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LABORATORY INVESTIGATION - HUMAN/ANIMAL TISSUE

Expression profile of frizzled receptors in human medulloblastomas

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Abstract Secreted WNT proteins signal through ten receptors of the frizzled (FZD) family. Because of the relevance of the WNT/β-catenin (CTNNB1) signaling pathway in medulloblastomas (MBs), we investigated the expression of all ten members of the *FZD* gene family (FZD1-10) in 17 human MBs, four MB cell lines and in normal human cerebellum, using real-time PCR. We found that *FZD2* transcript was over-expressed in all MBs and MB cell lines. Western blot analysis confirmed the expression of FZD2 at the protein level. Moreover, the levels of *FZD2* transcript were found to correlate with those of *ASPM* transcript, a marker of mitosis essential for mitotic spindle function. Accordingly, *ASPM* mRNA was expressed at a very low level in the adult, post-mitotic, human cerebellum, at higher levels in fetal cerebellum and at highest levels in MB tissues and cell lines. Unlike FZD2, the other FZDs were overexpressed (e.g., *FZD1*, *FZD3* and *FZD8*) or underexpressed (e.g., *FZD7*, *FZD9* and *FZD10*) in a case-restricted manner. Interestingly, we did not find any nuclear immuno-reactivity to CTNNB1 in four MBs over-expressing both FZD2 and other FZD receptors, confirming the lack of nuclear CTNNB1 staining in the presence of increased FZD expression, as in other tumor types. Overall, our results indicate that altered expression of FZD2 might be associated with a proliferative status, thus playing a role in the biology of human MBs, and possibly of cerebellar progenitors from which these malignancies arise.

Keywords ASPM · β-catenin · CTNNB1 · Frizzled · FZD2 · Medulloblastoma

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Introduction

WNT signaling pathways have been implicated in several biological processes, including cell proliferation, determination of cell fate, and generation of cell polarity [1, 2]. In humans, there are 19 identified WNT-secreted glycoproteins (i.e., WNT1, 2, 2B/13, 3, 3A, 4, 5A, 5B, 6, 7A, 7B, 8A, 8B, 9A/14, 9B/15, 10A, 10B/12, 11, and 16) that function as ligands to govern these processes. These WNT ligands signal through ten receptors of the frizzled (FZD) family, FZD1-10, and two co-receptors, LRP5 and LRP6. FZD receptors transduce the WNT signals via at least three intracellular pathways, that are distinct as 'canonical' and 'non-canonical'.

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role in promoting deregulation of WNT signaling in MB tissue and cell lines is difficult to infer.

Intriguingly, it should be noted that: (1) only the four MBs (all pediatric) over-expressing *MYC*, also over-express *FZD1*, which is a FZD receptor that signals by the canonical WNT pathway; (2) *MYC* is also over-expressed in the two MB cell lines (MB4 and 16) over-expressing *FZD1*; (3) *MYC* over-expression in the four MBs and in the MB4 and MB16 cell lines is not due to *MYC* amplification, while *MYC* over-expression in the D283 and D341 cell lines, which do not over-express *FZD1*, is associated with *MYC* amplification [22]. The meaning of this finding remains uncertain for the following reasons: (1) the sample size is very small; (2) *MYC* over-expression may be due to canonical WNT pathway gene mutations (e.g., *CTNNB1*); (3) *MYC* over-expression may be independent from the canonical WNT pathway activation. However, this observation may also suggest that FZD1 over-expression leads to canonical WNT pathway activation. This might be true even if no nuclear β-catenin immunoreactivity was found in the two MBs that over-expressed both *FZD1* and *MYC* and were investigated for β-catenin positivity (samples 9 and 16 in Table 1). Indeed, it has been previously proved that negative immunohistochemical results do not eliminate the possibility of increased nuclear localization of CTNNB1—with consequent WNT/CTNNB1 pathway activation—because weak nuclear staining may be difficult to evaluate [23]. Moreover, the lack of nuclear β-catenin staining has been reported in tumors arising from WNT/CTNNB1 pathway deregulation, and a variety of WNT/CTNNB1 pathway target genes, such as *MYC*, may be up-regulated even in the absence of nuclear β-catenin immunoreactivity [12, 23]. Finally, as observed by Merle et al. in human hepatocellular carcinomas, over-expression of FZD receptors might lead to levels of nuclear β-catenin sufficient to induce target genes, but not strong enough to be detected by immunohistochemistry [6].

FZD10 transcript is under-expressed in a variety of human MBs and in all MB cell lines examined. FZD10 appears to interact with Wnt1 (but not Wnt3a) as a ligand:receptor pair, and promote neuronal differentiation [24]. Hence, the under-expression of *FZD10* in primary tumors and cell lines of human MBs might reflect the less differentiated state of tumor cells. However, it remains to be investigated whether this observation has some biological relevance, considering the low levels of *FZD10* also present in normal adult cerebellum, and whether FZD10 plays some role in the neuronal differentiation of cerebellar progenitors, from which MBs arise. Moreover, *FZD10* was found to be 'enriched' in the WNT subgroup of MBs (see table 1 in Northcott et al. [5]).

FZD3 expression levels were the highest in both normal cerebellum and human MBs compared to the other *FZDs*, but *FZD3* was found to be over-expressed only in a subset of human MBs, and, most importantly, in none of the MB cell lines. Thus, the meaning of *FZD3* over-expression in MB tumorigenesis is unclear. *Fzd3*, *Fzd4* and *Fzd7* transcripts were all found up-regulated together in MBs from Ptch1$^{+/-}$ mice, suggesting that the expression of these receptors might be associated with deregulation of the Sonic Hedgehog pathway [25]. However, in nine cases of MB over-expressing *PTCH1* and *GLI1* mRNA—PTCH1 and GLI1 are downstream effectors of the SHH pathway—we found *FZD3* over-expression only in two (samples 8 and 15, both of the adult age and with no *FZD4* nor *FZD7* over-expression associated) (unpublished data). Hence, our data do not convincingly support any association between SHH pathway activation and *FZD3*, *FZD4*, and/or *FZD7* over-expression in human MBs.

FZD8, which may activate the oncogenic WNT/CTNNB1 signalling pathway [14], was over-expressed in fetal cerebellum and in some cases of MBs, but not in MB cell lines, as for FZD3.

None of the other FZDs (FZD4, FDZ5, FZD6, FZD7 and FZD9) showed consistent over- or under-expression in human MBs and MB cell lines. However, we highlight that *FZD6*, which is another gene 'enriched' in the WNT subgroup of MBs [5], was found to be over-expressed in three MBs and in the MB cell line (MB16) derived from one of them (sample 10); in contrast, *FZD7*, which is a WNT pathway gene that was found to be 'enriched' in the SHH subgroup of MBs [5], was found to be under-expressed in three MBs, and over-expressed only in one.

Overall, our results indicate that FZD2—which may be associated to non-canonical WNT pathways—may play a more important role than the other FZD receptors in human MBs, and in the cerebellar progenitors from which these malignancies derive. In contrast, the role of the other FZD receptors in the formation and maintenance of these malignancies, if any, remains uncertain, and probably redundant.

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Conflicts of interest None.

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