Molecular stratification of medulloblastoma: comparison of histological and genetic methods to detect Wnt activated tumours

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Aims: Wnt activation in medulloblastomas is associated with good outcome. Upfront testing and risk-adapted stratification of patients will be done in future clinical studies. In a cohort of 186 paediatric medulloblastomas our aim was to identify the optimal methods in standard clinical practice to detect this subgroup. Methods: Nuclear accumulation of β-catenin was analysed by immunohistochemistry (IHC). DNA of FFPE tissue was amplified by PCR for single-strand conformation polymorphism analysis and direct sequencing of CTNNB1 exon 3. Copy number of chromosome 6 was analysed by multiplex ligation-dependent probe amplification and molecular inversion profiling. Results: Different automated immunostaining systems showed similar results. Twenty-one of 186 samples had nuclear accumulation in ≥5% of cells. 17 samples showed <5% β-catenin positive nuclei. None of these 17 cases had CTNNB1 mutations, but 18 of 21 cases with ≥5% accumulation did, identifying these 18 cases as Wnt-subgroup medulloblastomas. Fifteen of 18 mutated cases showed monosomy 6, 3 had balanced chromosome 6. On the contrary, none of the CTNNB1 wild-type tumours had monosomy 6. Conclusions: Standard neuropathological evaluation of medulloblastoma samples should include IHC of β-catenin because tumours with high nuclear accumulation of β-catenin most probably belong to the Wnt subgroup of medulloblastomas. Still, IHC alone may be insufficient to detect all Wnt cases. Similarly, chromosome 6 aberrations were not present in all CTNNB1-mutated cases. Therefore, we conclude that sequencing analysis of CTNNB1 exon 3 in combination with β-catenin IHC (possibly as pre-screening method) is a feasible and cost-efficient way for the determination of Wnt medulloblastomas.

Keywords: β-catenin, immunohistochemistry, medulloblastoma, mutation analysis, sequencing, Wnt signalling.

Introduction

Within the last few years it became clear that medulloblastoma, the most common malignant paediatric brain tumour, comprises at least four distinct molecular subgroups (Wnt, SHH, Group 3 and Group 4) [1,2]. But even
though several methods have been published for rapid subgrouping [1,3–7], none of them found its way into routine clinical practice yet and most treatment protocols are still based on clinical features like age of patient, extent of surgical resection and metastatic status as well as on histopathological features. For Group 3 tumour patients, for example, an intensification of therapy might be beneficial, whereas patients with activated Wnt pathway have a generally favourable prognosis [8,9] and may benefit from a reduction or complete omission of chemotherapy or cranio-spinal radiotherapy, which would decrease neurological side-effects.

The Wnt subgroup represents a well-characterized and homogenous subgroup representing 10–18% of all medulloblastomas. Tumour samples show mostly classic histology, patients are generally older than five years at diagnosis and leptomeningeal dissemination is rare. Somatic point mutations in the\textit{CTNNB1} gene encoding for β-catenin have first been identified in medulloblastomas by Zurawel\textit{et al.} [10] and have been found later in more than 90% of Wnt tumours (resp. medulloblastomas with nuclear β-catenin accumulation) in most publications [5,6,11–17]. Other groups reported mutation rates of only about 60–70% [9,18,19]. Instead of\textit{CTNNB1} mutations other signalling pathway members like AXIN1, AXIN2/Conductin or APC were also found mutated or showed deletions in some cases leading to pathway activation [12,20–23].

The\textit{CTNNB1} mutations are exclusively found in exon 3 at or adjacent to four phosphorylation sites. The phosphorylation at these sites targets the β-catenin protein for ubiquitination and subsequent degradation. Mutations in exon 3 lead to cytoplasmic β-catenin accumulation and finally to translocation into the nucleus, where this leads to expression of specific target genes after binding and activating the transcription factors TCF/LEF [24]. The first publication showing that tumours with activated canonical Wnt pathway are associated with a favourable prognosis was based on immunohistochemistry (IHC) for nuclear β-catenin staining [8]. Retrospective analyses of medulloblastoma cohorts by molecular genetic analyses and integrative genomic subgrouping also showed a superior outcome of this group.

Another genomic feature for most but not all Wnt subgroup tumours is monosomy of chromosome 6 [1,9,13]. This was only rarely seen in any of the other medulloblastoma subgroups [6,7,19].

