Tumor-Associated Macrophages in SHH Subgroup of Medulloblastomas

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Abstract

Purpose: Medulloblastoma in children can be categorized into at least four molecular subgroups, offering the potential for targeted therapeutic approaches to reduce treatment-related morbidities. Little is known about the role of tumor microenvironment in medulloblastoma or its contribution to these molecular subgroups. Tumor microenvironment has been shown to be an important source for therapeutic targets in both adult and pediatric neoplasms. In this study, we investigated the hypothesis that expression of genes related to tumor-associated macrophages (TAM) correlates with the medulloblastoma molecular subgroups and contributes to a diagnostic signature.

Methods: Gene-expression profiling using human exon array (n = 168) was analyzed to identify medulloblastoma molecular subgroups and expression of inflammation-related genes. Expression of 45 tumor-related and inflammation-related genes was analyzed in 83 medulloblastoma samples to build a gene signature predictive of molecular subgroups. TAMs in medulloblastomas (n = 54) comprising the four molecular subgroups were assessed by immunohistochemistry (IHC).

Results: A 31-gene medulloblastoma subgroup classification score inclusive of TAM-related genes (CD163 and CSF1R) was developed with a misclassification rate of 2%. Tumors in the Sonic Hedgehog (SHH) subgroup had increased expression of inflammation-related genes and significantly higher infiltration of TAMs than tumors in the Group 3 or Group 4 subgroups (P < 0.0001 and P < 0.0001, respectively). IHC data revealed a strong association between location of TAMs and proliferating tumor cells.

Conclusions: These data show that SHH tumors have a unique tumor microenvironment among medulloblastoma subgroups. The interactions of TAMs and SHH medulloblastoma cells may contribute to tumor growth revealing TAMs as a potential therapeutic target. Clin Cancer Res; 21(6); 1–9. ©2014 AACR.

Introduction

The role of inflammation in promoting tumor growth and regulation of the antitumor immune response is an important characteristic of cancer (1–3). Tumor-associated macrophages (TAM) are major contributors to the tumor microenvironment and are present in a variety of human cancers (4, 5). TAMs are now known to promote cancer via multiple mechanisms, including effects on tumor cell growth, survival, invasion, metastasis, angiogenesis, inflammation, and immunoregulation (6, 7). The presence of TAMs has been described in many adult malignancies, including central nervous system tumors (8–12). The first evidence of TAMs in childhood tumors and its correlation with tumor stage was recently shown in neuroblastoma, a peripheral nervous system tumor (13). However, little is known about the role of TAMs and the tumor microenvironment in childhood brain tumors.

Medulloblastoma is the most common malignant brain tumor in children. Craniospinal irradiation still remains an essential component of multimodality therapy for many young children putting these patients at risk for developmental neurotoxicity (14, 15). Medulloblastomas are a molecularly heterogeneous group of tumors that can be classified into at least four distinct subgroups: WNT, SHH (Sonic Hedgehog), Group 3, and Group 4 (16–19). Recent data have provided insight into the biology (20) and prognosis of medulloblastomas (21, 22); however, relatively little is known about the role of the tumor microenvironment with respect to these molecular subgroups. Large-scale genomic and gene-expression analyses using a number of different platforms have been shown to accurately identify medulloblastoma subgroups, but implementation in real-time for clinical application remains a challenge (16–19, 23–26).

In this study, we developed a novel and clinically applicable 31-gene TaqMan Low Density Array (TLDA) signature that...
Translational Relevance

Medulloblastoma is the most common malignant childhood brain tumor. Approximately 30% of patients remain incurable and current radiotherapy containing treatment protocols cause significant adverse long-term neuropsychiatric effects and endocrine dysfunction. The role of tumor microenvironment as an enabling characteristic of cancer and development of novel immunotherapeutics invokes the possibility of tumor-associated inflammatory cells as therapeutic targets. Here, we report distinct tumor microenvironments of medulloblastoma molecular subgroups and the presence of tumor-associated macrophages (TAM) in the Sonic Hedgehog subgroup of medulloblastomas. We developed a 31–gene-expression signature inclusive of inflammation-related genes that is clinically applicable and highly accurate in classifying medulloblastoma subgroups. We confirm presence of TAMs using immunohistochemistry and demonstrate their proximity to proliferating cells. Our work sheds light on the importance of the tumor microenvironment in childhood brain tumors and inhibition of TAMs, possibly through the CSF1R inhibitor, as a potential new therapeutic target in medulloblastomas.

