Journal of Neuro-Oncology (2006) 79: 221–227
DOI 10.1007/s11060-006-9132-2
© Springer 2006

Laboratory Investigation

Expression of Notch and Wnt pathway components and activation of Notch signaling in medulloblastomas from heterozygous *patched* mice

Gabriel D. Dakubo[1,2,4], Chantal J. Mazerolle[1,2] and Valerie A. Wallace[1,2,3]
[1] *Molecular Medicine Program, Ottawa Health Research Institute, 501 Smyth Road, K1H 8L6, Ottawa, ON, Canada;* [2] *Eye Institute, University of Ottawa, 451 Smyth Road, K1H 8M5, Ottawa, ON, Canada;* [3] *Department of Biochemistry, Microbiology, and Immunology,University of Ottawa, 451 Smyth Road, K1H 8M5, Ottawa, ON, Canada;* [4] *Genesis Genomics Inc., 310-1294 Balmoral St., P7B 5Z5, Thunder Bay, ON, Canada*

Key words: gene expression, Hedgehog, medulloblastoma, notch, patched, singling pathways, smoothened, wingless

Summary

Hedgehog (Hh), Notch, and Wingless (Wnt) signaling control normal development of the cerebellum, and dysregulation of these signaling pathways are associated with medulloblastoma (MB). As an initial step in the study of the role of interacting signaling pathways in MB pathogenesis, we demonstrate the expression of several components of the Notch and Wnt signaling pathways, and activation of Notch signaling in MB from *Ptch*$^{+/-}$ mice that have elevated Hh signaling. We also show a marked downregulation in the expression of *Notch2*, *Jagged1*, *Hes1*, *mSfrp1*, and *mFrz7* in cerebella of developing mice with reduced Hh signaling, suggesting that Hh signaling regulates the expression of these genes. Together with recent published data, these findings indicate that Hh signaling might synergize simultaneously with Notch and Wnt signaling in MB development by controlling Notch and Wnt pathway ligand, receptor and/or target gene expression.

Introduction

Medulloblastomas (MBs) or infratentorial primitive neuroectodermal tumors (PNET) are the most common malignant solid childhood brain tumors. Whilst recent advances in patient management have increased the 5-year survival rate, mortality from MB still remains high. The molecular mechanisms of the etiology and pathogenesis of MB are just being uncovered. The cell of origin of this tumor is unclear, but the emerging view is that at least a subset of MBs originates from progenitor cells in the external granular layer of the cerebellum [1]. A number of intercellular signaling pathways, including the Hh, Notch and Wnt signaling pathways, regulate the development of the cerebellum [2–6]. Since some tumors are thought to arise from immature stem-like cells whose development are controlled by developmental signaling pathways, dysregulation of such signaling pathways could potentially cause tumors [7–9]. Thus, the Hh, Notch, and Wnt pathways are implicated in MB tumorigenesis.

Shh is a principal mitogen for cerebella granule neuron precursor (GNP) cells [3–5]. Germline mutations in the Hh signaling pathway receptor and tumor suppressor gene, *PTCH*, underlie the pathology of Gorlin's syndrome, in which patients have increased risk of developing a number of malignancies, including MB. Mutations in *PTCH* are also detected in sporadic MBs [10]. The mechanism of MB initiation in *Ptch*$^{+/-}$ mice is not fully understood. For instance only about 14–16% of *Ptch*$^{+/-}$ mice develop MB, and postnatal granule precursor growth is not globally altered in *Ptch*$^{+/-}$ mice [11,12]. However, *Ptch* heterozygosity seems to predispose some granule precursor cells to persistent proliferation with dysregulated developmental gene expression [12]. Whereas some *Ptch*$^{+/-}$ mice have been shown to develop MB without loss of heterozygosity [11], it is more likely that *Ptch*$^{+/-}$ mice develop MB after losing expression of the wildtype allele due to DNA methylation [13]. Germline and somatic mutations in *Suppressor of Fused* (*SUFU*), a downstream negative regulator of the Hh signaling pathway have also been demonstrated in MBs, thus underscoring the importance of Hh signaling in MB pathogenesis [14].

Since we are interested in the cross-talk between signaling pathways in the development of medulloblastoma, we asked whether the Notch and Wnt pathways were dysregulated in MBs from *Ptch*$^{+/-}$ mice that have elevated Hh signaling. By a combination of RNA *in situ* hybridization, immunohistochemistry and semi-quantitative RT-PCR, we screened 56 genes and identified 30 that were differentially expressed at higher levels in MBs than wildtype postnatal day 21 (P21) cerebella. These included Hh pathway components, cell cycle genes, and glial lineage markers. Interestingly some members of the Notch and Wnt signaling pathways were upregulated in these tumors compared to wildtype cerebella. We also observed that *Notch2*, *Jagged1*, *Hes1*, *mSfrp1* and *mFrz7* were downregulated in cerebella of developing mice with reduced Hh signaling, suggesting that Hh signaling might directly or indirectly regulate the

expression of some components of the Notch and Wnt signaling pathway in MB from $Ptch^{+/-}$ mice.

