

Mutations in *SUFU* predispose to medulloblastoma

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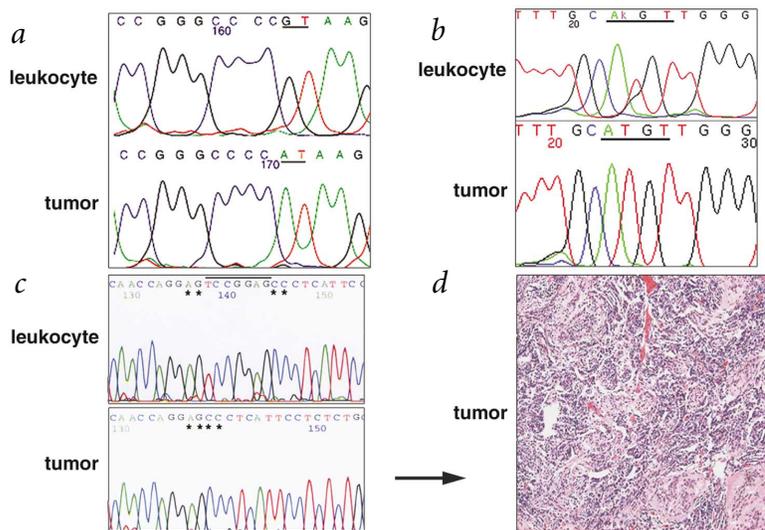
Published online: 17 June 2002, doi:10.1038/ng916

The sonic hedgehog (SHH) signaling pathway directs the embryonic development of diverse organisms and is disrupted in a variety of malignancies. Pathway activation is triggered by binding of hedgehog proteins to the multipass Patched-1 (PTCH) receptor, which in the absence of hedgehog suppresses the activity of the seven-pass membrane protein Smoothened (SMO). De-repression of SMO culminates in the activation of one or more of the GLI transcription factors that regulate the transcription of downstream targets. Individuals with germline mutations of the SHH receptor gene *PTCH* are at high risk of developmental anomalies and of basal-cell carcinomas, medulloblastomas and other cancers (a pattern consistent with nevoid basal-cell carcinoma syndrome, NBCCS). In keeping with the role of *PTCH* as a tumor-suppressor gene, somatic mutations of this gene occur in sporadic basal-cell carcinomas and medulloblastomas. We report here that a subset of children with medulloblastoma carry germline and somatic mutations in *SUFU* (encoding the human suppressor of fused) of the SHH pathway, accompanied by loss of heterozygosity of the wildtype allele. Several of these mutations encode truncated proteins that are unable to export the GLI transcription factor from nucleus to

cytoplasm, resulting in the activation of SHH signaling. *SUFU* is a newly identified tumor-suppressor gene that predisposes individuals to medulloblastoma by modulating the SHH signaling pathway through a newly identified mechanism.

SHH is a powerful mitogen for external granular cells of the cerebellum, which give rise to medulloblastomas, the most common malignant pediatric brain tumor^{1,2}. Mutations of the SHH receptor gene *PTCH* occur in 50% or more of individuals with NBCCS, who present at birth with an overgrowth syndrome that includes large body size, hypertelorism, frontal bossing and additional malformations such as a flat nasal bridge, bifid ribs, polydactyly and occasionally developmental delay³⁻⁶. Later in life, individuals with NBCCS may develop a spectrum of neoplasms, including basal-cell carcinoma (BCC), medulloblastoma and meningioma. Somatic mutations of the genes *PTCH* and *SMO* occur in a subset of sporadic BCCs and medulloblastomas⁷⁻¹³, and human *GLI* was initially identified as a gene amplified in a human glioblastoma¹⁴. These observations are consistent with those in mice: 14% of *Ptch*^{+/-} mice develop medulloblastomas^{15,16}, and overexpression of human *GLI*¹⁷, mouse *Gli2* (ref. 18) or *SHH*¹⁹ in mouse skin leads to BCCs.

Fig. 1 Mutations of *SUFU* in desmoplastic medulloblastomas. **a**, Conserved GT splice-donor site consensus sequence of exon 8 is mutated to AT in the tumor (IVS8+1G→A). No wildtype sequence was seen, suggesting LOH. Analysis by RT-PCR indicated splicing of exon 7 to exon 9 with a subsequent frameshift and premature stop codon (data not shown). **b**, There was a heterozygous mutation in the conserved AG splice-acceptor site of exon 2, which was mutated to AT (IVS1-1G→T) in the germline DNA. The wildtype was absent in a sample from the desmoplastic medulloblastoma of the same individual, consistent with LOH. Analysis by RT-PCR showed splicing of exon 1 to exon 4 with a subsequent frameshift and premature stop codon at the 3' end of exon 1 (data not shown). **c**, Sequence analysis of the tumor DNA showed a deletion of 7 nucleotides from exon 9 (1129delTCCGGAG) resulting in a frameshift and premature stop codon; the wildtype allele was absent. **d**, Hematoxylin and eosin stain showing histopathologic features of the tumor with the 1129delTCCGGAG mutation. This small blue cell tumor with nodular, reticulin-free zones was typical of a desmoplastic medulloblastoma.



