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G-Protein $G\alpha_s$ controls medulloblastoma initiation by suppressing sonic hedgehog signaling

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We identify $G\alpha_s$ as a novel tumor suppressor in medulloblastoma that functions principally by inhibition of sonic hedgehog signaling. $G\alpha_s$ 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 $G\alpha_s$ 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 G_s protein α-subunit ($G\alpha_s$) 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

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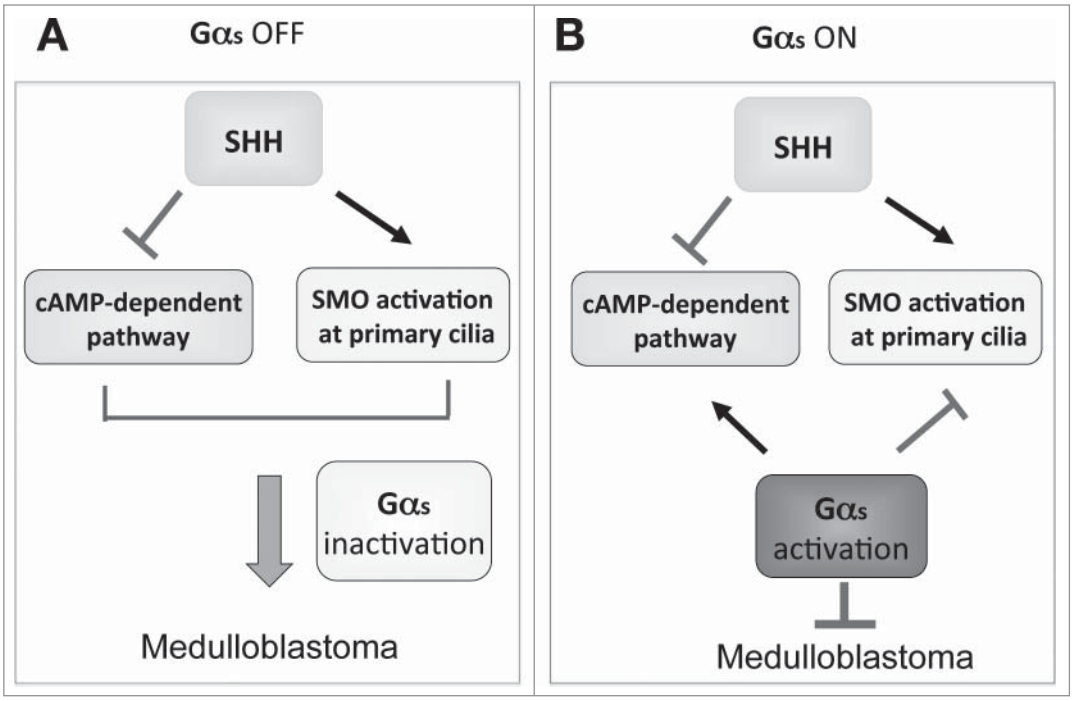

Figure 1. $G\alpha_s$ as a molecular switch to control sonic hedgehog signaling and medulloblastoma formation. (**A**) When $G\alpha_s$ GTPase activity is turned off due to $G\alpha_s$ inactivation or the presence of sonic hedgehog (SHH), intracellular cyclic adenosine monophosphate (cAMP) levels are reduced and the cAMP-dependent pathway is inhibited. Activation of smoothened (SMO) signaling then occurs in part through SMO translocation to primary cilia and Gli2 accumulation at the tip of primary cilia. The $G\alpha_s$ 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 $G\alpha_s$ GTPase activity increases cAMP levels, which activates the cAMP-dependent protein kinase A pathway to initiate downstream signaling cascades. $G\alpha_s$ is highly enriched in the primary cilia 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.

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 MB highlights the tumor heterogeneity with regard to the cellular origin and anatomical location.

We demonstrate that $G\alpha_s$ 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 $G\alpha_s$ activity. Gain- and loss-of-function studies demonstrate that $G\alpha_s$ can activate the cAMP-dependent pathway and enhance Gli3 processing into a Gli3R repressor form, which negatively regulates SHH signaling. Moreover, $G\alpha_s$ 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 cilia while maintaining positioning of the SMO inhibitor Patched1 at the primary cilium. This effect of $G\alpha_s$ on hedgehog component trafficking provides an additional level of regulation to control SHH signaling (**Fig. 1**).