Materials and methods

Transgenic mice

$Ptch^{+/-}$ mice [15] were maintained on a C57Bl/6 background and monitored on a daily basis until they showed signs of cerebella dysfunction and increased intracranial pressure, such as loss of coordination, poor feeding, lethargy, dehydration, and respiratory distress. Fifteen (15) adult sick $Ptch^{+/-}$ mice (3 months and older) and P21 C57Bl/6 wildtype mice were sacrificed and tissues frozen for RNA extraction, or processed for histology and *in situ* hybridization. Embryonic day 18 (E18) $Nestin^{Cre}$;$Smo^{n/c}$ and littermate wildtype control mice were obtained from A. P. McMahon (Department of Molecular and Cellular Biology, The Biolabs, Harvard University). Expression of Cre recombinase, which is regulated by the neural-specific enhancer of the *Nestin* promoter, induces recombination of floxed *Smoothened* (*Smo*) allele by E12.5 [16].

RNA in situ *hybridization and immunohistochemistry*

Ten of the fifteen sick animals and control P21 C57Bl/6 mice were used for *in situ* hybridization and immunohistochemistry. Animals were euthanized before perfusion with 4% Paraformaldehyde in 0.1 M PBS (pH 7.4), and the brains were removed and fixed overnight in 4% paraformaldehyde in 0.1 M PBS (pH 7.4), protected overnight in 30% sucrose/PBS and embedded in sucrose:OCT (Tissue-Tek®). Sections from controls and experimental tissues were processed on the same slide to allow fair comparison of signal intensities. *In situ* hybridization was performed essentially as previously described [17], with the respective digoxigenin-labeled antisense riboprobes. Immunohistochemistry with antibodies to GFAP, Neurofilament-3A10, β-catenin and calbindin was performed essentially according to Jensen and Wallace [18] with polyclonal anti-GFAP (from Martin Raff, MRC Laboratory for Molecular Cell Biology, the University College London), monoclonal anti-Neurofilament-3A10 (Developmental Studies Hybridoma Bank), polyclonal anti-β-catenin (Transduction Labs) and anti-calbindin (Sigma). Conjugated antibodies were detected with FITC (Sigma).

Semi-quantitative RT-PCR

Five frozen tumors were used for RT-PCR analysis. Total RNA was isolated from tissues with TRI REAGENT™ (Sigma) following manufacturer's instructions. cDNA was generated from 3 μg of total RNA using random hexamer and OligodT primers with SuperScript™ reverse transcriptase (Invitrogen). PCR was performed on 2 μl of undiluted and fourfold serial dilutions of the cDNA template using already published primers for Notch pathway components [6]. GAPDH served as loading controls.

Results

We have systematically examined the expression of components of the Notch and Wnt signaling pathways in a panel of 15 MBs from $Ptch^{+/-}$ mice in comparison to wildtype P21 cerebella. The features of the tumors we studied are similar to those already described for MBs from $Ptch^{+/-}$ mice [15]. Grossly, they appeared as smooth bulges in the posterior fossa with no foliation and the histopathology was indistinguishable from human medulloblastomas (Figure 1 and data not shown). By semi-quantitative RT-PCR and RNA *in situ* hybridization analysis, lower levels of *Ptch* and markedly high levels of *Gli1*, *Gli2* and *Ptch2* transcripts were detected in the tumors compared to wildtype P21 cerebella, indicating elevated Hh signaling in these tumors (Figure 1, Table 1 and data not shown). As well, the tumors expressed some markers of MBs including intermediate filament proteins such as neurofilament-3A10 (Developmental Studies Hybridoma Bank) and GFAP, as well as the transcription factor, *NeuroD* (Figure 1, Table 1 and data not shown). Consistent with their postulated granule precursor cell of origin, and distinguishing them from supratentorial PNET, we did not observe expression of *Mash1* in these tumors. Cell cycle genes were downregulated in wildtype adult cerebella; however, the highly proliferative MB cells expressed very high levels of a number of G1–S phase cell cycle genes including *Ccnd1, Ccnd2, Ccne1, Ccne2*, *Cdc25A*, and the proto-oncogene *N-myc* (Figure 1, Table 1 and data not shown).