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Table 1 • Summary of *SUFU* mutations in medulloblastomas and cell lines

Case	Phenotype	Morphology	Nucleotide	Tumor or germline	LOH in tumor	RT-PCR	Predicted protein	Comments
1	NBCCS-like; severe cognitive impairment	desmoplastic	2.5-Mb deletion	germline	NA	ND	NA	contiguous deletion syndrome in carrier mutation
			IVS8+1G→A splice donor	second hit in tumor	yes	E7 spliced to E9	frameshift at 5' and termination at 3' end of E9	
2	MB	desmoplastic	E1 143insA	germline	yes	ND	frameshift followed by termination codon 69 bp 3' to mutation	mutation
3	MB	desmoplastic	1129del TCCGGAG	tumor	yes	same as wild type	termination at 5' end of E10	mutation
4	MB	desmoplastic	E2 splice acceptor; IVS1-1A→T	germline	yes	E1 spliced to E4	termination at junction E1-E4	mutation
5	MB	unknown	C44T	tumor	yes	normal	P15L	probable polymorphism
6	MB	desmoplastic	G1018T	germline	no	normal	A340S	polymorphism

MB, medulloblastoma; NA, not applicable; ND, not determined.

Frequent loss of heterozygosity (LOH) on chromosome 10q24 in medulloblastomas suggests that this region contains one or more tumor-suppressor genes²⁰. We mapped *SUFU* to chromosome 10q24.3 by fluorescent *in situ* hybridization (FISH). Radiation-hybrid mapping of *SUFU* and *BTRC* (the latter encoding a ubiquitin ligase involved in both SHH and Wnt signaling) showed an identical map location for both genes at chromosome 10q24.3 (data not shown) distal to the tumor-suppressor gene *PTEN* on chromosome 10q23.31. Human genome sequence data indicated that *BTRC* is approximately 1 megabase (Mb) centromeric to *SUFU*. We screened the Roswell Park human genomic bacterial artificial chromosome (BAC) library with a *SUFU* cDNA probe and identified two BACs containing genomic *SUFU*: 2F13 and 124G18. Subcloning and sequence analysis of these BACs revealed that *SUFU* has 12 exons.

Mutational analyses of *PTEN* and *BTRC* revealed no mutations in a series of 21 and 36 sporadic medulloblastomas, respectively. We identified truncating mutations of *SUFU* in 4 of 46 samples (9%) by sequence analysis of exons 1–12 and their surrounding intronic sequences. This mutation frequency is comparable to that observed for *PTCH* (9%) and *CTNNB1* (encoding β -catenin; 5%)^{7,21}. Sequence analysis and RT-PCR analyses revealed that these four *SUFU* mutations predicted truncated protein products (Fig. 1 and Table 1). We also observed two missense mutations: the non-conservative P15L near the amino terminus and the relatively conservative A340S (Table 1). All four truncating mutations and the P15L mutation were accompanied by LOH, loss of the wildtype allele or both (Fig. 1a–c and Table 1), but we did not detect LOH of the A340S variant. All four medulloblastomas with *SUFU* truncating mutations were of the desmoplastic subtype (Fig. 1d and Table 1). Desmoplas-

tic tumors make up about 20–30% of medulloblastomas, have a more nodular architecture than 'classical' medulloblastoma and may have a better prognosis. Activation of the SHH pathway is particularly high in desmoplastic medulloblastomas, as shown by increased expression of the SHH target genes *GLI*, *SMOH* and *PTCH*^{12,22}. We conclude that *SUFU* functions as a tumor-suppressor gene in a subset of desmoplastic medulloblastomas.