allows accurate identification of medulloblastoma subgroups and examined the expression of inflammation-related genes with respect to each subgroup. We identified evidence of intratumoral inflammation and presence of TAMs in the SHH subgroup of medulloblastomas correlating with regions of proliferation. Our findings provide the first report of TAMs in pediatric brain tumors and introduce the possibility of using targeted therapies against TAMs, thereby decreasing the reliance on current radiation-based therapies, which are associated with potentially devastating long-term neurocognitive sequelae in young children.

Materials and Methods

Samples were collected from patients with medulloblastoma (primary samples n = 85 and relapse samples n = 2) treated at Children’s Hospital Los Angeles (CHLA; Los Angeles, CA) or Cincinnati Children’s Hospital Medical Center (Cincinnati, OH) between 1989 and 2012 with available adequate fresh-frozen tissue for evaluation. All samples underwent pathologic review by two neuropathologists to confirm the diagnosis. The patient and tumor characteristics are provided in Supplementary Table S1. Sixty-five samples underwent Affymetrix Human Exon 1.0 ST Array (HuEx) analysis. The data from these 65 HuEx data were analyzed in combination with data from a previously published cohort of 103 samples (Supplementary Fig. S1 and Supplementary Table S3; ref. 18). Additional 36 samples and a subset of HuEx samples with sufficient RNA (n = 47 of 65) were analyzed using a custom medulloblastoma-specific TLDA assay (total n = 83, Supplementary Table S2). The details of analyses performed on the HuEx microarray and the custom TLDA assay data are provided in the Supplementary Materials and Methods. In brief, the molecular subgroups were identified in an unsupervised manner using the HuEx data and performing 1,000 runs of nonnegative matrix factorization (NMF) clustering on several subsets of genes with high coefficient of variation (27). Silhouette analysis (28) was used to identify samples with high silhouette width for a given subgroup’s cluster, indicating higher similarity to their own subgroup than to any other molecular subgroup (Supplementary Table S3). These samples with large silhouette width along with two samples designated as the WNT group based on mutational analysis of the CTNNB1 (exon 3) gene (29, 30) were used as core samples or true positives in constructing and validating the TLDA signature. The molecular subgroup of the remaining samples was predicted using the TLDA signature. Supplementary Fig. S1 provides a schematic outline of the experimental design and samples used for generating the TLDA signature.

Macrophages were identified using immunohistochemical (IHC) analysis of 54 of the 83 medulloblastoma samples for which molecular subgroups had been determined using an antibody directed against CD163 as previously described (13). Paraffin tissue section scores ranged from 0 to 3, with higher scores, indicating a greater proportion of positive cells. Two neuropathologists independently scored all samples and the mean of the scores was used for further analyses. Twenty-three samples were also stained using an antibody against Ki-67 to assess association of macrophages and cell proliferation.

Statistical analysis

Common statistical analyses, including ANOVA with linear contrast, $\chi^2$, and Spearman rank correlation coefficient, were used where appropriate and are indicated in the text and Supplementary Data. Statistical computations were performed using the R project (http://www.r-project.org) or Stata 11 (StataCorp. 2009. Stata Statistical Software: Release 11: StataCorp LP).

Results

Identification of the molecular subgroup using HuEx assay

Molecular subgroups of medulloblastomas were identified using HuEx microarray data from 168 samples (65 study patients and 103 from a previously published cohort) using an algorithm based on NMF (Fig. 1A and Supplementary Fig. S2). There was an extremely high concordance (92%) between the molecular subgroup designation of the 103 patients previously published and results obtained with our combined analysis (Supplementary Table S3), with majority of discordant findings occurring between Groups 3 and 4. The patient characteristics and distribution of tumors among molecular subgroups for the 65 CHLA patients were similar to previous published reports (Supplementary Table S3; refs. 17–19).