The binding of a Notch receptor to its Delta-like or Jagged ligand leads to the proteolytic cleavage and nuclear translocation of the cytoplasmic domain of Notch ($Notch^{intra}$). In the nucleus, $Notch^{intra}$ forms a complex with CBF1/RBPJκ DNA binding protein and activates transcription of Notch target genes such as *Hes1* and *Hes5*. Aberrant Notch signaling is associated with a number of cancers, but in different ways [19]. Constitutive Notch1 signaling causes acute lymphoblastic leukemia [20], however, loss of Notch1 function in the skin causes basal cell carcinoma, suggesting Notch1 functions as a tumor suppressor gene in the skin [21]. Given the importance of Notch signaling in cerebella development and malignant transformation, we examined the expression of Notch pathway components in MBs from $Ptch^{+/-}$ mice. By semi-quantitative RT-PCR analyses, we observed elevated expression of *Notch2*, *Notch3* (lower levels than *Notch2*), *Jagged1 Hes1*, *Hes5* and *Rbpj*κ in MBs compared to wildtype cerebella (data not shown). To examine the distribution of these transcripts in the tumors, we performed RNA *in situ* hybridization on sections from five different MBs and control P21 wildtype cerebella. Consistent with our RT-PCR data, the aforementioned genes were all expressed at a higher level in medulloblastoma compared with wildtype cerebellum (Figure 2, Table 1). *Hes5* transcripts showed a grainy pattern, suggesting only some tumor cells were responding to Notch signaling.

The canonical Wnt signaling pathway is activated by binding of Wnt ligand with their Frizzled (Frz) receptors, which inhibits Casein Kinase 1 (CK1), and Glycogen

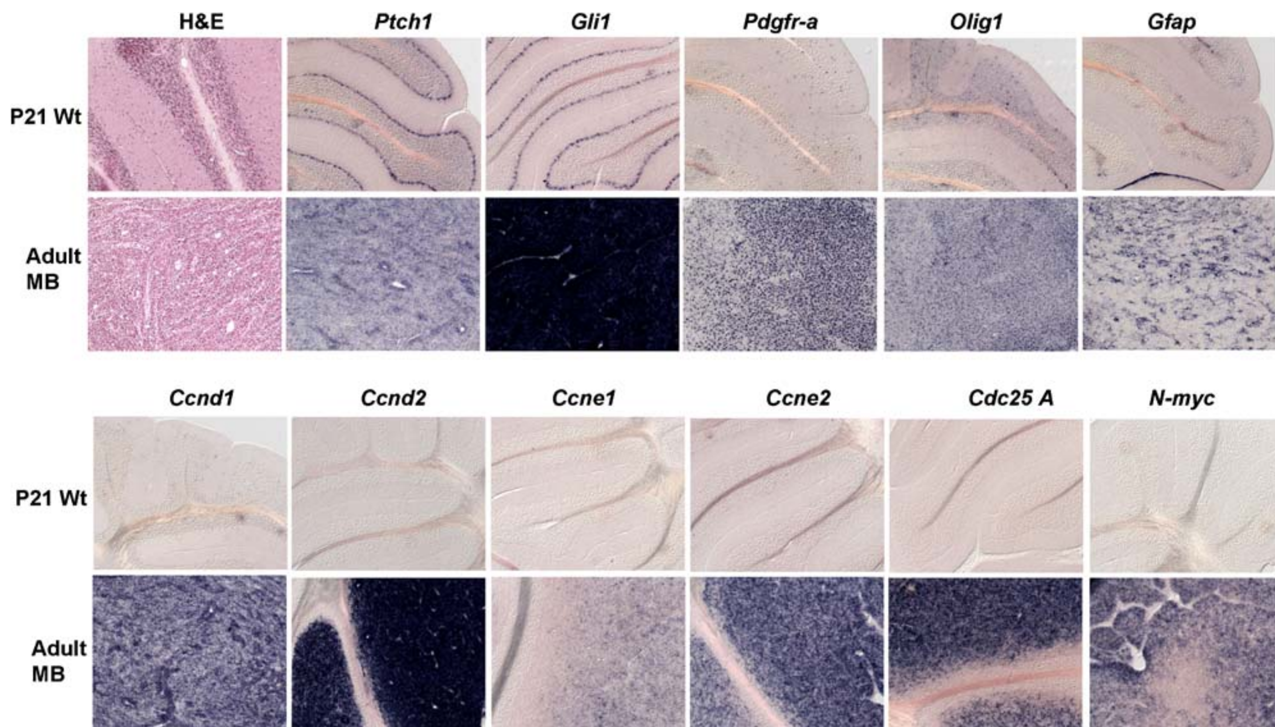

Figure 1. Histology and expression of Hh, glial lineage and cell cycle genes in MB from Ptch+/– mice.