Still, despite these three features clearly related to the Wnt subgroup – nuclear β-catenin accumulation,\textit{CTNNB1} exon 3 mutations and monosomy 6 – a rapid and clear diagnosis of Wnt medulloblastomas in standard clinical practice is not yet feasible. Upcoming clinical studies, however, aim to reduce the treatment for Wnt subgroup medulloblastoma patients. Our aim was, therefore, to identify the optimal methods in standard clinical practice to detect Wnt subgroup medulloblastomas.

Material and methods

Tissue samples, analysis of β-catenin by immunohistochemistry, DNA extraction, and sequencing of\textit{CTNNB1} exon 3

A cohort of 186 medulloblastoma samples from paediatric and adolescent patients aged between 0 and 21 years (median age: 7 years) was analysed, comprising 68 female and 118 male cases, 134 classic, 36 desmoplastic, 7 medulloblastomas with extensive nodularity (MBEN), 8 anaplastic and 1 large cell medulloblastoma. For further informations, we refer to Table S1 and a recent publication on prognostic markers in the HIT2000 clinical trial [25]. The study was approved by institutional review boards and informed consent was obtained from the legal representatives of the patients.

Formalin-fixed and paraffin-embedded (FFPE) tissue slides were stained on BenchMark XT (Roche-Ventana, Darmstadt, Germany), two functionally identical Autostainer [Medac (Hamburg, Germany) and DAKO (Glostrup, Denmark)] and Techmate (DAKO) systems in three independent laboratories always using the monoclonal anti-β-catenin antibody clone 14 (MAb 14) from BD Transduction Laboratories™ (Franklin Lakes, NJ, USA) at predefined, optimized concentrations. The stainings from both Autostainer systems were evaluated by three investigators, the other two series by one experienced neuropathologist.

The percentage of nuclear positive cells was evaluated. In particular, cut-off scores of 5% and 10% were considered. For comparison with genetic findings, we categorized the samples into three groups: (1) nuclear accumulation in ≥5% of tumour cells; (2) nuclear accumulation in <5% of tumour cells; (3) no nuclear accumulation.

DNA from FFPE tumour tissue containing >80% tumour cells was extracted using the QIAamp DNA Mini...
Tissue Kit (Qiagen, Düsseldorf, Germany), according to the manufacturer’s instructions.

Direct sequencing (Sanger) of exon 3 of CTNNB1 was performed as previously described [12]. Cases showing nuclear accumulation of β-catenin in ≥5% of tumour cells but without CTNNB1 mutation were sequenced to identify alternative mutations in the APC binding sites of AXIN1 and AXIN2/Conductin as described before [21,22].

**Single-strand conformation polymorphism (SSCP)**

PCR products were loaded on 10% and 14% polyacrylamide gels with different acrylamide : bisacrylamide ratios with and without 5% glycerol as previously described [12].

**Multiplex ligation-dependent probe amplification (MLPA)**

MLPA was performed as previously described [26] for all cases by using the SALSA P301-A1 medulloblastoma mix (MRC Holland, Amsterdam, Netherlands), which contains probes for eight loci on chromosome 6 (3 probes for chromosome arm 6p and 5 probes for chromosome arm 6q) and several reference loci (Fig. S1). PCR products were analysed on an ABI 3130 capillary sequencer (Applied Biosystems, Foster City, CA, USA). Data were normalized against reference samples using the GeneMapper software (version 3.7; Applied Biosystems). Gains and losses are regarded to be statistically significant if the calculated, normalized values were higher/lower than mean ± 3 SD of DNA derived from normal cerebellar FFPE tissue.

**Molecular inversion profiling**

To identify copy number gains and losses, the custom-designed OncoScan FFPE Express 330K molecular inversion profiling platform, version 2 (Affymetrix, Santa Clara, CA, USA) was used. The molecular inversion profiling assay was performed as described [27].

**DNA methylation array data generation**

DNA methylation profiling using the Illumina Infinium HumanMethylation450 BeadChip array was performed according to the manufacturer's instruction as previously described [3].

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**Results**

**Evaluation of nuclear β-catenin accumulation by immunohistochemistry**

First we performed IHC for β-catenin to test for nuclear accumulation in the tumour cells. To compare different staining systems, we stained slides from 180 cases on four platforms and six further cases on three platforms. Even though the staining intensities varied on the three systems the results of all stainings were mostly in good concordance. The results of the IHC evaluation for nuclear β-catenin accumulation using scoring of <5% vs. ≥5% of positive cells was concordant in 173/180 cases (96%). If a cut-off level of 10% was chosen, the concordance rate was 98%.