Inflammation-related genes in medulloblastoma molecular subgroups

We sought to identify inflammation and immunology-related genes that were differentially expressed among the molecular subgroups using HuEx gene-expression data (n = 168). We identified greater expression of inflammation-related genes (CD14, PTX3, CD4, CD163, CSF1R, and TGFb2) in tumors of the SHH molecular subgroup compared with those of the Group 3 and Group 4 subgroups (Fig. 1C). Several of these genes have been shown to play an important role in the microenvironment of the developing cerebellum or other tumors types. CD14, a monocytic marker present on both circulating and resident monocytes, has been show to correlate with tumor grade in gliomas.
Murine experiments demonstrate that increased levels of TGFB2 are associated with the presence of proliferating, undifferentiated cerebellar neurons (31). CD163 is a well-described marker of TAMs, and CSF1R is an important receptor that along with its ligand CSF1 controls the production, differentiation, and function of TAMs (4, 6, 32, 33). PTX3 is produced by macrophages that have been polarized to the M2-like phenotype via their interaction with CD4+ T regulatory cells (32).

Expression levels of TAM markers, CD163 and CSF1R, varied significantly among molecular subgroups (CD163 P < 0.0001; CSF1R P < 0.0001) and were significantly greater in tumors of the SHH and WNT subgroups compared with those in Groups 3 and 4 (CD163, ANOVA with linear contrast P < 0.0001 for SHH or WNT compared with Groups 3 and 4; CSF1R, ANOVA with linear contrast P < 0.0001 for SHH or WNT compared with Groups 3 and 4). There was no statistically significant difference in CD163 or CSF1R expression between SHH tumors and WNT tumors (CD163 P = 0.97; CSF1R P = 0.50; Fig. 1B and Supplementary Fig. S3). Additional unbiased analysis was performed to assess association between expression levels of macrophage-related genes to medulloblastoma subgroups. Unsupervised clustering of 40 genes identified through Gene Ontology search to be related to macrophage biology demonstrated distinct clustering of SHH and WNT subgroups from Group 3 and 4 samples (Supplementary Fig. S9). These data suggest that expression of inflammation-related genes, especially those related to TAMs, can distinguish the tumor microenvironment of the SHH and WNT subgroups of medulloblastomas from Groups 3 and 4.

Expression of inflammation- and tumor cell–related genes comprises a molecular subgroup signature

To identify the subgroups in a larger cohort of medulloblastoma patients and to validate expression of inflammation-related genes, we developed a robust and clinically applicable assay using the TLDA technology, a system currently being evaluated in neuroblastoma and used in breast cancer clinical trials (34, 35). We built a medulloblastoma-specific TLDA card containing 39 tumor-related and six inflammation-related genes (CD163, CSF1R, MMD, CD4, ALCAM, and CXCR4) that were observed as significantly deregulated among medulloblastoma subgroups in our HuEx microarray analysis (Supplementary Table S2). The TLDA gene-expression profiles of medulloblastomas were then used to build and validate a 31-gene signature that could accurately predict the four molecular subgroups in 83 samples, including two matched relapse cases (Table 2 and Fig. 2A). The estimated leave-one-out cross-validated error rate of the 31-gene signature was 2% with classification errors occurring only in...
samples identified as Group 4 (Supplementary Tables S4–S6). The patient characteristics and distribution of tumors among molecular subgroups for the 81 CHLA patients were again similar to previous published reports with 4% WNT, 31% SHH, 26% Group 3, and 39% Group 4 (Table 1). The molecular subgroups of the two relapse cases were the same as their diagnostic counterparts. All patients identified as the WNT subgroup in our study cohort enjoyed long-term progression-free survival (PFS), similar to previously published reports (Supplementary Fig. S4).

Among the 31 genes included in our signature, increased expression of \( \text{WIF1}, \text{DKK2}, \text{PGY1}, \text{and TNC} \) was associated with WNT tumors, \( \text{HHIP}, \text{PDLIM3}, \text{SERP1}, \text{and GLI1} \) associated with SHH tumors, \( \text{MYC}, \text{IMP2}, \text{NPR3} \) associated with Group 3, and \( \text{KCNA}, \text{MPP3}, \text{and EOMES} \) associated with Group 4 tumors. These data are consistent with previous microarray-based publications, indicating differential expression of these genes among molecular groups of medulloblastomas (17–19, 23, 36).