Synthase Kinase-3β (GSK-3β)-mediated phosphorylation and degradation of the Wnt-pathway transcriptional activator, β-catenin. Thus, unphosphorylated β-catenin accumulates in the cytoplasm and translocates to the nucleus where it forms a complex with the T cell factor (TCF) and lymphoid-enhancing factor (LEF) family of transcription factors resulting in activation of target genes including *Ccnd1*, *c-myc*, *Sfrp2*, and possibly *Lef1* [22]. Wnt signaling regulates cerebellum development, and is implicated in the etiology of MB. Given the possibility that the Hh and Wnt pathways might communicate in at least a subset of MBs, we examined by RNA *in situ* hybridization the expression of components of the Wnt signaling pathway in MBs from $Ptch^{+/-}$ mice. We show that *Wnt1*, *Wnt8*, *mFrz3*, *mFrz4*, *mFrz7*, *mSfrp1*, *mSfrp2* and *Lef1* are highly expressed in MBs in comparison to wildtype cerebella (Figure 2, Table 1). The upregulation of *Lef1* in tumors, but not in normal adult cerebella is intriguing in view of a recent study that implicates *Lef1* as a direct target of Wnt signaling in colonic cancers [22]. This finding suggests there might be active Wnt signaling in MBs from $Ptch^{+/-}$ mice. We thus examined β-catenin expression and the presence of β-catenin in cytoplasm and/or nucleus, an indicator of active Wnt signaling. Immunohistochemistry for total β-catenin revealed elevated levels in the tumors, but this appeared to be membrane associated (data not shown). Nuclear β-catenin was not observed in any of the three tumors examined. This finding is consistent with recent observation in some human MBs [23,24].

The above expression analysis suggests that Notch and Wnt signaling pathways could be downstream of the Hh pathway; however, whether the expression of components of these signaling pathways is controlled by Hh signaling is unclear. Since gene expression profiles of MB are similar to those in the perinatal cerebellum [25], we asked whether these genes were similarly regulated in the embryonic cerebellum with Hh pathway modulation. We examined the cerebella of E18 $Nestin^{Cre}$; $Smo^{n/c}$ mutant mice in which the Hh pathway was downregulated due to disruption of the Hh signal transducer, *Smoothened* (*Smo*) by Cre-mediated recombination of *Smo* floxed allele. Hh signaling is inactivated in neural precursor cells of $Nestin^{Cre}$; $Smo^{n/c}$ mice by E12.5 [16]. The expression of Hh target genes, such as *Gli1*, *Ptch1*, and *Ccnd1* were markedly downregulated in the cerebella of these mice, confirming that the Hh pathway was inhibited in this region of the CNS (Figure 3a, and data not shown). In addition, proliferation in the external granule layer was markedly reduced resulting in a thinner external granule layer. In wildtype cerebellum at this developmental stage, Purkinje cells are usually organized into a distinct layer composed of a few cells; however in mutant mice, the Purkinje cells were completely disorganized and widely dispersed [26] (Figure 3a). By RNA *in situ* hybridization, we demonstrate a marked downregulation of *Notch2*, *Jagged1*, *Hes1*, *mSfrp1* and *mFrz7* in cerebella of $Nestin^{Cre}$; $Smo^{n/c}$ mice in comparison to wildtype littermates (Figure 3b,c). Although clearly present, the external granule layer of $Nestin^{Cre}$; $Smo^{n/c}$ mice is reduced (Figure 3a, egl). Therefore, the downregulation of *mFrz7* and *mSfrp1* should be interpreted with caution. However, downregulation of *Notch2*, *Jagged1* and *Hes1* in Purkinje cells that are specified in these mutant mice (evidenced by anti-calbindin staining and expression of *mFrz4* in this layer), shows these genes might be directly or indirectly controlled by Hh signaling.

Table 1. Genes that are upregulated[a] in MBs

Genes	P21 cerebellum	Medulloblastoma
Hh pathway		
Ptch	+ +	+
Ptch2	–	+ +
Gli1	+ +	+ + +
Gli2	–	+ +
Cell cycle		
Ccnd1	–	+ + +
Ccnd2	–	+ + +
Ccne1	–	+
Ccne2	–	+ +
Cdc25	–	+ + +
Nmyc	–	+ +
Glial lineage		
Pdgfra	+	+ + +
Olig1	+	+ +
Gfap	+	+ +
GluSyth	+	+ +
Notch pathway		
Notch2	+	+ +
Notch3	–	+
Jag1	–	+ +
Hes1	–	+ +
Hes5	–	+ +
Rbpjk	–	+ +
Wnt pathway		
Wnt1	–	+
Wnt8	–	+
mFrz3	+	+ + +
mFrz4	+	+ +
mFrz7	–	+ + +
mSfrp1	+	+ + +
mSfrp2	+	+ + +
Tcf4	+	+
Lef1	–	+ +
β-catenin	–	+ +

[a]Data based on *in situ* hybridization analysis. Strength of gene expression was scored as; (–), undetectable; (+), weak expression; (+ +), intermediate expression; (+ + +), strong expression (see examples in Figure 2).

Discussion

There is ample evidence to suggest that Hh signaling interacts with other signaling pathways during MB tumorigenesis. First, the Hh pathway target genes, *Gli1* and *Ptch2*, are upregulated in mouse MBs that develop from loss of function in cell cycle regulatory genes [25], suggesting that the Hh pathway interacts with cyclin-dependent kinases in MB. Second, Sufu negatively controls β-catenin, a component of the Wnt signaling pathway [27]. Third, loss of *p53* augments MB development in *Ptch*$^{+/-}$ mice [28], and mice lacking both p53 and poly (ADP-ribose) polymerase (PARP) develop MB with activated Hh signaling [29]. Fourth, Shh synergizes with insulin-like growth factor in inducing MB formation [30,31]. Finally, recent findings indicate that Hh signaling activates the Notch pathway in MB and that Notch signaling contributes to tumor behavior [32,33]. Thus, the interplay of signaling pathways may underlie the initiation and/or pathogenesis of a subset of MBs.

