Introduction

Medulloblastoma, a primitive neuro-ectodermal tumor of the cerebellum, is the most common malignant brain tumor of childhood. Five-year overall survival rates have increased over recent years to 70% to 80% for standard-risk patients. However, for high-risk patients (infants <3 years, cases with metastatic disease at diagnosis or incomplete surgical resection), current treatments only cure around 40% to 60% of cases. In addition, many survivors exhibit long-term therapy-associated late effects. The development of novel targeted treatments, alongside refined patient stratification, will be essential to increase survival rates and reduce adverse sequelae (1). Improvements in understanding of the molecular basis of medulloblastoma will be fundamental to such advances.

The constitutive activation of developmental signaling pathways plays a key role in medulloblastoma pathogenesis, and pathway components represent the major mutational targets identified in the disease to date. The Sonic Hedgehog (SHH) pathway plays an essential role in normal cerebellar development, is activated by PTCH1 mutation in around 10% of human primary medulloblastomas, and promotes medulloblastoma development in mouse models of the disease (2–4). Similarly, mutations in components of the canonical Wnt/Wingless (WNT) signaling pathway have been described in up to 20% of cases (5–7). Importantly, these pathways appear to have therapeutic significance; WNT-active cases are associated with a favorable prognosis...
best documented, and is distinguished by nuclear supported by these studies (12, 13). The WNT subgroup is characterized respectively by activation and mutation of related clinical disease features. Two disease groupings, distinguished by their gene expression profiles, and display tumoric investigations in medulloblastoma have identified early-clinical activity against the disease (10, 11). Culicine inhibitors of the SHH pathway show preclinical and (14). Recent array-based genome-wide genomic and transcriptomic investigations in medulloblastoma have identified distinct molecular disease subgroups, which are distinguished by their gene expression profiles, and display related clinical disease features. Two disease groupings, characterized respectively by activation and mutation of the WNT and SHH signaling pathways, are consistently supported by these studies (12, 13). The WNT subgroup is best documented, and is distinguished by nuclear β-catenin immunostaining, CTNNB1 mutations, and chromosome 6 loss (5, 13–15), alongside its associated favorable prognosis (8, 9). The SHH subgroup is, however, less well characterized; PTCH1 mutations are only identified in a subset of SHH cases, indicating a role for other activating mechanisms and correlates. A series of putative mechanisms of SHH activation [e.g., PTCH1 hypermethylation, SUFU/SMO mutation, and REN (KCDT11) genetic loss] have been reported in medulloblastoma (16–19), but any relationships to the SHH subgroup remain to be established. Moreover, SHH subgroup clinical features require clarification; SHH activation has been associated with the desmoplastic/nodular (DN) disease histologic variant in some studies, but not others, and associations with infant cases have also been postulated (12, 17, 20, 21).

Identification of these medulloblastoma molecular subgroups has significant potential to (i) improve clinical management, through molecular disease-risk stratification strategies and the identification of patients who could benefit from SHH and WNT targeted molecular therapeutics; and (ii) provide a basis for biological investigations to further understand disease molecular pathogenesis and its therapeutic applications. However, subgroup identification has so far relied on advanced genomics (i.e., microarray) technologies, which are relatively expensive and have used different gene expression data collection and analysis methodologies. The development of robust biomarkers and assays for subgroup detection, which can be routinely tested in clinical material across multiple treatment centers and are informative in small tumor biopsies, will therefore be essential for their future study and any clinical application. Moreover, studies in modestly sized cohorts have limited comprehensive investigation of subgroup clinical characteristics and their molecular basis.

In this study, we report the development and validation of minimal mRNA expression signatures, which are robust for the routine diagnosis of the SHH and WNT subgroups in clinical material and can be applied rapidly and cost-effectively using array-independent methodologies. Using these signatures, we show that equivalent disease subgroups are detected in 4 independent medulloblastoma cohorts, independent of gene expression assay used. We then use these signatures to assign subgroup affiliation in this large combined cohort of 173 medulloblastomas and use these data as the basis for comprehensive investigations to define the clinical and molecular characteristics of each subgroup, and their utility for improved disease management.

