N-myc Can Substitute for Insulin-Like Growth Factor Signaling in a Mouse Model of Sonic Hedgehog–Induced Medulloblastoma

Samuel R. Browd, Anna M. Kenney, Oren N. Gottfried, Joon Won Yoon, David Walterhouse, Carolyn A. Pedone, and Daniel W. Fults

Abstract

Medulloblastoma is a malignant brain tumor that arises in the cerebellum in children, presumably from granule neuron precursors (GNP). Advances in patient treatment have been hindered by a paucity of animal models that accurately reflect the molecular pathogenesis of human tumors. Aberrant activation of the Sonic hedgehog (Shh) and insulin-like growth factor (IGF) pathways is associated with human medulloblastomas. Both pathways are essential regulators of GNP proliferation during cerebellar development. In cultured GNPs, IGF signaling stabilizes the oncogenic transcription factor N-myc by inhibiting glycogen synthase kinase 3β–dependent phosphorylation and consequent degradation of N-myc. However, determinants of Shh and IGF tumorigenicity in vivo remain unknown. Here we report a high frequency of medulloblastoma formation in mice following postnatal overexpression of Shh in cooperation with N-myc. Over-expression of N-myc, alone or in combination with IGF signaling mediators or with the Shh target Gli1, did not cause tumors. Thus, Shh has transforming functions in addition to induction of N-myc and Gli1. This tumor model will be useful for testing novel medulloblastoma therapies and providing insight into mechanisms of hedgehog-mediated transformation.

Introduction

Medulloblastoma is a malignant brain tumor that arises in the cerebellum in children. Several lines of evidence implicate granule neuron precursors (GNP) in the external granule layer (EGL) of the developing cerebellum as likely cells of origin for certain classes of medulloblastomas (reviewed in ref. 1). For example, cells that compose a preneoplastic stage of medulloblastoma colocalize with GNPs in the EGL and they express molecular markers of immature granule neurons (2). Recently, another possible medulloblastoma cell of origin has been identified: a neural progenitor located in the cerebellar white matter and expressing both nestin and prominin (3). Signal transduction pathways that stimulate proliferation and inhibit differentiation of GNPs and other neural progenitor cells during development have been implicated in medulloblastoma. Thus, understanding the mitogenic functions of these pathways will yield insights into medulloblastoma formation.

In mice and humans, GNPs undergo a rapid, postnatal expansion phase in the EGL (reviewed in ref. 4). This presents a vulnerable setting for transforming events leading to tumorigenesis. GNP proliferation requires Sonic hedgehog (Shh) signaling (reviewed in ref. 5). GNP expansion and survival are also promoted by insulin-like growth factor (IGF) signaling (reviewed in ref. 6). Mutations that activate the Shh pathway occur in human medulloblastoma (7, 8). Increased IGF pathway activity has also been observed in medulloblastoma, suggesting that the pro-proliferative effects of this pathway may play an accessory or permissive role in medulloblastoma formation or maintenance (9).

Efforts to model medulloblastoma formation in mice have suggested conservation of the developmental and tumorigenic roles of the Shh and IGF pathways. For example, genetic manipulations that activate Shh signaling in the developing mouse cerebellum induce tumors that closely resemble human medulloblastoma. Targeted disruption of the Patched gene, which encodes the inhibitory component of the Shh response machinery, predisposes Patched–/– mice to medulloblastoma formation (10). Retroviral transfer and expression of Shh itself in the cerebellum induce EGL hyperplasia and medulloblastoma (11, 12). Consistent with these results, enhancing activity of Smoothened, the positively responding component of the Shh receptor apparatus, also promotes medulloblastoma development in transgenic mice (13). Enhanced IGF pathway activity causes cerebellar hyperplasia in mice (14) and contributes to Shh-mediated medulloblastoma formation (15).