We also identified several novel genes, which were differentially expressed among medulloblastoma molecular subgroups and contributed to their accurate identification (Table 2). Notably, the TAM-associated genes \( \text{CD163} \) and \( \text{CSF1R} \) were differentially expressed among molecular subgroups with increased expression in tumors of the SHH subgroup compared with those in Groups 3 and 4 (\( CD163 \) \( P < 0.0001 \); \( CSF1R \) \( P < 0.0001 \) for all pairwise comparisons) and contributed to the 31-gene signature predictive of molecular subgroups (Fig. 2B and C). A gene–gene correlation was observed between \( CD163 \) and \( CSF1R \) (Spearman \( r = 0.67 \), Fig. 2D), suggestive of coexpression of these two genes most likely by TAMs. There was no difference in \( CD163 \) expression in SHH tumors with desmoplastic histology compared with those with classic histology (Supplementary Fig. S5). The median gene expression of \( CD163 \) among the 22 patients with SHH medulloblastoma was used to define low and high \( CD163 \) expressers. There was no difference in the 10-year PFS for patients in these two groups (\( P = 0.57 \)); however, the 10-year overall survival (OS) for patients in these two groups trended toward but did not reach statistical significance (Supplementary Fig. S6; \( CD163 \) high vs. low expresser, 58% vs. 100%, respectively, \( P = 0.08 \)).

With regard to WNT tumors, there was a statistically significant difference in the expression of \( CD163 \) when compared with Group 4 (\( P = 0.04 \)), but not compared with Group 3 tumors (\( P = 0.18 \)) and no difference in expression of \( CSF1R \) (\( P = 0.83 \) for Group 3 and \( P = 0.48 \) for Group 4). Only three WNT tumors were available for analysis with TLDA, so we cannot conclude whether there is increased expression of \( CD163 \) or \( CSF1R \) in WNT tumors compared with Group 3 or Group 4 tumors.

Pattern of infiltration of macrophages in SHH medulloblastoma

We next performed IHC analysis of 54 paraffin-embedded medulloblastoma tumors using antibodies directed against \( CD163 \), to assess the extent and pattern of TAM infiltration in medulloblastomas. There was a significant difference in macrophage infiltration among molecular subgroups (\( P < 0.0001 \)) with significantly greater numbers of macrophages observed in tumors of the SHH subgroup compared with those in Groups 3 and 4 (\( P < 0.0001 \) for both comparisons; Fig. 3). There was a statistically significant difference in the number of intratumoral \( CD163^- \) macrophages between WNT tumors and Group 3 and
Group 4 tumors ($P = 0.04$ for both groups) and no statistically significant difference was found between the WNT and SHH tumors ($P = 0.80$); however, there were only two WNT samples available for evaluation. Among tumors with an IHC score $\geq 2.5$ ($n = 16$), 94% were SHH tumors and the remaining 6% were WNT tumors. The SHH tumors with desmoplastic

### Table 1. Patient characteristics by the molecular subgroup

<table>
<thead>
<tr>
<th>Molecular Subgroup</th>
<th>Number of Patients (%)</th>
<th>Number of Patients (%)</th>
<th>Number of Patients (%)</th>
<th>Number of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WNT</strong></td>
<td>Total</td>
<td>Age group, y</td>
<td>Sex</td>
<td>M Stage</td>
</tr>
<tr>
<td></td>
<td>3 (4)</td>
<td>&lt;3</td>
<td>Male</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>25 (31)</td>
<td>3–6</td>
<td>Female</td>
<td>M1</td>
</tr>
<tr>
<td></td>
<td>21 (26)</td>
<td>&gt;10</td>
<td></td>
<td>M2</td>
</tr>
<tr>
<td></td>
<td>32 (39)</td>
<td></td>
<td></td>
<td>M3</td>
</tr>
<tr>
<td><strong>SHH</strong></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>13 (52)</td>
<td>3 (100)</td>
</tr>
<tr>
<td></td>
<td>15 (60)</td>
<td>5 (20)</td>
<td>14 (67)</td>
<td>12 (48)</td>
</tr>
<tr>
<td></td>
<td>5 (24)</td>
<td>6 (29)</td>
<td>7 (33)</td>
<td>7 (33)</td>
</tr>
<tr>
<td></td>
<td>2 (6)</td>
<td>11 (34)</td>
<td>25 (78)</td>
<td>7 (22)</td>
</tr>
<tr>
<td><strong>Group 3</strong></td>
<td>2 (67)</td>
<td>4 (16)</td>
<td>6 (29)</td>
<td>4 (19)</td>
</tr>
<tr>
<td></td>
<td>1 (33)</td>
<td>1 (4)</td>
<td>14 (67)</td>
<td>9 (28)</td>
</tr>
<tr>
<td><strong>Group 4</strong></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

