G-Protein Gαs controls medulloblastoma initiation by suppressing sonic hedgehog signaling

Xuelian He and Q. Richard Lu*

Department of Pediatrics, Brain Tumor Center; Divisions of Experimental Hematology and Cancer Biology & Developmental Biology; Cancer and Blood Diseases Institute; Cincinnati Children’s Hospital Medical Center; University of Cincinnati College of Medicine; Cincinnati, OH USA

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We identify Gαs as a novel tumor suppressor in medulloblastoma that functions principally by inhibition of sonic hedgehog signaling. Gαs not only stimulates cyclic adenosine monophosphate (cAMP)-dependent signaling but also inhibits ciliary trafficking of hedgehog components. Elevation of cAMP inhibits medulloblastoma growth and augments inhibition of smoothened to decrease tumor cell proliferation, thus highlighting Gαs as a potential therapeutic target.

Medulloblastoma (MB) is the most common malignant brain tumor in children, accounting for 25% of all pediatric brain cancers. At present, the molecular events and signaling pathways that drive the initiation and progression of these tumors are not fully understood. This hinders vital improvement of current therapies, which are often associated with major long-term side effects and cause life-changing sequelae for survivors.1

Human MB can be classified into at least 4 principal subgroups based on gene expression profiles: wingless (WNT) group, sonic hedgehog (SHH) group, group 3, and group 4.2 Activation of the SHH pathway, defining the SHH group, is detected in ~30% of human MB cases. SHH signaling is mediated through activation of the G protein-coupled receptor (GPCR)-like 7-transmembrane protein, smoothened (SMO). However, mutations in SHH signaling components, including patched1, SMO, and suppressor-of-fused, account for only approximately 50% of cases of sporadic human MB exhibiting SHH pathway activation. Other gene alterations or signaling modifiers that drive MB tumorigenesis have not been fully elucidated and very few pathways that suppress MB formation have been identified. Recent genome-wide analyses of somatic mutations and copy number alterations identified GNAS as one of the most frequently mutated genes in a variety of human cancers, suggesting a wide-ranging role for GNAS in multiple cancer types.3 GNAS encodes the heterotrimeric Gα protein α-subunit (Gαs) that functions as a molecular switch to transmit various GPCR signals to control cell growth, survival, and motility.4 We found that low GNAS expression was tightly correlated with significantly decreased overall survival within SHH-group tumors.2 The prognostic impact of GNAS was not observed in WNT group, group 3, or group 4 tumors and across MB subgroups. Remarkably, a recent case report showed that a 14-month-old infant with a novel homozygous nonsense mutation within the GNAS coding region developed MB.5 These observations suggest that low expression or loss of GNAS specifically defines a subset of aggressive SHH-group MBs. Our study identifies GNAS as a prognostic biomarker in humans for the stratification of SHH-driven MB treatment. Moreover, a recent genome sequencing study showed that 8 cases out of 133 SHH-driven MBs carried GNAS mutations.6 These findings highlight GNAS dysregulation in MB formation as clinically significant.

Our studies using in vivo animal models demonstrate that GNAS is a potent tumor suppressor gene in SHH-driven MB.7 We provide the first evidence that loss of the single gene Gnas is sufficient to initiate formation of MB-like tumors in anatomically distinct progenitors of the murine developing hindbrain. Loss of function of Gnas-floxed alleles in human glial fibrillary acidic protein (GFAP) promoter-expressing neural progenitor cells, atonal homolog 1 (Atoh1) promoter-expressing cerebellar granular neuron progenitor cells (GNPs), or oligodendrocyte transcription factor 1 (Olig1)-expressing progenitors leads to formation of heterogeneously located SHH-associated MBs that recapitulate their human
counterparts. Thus, Gnas is a critical determinant of progenitor cell competency for MB initiation across disparate cells of origin. The identification of Olig1 progenitor cells as a novel cellular origin for a subset of an anatomically distinct malignant SHH-associated MBs highlights the tumor heterogeneity with regard to the cellular origin and anatomical location.

We demonstrate that Gαs employs a dual-mode regulation of SHH signal transduction through controlling both trafficking of SHH signaling components in the murine primary cilium of GNPs and the cyclic adenosine monophosphate (cAMP) signaling cascade, which reinforces the inhibition of SHH signaling by Gαs activity. Gain- and loss-of-function studies demonstrate that Gαs can activate the cAMP-dependent pathway and enhance Gli3 processing into a Gli3R repressor form, which negatively regulates SHH signaling. Moreover, Gαs activity modulates trafficking of SHH signaling components at the primary cilium by inhibiting ciliary translocation of SMO and Gli2 accumulation at the tip of primary cilium. The Gαs deletion phenocopies the gain-of-function SMO mutants by activating SHH signaling in cerebellar granular neuron progenitor cells (GNPs) and promotes medulloblastoma formation. (B) Upregulation of Gαs GTPase activity increases cAMP levels, which activates the cAMP-dependent protein kinase A pathway to initiate downstream signaling cascades. Gαs is highly enriched in the primary cilium of GNPs, and its activation blocks SMO ciliary translocation and maintains Patched1 positioning at the primary cilium to block SMO signaling activation and medulloblastoma initiation.

**Figure 1.** Gαs as a molecular switch to control sonic hedgehog signaling and medulloblastoma formation. (A) When Gαs GTPase activity is turned off due to Gαs inactivation or the presence of sonic hedgehog (SHH), intracellular cyclic adenosine monophosphate (cAMP) levels are reduced and the cAMP-dependent pathway is inhibited. Activation of smoothened (SMO) signaling then occurs in part through SMO translocation to primary cilium and Gli2 accumulation at the tip of primary cilium. The Gαs deletion phenocopies the gain-of-function SMO mutants by activating SHH signaling in cerebellar granular neuron progenitor cells (GNPs) and promotes medulloblastoma formation. (B) Upregulation of Gαs GTPase activity increases cAMP levels, which activates the cAMP-dependent protein kinase A pathway to initiate downstream signaling cascades. Gαs is highly enriched in the primary cilium of GNPs, and its activation blocks SMO ciliary translocation and maintains Patched1 positioning at the primary cilium to block SMO signaling activation and medulloblastoma initiation.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
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