A disadvantage to relying on transgenesis-mediated, ectopic gene expression or ablation for modeling human tumors is the difficulty in controlling temporal regulation of gene expression in a cell type–specific manner. This is particularly important with regard to medulloblastoma because the presumed cells of origin proliferate after birth. To target GNPs during their expansion phase, we have used the RCAS/tv-a system, which allows postnatal gene transfer in a cell type–specific manner (16). This system uses replication-competent avian leukosis virus (ALV) splice-acceptor (RCAS) vectors, derived from the avian retrovirus ALV (subgroup A), and a transgenic mouse line (Ntv-a) that produces TV-A (the receptor for ALV-A) under the control of the Nestin gene promoter. Nestin is an intermediate filament protein expressed by neuronal and glial progenitors (17). After TV-A-mediated viral infection, the RCAS provirus integrates into the host cell genome where the transgene is expressed from a spliced message under the control of the constitutive retroviral promoter, long terminal repeat. Combinations of genes can be transferred to individual cells by infecting them with multiple RCAS vectors carrying different genes. This makes it possible to determine whether different oncogenes cooperate to induce tumor formation in vivo.

Using the RCAS/tv-a system to target gene expression to nestin-expressing neural progenitors in newborn mice, we previously...
reported that activation of phosphoinositide 3-kinase (PI3K) signaling by IGF-II synergizes with Shh to enhance medulloblastoma formation (15). Working with cultured GNPs, Kenney et al. (18) described a two-pronged mechanism whereby Shh and PI3K signaling pathways cooperate to promote proliferation by converging on the oncogenic transcription factor N-myc. N-myc is a critical regulator of Shh-induced GNP proliferation (18–21) and its expression is up-regulated in human medulloblastomas (13, 22). Shh signaling directly stimulates N-myc gene transcription (19, 20). IGF-mediated PI3K signaling stabilizes the N-myc protein by inhibiting glycogen synthase kinase 3β (GSK3β)-dependent phosphorylation and consequent degradation of N-myc (18). Consistent with this model, we detected abundant, endogenous N-myc in medulloblastomas induced in mice by retroviral transfer of Shh and IGF-II (15).

To test the hypothesis that Shh induction of N-myc plays an important role in medulloblastoma tumorigenesis and that an important function of the IGF pathway in vivo is to stabilize N-myc, we used the RCAS/tv-a system to transfer N-myc to nestin-expressing cerebellar progenitors in mice. Targeting nestin+ cells enabled us to make direct comparisons with our previous results. We found an enhancing effect of N-myc on Shh-induced medulloblastoma formation. A mutant N-myc allele encoding a T50A amino acid substitution had a more potent enhancing effect than wild-type N-myc. This amino acid substitution stabilizes the N-myc protein by preventing GSK3β-catalyzed phosphorylation of N-myc (18). These results are consistent with a model in which IGF/PI3K signaling promotes medulloblastoma formation by stabilizing N-myc. Transfer of N-myc, alone or in combination with IGF signaling mediators or with the Shh target Gli1, did not induce tumors, suggesting that Shh has unique and essential transforming functions in GNPs distinct from induction of N-myc and Gli1.

Materials and Methods

Transgenic mice. Production of the Nte-a transgenic mouse line has been previously described (23). The mice used in these experiments are mixtures of the following strains: C57BL/6, BALB/C, FVB/N, and CD1.

Vector constructs. The RCAS-N-myc vector was constructed by ligating a PCR-generated cDNA sequence corresponding to exons 2 and 3 of the wild-type N-myc gene into parent retroviral vector, RCASB(P)A (16). RCAS-N-mycT50A was derived by site-directed mutagenesis as previously described (18). RCAS-N-mycT50A has a single Flag epitope (DYKDDDDKP) on the NH2 terminus of the encoded protein. To detect expression of the virus-transferred protein in formalin-fixed, paraffin-embedded tissue sections, we prepared an additional epitope-tagged version of RCAS-N-mycT50A by appending six repeats of the sequence encoding the human c-Myc epitope (MEQKLISEEDLN) recognized by monoclonal antibody 9E10. The Flag epitope sequence was not present in the Myc-tagged vector system to transfer N-myc to nestin-expressing cerebellar progenitors in mice. Targeting nestin+ cells was the goal, the cell pellet was prepared by mixing equal numbers of both retrovirus-producing cells. The Nestin promoter directs expression of TV-A to central nervous system progenitor cells, thereby ensuring that RCAS retroviruses infect immature precursor cells in the injected region of the cerebellum. At the developmental stage when the injections took place, the majority of cells expressing TV-A in the cerebellum are predicted to be GNPs (4). The recently discovered prominin/nestin+ progenitors, located in the cerebellar white matter, do not proliferate in response to Shh and may therefore represent cells of origin for medulloblastomas that are not associated with Shh pathway activity (3). Targeting ectopic Shh to these cells is unlikely to be tumorigenic.
Animals showing increased head circumference (a sign of internal hydrocephalus) or debilitation were sacrificed and immediately analyzed. All remaining mice were sacrificed for analysis 12 weeks after injection. To assess tumor formation, the brains were dissected, sectioned, and subjected to H&E staining as well as immunostaining for markers characteristic of immature neurons, mature neurons, and medulloblastoma. Consistent with our previous results (15), medulloblastomas were detected in 15% of mice injected with cells producing RCAS-Shh alone (Table 1). Interestingly, this is similar to the percentage of Patched+/− mice reported to develop medulloblastoma (10, 25). These results suggest that the effect of RCAS-mediated ectopic expression of Shh in GPNs is comparable to endogenous, constitutive pathway activation and that both methods of activating the Shh pathway render GPNs similarly susceptible to secondary transforming events.