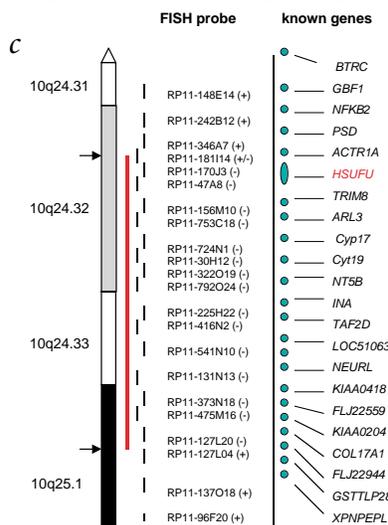
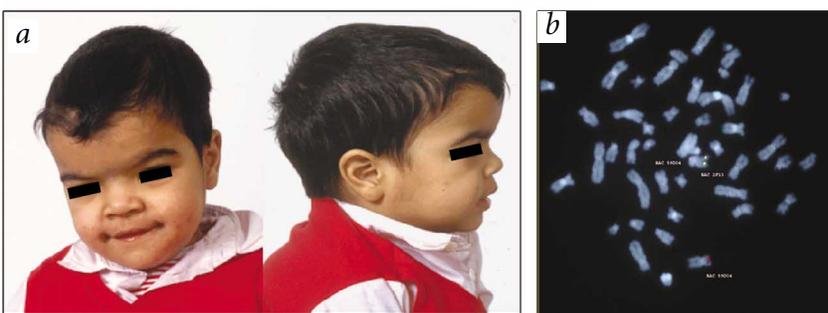


Fig. 2 Contiguous deletion encompassing *SUFU* in a child with developmental anomalies and a desmoplastic medulloblastoma. **a**, Anteroposterior and lateral photographs of a child with global developmental delay and a desmoplastic medulloblastoma. Facial features included frontal bossing, prominent jaw and hypertelorism, which were comparable to the facial features in NBCCS. **b**, Metaphase FISH on peripheral leukocytes from this child showed 4 red signals from rhodamine-labeled BAC RP11-59D04 on chromosome 10p15 but only 2 green signals from fluorescein-labeled BAC RP11-2F13 on chromosome 10q24, which encompassed the *SUFU* locus. Both of the child's parents showed normal signals from chromosome 10q24 (data not shown). **c**, Diagram of the deletion on chromosome 10q in the child. Multiple FISH experiments using contiguous BAC clones showed that the deletion was 2.5–2.8 Mb in size and included at least 28 genes (not all shown here). FISH using a BAC probe that encompassed *BTRC* indicated that this gene was not included in the deletion (most centromeric gene in this figure).

The individual with a medulloblastoma bearing the IVS8+1G→A intron 8 truncating mutation was a 4-year-old boy with some phenotypic characteristics suggestive of NBCCS, including frontal bossing, prominent jaw and hypertelorism (Fig. 2a). He did not exhibit odontogenic cysts, falx calcification, skin pits or BCCs, but these characteristics may not arise until later in life in people with NBCCS. This child also had severe developmental delay: he was unable to walk and speak, and did not recognize his parents as his primary care-givers. Although some degree of cognitive impairment may follow treatment for medulloblastoma, the child's examination and history were more in keeping with a developmental syndrome. Neither parent had any evidence of NBCCS and there was no family history of cancer.

The IVS8+1G→A mutation detected in this boy's medulloblastoma was not present in his germline DNA. Southern blotting using an *SUFU* cDNA probe revealed no differences in the child's DNA compared with controls (data not shown). We carried out metaphase FISH using a BAC clone encompassing *SUFU*

in its entirety on lymphoblastoid cell lines from both parents and child. We observed only one chromosome 10q24 signal for the child (Fig. 2b) but two signals for each parent (data not shown); FISH of the child's peripheral blood lymphocytes confirmed this finding. Haplotyping indicated that the loss had occurred in the paternal chromosome (data not shown). Additional FISH experiments using contiguous BAC clones in the region of chromosome 10q24.3 showed that the child had a deletion of 2.5–2.8 Mb that encompassed chromosomal sub-bands 10q23.32–10q25.1, including at least 28 genes but sparing the centromeric *BTRC* (Fig. 2c). Some individuals with cytogenetic interstitial deletions of 10q22–10q26 share some of the phenotypic characteristics observed in the child with the somatic IVS8+1G→A mutation, including psychomotor retardation, hypertelorism and a broad nasal bridge²³. In this child, loss of contiguous genes at 10q—including *SUFU*—was therefore associated with both medulloblastoma and a NBCCS-like phenotype with profound developmental delay.