The goal of this study was to test the hypothesis that components of Notch and Wnt signaling pathway are abnormally expressed in MB from *Ptch*$^{+/-}$ mice. Although microarray gene expression profiling of MB from *Ptch*$^{+/-}$ mice have been conducted [25], our focused analysis of 15 MBs from these mice allowed us to corroborate previous observations and to extend these findings by demonstrating that other members of the Notch and Wnt pathways such as *Notch3*, *Jagged1*, *Rbpjκ*, *Wnt1, Wnt8*, *mFrz3*, *mFrz7* and *Left1*, that have hitherto not been demonstrated in MBs from *Ptch*$^{+/-}$ mice are highly expressed in these tumors. In addition, our observation that the expression of some members of these pathways are reduced in the external granule layer and/or Purkinje cell layer of E18 mice with reduced Hh signaling further suggests that the Hh pathway might interact with these pathways at these levels.

The expression of Notch target genes *Hes1* and *Hes5* in MB from *Ptch*$^{+/-}$ mice implies there is active Notch signaling in these tumors, which is consistent with two recent studies that demonstrate an important role for Notch signaling in MB tumorigenesis [32,33]. Shh pathway activation (*Smo*-induced activation*)* was sufficient to activate Notch signaling in MB, and Notch signaling was associated with increased MB proliferation and survival [32,33]. Moreover, *Hes1* expression in human MB is suggested to be a prognostic factor [32,33]. The mechanism by which Hh signaling controls Notch pathway is not fully understood. However, this could occur at the level of *Notch2*, *Jagged1*, and *Hes1* as revealed by our mutant analysis. Thus, the roles of other Notch signaling components such as *Notch3*, *Jagged1*, and *Rbpjκ* that are elevated in MB from Hh pathway activation require an indebt study.

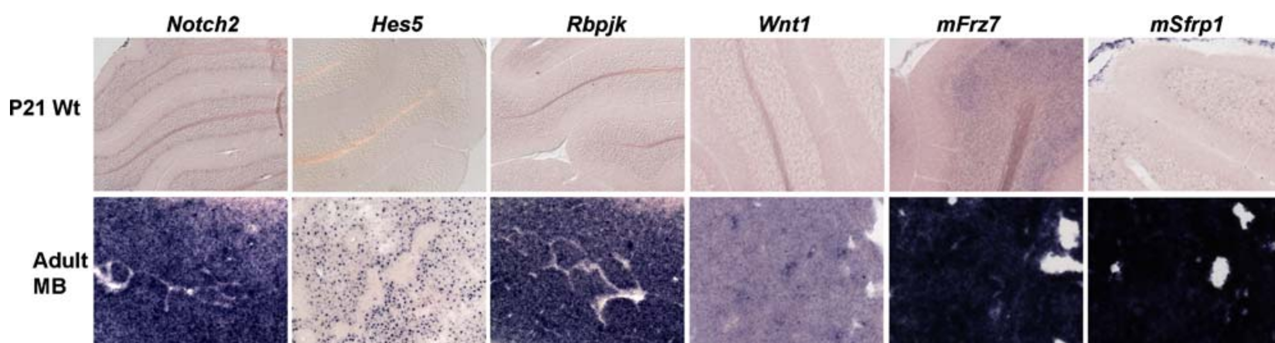

Figure 2. Expression profiles of some Notch and Wnt pathway genes in MB. A representation of how gene expressions were scored in Table 1 are; *Wnt1* (+); *Notch2, Rbpjκ* (+ +); and *mFrz7, mSfrp1* (+ + +).

