benefit of depriving stem-like cells of the niche signals they require for survival.

In summary, much remains to be defined regarding the relationship between medulloblastoma and the stem cells that form the cerebellum. However, it seems clear that at least some medulloblastoma derive from stem or progenitor populations, and that the same signaling pathways required in these non-neoplastic cells are frequently activated in embryonal brain tumors. These molecular pathways may represent an Achilles heel for cancer stem cells in medulloblastoma, and by targeting these cells, we may be able to circumvent their presumed resistance to standard therapies. Inhibition of Hh, Notch, and other developmental pathways therefore represents an exciting new tool in the therapeutic armamentarium, although detrimental effects on non-neoplastic stem cells will have to be closely monitored as new agents are moved into the clinic.

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Fig. 1.

Only true stem cells can self-renew indefinitely, and they give rise to a transiently proliferating group of progenitors that eventually differentiate. Evidence exists for transformation of both neural stem and progenitor cells into medulloblastoma, and it is possible that genetic changes could generate these tumors from a fully differentiated cell as well. It is not clear whether cancer stem cells in medulloblastoma derived from these various origins would have unique properties.

Fig. 2.

Mutations activating Wnt have been identified primarily in the classic medulloblastoma subtype, which is thought to derive primarily from stem and/or progenitor cells of the ventricular zone. The external germinal layer (EGL), in contrast, is thought to give rise to nodular/desmoplastic medulloblastoma commonly associated with Hedgehog (Hh) pathway activation. Other cerebellar stem and progenitor cells have been described and may also give rise to medulloblastoma (modified from Louis et al¹). MB, medulloblastoma; PNET, primitive neuroectodermal tumor.

Fig. 3. Notch pathway blockade seems to preferentially deplete stem-like medulloblastoma cells, leaving behind better-differentiated cells no longer able to form tumor masses. Both differentiation and cell death are involved in loss of the putative cancer stem cell (CSC) subpopulation.