We found 21 cases with ≥5% positive nuclei, among them 12 cases with widespread nuclear staining in more than 50% of tumour cells (Fig. 1a) and 9 cases with only 5–50% nuclear accumulation. Of these, 5 cases were scored between 5% and 10% (Table 1). Further 17 cases showed only few stained nuclei (<5% in at least 2 of the stainings; Fig. 2b). All other tumour samples showed only cytoplasmic or no significant β-catenin staining in tumour cells (Fig. 1c). Interestingly, in many but not all of these samples nuclei of endothelial cells were stained for β-catenin (Fig. 1d).

To assess the inter-observer variability, 2 series were evaluated by 3 independent investigators. Overall good concordance rates regarding scoring as significantly accumulated cases were found. In the Autostainer Bonn series, concordance rate was >95% for a cut-off level of 10% and >93% for a cut-off level of 5% of positive nuclei. In the Autostainer Vienna series, concordance was even higher [>98% (10% cut-off) and >96% (5% cut-off)]. For detailed informations on all evaluations we refer to Table S1.

**CTNNB1 mutation analysis by SSCP and sequencing**

To find out if all the cases with nuclear β-catenin staining in ≥5% of tumour cells indeed represented Wnt subgroup medulloblastomas, we searched for mutations in the CTNNB1 exon 3 gene. First, we screened the whole cohort for mutated alleles showing aberrant migration in SSCP gels (Fig. 2a), but found only three cases representing two different mutations in codon 32 and 33 (1×D32G and 2×S33C). In addition, the whole cohort was analysed by
Figure 1. Immunohistochemistry for β-catenin sections of tumour samples from four different patients with nuclear accumulation in ≥5% (widespread) of tumour cells (a), <5% (b, arrows indicate β-catenin positive nuclei), no nuclear accumulation (c) and nuclear accumulation in endothelial cells (d, arrows).

Table 1. Overview over the results for β-catenin immunohistochemistry (percentage of tumour cells with nuclear β-catenin accumulation), CTNNB1 exon 3 mutations (direct Sanger sequencing), chromosome 6 aberrations (MLPA and molecular inversion profiling) and subgroup informations (450k methylation array) in samples with ≥5% nuclear β-catenin accumulation

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>IHC scoring (%)</th>
<th>CTNNB1 exon 3 mutation</th>
<th>MLPA chromosome 6</th>
<th>Molecular inversion profiling chromosome 6</th>
<th>Subgroup (450k)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>&gt;50</td>
<td>G34E</td>
<td>Balanced</td>
<td>No allele loss/no copy number loss</td>
<td>Wnt</td>
</tr>
<tr>
<td>24</td>
<td>&gt;50</td>
<td>D32V</td>
<td>Monosomy</td>
<td>Not done</td>
<td>Wnt</td>
</tr>
<tr>
<td>65</td>
<td>20–50</td>
<td>D32G</td>
<td>Monosomy</td>
<td>Allele loss/copy number loss</td>
<td>Wnt</td>
</tr>
<tr>
<td>78</td>
<td>5–10</td>
<td>–</td>
<td>Balanced</td>
<td>No allele loss/no copy number loss</td>
<td>Wnt</td>
</tr>
<tr>
<td>83</td>
<td>15–30</td>
<td>S33F</td>
<td>Monosomy</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>86</td>
<td>20–50</td>
<td>S33C</td>
<td>Monosomy</td>
<td>Allele loss/copy number loss</td>
<td>Wnt</td>
</tr>
<tr>
<td>92</td>
<td>&gt;50</td>
<td>S37P</td>
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<td>Not done</td>
</tr>
<tr>
<td>99</td>
<td>&gt;50</td>
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<td>Allele loss/copy number loss</td>
<td>Wnt</td>
</tr>
<tr>
<td>108</td>
<td>&gt;50</td>
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<td>Allele loss/copy number loss</td>
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</tr>
<tr>
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<td>Wnt</td>
</tr>
<tr>
<td>126</td>
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<td>Balanced</td>
<td>No allele loss/no copy number loss</td>
<td>Wnt</td>
</tr>
<tr>
<td>127</td>
<td>5–10</td>
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<td>Monosomy</td>
<td>Allele loss/copy number loss</td>
<td>Wnt</td>
</tr>
<tr>
<td>141</td>
<td>20–50</td>
<td>D32Y</td>
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<td>Allele loss/copy number loss</td>
<td>Wnt</td>
</tr>
<tr>
<td>143</td>
<td>&gt;50</td>
<td>S33C</td>
<td>Monosomy</td>
<td>Allele loss/copy number loss</td>
<td>Wnt</td>
</tr>
<tr>
<td>149</td>
<td>&gt;50</td>
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<td>Allele loss/copy number loss</td>
<td>Wnt</td>
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<tr>
<td>157</td>
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<td>Allele loss/copy number loss</td>
<td>Wnt</td>
</tr>
<tr>
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<td>Not done</td>
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<tr>
<td>167</td>
<td>5–10</td>
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<td>Allele loss/copy number loss</td>
<td>Wnt</td>
</tr>
<tr>
<td>172</td>
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<td>–</td>
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<td>4</td>
</tr>
<tr>
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<td>–</td>
<td>Balanced</td>
<td>Not done</td>
<td>3</td>
</tr>
<tr>
<td>198</td>
<td>&gt;50</td>
<td>T41A</td>
<td>6p loss</td>
<td>Not done</td>
<td>Wnt</td>
</tr>
</tbody>
</table>