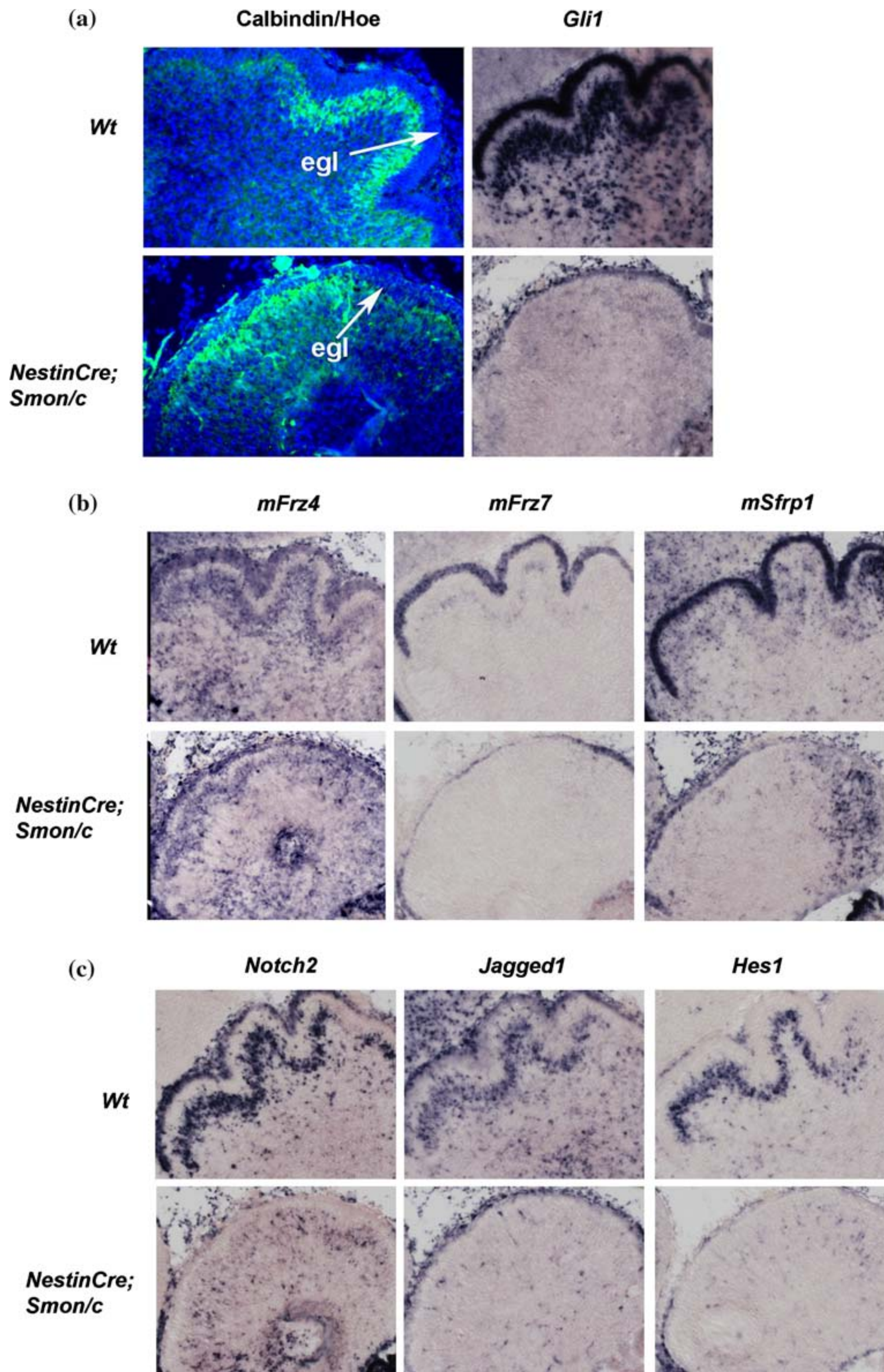

Figure 3. Downregulation of some components of Notch and Wnt pathways in mice with reduced Shh signaling. (a) Loss of Hh signaling in cerebellum of $Nestin^{Cre}$;$Smo^{n/c}$ mice is indicated by downregulation of *Gli1*. Purkinje cells in mutants are scattered and occupy a much broader area. (b) Whereas expression of *mFrz4* is preserved, those of *mFrz7* and *mSfrp1* seem to be much reduced in cerebellum of mutants compared to littermates. (c) *Notch2*, *Jagged1* and *Hes1* are markedly downregulated in cerebellum of mutant compared to wildtype littermate. Egl, external granule layer.

Wnt signaling plays important role in MB pathogenesis. Germline mutations in the tumor suppressor gene, *Adenomatous Polyposis Coli* (*APC*), a component of the Wnt pathway underlies Turcot syndrome, a familial cancer syndrome in which patients have increased predisposition to developing colonic cancers and MB. Activating mutations in the Wnt pathway components, including mutations in members of the Wnt pathway inhibitory complex, APC, Axin, β-catenin and GSK-3β, have been found in sporadic MBs [34–36]. Although there is sufficient evidence that indicate a possible cross-talk between the Hh and Wnt pathways in development

and disease, the interactions of these pathways in MB is not well understood. Thus the observed expression of several components of the Wnt pathway in MB from $Ptch^{+/-}$ mice suggest they might interact in MB development ([25] and this study).

In summary, we demonstrate an elevated expression of several components of the Notch and Wnt pathways in MB from $Ptch^{+/-}$ mice. In addition, we provide evidence that some components of the Notch and Wnt signaling pathways might be controlled by Hh signaling, suggesting that Hh signaling might directly or indirectly induce the upregulation of these genes in MB from $Ptch^{+/-}$ mice. Since the interaction of signaling pathways can occur at several levels, further work is required to elucidate the relevance of interacting signaling pathways in tumor initiation and/or progression.