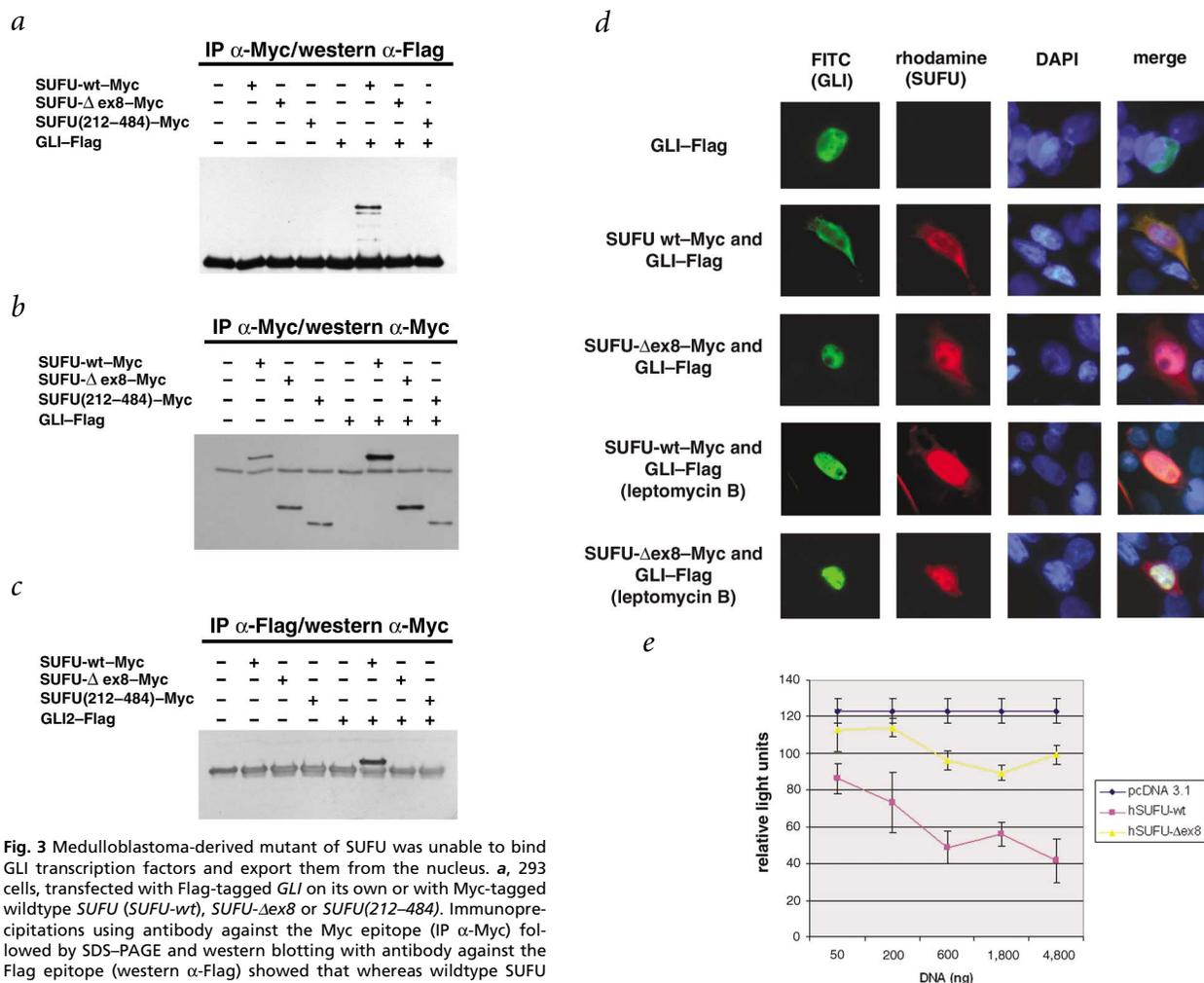


Fig. 3 Medulloblastoma-derived mutant of *SUFU* was unable to bind GLI transcription factors and export them from the nucleus. **a**, 293 cells, transfected with Flag-tagged *GLI* on its own or with Myc-tagged wildtype *SUFU* (*SUFU*-wt), *SUFU*-Δex8 or *SUFU*(212–484). Immunoprecipitations using antibody against the Myc epitope (IP α-Myc) followed by SDS-PAGE and western blotting with antibody against the Flag epitope (western α-Flag) showed that whereas wildtype *SUFU* (*SUFU*-wt) could bind *GLI*, the Δex8 mutant and the *SUFU*(212–484) mutant could not. **b**, The presence of equal amounts of immunoprecipitated wildtype *SUFU*, Δex8 and *SUFU*(212–484) was confirmed by stripping and reprobing the IP α-Myc, western α-Flag blot from Fig. 4a with an antibody against Myc. **c**, We then transfected 293 cells, co-transfected alone and in combination with Flag-tagged *GLI2* and Myc-tagged wildtype *SUFU*, *SUFU*-Δex8 and *SUFU*(212–484). Immunoprecipitations against the Flag epitope followed by SDS-PAGE and western blotting against the Myc epitope showed that whereas *GLI2* immunoprecipitated *SUFU*-wt, it did not bind *SUFU*-Δex8 or *SUFU*(212–484). **d**, C3HT101/2 cells were transfected with Flag-*GLI* and either Myc-*SUFU*-wt or Myc-*SUFU*-Δex8, and stained for immunofluorescence with monoclonal antibody against Flag and polyclonal antibody against Myc followed (as secondary antibodies) by rhodamine-labeled antibody against rabbit IgG and FITC-labeled antibody against mouse IgG. Cells were also stained with DAPI for visualization of the nucleus. **e**, C3HT101/2 cells were transfected with a Gli-responsive promoter construct (*8*Gli*) and various doses of *GLI*, and either the empty pcDNA 3.1(+) vector (control), *SUFU*-wt or *SUFU*-Δex8. *SUFU*-wt strongly inhibited transcription promoted by *GLI* at very low dosages, whereas *SUFU*-Δex8 had no effect as compared with the control. Very high doses of *SUFU*-Δex8 blocked transcription from the *8*Gli* reporter to a moderate degree, but not nearly as much as *SUFU*-wt. Error bars, ± s.e.m.