As shown in Table 1, tumor incidence was increased in mice infected with RCAS-Shh and RCAS-N-myc together (47%; P = 0.0014). The incidence of tumor formation was comparable to that we previously reported after transfer of Shh in combination with upstream and downstream components of the PI3K pathway [i.e., IGF-II (39%) and activated Akt (48%); ref. 15]. No tumors were found, however, in mice injected with RCAS-N-myc alone (Table 1). Thus, N-myc can substitute for PI3K pathway activation in enhancing Shh-dependent medulloblastoma formation but N-myc alone is not sufficient for GNP transformation.

**Tumors induced by Shh + N-myc arise from neuronal precursors and resemble human medulloblastomas.** The tumors induced by Shh + N-myc arose in the dorsolateral cerebellum at the injection sites (Fig. 1A and B). Microscopically, these tumors closely resembled human medulloblastomas and they appeared to be identical to tumors induced by Shh alone. They were composed of homogeneous, densely packed sheets of cells with hyperchromatic nuclei and scant cytoplasm (Fig. 1C) with frequent mitotic figures (Fig. 1C, arrows). We observed foci of EGL hyperplasia adjacent to some of the tumors as we previously reported after transfer of Shh in combination with external and downstream components of the PI3K pathway [i.e., IGF-II (39%) and activated Akt (48%); ref. 15]. No tumors were found, however, in mice injected with RCAS-N-myc alone (Table 1). Thus, N-myc can substitute for PI3K pathway activation in enhancing Shh-dependent medulloblastoma formation but N-myc alone is not sufficient for GNP transformation.

To assess the differentiation status of tumors induced by Shh + N-myc, we carried out an immunocytochemical analysis using antibodies for neuronal differentiation antigens. All tumors showed abundant βIII tubulin in the cytoplasm and NeuN in the nucleus (Fig. 1D-E), markers of early neuronal differentiation (26, 27). We also observed cytoplasmic TrkC, a high-affinity neurotrophin receptor, which is expressed in human medulloblastomas (Fig. 1F; ref. 28). All tumors showed focal expression of synaptophysin, a neurosecretory protein expressed in 94% of human medulloblastomas (ref. 29; Fig. 1G). We did not detect expression of neurofilament protein, a marker of terminally differentiated neurons, in any tumor. As previously reported (12), immunoreactivity for the astrocytic marker GFAP was visible only in processes of entrapped astrocytes and was not seen in tumor cells. The preferential expression of neuronal markers supports an origin of the induced tumors from neuronal precursors.

It is possible that ectopic expression of Shh may drive nestin+ cerebellar progenitors away from a glial fate, concurrent with
enhancing proliferation and promoting transformation. Several studies from the developmental biology literature, however, suggest that this is unlikely. In the developing spinal cord, Shh drives expression of early oligodendrocyte markers (30). In the cerebellum, Shh promotes postnatal differentiation of an already committed astrocytic lineage, Bergman glia (31). Moreover, exposure of GNPs, isolated from the postnatal EGL, to Shh and bone morphogenetic proteins promotes astroglial differentiation (32).