Materials and Methods

Tissue samples

A representative cohort of 55 medulloblastoma samples was analyzed, comprising 39 classic, 5 large cell/anaplastic (LCA), and 11 tumors of the DN histopathologic subtype (22), 21 female and 34 male cases, 11 infants (≤3 years), 41 children (>3–15 years), and 3 adults (>16 years). DNA (n = 55) and RNA (n = 39) were extracted from these snap-frozen tissues using standard methods. A panel of constitutional DNA samples from 100 normal individuals was obtained from the North Cumbria Community Genetics Project in the United Kingdom. Research Ethics Committee approval has been obtained for the collection, storage, and biological study of all material.

Mutation screening

All coding exons of the PTCH1 and SMO genes were PCR amplified using the primers and conditions shown in Supplementary Table S1. Mutation screening methods for the SUIFU gene have been described previously (23). Mutation screening was performed by analysis of PCR products for heteroduplex formation, before and after ‘spiking’ with equal amounts of control wild-type DNA using denaturing high performance liquid chromatography (Wave DNA Fragment Analysis System, Transgenic) according to the manufacturer’s instructions. Products detected as containing a heteroduplex were sequenced directly on an ABI
377 sequencer (Applied Biosystems). In reported studies including our own, denaturing high-performance liquid chromatography has been reported to identify >90% of sequence variants (23). Mutation analysis of the CTNNB1 and APC genes was performed as previously described (8).

Analysis of promoter methylation status

Two promoter-associated CpG islands of the PTCH1 gene, spanning exons 1a and 1c (16), were identified and characterized using the Emboss CpGPlot website (http://www.ebi.ac.uk/emboss/cpgplot/): 1a methylation status was determined by methylation-specific PCR (MSP), and 1c by bisulphite sequencing using previously published primers (16). A CpG island of the SUFU gene was also identified, and its methylation status analyzed by MSP (24). COL1A2 methylation status was assessed by bisulphite sequencing, and has been previously reported (25). Bisulphite treatment, methylated, and unmethylated controls have been described previously (23). Primers and conditions for analysis of PTCH1 CpG island 1a are shown in Supplementary Table S2. Methylation status was designated as methylated or unmethylated, as previously described (26). For loci assessed by MSP, any sample showing a visible PCR product using primers specific for the methylated sequence was classed as showing evidence of methylation (i.e., methylated). For loci assessed by bisulphite sequencing, the relative peak intensities at each CpG residue were determined. Samples in which the methylated peak represented >25% of the total peak height in greater than 25% of the analyzed CpG sites were classed as showing evidence of methylation (i.e., methylated).

Loss of heterozygosity analysis

Loss of heterozygosity (LOH) of chromosome regions 9q22.3 and the p-arm of chromosome 17 was analyzed using polymorphic microsatellite markers D9S1689, D9S1816, D9S287, D9S1809, D9S1786, D9S1851, and D17S2196, D17S936, D17S969, D17S974, D17S1866, D17S1308, respectively (www.ncbi.nlm.nih.gov/genome/sts), as previously described (27). LOH of chromosome 6 was analyzed using previously described markers and methods (14).

A multigene mRNA expression signature to identify SHH and WNT pathway activated tumors

A 13-gene multiplex mRNA expression assay (GeXP; Beckman Coulter; ref. 28) was developed and used to test tumors for membership of the SHH or WNT medulloblastoma subgroups in 39 cases. Detailed clinical and pathologic data for individual cases are summarized in Table 1. Two previously reported independent medulloblastoma expression microarray data sets (12, 13) were used to design SHH and WNT subgroup signatures. Data were respectively downloaded from the St Jude Research website (http://www.stjuderesearch.org/site/data/medulloblastoma) and the Gene Expression Omnibus (GEO; ref. 29). Data were rma normalized (30) using R and Bioconductor. Using t tests, we identified probes differential for the WNT (group A) or SHH (group B) subgroups, defined by Kool and colleagues (12), which were significant in both data sets. Previous work had validated 3 SHH-associated genes (GLI1, PTCH2, and SFRP1) and 2 WNT-associated genes (DKK2, WIF1) by quantitative reverse transcriptase real-time PCR (13) and these genes were also considered for inclusion in the signatures. Putative signature genes were assessed for assay suitability, and assays were subsequently performed using 50 ng total RNA per replicate, according to manufacturer’s instructions. To eliminate the possibility of detecting genomic DNA, PCR products were designed across exon boundaries; products were also designed where possible to overlap the Affymetrix probes from which they were derived. For genes with multiple transcripts, amplification was designed that detected all known transcripts. Test gene expression was normalized to 28S rRNA, and results displayed are means based on independent assessment in triplicate. The presence of SHH or WNT expression signatures was used to identify sample subgroup membership (see next section). Signature gene selection is summarized in Supplementary Table S3, and PCR primer sequences are listed in Supplementary Table S4.