We sequenced peripheral blood DNA from individuals with medulloblastomas bearing *SUFU* mutations and demonstrated that the exon 2 splice-site mutation (IVS1-1A→T) and the exon 1 insertion (143insA) were present in lymphocyte DNA. In each case, the wildtype allele was either deleted or mutated in the corresponding tumor (Table 1). The individual with the IVS1-1A→T mutation was adopted, and his biological family history was unavailable. The individual with the 143insA mutation (Table 1) had no known family history of any cancer. Six years after radiotherapy for his medulloblastoma, however, he developed a meningioma in the region of the radiation field. These two individuals with heterozygous germline point mutations of *SUFU* had no discernible developmental abnormalities; this is consistent with the very subtle phenotypic alterations in wing patterning seen in *Drosophila* homozygous *SUFU* mutants²⁴. We screened 29 DNA samples from persons with NBCCS lacking germline mutations of *PTCH* but detected no mutations of *SUFU*, concluding that alterations of *SUFU* alone were not a proximate cause of NBCCS.

To study the functional consequences of the *SUFU* truncation mutants, we employed the *SUFU* IVS8+1G→A variant. The corresponding protein lacks the carboxy-terminal half of *SUFU* (designated *SUFU*- Δ ex8). We also constructed a corresponding N-terminal deletion mutant protein, designated *SUFU*(212-484)-Myc. Transfection of epitope-tagged wildtype *SUFU*, Δ ex8 or *SUFU*(212-484) mutants together with either *GLI* or *GLI2* shows that wildtype *SUFU* can bind *GLI* and *GLI2*, whereas the Δ ex8 and *SUFU*(212-484) mutants cannot (Fig. 3a-c). We studied the distribution of *SUFU* and *GLI* in the nucleus and cytoplasm by transfecting expression vectors bearing either wildtype or Δ ex8 mutant *SUFU* together with one bearing *GLI* into C3HT101/2 cells. Fluorescence microscopy showed that *GLI* transfected by itself was predominantly localized in the nucleus. Wildtype *SUFU* was primarily found in the cytoplasm, and in its presence, *GLI* was also localized in the cytoplasm and hence inactive (Fig. 3d). In contrast, the Δ ex8 mutant was localized in the nucleus, and in its presence, *GLI* accumulated substantially in the nucleus in all cells observed (Fig. 3d). Administration of leptomycin B, a CRM-1-dependent inhibitor of nuclear export, blocked the effects of wildtype *SUFU* so that both wildtype *SUFU* and *GLI* accumulated in the nucleus (Fig. 3d). As predicted, leptomycin B has no effect on *SUFU*- Δ ex8 and *GLI* co-transfectants, as both proteins were already localized in the nucleus (Fig. 3d). Finally, transfection of wildtype *SUFU* repressed transcriptional activation by *GLI* from a *GLI*-responsive promoter (8*3'*GLI*-BS-Luc) with very small amounts of input DNA, whereas transfection of *SUFU*- Δ ex8 produced results no different from transfection of an empty vector control (Fig. 3e). We deduced that the *SUFU*- Δ ex8 mutant protein does not block *GLI*-mediated transcriptional activation at physiological doses²⁵.

The *SUFU* protein can repress Wnt signaling by binding β -catenin and exporting it from the nucleus²⁶. We have shown that the medulloblastoma-derived *SUFU*- Δ ex8 mutant does not suppress Wnt signaling (M.D.T. *et al.*, manuscript in preparation). Our data support a model in which the tumor-derived *SUFU*- Δ ex8 is unable to bind *GLI* transcription factors and export them from the nucleus, resulting in activation of *SHH* target genes. This is a new mechanism of tumor suppression that entails modulation of the nuclear-cytoplasmic shuttling of transcription factors.