Nestin is not restricted to neural progenitor cells and is also found in embryonic capillaries in brain and other organs (33). We cannot exclude the possibility that our RCAS vectors infected immature endothelial cells. Nevertheless, expression of neural antigens in the induced tumors indicates that the most likely target cells are undifferentiated neural progenitors, not vascular stem cells. Furthermore, when we probed tumors induced by Shh and Shh + N-myc with antibodies against vascular markers, von Willebrand Factor and PECAM-1 (CD31), we found immunoreactive staining only in mature blood vessels and not in tumor cells (Fig. 1H).

**Stabilized N-myc strongly enhances Shh-induced tumor formation.** Highly conserved, NH2-terminal phospho-acceptor sites have been implicated in Myc protein turnover. Phosphorylation of c-Myc at T58 or N-myc at T50 by GSK3β precedes ubiquitination and degradation (18, 34, 35). Mutations surrounding the GSK3β phosphorylation site in c-Myc occur in human malignant lymphomas and cause highly aggressive tumors in a mouse model of Burkitt’s lymphoma, indicating conservation of this regulatory mechanism in mouse models of human disease (36). Mutation of T50 or S54, the priming site for GSK3β, enhances N-myc stability and promotes proliferation of cultured GNPs (18). Because IGF activates Akt to inhibit GSK3β, we speculated that the enhancing effect of IGF-II and Akt on Shh-induced medulloblastoma formation (15) might result from the ability of PI3K pathway activation to stabilize N-myc by inhibiting GSK3β.

We asked how N-myc with a nonphosphorylatable alanine substitution at T50 affected Shh-mediated medulloblastoma formation. Tumor incidence increased to 78% \((P = 0.0018)\) when we used the RCAS-N-mycT50A vector to express this stabilized allele of N-myc in nestin-expressing neural progenitor cells together with RCAS-Shh (Table 1). The induced tumors were highly aggressive. The median survival for mice that developed tumors was shorter following transfer of Shh + N-mycT50A (22 days) compared with Shh + N-myc (47 days; \(P = 0.0006\) by \(t\) test). A trend toward increased proliferation was shown by immunostaining for Ki67, a nuclear protein expressed by actively dividing cells. The percentage of Ki67+ cells (mitotic index) was 37% \((\pm 7\%)\) in Shh + N-mycT50A–induced tumors compared with 17% \((\pm 3\%)\) in Shh + N-myc–induced tumors \((P = 0.07 \text{ by } \text{t test}; \text{Table 1}).\)

Immunoperoxidase staining with N-myc-specific antibody C19 of tumor-bearing brain sections from mice injected with RCAS-Shh + RCAS-N-mycT50A showed nuclear or cytoplasmic immunoreactivity in 100% of tumor cells (Fig. 2A). To detect ectopic (RCAS-mediated) gene expression, we induced medulloblastomas in Ntv-a mice with epitope-tagged versions of N-mycT50A and Shh (c-Myc-tagged N-mycT50A and HA-tagged Shh). When we probed brain tissue sections with antibodies directed against the tags, we found expression of RCAS-N-mycT50A (nuclear or cytoplasmic) in ~50% of tumor cells (Fig. 2B and C) and RCAS-Shh (cytoplasmic) in <30% (Fig. 2D).

Thus, the induced tumors are mixtures of clones originating from cells infected with Shh alone or in combination with N-myc. Consistent with our previous report on medulloblastomas induced by Shh + c-Myc, these results suggest that Shh, a secreted protein, may exert a paracrine effect on neighboring cells (12). Figure 2C and D shows ectopic (RCAS-mediated) expression of Shh and N-myc in tumor cells abutting the cortical surface of the cerebellum. We did not see transgene expression in deeper layers of the cerebellum, except for tumors cells infiltrating the molecular layer. These findings support an origin of the induced tumors from GNPs in the EGL.

To obtain additional evidence for transfer and expression of retroviral N-myc, we removed the brains from three mice, injected 3 months earlier with Flag-tagged RCAS-N-mycT50A in combination with untagged RCAS-Shh. The brain from one mouse (D3.1) showed a large tumor that replaced half of the cerebellum. A second mouse (A3) showed a smaller, but still grossly visible, tumor. No tumor was visible in the third mouse (D3.2).