Integration and analysis of combined gene expression data sets

An additional medulloblastoma expression microarray data set, reported by Fattet and colleagues (15), was normalized as described earlier. All 3 publicly available data sets have linked clinical (age at diagnosis, pathology subtype, and gender) and PTCH1/CTNNB1 mutational data available [except PTCH1 mutation data not available for the Fattet and colleagues (15) data set]. Expression data of signature genes from all 3 data sets, together with our own, were integrated. First, our GeXP expression data were log2 transformed as for the expression microarray data and, before joining, all data sets were scaled separately, on a per-gene basis, to give a mean of 0, with variance of 1. Unsupervised hierarchical clustering, supported by principal component analysis (PCA), was used to assign subgroup membership status (Fig. 1), and these data were used in conjunction with stacked bar plots (Fig. 2) and silhouette plots (ref. 31; Supplementary Fig. S1) to assess performance of the signatures. There was some sample overlap between studies: 11 samples were assessed by expression array by Thompson and colleagues (13) and GeXP; 3 samples were assessed by expression array by Kool and colleagues (12) and GeXP, which enabled the comparison of subgroup assignment in individual samples using our signatures, when evaluated using different gene expression assays. Clinical and genomic correlates were also combined for the joined cohort. Duplicate analyses were removed from all correlative investigations.

Statistical analysis

Fisher’s exact and $\chi^2$ tests were used to identify relationships between expression signatures and molecular and clinical features of the disease in the primary investigative
Table 1. Associations between molecular subgroups and medulloblastoma genomic, epigenomic, and clinical disease features

<table>
<thead>
<tr>
<th>Molecular subgroup</th>
<th>Genotype</th>
<th>Hypermethylation</th>
<th>Chromosomal loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHH</td>
<td>WNT</td>
<td>WNT/SHH-independent</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Cases are shown arranged by signature status (SHH, WNT, WNT/SHH-independent) as determined in Fig. 1, and then by ascending age, molecular subgroup; mutation status; hypermethylation status (black, methylated; white, unmethylated); and chromosomal loss (black, allelic; white, no loss). Age is shown in years and categorized into infants (<3 years, black) and noninfants (≥3 years, white). Pathology variant is indicated by a white square (classic), black square (DN) or gray square (LCA). Gender is indicated by black squares (F, female) and white squares (M, male). M stage 0/1 is indicated by white squares and M stage 2/3 by black squares. Raw P and P values corrected for multiple hypothesis testing are shown for relationships between molecular/clinical correlates and subgroup membership (c2 tests, Bonferroni correction). Significant (*P < 0.05) and marginally significant (**P = 0.051–0.10) associations are marked. Diagonally hatched boxes indicate unavailable data.

**Abbreviations:** FS, frameshift; MS, missense mutation; ns, not significant.

Schwalbe et al.  
Clin Cancer Res; 17(7) April 1, 2011

Clinical Cancer Research

Published OnlineFirst February 16, 2011; DOI: 10.1158/1078-0432.CCR-10-2210

Downloaded from clinanceres.aacrjournals.org on April 13, 2017. © 2011 American Association for Cancer Research.
Results

A diagnostic multigene mRNA expression signature assay for SHH and WNT medulloblastoma subgroups