Methods

Tumor samples and isolation of nucleic acids. We collected samples of pediatric medulloblastoma under the guidelines of the Hospital for Sick

Children Research Ethics Board, flash-froze tissue directly after surgical removal and stored the samples in liquid nitrogen. Additional tumor samples came from the Brain Tumor Tissue Bank (London, Ontario, Canada), the Pediatric Co-operative Human Tissue Network of the US National Cancer Institute (Bethesda, Maryland), the Tissue Bank of the Brain Tumor Research Center at the University of California, San Francisco and the Children's National Medical Center (Washington, DC). Individuals with NBCCS but no mutations in *PTCH* were identified in a National Cancer Institute study^{6,27} or at the University of Queensland (Brisbane, Australia)²⁸. In every case, affected individuals or their guardians provided informed consent as outlined in protocols approved by local or national institutional review boards.

We isolated total RNA using Trizol reagent (Gibco-BRL), and isolated genomic DNA by overnight digestion in proteinase K followed by standard phenol/chloroform extraction. After dissecting tumor tissue from paraffin slides and preparing DNA by xylene extraction, we carried out digestion in proteinase K at 55 °C overnight.

Determination of genomic structure and chromosomal location of *SUFU* and *BTRC*. Using *SUFU* cDNA as a probe, we identified clones 124G18 and 2F13 from the Roswell Park human genomic BAC library, and sequenced subclones to determine intron-exon boundaries. We used PCR to screen a BAC library specific for chromosome 10q (Genome Systems) for clones containing human *BTRC*, and carried out radiation hybrid mapping of *SUFU* and *BTRC* with the GB4 panel (Research Genetics). All primer sequences are available on request.

RT-PCR. We generated cDNAs using SuperScript II RT (Gibco-BRL) with both oligo-dT and *SUFU*-specific primers. We performed 2 nested PCRs to amplify exons 1-4 and exons 4-12, respectively, subcloned the PCR products with the TA cloning Kit (Invitrogen) and sequenced them using the Cy-5/5.5-labeled M13 primer.

Fluorescence *in situ* hybridization. We cultured lymphocytes from peripheral blood or cell lines infected with Epstein-Barr virus and incubated the cells for 30 min with colcemid (0.1 μ g/ml) before harvesting. We labeled a contiguous series of BAC clones from chromosome 10q24-q25 with biotin using the BioNick Labeling system (Invitrogen), and labeled BAC RP11-59D04 on chromosome 10p15 with digoxigenin as a control. FISH signals were detected with fluorescein-avidin D (Vector) and fluorescein-labeled antibody against avidin D (Vector) for biotin-labeled probes, and with antibody against digoxigenin, digoxigenin-labeled antibody against mouse Ig, and rhodamine-labeled antibody against digoxigenin (Boehringer Mannheim) for digoxigenin-labeled probes.

Mutational analysis. Using intronic primers designed to include a 5'-M13 sequencing cassette for all 12 exons of *SUFU*, we carried out PCR reactions in a PTC-100 Programmable Thermal Controller for 35-40 cycles, usually at an annealing temperature of 60 °C. In some cases, we performed nested PCR using a second set of intronic primers. Amplified products were sequenced as previously described²⁹. Primer sequences and protocols are available on request.

Using single-strand conformation polymorphism (SSCP) as previously described³⁰, we screened 36 medulloblastomas for each exon of *BTRC* and sequenced exons that migrated aberrantly through the SSCP gel (11 of 38). We tested for LOH of the markers D10S215, D10S520 and D10S540 near *PTEN* in 21 medulloblastomas as previously described, and sequenced genomic DNA for all coding exons of *PTEN* from the corresponding tumors³⁰.

***SUFU* expression constructs.** We amplified *SUFU* cDNA from the Marathon fetal brain cDNA (Clontech) into pT-Adv (Clontech), created an in-frame N-terminal Myc tag by PCR of the *SUFU* cDNA, and cloned the *Myc-SUFU* cDNA into pcDNA3.1 (Invitrogen). Then we shuttled the mutant *SUFU*- Δ ex8 transcript (amplified from cDNA of medulloblastoma HMB2) into the pcDNA3.1 wildtype *SUFU*-Myc construct. We constructed the *SUFU*(212-484)-Myc expression vector by PCR and sequenced both strands of each expression construct to rule out PCR errors. pCMV-Flag-GLI1 and pCMV-Flag-GLI2 have previously been described³¹. Primer sequences are available on request.