Acknowledgements

We thank A. P. McMahon for kindly providing us with $Nestin^{Cre}$;$Smo^{n/c}$ mice. This work was supported by funding from the National Cancer Institute of Canada and the Canadian Institutes of Health Research to VAW who is also the recipient of a CIHR New Investigator Award. G. D. Dakubo was a recipient of an M. S. Society Postdoctoral Fellowship at the time that these experiments were performed.

References

1. Kadin ME, Rubinstein LJ, Nelson JS: Neonatal cerebellar medulloblastoma originating from the fetal external granular layer. J Neuropathol Exp Neurol 29: 583–600, 1970
2. Brault V, Moore R, Kutsch S, Ishibashi M, Rowitch DH, McMahon AP, Sommer L, Boussadia O, Kemler R: Inactivation of the beta-catenin gene by Wnt1-Cre-mediated deletion results in dramatic brain malformation and failure of craniofacial development. Development 128: 1253–1264, 2001
3. Wallace VA: Purkinje-cell-derived Sonic hedgehog regulates granule neuron precursor cell proliferation in the developing mouse cerebellum. Curr Biol 9: 445–448, 1999
4. Dahmane N, Ruiz-i-Altaba A: Sonic hedgehog regulates the growth and patterning of the cerebellum. Development 126: 3089–3100, 1999
5. Wechsler-Reya RJ, Scott MP: Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. Neuron 22: 103–114, 1999
6. Solecki DJ, Liu XL, Tomoda T, Fang Y, Hatten ME: Activated Notch2 signaling inhibits differentiation of cerebellar granule neuron precursors by maintaining proliferation. Neuron 31: 557–568, 2001
7. Galderisi U, Cipollaro M, Giordano A: (2005) Stem cells and brain cancer. Cell Death Differ
8. Lee A, Kessler JD, Read TA, Kaiser C, Corbeil D, Huttner WB, Johnson JE, Wechsler-Reya RJ: Isolation of neural stem cells from the postnatal cerebellum. Nat Neurosci 8: 723–729, 2005
9. Golestaneh N, Mishra B: TGF-beta, neuronal stem cells and glioblastoma. Oncogene 24: 5722–5730, 2005
10. Xie J, Johnson RL, Zhang X, Bare JW, Waldman FM, Cogen PH, Menon AG, Warren RS, Chen LC, Scott MP, Epstein EH Jr: Mutations of the PATCHED gene in several types of sporadic extracutaneous tumors. Cancer Res 57: 2369–2372, 1997
11. Wetmore C, Eberhart DE, Curran T: The normal patched allele is expressed in medulloblastomas from mice with heterozygous germ-line mutation of patched. Cancer Res 60: 2239–2246, 2000
12. Kim JY, Nelson AL, Algon SA, Graves O, Sturla LM, Goumnerova LC, Rowitch DH, Segal RA, Pomeroy SL: Medulloblastoma tumorigenesis diverges from cerebellar granule cell differentiation in patched heterozygous mice. Dev Biol 263: 50–66, 2003
13. Berman DM, Karhadkar SS, Hallahan AR, Pritchard JI, Eberhart CG, Watkins DN, Chen JK, Cooper MK, Taipale J, Olson JM, Beachy PA: Medulloblastoma growth inhibition by hedgehog pathway blockade. Science 297: 1559–1561, 2002
14. Taylor MD, Liu L, Raffel C, Hui CC, Mainprize TG, Zhang X, Agatep R, Chiappa S, Gao L, Lowrance A, Hao A, Goldstein AM, Stavrou T, Scherer SW, Dura WT, Wainwright B, Squire JA, Rutka JT, Hogg D: Mutations in SUFU predispose to medulloblastoma. Nat Genet 31: 306–310, 2002
15. Goodrich LV, Milenkovic L, Higgins KM, Scott MP: Altered neural cell fates and medulloblastoma in mouse patched mutants. Science 277: 1109–1113, 1997
16. Machold R, Hayashi S, Rutlin M, Muzumdar MD, Nery S, Corbin JG, Gritli-Linde A, Dellovade T, Porter JA, Rubin LL, Dudek H, McMahon AP, Fishell G: Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. Neuron 39: 937–950, 2003
17. Wallace VA, Raff MC: A role for Sonic hedgehog in axon-to-astrocyte signalling in the rodent optic nerve. Development 126: 2901–2909, 1999
18. Jensen AM, Wallace VA: Expression of Sonic hedgehog and its putative role as a precursor cell mitogen in the developing mouse retina. Development 124: 363–371, 1997
19. Weng AP, Aster JC: Multiple niches for Notch in cancer: context is everything. Curr Opin Genet Dev 14: 48–54, 2004
20. Weng AP, Ferrando AA, Lee W, Morris JPt, Silverman LB, Sanchez-Irizarry C, Blacklow SC, Look AT, Aster JC: Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 306: 269–271, 2004
21. Nicolas M, Wolfer A, Raj K, Kummer JA, Mill P, van Noort M, Hui CC, Clevers H, Dotto GP, Radtke F: Notch1 functions as a tumor suppressor in mouse skin. Nat Genet 33: 416–421, 2003
22. Hovanes K, Li TW, Munguia JE, Truong T, Milovanovic T, Lawrence Marsh J, Holcombe RF, Waterman ML: Beta-catenin-sensitive isoforms of lymphoid enhancer factor-1 are selectively expressed in colon cancer. Nat Genet 28: 53–57, 2001
23. Utsuki S, Oka H, Sato Y, Tsutiya B, Kondo K, Tanizaki Y, Tanaka S, Fujii K: E, N-cadherins and beta-catenin expression in medulloblastoma and atypical teratoid/rhabdoid tumor. Neurol Med Chir (Tokyo) 44: 402–406, 2004discussion 407
24. Ellison DW, Onilude OE, Lindsey JC, Lusher ME, Weston CL, Taylor RE, Pearson AD, Clifford SC: Beta-Catenin status predicts a favorable outcome in childhood medulloblastoma: the United Kingdom Children's Cancer Study Group Brain Tumour Committee. J Clin Oncol 23: 7951–7957, 2005
25. Lee Y, Miller HL, Jensen P, Hernan R, Connelly M, Wetmore C, Zindy F, Roussel MF, Curran T, Gilbertson RJ, McKinnon PJ: A molecular fingerprint for medulloblastoma. Cancer Res 63: 5428–5437, 2003
26. Lewis PM, Gritli-Linde A, Smeyne R, Kottmann A, McMahon AP: Sonic hedgehog signaling is required for expansion of granule neuron precursors and patterning of the mouse cerebellum. Dev Biol 270: 393–410, 2004
27. Meng X, Poon R, Zhang X, Cheah A, Ding Q, Hui CC, Alman B: Suppressor of fused negatively regulates beta-catenin signaling. J Biol Chem 276: 40113–40119, 2001
28. Wetmore C, Eberhart DE, Curran T: Loss of p53 but not ARF accelerates medulloblastoma in mice heterozygous for patched. Cancer Res 61: 513–516, 2001
29. Tong WM, Ohgaki H, Huang H, Granier C, Kleihues P, Wang ZQ: Null mutation of DNA strand break-binding molecule poly(ADP-ribose) polymerase causes medulloblastomas in p53(−/−) mice. Am J Pathol 162: 343–352, 2003
30. Rao G, Pedone CA, Valle LD, Reiss K, Holland EC, Fults DW: Sonic hedgehog and insulin-like growth factor signaling synergize to induce medulloblastoma formation from nestin-expressing neural progenitors in mice. Oncogene 23: 6156–6162, 2004