We first developed and validated multigene mRNA expression signatures to facilitate routine identification of the SHH and WNT medulloblastoma molecular disease subgroups and assessment of their molecular basis, associated biomarkers, and clinical relevance. The 8-gene SHH (BCHE, GLI1, ITIH2, MICAL1, PDLIM3, PTCH2, RAB33A, and SFRP1) and 5-gene WNT (CCDC46, DKK2, PYGL, TNC, and WIF1) subgroup signatures developed were initially assessed by GeXP assay in our primary sample cohort (n = 39) and were unequivocal for all samples, independent of data analysis method used (stacked bar plots, PCA, or unsupervised hierarchical clustering; Figs. 1 and 2). Validation of these signatures in 3 independent medulloblastoma expression microarray data sets [Thompson and colleagues (n = 46; ref. 13); Kool and colleagues (n = 62; ref. 12); and Fattet and colleagues (n = 40; ref. 15)] showed the signatures could be successfully interrogated (GeXP) cohort (n = 39) and in the combined cohort (n = 173). Bonferroni corrections for multiple hypothesis testing were applied where appropriate. Additional patient age data were kindly provided by Dr. M. Kool (Academic Medical Centre, Amsterdam, the Netherlands).
by hierarchical cluster analysis and PCA, were diagnostic in all cases, independent of cohort or gene expression assay used (Fig. 1), and showed close consistency with stacked bar plot data (Fig. 2). Signature positivity was concordant with the disease subgroups apparent after independent clustering of the most variable probes within each entire array data set, and correctly classified disease subgroup (i.e., as SHH, WNT, or WNT/SHH independent) in 99% (146/148) of cases overall (Fig. 1). Data sets similarly showed complete concordance in subgroup assignment, using all analytical methods, of the 14 cases (4 SHH, 2 WNT, and 8 WNT/SHH independent cases) where signatures were assessed in parallel by microarray and GeXP assays.

**Integration of cohorts and gene expression data sets: molecular subgroup incidence**

Based on our validated gene expression signatures and data analysis methods, the molecular subgroup data from all 4 cohorts could be combined for meta-analysis with the mutational and clinical data which were consistently reported for all studies. A total cohort of 173 cases was assessed in this analysis (Supplementary Table S5). Clinical demographics available for the combined cohort were consistent with previously reported groupwide estimates for medulloblastoma (1); male:female ratio (1.42:1), histology [115 classic (68%), 39 (23%) DN, 16 (9%) LCA, 3 cases with data not available; pathologic classifications were those reported in the original publications.

**Figure 2. Identification of SHH and WNT subgroup medulloblastomas using diagnostic expression signatures.** Data are illustrated from the 4 independent data sets using stacked bar plots [A, primary investigation cohort; B, Kool and colleagues (n = 62; ref. 12); C, Thompson and colleagues (n = 46; ref. 13); and D, Fattet and colleagues (n = 40; ref. 15)]. WNT signature genes (red) and SHH signature genes (blue) in combination clearly define subgroup membership. Vertical dashed lines delineate sample groups positive for WNT and SHH signatures by hierarchical clustering and PCA analysis in Fig. 1. Right-hand panel indicates stacked order of genes for each signature. Before generation of bar plots, expression data from each cohort were scaled on a per gene basis to a mean of zero and a variance of 1. Samples expressing all or most signature genes at above average levels will show bars of greater positive magnitude.
were excluded in SHH and WNT subgroup cases, respectively, across all 4 cohorts, where data were available [combined cohort, n = 39; Table 1], indicating no major role in WNT cases with available data (P < 3 × 10^{-16}; Fisher's exact), but this relationship was not exclusive. Chromosome 6 loss was also detected in occasional non-WNT cases (2/145). Eight of 35 tumors tested (23%) showed evidence of genetic loss at the 9q22.3 region surrounding the PTCH1 locus in our primary cohort (Table 1). Four of 8 were in the SHH subgroup and 2 of 4 tumors with PTCH1 mutations showed LOH of 9q22.3; however neither association reached significance (P = 0.09 and 0.22 respectively, Fisher's exact test). A significant inverse association between 17p loss and membership of the SHH and WNT subgroups was observed; 17p losses were exclusively observed in WNT-/SHH-independent cases (17p LOH in 0/12 SHH or WNT cases vs. 9/25 WNT-/SHH-independent cases [17p LOH in WNT-/SHH-independent cases vs. 9/25 WNT-/SHH-independent cases (P = 0.02, Fisher’s exact test)]. In addition, COL1A2 hypermethylation was detected in 76% (25/33) of cases; an absence of COL1A2 methylation was significantly associated with the SHH subgroup (P = 0.01, χ² test), but this relationship was not maintained when a correction for multiple hypothesis testing was applied (Table 1).