Co-precipitation assay. We transfected 293 cells with pCMV–Flag–GLI1 or pCMV–Flag–GLI2 and either pcDNA 3.1–Myc–SUFU, pcDNA 3.1–Myc–SUFU Δ ex8 or pcDNA 3.1–Myc–SUFU(212–484) using Superfect (Qiagen). We lysed cells in 500 μ l lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100 and 1 mM EDTA), and incubated the lysates overnight at 4 °C with 2 μ l monoclonal antibody against Myc (UBI), or 2 μ l monoclonal antibody against Flag M2 (Sigma) and 50 μ l 20% protein G–Sepharose beads (Sigma). After washing the beads 5 times in IP washing buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 100 μ M ZnCl₂, 2 mM EDTA, 1% Nonidet P-40 and 10% glycerol), we resuspended and boiled them in Laemmli sample buffer. We resolved the samples by SDS–PAGE (immunoprecipitate/lysate ratio 50:1), transferred the gel to polyvinylidene difluoride membranes (Immobilon-P), probed membranes with monoclonal antibody against Flag-M2 (Sigma) or monoclonal antibody against Myc (UBI) and visualized the signals using enhanced chemiluminescence.

Immunofluorescence. We grew C3H10T1/2 cells on glass cover slips and transfected them with plasmid DNA using Fugene 6 (Roche). After 24 h, we fixed the cells in 4% paraformaldehyde at 37 °C for 10 min and rendered them permeable by incubation in methanol for 2 min. Then we incubated the samples in blocking solution (PBS with 10% goat serum) for 1 h at room temperature, with primary antibodies (rabbit antibody against Myc (Santa Cruz) and/or mouse antibody against Flag-M5 (Sigma)) for 1 h at room temperature, and with secondary antibodies (FITC-labeled goat antibody against mouse IgG and rhodamine-labeled goat antibody against rabbit IgG) overnight at 4 °C. After counterstaining nuclei with DAPI, we acquired fluorescence images using a Leica DMIRE2 inverted fluorescence microscope with Open Lab software (Improvision).

Promoter assay. We plated C3H10T1/2 cells at a density of 5×10^4 cells/well in 6-well plates and transfected them using Fugene (Roche) with the reporter gene 8*GLI–BS–Luc²⁴, a reference plasmid pCMV– β –gal, appropriate expression constructs and sufficient pcDNA 3.1 to achieve an equal amount of DNA in each well. At 36 h after transfection, we harvested cells and normalized luciferase activity with respect to β –galactosidase activity. All transfections were repeated in at least two independent experiments, which gave reproducible results.

GenBank accession numbers. The exons and surrounding intronic sequences for *SUFU* have been submitted to GenBank under the accession numbers AY081818, AY081819, AY081820, AY081821, AY081822, AY081823, AY081824, AY081825, AY081826, AY081827, AY081828 and AY081829.

Acknowledgments

We thank the individuals and families who agreed to take part in these studies, and members of the McGlade laboratory for helpful discussions. This work was supported by the National Cancer Institute of Canada (NCIC) and the Michael Young Melanoma Fund (D.H.), a Terry Fox New Frontiers Award from the NCIC (C.C.H. and J.T.R.), the Canadian Institutes of Health Research (J.T.R. and C.C.H.) and Brainchild (J.T.R.). M.D.T. and T.G.M. were supported by Terry Fox fellowships from the NCIC with funds from the Terry Fox run. M.D.T. was subsequently supported by a fellowship from the Neurosurgery Research and Education Foundation.

Competing interests statement

The authors declare that they have no competing financial interests.

Received 7 March; accepted 17 May 2002.