The three cases with grey background are those without CTNNB1 mutations. The three mutations in bold are those identifiable with SSCP.
direct Sanger sequencing of CTNNB1 exon 3 and this analysis revealed 18 cases with somatic point mutations (9.7%; Fig. 2b,c). Most of these mutations were located at or adjacent to codons 33 and 37 (both serine), while a single case with a mutation at codon 41 (threonine) was found.

All 18 cases with mutations showed nuclear β-catenin accumulation in ≥5% of tumour cells, whereas none of the 17 samples with less than 5% as well as none of the samples without nuclear accumulation were found mutated in CTNNB1 exon 3. Interestingly, none of the residual three cases without CTNNB1 mutation but with ≥5% nuclear accumulation in the tumour cells (sample no. 78, 172 and 189) showed a widespread staining in the majority of nuclei as was seen in most of the mutated cases and actually all three cases were categorized <5% or even negative in two of the four stainings. However, as mentioned before, not all the CTNNB1-mutated cases showed a widespread nuclear staining pattern with β-catenin positive nuclei in most of the tumour cells and in 2 of the 9 cases without widespread staining (sample no. 126 and 161) we found only between 5 and 10% positive nuclei.

Sequencing of AXIN1 and AXIN2/Conductin

We also looked for mutations in the APC-binding domains of AXIN1 and AXIN2/Conductin in the three samples with ≥5% nuclear β-catenin accumulation but lacking CTNNB1 exon 3 mutations, but could not find any (not shown).

Evaluation of monosomy 6 by MLPA and molecular inversion profiling

The whole cohort was screened by MLPA. We found 14 cases with monosomy 6 and one case with isolated 6p loss, all showing nuclear β-catenin accumulation in more than 5% of tumour cells and harbouring CTNNB1 mutations. An example is given in Fig. S1. The other three of the CTNNB1-mutated cases as well as all CTNNB1 wild-type tumours had balanced chromosome 6. To verify the MLPA results in cases suspected to represent the Wnt subgroup (≥5% nuclear β-catenin accumulation) we performed a molecular inversion profiling of 15 of the 21 cases where enough material was available. All of the 15 analysed samples showed identical results as the MLPA (Table 1).
Methylation profiling by 450k array

For an additional confirmation that only the CTNNB1 mutated medulloblastomas belonged to the Wnt subgroup we analysed nearly the whole cohort (178 from 186) with 450k arrays, which clearly grouped the 3 critical cases lacking CTNNB1 mutations but showing ≥5% nuclear β-catenin accumulation into other subgroups while all analysed CTNNB1-mutated cases (15 of 18) were clustered to the Wnt subgroup. No additional Wnt cases without CTNNB1 mutations were detected (Table S1).

Discussion

The secure selection of Wnt subgroup medulloblastoma patients for upcoming clinical trials that aim to reduce treatment is very important as these trials will be stopped if a patient will die due to this therapy de-escalation. Also a recent study found out that parents of paediatric medulloblastoma patients would need to be very confident that their child was correctly categorized as having a good prognosis before they would be willing to decrease therapy [28].

Ellison et al. showed for the first time in the PNET3 medulloblastoma trial cohort that patients with medulloblastomas characterized by nuclear accumulation in a significant fraction of cells had a significantly better outcome compared to medulloblastomas lacking this feature [8].