Medulloblastoma molecular subgroups display distinct clinical features

Analysis of the medulloblastoma molecular subgroups defined by our gene expression signatures, in all 173 cases of the combined cohort, revealed marked differences in their clinical disease features. WNT-/SHH-independent tumors made up the majority of cases, and had their peak incidence in the 3- to 6-year age group, but were extremely rare in the first 2 years of life (Fig. 3). In contrast, SHH subgroup tumors had their major peak of incidence in infancy [50% (21/42) of SHH cases were ≤3 years of age]. The SHH subgroup tumors represented the majority of
the infant clinical group [62% (21/34) of cases ≤3 years of age], but were less common in noninfant children [>3–15 years; 15% (16/127)], and had a further increased incidence in adult cases [≥16 years (45%; 5/11P); overall P = 5.8 × 10−9, χ² test]. Almost all cases <2 years of age [92% (11/12)] were SHH-positive. WNT subgroup cases were not observed in infants (minimum age observed, 5 years) and had a bi-modal age distribution with major and minor peaks at 10 and 20 years, respectively.

Significant differences were also observed in the distribution of medulloblastoma histologic subtypes between the molecular subgroups (P = 3.1 × 10−11, χ² test; Figs. 4 and 5). WNT subgroup cases exclusively displayed classic histology (P = 0.0003, Fisher’s exact test), and WNT-/SHH-independent tumors were also predominantly of the classic subtype, but DN and LCA cases were also observed. Consistent with previous studies (3, 12, 13, 21), SHH cases were overall strongly associated with DN histology (P = 1.1 × 10−9, Fisher’s exact test). However, this relationship was not absolute and LCA and classic cases were also observed in the SHH group. Most notably, examination of this large cohort revealed the relationship between SHH activation and DN pathology to be age dependent (Figs. 4 and 5); DN cases made up the majority of infant (≤3 years old) SHH subgroup cases; all DN cases in this infant group displayed SHH activation. DN pathology may therefore serve as a surrogate marker of SHH activation in the infant group. In contrast, there were almost equal proportions of DN, LCA, and classic cases in SHH-expressing noninfant cases, and the majority of noninfant DN tumors were not SHH activated (P = 8.6 × 10−5, Fisher’s exact test). No significant evidence for differences in metastatic stage [WNT 6% (1/16) M stage 2/3, SHH 16% (5/32), and WNT-/SHH-independent 24% (20/82)] was observed between the different expression subgroups (P = 0.20, χ² test).

Figure 3. Medulloblastoma molecular subgroups show distinct age of incidence distributions. Data for the WNT (gray), SHH (black), and WNT-/SHH-independent (hatched) subgroups are shown, based on a combined cohort of 173 medulloblastomas. A, density plots show subgroup-dependent ages of incidence. Case density represents the smoothed frequency of incidence within each of the 3 subgroups. Gray dotted line is plotted at 3 years of age. B, bar plots show age distribution of data set. C, bar plot shows age distribution of cases aged ≤6 years at diagnosis. F, frequency.
Discussions

The identification of distinct medulloblastoma molecular subgroups (12, 13, 15) offers significant potential to improve our understanding of disease biology and clinical management. Here, we report the development and validation of minimal diagnostic gene expression signatures which can be routinely applied to identify the SHH, WNT, and WNT/SHH-independent medulloblastoma disease subgroups. These gene expression signatures are robust and informative for subgroup identification in RNA extracted from snap-frozen tumor material, and using different gene expression assays. In particular, the GeXP multiplex expression assay reported offers a number of significant advantages over microarray methodologies for the routine assignment of subgroup affiliation; analysis can be undertaken straightforwardly, rapidly (in 1 working day, compared with 2–3 days for a microarray experiment) and cost-effectively (approximately one tenth of microarray analysis costs) and, importantly, can be performed on small amounts of total RNA (150 ng, compared with 500 ng to 5 µg for a typical microarray expression analysis), a common limitation when only small amounts of biopsy material are available. This removal of the need for relatively time-consuming, complex, and expensive array analysis platforms for subgroup assignment provides a strong basis for their clinical application; these methods are feasible for investigation in real time across multiple treatment centers during clinical treatment and in future clinical trials.