- Dahmane, N. & Ruiz-i-Altaba, A. Sonic hedgehog regulates the growth and patterning of the cerebellum. *Development* **126**, 3089–3100 (1999).
- Wechsler-Reya, R.J. & Scott, M.P. Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. *Neuron* **22**, 103–114 (1999).
- Hahn, H. *et al.* Mutations of the human homolog of *Drosophila patched* in the nevoid basal cell carcinoma syndrome. *Cell* **85**, 841–851 (1996).
- Johnson, R.L. *et al.* Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science* **272**, 1668–1671 (1996).
- Gorlin, R.J. Nevoid basal-cell carcinoma syndrome. *Medicine (Baltimore)* **66**, 98–113 (1987).
- Kimonis, V.E. *et al.* Clinical manifestations in 105 persons with nevoid basal cell carcinoma syndrome. *Am. J. Med. Genet.* **69**, 299–308 (1997).
- Raffel, C. *et al.* Sporadic medulloblastomas contain PTCH mutations. *Cancer Res.* **57**, 842–845 (1997).
- Uden, A.B. *et al.* Mutations in the human homologue of *Drosophila patched* (PTCH) in basal cell carcinomas and the Gorlin syndrome: different *in vivo* mechanisms of PTCH inactivation. *Cancer Res.* **56**, 4562–4565 (1996).
- Wolter, M., Reifenberger, J., Sommer, C., Ruzicka, T. & Reifenberger, G. Mutations in the human homologue of the *Drosophila* segment polarity gene patched (PTCH) in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. *Cancer Res.* **57**, 2581–2585 (1997).
- Zurawel, R.H. *et al.* Analysis of PTCH/SMO/SHH pathway genes in medulloblastoma. *Genes Chromosom. Cancer* **27**, 44–51 (2000).
- Taylor, M.D., Mainprize, T.G. & Rutka, J.T. Molecular insight into medulloblastoma and central nervous system primitive neuroectodermal tumor biology from hereditary syndromes: a review. *Neurosurgery* **47**, 888–901 (2000).
- Reifenberger, J. *et al.* Missense mutations in *SMO*H in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. *Cancer Res.* **58**, 1798–1803 (1998).
- Xie, J. *et al.* Activating Smoothed mutations in sporadic basal-cell carcinoma. *Nature* **391**, 90–92 (1998).
- Kinzler, K.W. *et al.* Identification of an amplified, highly expressed gene in a human glioma. *Science* **236**, 70–73 (1987).
- Goodrich, L.V., Milenkovic, L., Higgins, K.M. & Scott, M.P. Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science* **277**, 1109–1113 (1997).
- Hahn, H. *et al.* Rhabdomyosarcomas and radiation hypersensitivity in a mouse model of Gorlin syndrome. *Nature Med.* **4**, 619–622 (1998).
- Nilsson, M. *et al.* Induction of basal cell carcinomas and trichoepitheliomas in mice overexpressing GLI-1. *Proc. Natl Acad. Sci. USA* **97**, 3438–3443 (2000).
- Grachtchouk, M. *et al.* Basal cell carcinomas in mice overexpressing Gli2 in skin. *Nature Genet.* **24**, 216–217 (2000).
- Oro, A.E. *et al.* Basal cell carcinomas in mice overexpressing sonic hedgehog. *Science* **276**, 817–821 (1997).
- Bayani, J. *et al.* Molecular cytogenetic analysis of medulloblastomas and supratentorial primitive neuroectodermal tumors by using conventional banding, comparative genomic hybridization, and spectral karyotyping. *J. Neurosurg.* **93**, 437–448 (2000).
- Zurawel, R.H., Chiappa, S.A., Allen, C. & Raffel, C. Sporadic medulloblastomas contain oncogenic β -catenin mutations. *Cancer Res.* **58**, 896–899 (1998).
- Pomeroy, S.L. *et al.* Prediction of central nervous system embryonal tumour outcome based on gene expression. *Nature* **415**, 436–442 (2002).
- Lobo, S., Cervenka, J., London, A. & Pierpont, M.E. Interstitial deletion of 10q: clinical features and literature review. *Am. J. Med. Genet.* **43**, 701–703 (1992).
- Ohlmeyer, J.T. & Kalderon, D. Hedgehog stimulates maturation of Cubitus interruptus into a labile transcriptional activator. *Nature* **396**, 749–753 (1998).
- Sasaki, H., Hui, C., Nakafuku, M. & Kondoh, H. A binding site for Gli proteins is essential for HNF-3 β floor plate enhancer activity in transgenics and can respond to Shh *in vitro*. *Development* **124**, 1313–1322 (1997).
- Meng, X. *et al.* Suppressor of fused negatively regulates β -catenin signaling. *J. Biol. Chem.* **276**, 40113–40119 (2001).
- Chidambaram, A. *et al.* Mutations in the human homologue of the *Drosophila patched* gene in Caucasian and African-American nevoid basal cell carcinoma syndrome patients. *Cancer Res.* **56**, 4599–4601 (1996).
- Wicking, C. *et al.* Most germ-line mutations in the nevoid basal cell carcinoma syndrome lead to a premature termination of the PATCHED protein, and no genotype–phenotype correlations are evident. *Am. J. Hum. Genet.* **60**, 21–26 (1997).
- Liu, L. *et al.* Mutation of the CDKN2A 5' UTR creates an aberrant initiation codon and predisposes to melanoma. *Nature Genet.* **21**, 128–132 (1999).
- Raffel, C. *et al.* Analysis of oncogene and tumor suppressor gene alterations in pediatric malignant astrocytomas reveals reduced survival for patients with PTEN mutations. *Clin. Cancer Res.* **5**, 4085–4090 (1999).
- Ding, Q. *et al.* Mouse suppressor of fused is a negative regulator of sonic hedgehog signaling and alters the subcellular distribution of Gli1. *Curr. Biol.* **9**, 1119–1122 (1999).