Furthermore, we show that elevation of the levels of cAMP, an effector of $G\alpha_s$ signaling, by Rolipram possesses strong efficacy as an inhibitor of tumor growth in *Gnas* mutants and extends the life expectancy of *Gnas* mutant mice. Thus, our study reveals that an existing drug active against $G\alpha_s$, which has been used as an antidepressant in humans with clinical approval in Japan and Europe,[8] displays high efficacy in mitigating a subset of aggressive SHH-associated MBs. Rolipram enhances the efficacy of a smoothened antagonist in blocking GNP proliferation, suggesting that combinatorial treatment using cAMP-elevating agents together with smoothened receptor inhibitors might be particularly useful in MB.

$G\alpha_i$ was reported to mediate SMO signaling in *Drosophila*, yet the role of G protein-coupled SHH signaling in mammalian development and cancer formation remains controversial.[9] We provide the first demonstration that the GPCR signal transducer $G\alpha_s$ is able to control hedgehog signaling in the mammalian nervous system. Since activating *GNAS* mutations are associated with most somatic tumor types, our analysis of MB patients with *GNAS*-attenuating/inactivating mutations and loss-of-function studies in animal models unexpectedly reveal that *GNAS* is a potent tumor suppressor gene in SHH-driven MB, representing a paradigm shift in MB tumorigenesis and treatment. $G\alpha_s$-mediated signaling control may be a point of signaling convergence for GPCR-like SMO, and perhaps other GPCRs, during MB initiation, and indicates that co-targeting of SMO and G-protein signaling may circumvent the drug resistance seen with SMO antagonists alone[1,10] and could be beneficial in the treatment of these deadly pediatric tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Ng JM, Curran T. The Hedgehog's tale: developing strategies for targeting cancer. Nature Rev Cancer 2011; 11:493-501; PMID:21614026; http://dx.doi.org/10.1038/nrc3079
2. Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, Eberhart CG, Parsons DW, Rutkowski S, Gajjar A, et al. Molecular subgroups of medulloblastoma: the current consensus. Acta neuropathologica 2012; 123:465-472; PMID:22134537; http://dx.doi.org/10.1007/s00401-011-0922-z
3. Kan Z., Jaiswal BS, Stinson J, Janakiraman V, Bhatt D, Stern HM, Yue P, Haverty PM, Bourgon R, Zheng J, et al. Diverse somatic mutation patterns and pathway alterations in human cancers. Nature 2010; 466:869-873; PMID:20668451; http://dx.doi.org/10.1038/nature09208
4. Neves SR, Ram PT, Iyengar R. G protein pathways. Science 2002; 296:1636-1639; PMID:12040175; http://dx.doi.org/10.1126/science.1071550
5. Huh JY, Kwon MJ, Seo KY, Kim MK, Chae KY, Kim SH, Ki CS, Yoon MS, Kim DH. Novel nonsense GNAS mutation in a 14-month-old boy with plate-like osteoma cutis and medulloblastoma. J Dermatol 2014; 41:319-21; PMID:24517547; http://dx.doi.org/10.1111/1346-8138.12284
6. Kool M, Jones DT, Jager N, Northcott PA, Pugh TJ, Hovestadt V, Piro RM, Esparza LA, Markant SL, Remke M, et al. Genome sequencing of SHH Medulloblastoma predicts Genotype-Related response to smoothened inhibition. Cancer cell 2014; 25:393-405; PMID:24651015; http://dx.doi.org/10.1016/j.ccr.2014.02.004
7. He X, Zhang L, Chen Y, Remke M, Shih D, Lu F, Wang H, Deng Y, Yu Y, Xia Y, et al. The G protein alpha subunit Galphas is a tumor suppressor in Sonic hedgehog-driven medulloblastoma. Nat Med 2014; 20:1035-1042; PMID:25150496; http://dx.doi.org/10.1038/nm.3666
8. Sengupta R, Sun T, Warrington NM, Rubin JB. Treating brain tumors with PDE4 inhibitors. Trends Pharmacol Sci 2011; 32:337-344; PMID:21450351; http://dx.doi.org/10.1016/j.tips.2011.02.015
9. Philipp M, Caron MG. Hedgehog signaling: is SMO a G protein-coupled receptor? Curr Biol 2009; 19:R125-127; PMID:19211052; http://dx.doi.org/10.1016/j.cub.2008.12.010
10. Yauch RL, Dijkgraaf GJ, Alicke B, Januario T, Ahn CP, Holcomb T, Pujara K, Stinson J, Callahan CA, Tang T, et al. Smoothened mutation confers resistance to a Hedgehog pathway inhibitor in medulloblastoma. Science 2009; 326:572-574; PMID:19726788; http://dx.doi.org/10.1126/science.1179386