We have shown that the disease subgroups recognized by these signatures are equivalent and consistently identified in 4 independent medulloblastoma cohorts, allowing their assembly into a large combined cohort. Coupled with an extensive analysis of our novel primary cohort, this has allowed significant insights into the underlying molecular mechanisms, associated biomarkers, and clinical characteristics of these molecular disease subgroups.

Our systematic investigation of specific medulloblastoma genetic and epigenetic defects in this study has informed their roles as determinants or correlates of the molecular subgroups identified. Consistent with previous studies (12–14), CTNNB1 mutations were identified as the primary pathway-activating event present in almost all WNT subgroup tumors, with chromosome 6 losses also affecting the majority of these cases. PTCH1 mutation was the major mechanistic correlate of SHH activation, identified in ~34% of SHH cases. SHH-associated PTCH1 mutations were detected both in conjunction with chromosome 9q loss, and in the heterozygous state, indicating disruption of a single PTCH1 allele can be sufficient to cause SHH pathway disruption in medulloblastoma. An absence of COL1A2 hypermethylation was also significantly associated with SHH subgroup medulloblastomas, most strongly in infant cases. Notably, a number of the previously reported PTCH1 mutations have been reviewed.

The presence of some 17 genes in these cases. This disease subgroup, however, remains the least well characterized at the molecular level. Subdivision of this group has been proposed on the basis of its transcriptomic and genomic patterns; however, unlike the SHH and WNT groups, inconsistent results

Published OnlineFirst February 16, 2011; DOI: 10.1158/1078-0432.CCR-10-2210

www.aacjrournals.org
have been reported from different studies (12, 13), and the identification of specific genes and pathways associated with the pathogenesis of this subgroup will be critical to future advances in our understanding of its molecular basis and any underlying heterogeneity.

The significant associations observed between medulloblastoma molecular subgroups and specific gene, pathway, and chromosomal defects (i) strongly support the existence of molecularly distinct SHH and WNT subgroups, (ii) inform the contributory mechanisms involved in their pathogenesis, and (iii) provide potential biomarkers for subgroup identification. When assessed for suitability as primary biomarkers, only CTNNB1 mutations, which were specifically observed in all but 1 WNT subgroup case, have sufficient sensitivity and specificity to have utility. Nuclear localization of β-catenin has also been widely reported as a positive marker of WNT pathway activation (5, 8, 14), although its relationship to our WNT expression signature and CTNNB1 mutations could not be investigated in this study due to tissue limitations. Similarly, COL1A2 status may have utility in the identification of SHH subgroup infant desmoplastic medulloblastomas [this study and (25)], particularly in cases where biopsy limitations do not allow assessment of the DN pathologic variant. The remainder of gene and chromosomal defects investigated were not suitable as primary biomarkers for positive subgroup discrimination, as a result of their (i) nonexclusivity, (ii) limitation to subsets of subgroup cases, or (iii) inverse correlation with pathway activation. In comparison, gene expression signatures positively identified all subgroup cases and provide an accurate diagnostic test for subgroup membership. The genomic markers examined may therefore provide useful secondary or confirmatory markers, when used in conjunction with these signatures. It is not presently clear whether the expression signatures reported translate into subgroup-specific protein expression. Protein expression markers, which are testable by immunohistochemistry, may have utility for routine subgroup assignment in the diagnostic setting, however careful investigation and validation of their sensitivity, robustness, and reflection of expression array subgroup data, will be essential before their application.