We prepared protein lysates from the cerebellum and forebrain of each mouse and probed an immunoblot with an antibody directed against the Flag epitope. Flag immunoreactivity was detected in the tumor-bearing cerebellar but not in cerebellum or
forebrain tissue from mouse D3.2, which lacked a visible tumor (Fig. 2E). Taken together, these results indicate that stabilization of N-myc, by preventing GSK3β-mediated phosphorylation and degradation, powerfully enhanced medulloblastoma formation.

N-myc positively regulates G1 cyclins to enhance proliferation in medulloblastoma. Overexpressing N-myc in cultured GNPs promotes cell cycle progression in the absence of Shh signaling (19). This can be attributed at least partly to N-myc induction of D-type cyclins, which promote progression through the G1 phase of the cell cycle (37). Impaired cerebellar neurogenesis occurs in mice lacking cyclin D2, and this cyclin is strikingly down-regulated in mice lacking N-myc in the central nervous system (21, 38). We found high levels of cyclin D2 in the grossly visible mouse tumors induced by Shh + N-mycT50A (Fig. 2E). Immunocytochemistry showed intense cyclin D2 staining in the majority of tumor cell nuclei (Fig. 2F). These data suggest that up-regulation of N-myc in medulloblastoma promotes continuous cell cycle progression, at least partly through prolonged G1 cyclin up-regulation. N-myc is likely to have additional downstream effectors promoting GNP proliferation because overexpression of D-type cyclins alone is not sufficient for GNP proliferation in vitro (20).

Shh is required for GNP transformation. In proliferating GNPs, Shh signaling induces N-myc gene transcription (19, 20) and IGF/Pi3K signaling stabilizes the N-myc protein (18). Because Shh and IGF cooperate to promote medulloblastoma formation in mice (15), we wished to determine whether their combined effects on N-myc expression and stability were the principle determinants of tumorigenesis in vivo or if additional elements were required. We injected the cerebella of newborn Ntv-a mice with cell pellets producing RCAS-N-mycT50A viruses alone. We did not detect tumors in these animals. Thus, whereas both N-myc and N-mycT50A strongly synergized with Shh in promoting medulloblastoma formation, increased levels of N-myc alone were not sufficient to induce tumor formation.

These results suggest that Shh may have transforming effects on GNPs, distinct from increasing levels of N-myc mRNA. Alternatively, Shh and IGF may both provide transforming cues and N-myc may cooperate with either pathway to render GNPs susceptible to transformation. To address this possibility, we asked whether N-myc could cooperate with IGF to induce medulloblastoma. We could not induce tumors by injecting mice with RCAS-N-myc in combination with RCAS-IGF-II or its downstream effector, RCAS-Akt (Table 1). These data indicate that Shh has transforming functions that are not supplied by IGF/Pi3K signaling in GNPs.

Members of the Gli family of transcription factors are well-known transcriptional targets of Shh and they are required for mediating the effects of Shh during embryonic development (reviewed in ref. 39). Gli1, a positive regulator of Shh signaling, has been implicated in spontaneous medulloblastoma formation in Patched-deficient mice (10, 25). However, Gli1 is not necessary for induction of medulloblastoma in a different mouse model involving ectopic expression of Shh in utero (11). Both of these models rely on fetal alteration of gene expression whereas medulloblastomas in humans arise during postnatal cerebellar development. We wished to address the role of Gli1 in GNP transformation during the appropriate developmental window using RCAS-mediated gene transfer. Full-length Gli1 cDNA is too large for efficient RCAS packaging (3.3 kb). Therefore, we designed an RCAS vector to transfer and express a deleted version of Gli1, termed Gli1(-AT), which retains full transcriptional activity (ref. 24; Fig. 3A). GLI(-AT) retroviruses were also highly infectious and expressed in DF-1 cells (Fig. 3B).

In contrast to Shh alone, which promotes medulloblastoma formation in ~15% of injected mice, RCAS-Gli1(-AT) did not induce medulloblastoma in any animals (Table 1). This suggests that Shh promotes oncogenesis through mechanisms separate from or in addition to induction of Gli1. One of these mechanisms may involve up-regulation of N-myc. Whether Shh induces N-myc through Gli1 signaling is not yet resolved. Thus, we next asked if Gli1 could cooperate with N-myc to promote medulloblastoma formation. However, we found that this was not the case (Table 1). We concluded from these results that Gli1 is not a critical mediator of Shh tumorigenic effects in GNPs.
Discussion

We show here that N-myc, a transcriptional target of Shh in GNPs, can replace IGF/Pi3K signaling in cooperating with Shh to promote medulloblastoma formation in mice in vivo. Ectopic expression of an N-myc mutant, resistant to the proteolytic effects of GSK3β, significantly enhanced Shh-induced medulloblastoma formation. This suggests that an important role of the IGF/Pi3K pathway is to increase levels of N-myc, at least partly through its stabilizing effects resulting from GSK3β inhibition.