31. Hartmann W, Koch A, Brune H, Waha A, Schuller U, Dani I, Denkhaus D, Langmann W, Bode U, Wiestler OD, Schilling K, Pietsch T: Insulin-like growth factor II is involved in the proliferation control of medulloblastoma and its cerebellar precursor cells. Am J Pathol 166: 1153–1162, 2005
32. Hallahan AR, Pritchard JI, Hansen S, Benson M, Stoeck J, Hatton BA, Russell TL, Ellenbogen RG, Bernstein ID, Beachy PA, Olson JM: The SmoA1 mouse model reveals that notch signaling is critical for the growth and survival of sonic hedgehog-induced medulloblastomas. Cancer Res 64: 7794–7800, 2004
33. Fan X, Mikolaenko I, Elhassan I, Ni X, Wang Y, Ball D, Brat DJ, Perry A, Eberhart CG: Notch1 and notch2 have opposite effects on embryonal brain tumor growth. Cancer Res 64: 7787–7793, 2004
34. Dahmen RP, Koch A, Denkhaus D, Tonn JC, Sorensen N, Berthold F, Behrens J, Birchmeier W, Wiestler OD, Pietsch T: Deletions of AXIN1, a component of the WNT/wingless pathway, in sporadic medulloblastomas. Cancer Res 61: 7039–7043, 2001
35. Yokota N, Nishizawa S, Ohta S, Date H, Sugimura H, Namba H, Maekawa M: Role of Wnt pathway in medulloblastoma oncogenesis. Int J Cancer 101: 198–201, 2002
36. Koch A, Waha A, Tonn JC, Sorensen N, Berthold F, Wolter M, Reifenberger J, Hartmann W, Friedl W, Reifenberger G, Wiestler OD, Pietsch T: Somatic mutations of WNT/wingless signaling pathway components in primitive neuroectodermal tumors. Int J Cancer 93: 445–449, 2001

Address for offprints: Gabriel D. Dakubo, Genesis Genomics Inc., 310-1294 Balmoral St., P7B 5Z5, Thunder Bay, ON, Canada; Tel.: +1-807-346-8100; Fax: +1-807-346-8105; E-mail: gabriel.dakubo @genesisgenomics.com.