The observation of 2 of 148 cases which were not consistently classified using the presently reported signatures and their respective array data sets (Fig. 1) indicates potential difficulties in the robust classification of a small subset (<2%) of cases. Inspection of the microarray expression data for these 2 cases revealed markedly reduced expression of pathway signature genes relative to the other pathway positive cases in their respective cohorts, despite their subgroup positivity using our signatures (refs. 12, 13, 15; Fig. 2). In addition, the stacked bar plot analyses revealed 2 further SHH-positive samples (T27, K452; Fig. 2) which, although clustered consistently between signature and array on hierarchical cluster and PCA analysis, could be classified as indeterminate based on the application of quantitative criteria to the individual expression data in stacked bar plot analysis [i.e., cumulative stacked bar plot expression score >8 (for WNT expression signature positive cases) or >4 (SHH cases)]. For such cases, additional markers of pathway activation (CTNNB1, PITCH1 mutation) could aid definitive subgroup assignment, and it is notable that 1 of these 2 samples also harbored a PITCH1 mutation, further supporting SHH subgroup membership. Silhouette analysis supported the robust assignment of subgroup status using our signatures (Supplementary Fig. S1) but, consistent with the other analytical methods applied, did not support the subgroup assignment made for 3 of the 4 discrepant cases described earlier, further highlighting difficulties in their classification. Thus, in diagnostic applications, a "nonclassified" designation could be reserved for these rare cases which do not classify consistently across all analyses performed on the signature data, particularly where subgroup assignment may impact clinical or therapeutic decisions.

The combination of molecular and clinicopathologic data from 4 independent cohorts for meta-analysis, totaling 173 cases, has facilitated clear and significant insights to the clinical features of the medulloblastoma molecular subgroups, which have either not been apparent or not shown statistical significance in individual analyses of the smaller component cohorts reported to date (12, 13, 15). The SHH (24% of cases), WNT (12%), and WNT/SHH-independent (64%) groups show different age distributions and relationships to disease histopathology. SHH subgroup tumors peak in infancy and are intimately correlated with DN pathology in this group, to the extent that DN pathology may be considered as a surrogate marker for SHH activation in medulloblastomas arising in infants <3 years old at diagnosis, although classic and LCA cases also constitute a minority of SHH subgroup cases in this age group. This relationship breaks down in noninfants (>3 years at diagnosis), where SHH tumors are less common, and shows equivalent proportions of DN, classic, and LCA disease; SHH-independent DN cases are also commonly observed in this age group. These data strongly indicate that (i) SHH subgroup and (ii) DN tumors, arising in the infant and noninfant age groups, have different biological and clinical characteristics, and that SHH-positive DN tumors of infancy represent a unique disease subgroup associated with a favorable clinical behavior (35–37), and a characteristic molecular pathogenesis [COL1A2 unmethylated (ref. 25)] and mutational spectrum [SUFU (refs. 17, 34)]. Conversely, WNT tumors display classic pathology and occur in noninfants. Notably, both the SHH and WNT subgroups show at least 2 different incidence peaks in their age distribution (both have second peaks in adults), suggesting additional clinical and molecular heterogeneity within these groups. The inclusion of cohort-wide central pathology review in future studies may aid the further refinement of the associations observed.

The lack of association between M stage and molecular subgroups (P = 0.20) is in disagreement with the previous study by Kool and colleagues (12), who reported metastatic tumors being less common in WNT and SHH pathway-activated medulloblastomas. This could be due to the
different measurement criteria for metastasis between the studies (this study compared M0 vs. M2/3, whereas Kool and colleagues compared M0 vs. M1/M2/M3). Alternatively, the increased numbers in this study (130 vs. 58 with M stage data in the Kool and colleagues study) may have enabled a more accurate characterization of the relationship between signaling pathway activation and metastasis, and future large clinically controlled studies which include central review of metastatic status should be informative in this regard.