Mice in which the N-myc gene has been constitutively knocked out fail to survive beyond midgestation because multiple defects arise during organogenesis (40, 41). These developmental defects are particularly severe in the nervous system. A vital role for N-myc in governing cerebellar development was shown by Knoepfler et al. (21) who created conditional knockout mice in which N-myc was deleted in nestin-expressing, neural progenitors. These mice survived to adulthood, but with marked reduction in brain size, which was strongly evident in the cerebellum. Cerebellar hypoplasia correlated with progenitor cell depletion and increased levels of cyclin-dependent kinase inhibitors. In cultured GNPs, blocking N-myc function results in reduced proliferation (19, 20). Conversely, ectopic expression of N-myc enhances Shh-induced proliferation in vitro, indicating that GNPs are highly sensitive to levels of N-myc in regulating proliferation (18). Our finding that enhancing levels of N-myc increases Shh-induced medulloblastoma formation suggests that heightened or protracted expression of N-myc makes GNPs highly susceptible to additional transforming events.

Hyperactive IGF signaling plays a supporting role in medulloblastoma formation but it is not sufficient for tumor induction (15). Transgenic mice in which IGF-I expression is driven to high levels by the neurologically active Igf2 gene promoter do not develop tumors. Instead, they develop enlarged cerebella containing dramatically increased numbers of granule neurons (14). This hyperplastic response stems from the ability of IGF to promote both survival and proliferation of GNPs (42, 43). It is possible that the complementary transforming function supplied by Shh is its ability to suppress differentiation, an important role of Shh signaling in GNPs (44). Our observation that N-myc is a potent substitute for IGF/Pi3K signaling in cooperating with Shh to induce medulloblastoma is consistent with the in vitro finding that PI3K signaling in GNPs inhibits GSK3β-dependent phosphorylation and turnover of N-myc protein and thus increases accumulation of this oncogenic, cell cycle–stimulating transcription factor (18). Alternatively, IGF and N-myc might independently cooperate with Shh to enhance tumor formation through undetermined mechanisms.

In cultured GNPs, retrovirally expressed N-myc can replace Shh in promoting cell cycle progression, suggesting that N-myc transcriptional targets are likely to be downstream mediators of Shh proliferative effects (19, 20). However, ectopic expression of N-myc or upstream PI3K pathway activators, IGF-II and Akt, was not sufficient to induce medulloblastoma formation without coexpression of Shh. This suggests that Shh has additional targets that complement the pro-proliferative effects of N-myc to induce neoplastic transformation in vivo. Transcriptional profiling studies have shown that stimulation of GNPs by Shh up-regulates the expression of broad classes of genes governing such diverse functions as cell cycle progression, differentiation, DNA replication, and ribosome assembly (20, 45). It is not known which of these Shh target genes might provide the complementary oncogenic functions to N-myc.

The transcription factor Gli1, a direct target of Shh, has been implicated in medulloblastoma (10). However, the role of Gli1 in GNP proliferation is questionable because mice lacking Gli1 show no cerebellar abnormalities (46). It has recently been suggested that Gli1 plays a role in tumor formation in Patched+/− mice (47). However, these authors also found evidence of increased Gli2 activity in medulloblastomas from Patched+/− mice, and others have shown that Gli2 mediates effects of aberrant Shh pathway activation during development (49). Gli1 is dispensable for Shh-mediated medulloblastoma formation in Patched-wild-type mice (11), which is consistent with our inability to induce medulloblastoma by RCAS-mediated expression of Gli1. Thus, resolving the individual roles of Gli1 and Gli2 in mediating the transforming functions of Shh in GNPs will require further investigation.