This study was based on IHC only performed as retrospective analysis in a single laboratory. If β-catenin IHC will be used as method to rapidly identify nuclear accumulation and subsequent patient stratification the problem of inter-laboratory (inter-platform) and inter-observer variability should be considered. Although the inter-platform concordance and inter-observer concordance to call a case positive for nuclear β-catenin accumulation in this study was documented to be >95% (cut-off level 10% of positive nuclei), some cases with borderline fraction of tumour cells might be wrongly categorized. Inter-observer discrepancies might furthermore be explained by the adjustment of a neuropathologist to the staining of his or her laboratory so that unaccustomed stainings might not be scored correctly. The immunohistochemical evaluation of Wnt medulloblastomas can be difficult even from perfectly stained tissue slides and even for experienced neuropathologists and the use of IHC as subgrouping method [1,7] has proved difficult to standardize across neuropathology laboratories [3]. Further reasons why β-catenin IHC alone might be insufficient for the determination of Wnt subgroup cases can be low quality of paraffin tissue or technical problems that might lead to false evaluation of the stainings. If it is not clear whether a staining failed we would first recommend to repeat the staining. This should be the case if neither cytoplasmic staining nor nuclear accumulation in endothelial cells is visible at all. However, absence of nuclear β-catenin in endothelial cells occurs in a fraction of medulloblastomas and may not per se indicate staining failure.

Because of these reasons we wanted to evaluate the usefulness of incorporating additional methods to identify Wnt medulloblastomas. These analytic methods should be robust, fast and cost-efficient if included in standard primary diagnostics. In this study we have compared CTNNB1 sequencing, SSCP screening, analysis of chromosome 6 monosomy using MLPA (and molecular inversion profiling in a subgroup of cases) as well as 450k array methylation profiling.

First, we sequenced all cases to identify CTNNB1 exon 3 activating mutations as frequent cause for Wnt activation and nuclear accumulation of β-catenin protein. In our cohort we detected 18 medulloblastomas harbouring CTNNB1 mutations (9.7% of the cohort). By IHC, all of these showed nuclear accumulation in more than 5% of cells. Three further cases with nuclear accumulation were found. This was not unexpected because most publications reported mutation rates in medulloblastomas with nuclear accumulation in about 90% or even less. The presence of a CTNNB1 mutation is not considered a prerequisite for Wnt medulloblastomas. Besides the possibility of AXIN1 or AXIN2/Conductin mutations activated Wnt signalling can also occur due to mutations in APC [12,21,23]. However, sequencing of the APC-binding sites of AXIN1 and AXIN2/Conductin did not uncover mutations in these 3 cases. 450k array methylation profiling in fact indicated that these three medulloblastomas with nuclear accumulation but CTNNB1 wild-type status were related to the biological groups 3 in two cases and 4 in one case. Tumours of these groups may show a more aggressive course of disease and their inclusion in treatment protocols with reduced dosage may lead to stopping because of relapses.

An additional typical genetic feature of many Wnt medulloblastomas is monosomy 6. Therefore, an alternative method to identify Wnt medulloblastomas is the...
determination of chromosome 6 status. However, Wnt medulloblastomas only have monosomy 6 in 80–90% of cases [1,6,13,15,19]. For detection of monosomy 6 we performed MLPA with probes covering the short and long arms of chromosome 6 in the whole cohort, and detected 14 cases with monosomy 6 and one case with 6p loss only. All cases with monosomy 6 (and the one with 6p loss) had CTNNB1 mutations and therefore can be considered as typical Wnt cases, but in our series no other cases showed monosomy 6. This indicates a high specificity (100%) but restricted sensitivity (15/18 cases) of this assay. MLPA is a robust, DNA-based method which is suitable to analyse low amounts of FFPE-derived degraded DNA. In this series, we had no test failures. To validate the MLPA findings, 15 cases were analysed by genome-wide molecular inversion profiling. The results of both methods matched perfectly (Table 1). As molecular inversion profiling requires more DNA as test input and is more time-intensive and much less cost-efficient if only monosomy 6 is assessed, we think that MLPA analysis is a feasible method for the determination of chromosome 6 status from FFPE-derived DNA in medulloblastomas. Although we have not compared our MLPA results to FISH-derived data in this cohort, FISH testing usually show a higher percentage of tumour cells with nuclear accumulation but the cut-off level may help to detect Wnt cases with lower percentages. For detection of monosomy 6 we performed MLPA with probes covering the short and long arms of chromosome 6 in the whole cohort, and detected 14 cases with monosomy 6 and one case with 6p loss only. All cases with monosomy 6 (and the one with 6p loss) had CTNNB1 mutations and therefore can be considered as typical Wnt cases, but in our series no other cases showed monosomy 6. This indicates a high specificity (100%) but restricted sensitivity (15/18 cases) of this assay. MLPA is a robust, DNA-based method which is suitable to analyse low amounts of FFPE-derived degraded DNA. In this series, we had no test failures. To validate the MLPA findings, 15 cases were analysed by genome-wide molecular inversion profiling. The results of both methods matched perfectly (Table 1). As molecular inversion profiling requires more DNA as test input and is more time-intensive and much less cost-efficient if only monosomy 6 is assessed, we think that MLPA analysis is a feasible method for the determination of chromosome 6 status from FFPE-derived DNA in medulloblastomas. Although we have not compared our MLPA results to FISH-derived data in this cohort, FISH testing usually show a higher failure rate if chromosomal alterations are assessed in FFPE material. If FISH is used at least two probes for chromosome arms 6p and 6q should be used to avoid false positive calling due to local losses.