The identification of medulloblastoma molecular subgroups has significant prognostic and predictive potential to improve therapeutic delivery and disease outlook in the clinical setting and could represent a first step in the molecular diagnostic triage of medulloblastomas to guide treatment decisions. In addition to distinct clinical features, molecular subgroups also appear to have characteristic clinical behaviors; the favorable prognosis of WNT subtype medulloblastomas is now established in multiple clinical cohorts (8, 9, 15, 38) and will form the basis of treatment reductions in forthcoming international molecularly driven clinical trials (1). Combined data from this and other studies indicate SHH-positive DN tumors arising in infants represent a similarly favorable prognosis subgroup with a distinct molecular basis (25, 35–37). The nonavailability of outcome data for the 4 cohorts assessed in our meta-analysis have limited any direct assessment of survival associations in these cohorts. Moreover, their retrospective, heterogeneously treated nature would likely confound such analyses. Robust assessment of the prognostic impact of the medulloblastoma molecular subgroups will therefore now be essential, ideally within the context of adequately powered, centrally reviewed, and uniformly treated clinical trials cohorts, to determine their utility to direct the selection of adjuvant therapy. The molecular signatures we report will have significant utility in this regard. Molecularly targeted SHH inhibitors are also currently under preclinical and clinical development, and have shown early evidence of activity in medulloblastoma (10, 11). The ability to accurately diagnose the SHH molecular subgroup will thus be important for the targeted delivery of these novel agents, and our findings have identified SHH-positive subgroups of medulloblastomas which would be predicted to benefit most from SHH inhibition strategies. However, the SHH pathway plays a key role in normal, including cerebellar, development, and its transient inhibition in young mice causes permanent defects in growth plate formation and bone structure (39). In view of such potential toxicities, we recommend caution in the application of our data, particularly in the infant age group where SHH subgroup tumors predominate.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Medulloblastomas investigated in this study include samples provided by the UK Children’s Cancer and Leukaemia Group (CCLG) as part of CCLG-approved biological study BS-2007-04. This study was conducted with ethics committee approval from Newcastle/North Tyneside REC (study reference 07/Q0905/71).

Grant Support

This work was supported by grants from The Katie Trust (to S.C. Clifford), The Samantha Dickson Brain Tumour Trust (to S.C. Clifford), and Cancer Research UK (to S.C. Clifford and D.W. Ellison). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 17, 2010; revised January 6, 2011; accepted January 10, 2011; published OnlineFirst February 16, 2011.

References

14. Clifford SC, Lusher ME, Lindsay JC, Langdon JA, Gilbertson RJ, Straughton D., et al. Wnt/Wingless pathway activation and chromo-

some 6 loss characterize a distinct molecular sub-group of medullo-

16. Diele SJ, Guenthero J, Geng LN, Mahoney SE, Marotta M, Olson JM, et al. DNA methylation of developmental genes in pediatric medul-

loblastomas identified by denaturation analysis of methylation differ-


24. Herman JG, Graff RJ, Myohanen S, Nekin BD, Baylin SB. Methyla-

25. Anderton JA, Lindsay JC, Lusher ME, Gilbertson RJ, Bailey S, Ellison DW, et al. Global analysis of the medulloblastoma epigenome identi-

fies disease-subgroup-specific inactivation of COL1A2. Neuro-


loblastoma development by hypermethylation profiling. Carcinogen-

27. Langdon JA, Lamont JM, Scott DK, Dyer S, Prebble E, Bown N, et al. Combined genome-wide allelotyping and copy number analysis iden-

ify frequent genetic losses without copy number reduction in medul-

28. Rai AJ, Kamath RM, Gerald W, Fleisher M. Analytical validation of the GeXP analyzer and design of a workflow for cancer-biomarker dis-

32. Kogerman P, Krause D, Rahnama F, Kogerman L, Unden AB, Zaphir-


Rapid Diagnosis of Medulloblastoma Molecular Subgroups

Ed C. Schwalbe, Janet C. Lindsey, Debbie Straughton, et al.

Clin Cancer Res 2011;17:1883-1894. Published OnlineFirst February 16, 2011.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-2210

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2011/03/31/1078-0432.CCR-10-2210.DC1

Cited articles
This article cites 36 articles, 17 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/17/7/1883.full.html#ref-list-1

Citing articles
This article has been cited by 12 HighWire-hosted articles. Access the articles at:
/content/17/7/1883.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.