As shown in Fig. 4, it is possible that Shh signaling is involved in tumor initiation but subsequent expansion of tumor cells is driven by Shh-mediated up-regulation of the IGF/Pi3K pathway. The ability of Shh to activate this pathway is supported by the observations that IGF-II is required for medulloblastoma formation in Patched-deficient mice (49) and that PI3K signaling is activated in medulloblastomas induced by ectopic expression of Shh (15). Once activated, IGF/Pi3K signaling would then promote N-myc stabilization and consequent cell cycle progression, in addition to playing other oncogenic roles, such as enhancing cell survival. This mechanism can be initiated by excess Shh signaling alone and magnified by the addition of ectopic N-myc.

Although Fig. 4 depicts a linear pathway connecting IGF and Akt with N-myc, the oncogenic effects of IGFs in human brain tumors are probably more complex. IGFs can activate multiple downstream effectors, including the mitogen-activated protein kinase (MAPK) pathway. In cerebellar neurons, however, IGF-dependent survival is mediated by PI3K signaling, not MAPK signaling (42). Furthermore, a central role of PI3K signaling in mediating the oncogenic effects of IGF is supported by our previous report that activated Akt can functionally replace IGF in enhancing Shh-induced medulloblastoma formation (15). It will be difficult to unravel the distinct roles of these effectors in the context of initiating medulloblastoma.

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Proposed model for synergy between Shh and IGF/Pi3K signaling in medulloblastoma. IGF/Pi3K signaling inhibits GSK3β-mediated phosphorylation and consequent degradation of N-myc. Shh directly stimulates N-myc gene transcription. Shh may also activate the IGF/Pi3K pathway via unknown intermediary molecules. In GNPs, the combined effect of these events is to increase N-myc protein levels, thereby stimulating G1 cell cycle progression and promoting tumorgenesis. Medulloblastomas can be induced by RCAS-mediated ectopic expression of Shh, but not of IGF-II, Akt, or N-myc, indicating that Shh has unique and essential transforming function.
played by N-myc and IGF in Shh-induced tumorigenesis in vivo due to the neural defects in constitutive N-myc knockout mice (40, 41) and the marked cerebellar hypoplasia in conditional knockouts (21). Modeling signal transduction pathways using the RCAS/Av-a system not only provides insights into the molecular pathogenesis of human medulloblastoma but also identifies potential therapeutic targets. Independent studies have shown that N-myc mRNA and protein levels are elevated in human medulloblastomas (13, 22). A broader role for Myc proteins is suggested by our previous report that c-Myc also cooperates with Shh to enhance medulloblastoma formation (12). The histology of tumors induced by Shh + c-Myc is identical to that described in this report, suggesting that N-myc and c-Myc may perform similar functions in cooperating with Shh.

Because the intracellular concentration of c-Myc is regulated by GSK3β-catalyzed phosphorylation reactions analogous to those that regulate N-myc turnover, a general therapeutic strategy for medulloblastoma could involve suppression of Myc levels in tumor cells by inhibiting PK3 signaling.

Acknowledgments

Received 6/29/2005; revised 11/26/2005; accepted 12/14/2005.

Grant support: NIH Cancer Training Grant T32 CA093247 (S.R. Browd), The Southung Foundation (A.M. Kenney), The Brain Tumor Society (D.W. Fults), The Charlotte Geyer Foundation (D.W. Fults), and NIH grant CA108622 (D.W. Fults). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

N-myc Can Substitute for Insulin-Like Growth Factor Signaling in a Mouse Model of Sonic Hedgehog–Induced Medulloblastoma

Samuel R. Browd, Anna M. Kenney, Oren N. Gottfried, et al.


**Updated version**
Access the most recent version of this article at:
[http://cancerres.aacrjournals.org/content/66/5/2666](http://cancerres.aacrjournals.org/content/66/5/2666)

**Cited articles**
This article cites 49 articles, 30 of which you can access for free at:
[http://cancerres.aacrjournals.org/content/66/5/2666.full#ref-list-1](http://cancerres.aacrjournals.org/content/66/5/2666.full#ref-list-1)

**Citing articles**
This article has been cited by 15 HighWire-hosted articles. Access the articles at:
[http://cancerres.aacrjournals.org/content/66/5/2666.full#related-urls](http://cancerres.aacrjournals.org/content/66/5/2666.full#related-urls)

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.