450k methylation profiling of almost the whole cohort (178/186) did not identify additional Wnt type medulloblastoma cases in comparison to CTNNB1 mutational analysis and monosomy 6 assessment. This finding is in contrast to the study of Schwalbe et al. [5] in which 450k array methylation profiling identified 8/26 CTNNB1 wild-type medulloblastomas with a Wnt methylation profile, 7 of these 8 cases also lacked monosomy 6. This discrepancy may be related to different statistical methods of the analysis of 450k array methylation profiles. In principal, 450k array methylation profiling should be useful for the confirmation of the Wnt subgroup or designation to other medulloblastoma subgroups in IHC positive cases lacking detectable CTNNB1 mutations (or monosomy 6), but on the other hand, similar to molecular inversion profiling, is more time-consuming and cost-intensive.

Our results suggest that the employment of alternative genetic methods in addition to IHC of β-catenin increases the validity of Wnt subgroup calling, in particular for proper patient stratification. At least two different methods should be performed to make sure the tumour has indeed activated Wnt signalling.

We further wanted to address the question if the immunohistochemical cut-off of 10% nuclear accumulated cells to classify medulloblastomas [13,19] is appropriate in the setting of combination of alternative tests. We wanted to know if also cases with nuclear accumulation in 5–10% of the tumour cells can belong to the Wnt subgroup and carry CTNNB1 mutations and/or monosomy 6. Indeed, when we set our cut-off at 5%, two cases identified as having a nuclear accumulation between 5% and 10% carried CTNNB1 exon 3 mutations. Unfortunately, only from one of these samples enough DNA was available for the 450k methylation array analysis, but this case was grouped to Wnt medulloblastomas as expected. Therefore, with a cut-off at 10% patients might be missed who would profit from therapy de-escalation.

On the other hand three cases scored between 5% and 10% lacked CTNNB1 mutations, monosomy 6 and Wnt methylation profile so that these would represent false-positive calls by IHC alone. This indicates that lowering the cut-off level may help to detect Wnt cases with lower percentage of tumour cells with nuclear accumulation but should be only done when false-positive cases can be identified by additional genetic tests. Our data also showed that further lowering of the cut-off level below 5% did not identify additional cases which could be confirmed as Wnt activated.

In conclusion, based on our data as well as those from other similar studies [5,13,19], we recommend a combination of IHC analysis for nuclear β-catenin accumulation (possibly as a pre-screening method) with CTNNB1 exon 3 sequencing (in duplicate – forward and reverse) in at least all IHC positive cases (≥5% of nuclei). We think that this methodological approach represents a fast, feasible and cost-efficient way for the determination of Wnt subgroup medulloblastomas.

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References


Detection of Wnt activated medulloblastoma


Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Figure S1. MLPA-electropherograms of normal DNA (cerebellum, upper figure) and DNA of a tumour with monosomy 6 (lower figure); big black arrows indicate loci on chromosome 6 and thin red arrows indicate reference loci.
Table S1. Overview over the histological subtypes (diagnosis), age and gender of the patients and detailed informations on all immunohistochemistry evaluations from all 4 staining platforms (percentage of tumour cells with nuclear β-catenin accumulation). CTNNB1 exon 3 sequencing results, MLPA chromosome 6 data and sub-group informations identified by 450k methylation arrays. IHC grouping shows cases with <5% nuclear accumulation in tumour cells (yellow) and ≥5% (orange). Inv., investigator; M, male; F, female.

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