**Table 2. TLDA 31-gene signature**

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Gene location</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TERC</td>
<td>Telomerase RNA component</td>
<td>3q26</td>
<td>0.78</td>
</tr>
<tr>
<td>FOXG1</td>
<td>Forkhead box G1</td>
<td>14q13</td>
<td>0.95</td>
</tr>
<tr>
<td>PPP1R17</td>
<td>Protein phosphatase 1C, regulatory subunit 17</td>
<td>7p23</td>
<td>0.91</td>
</tr>
<tr>
<td>SLC6A5</td>
<td>Solute carrier family 6 (neurotransmitter transporter), member 5</td>
<td>11p15.1</td>
<td>0.82</td>
</tr>
<tr>
<td>BCA71</td>
<td>Branch chain amino-acid transaminase 1, cytosolic</td>
<td>1p11.21</td>
<td>0.72</td>
</tr>
<tr>
<td>CBLN3</td>
<td>Cerebellin 3 precursor</td>
<td>14q21</td>
<td>0.81</td>
</tr>
<tr>
<td>PDI1</td>
<td>Phosphotyrosine interaction domain containing 1</td>
<td>2q13.31</td>
<td>0.98</td>
</tr>
<tr>
<td>ERG1</td>
<td>Early growth response protein 1</td>
<td>5q23-q31</td>
<td>0.63</td>
</tr>
<tr>
<td>WIF1</td>
<td>WNT inhibitory factor 1</td>
<td>12q4.1</td>
<td>0.51</td>
</tr>
<tr>
<td>DKK2</td>
<td>Dickkopf WNT signaling pathway inhibitor 2</td>
<td>4q25</td>
<td>0.71</td>
</tr>
<tr>
<td>PYGL</td>
<td>Phosphorylase, glycogen, liver</td>
<td>1q42-q22</td>
<td>0.51</td>
</tr>
<tr>
<td>TNC</td>
<td>Tenascin C</td>
<td>9q33</td>
<td>0.82</td>
</tr>
<tr>
<td>PDLIM3</td>
<td>PDZ and LIM domain 3</td>
<td>4q35</td>
<td>0.89</td>
</tr>
<tr>
<td>HHIH</td>
<td>Hedgehog interacting protein</td>
<td>4q28-q32</td>
<td>0.93</td>
</tr>
<tr>
<td>SFRP1</td>
<td>Secreted frizzled-related protein 1</td>
<td>8p12.21</td>
<td>0.90</td>
</tr>
<tr>
<td>GLI1</td>
<td>GLI family zinc finger 1</td>
<td>12q3.2-q13.3</td>
<td>0.96</td>
</tr>
<tr>
<td>NPR3</td>
<td>Natriuretic peptide receptor C/guanylate cyclase C (arionatriuretic peptide receptor C)</td>
<td>5p14-p13</td>
<td>0.75</td>
</tr>
<tr>
<td>MYC</td>
<td>V-my cmyc avian myelocytomatosis viral oncogene homolog</td>
<td>8q24.21</td>
<td>0.52</td>
</tr>
<tr>
<td>IMPG2</td>
<td>Intersphincter phosphoglycan 2</td>
<td>3q12.2-q12.3</td>
<td>0.88</td>
</tr>
<tr>
<td>GABRA5</td>
<td>Gamma-aminobutyric acid (GABA) A receptor, alpha 5</td>
<td>1q52</td>
<td>0.88</td>
</tr>
<tr>
<td>EOMES</td>
<td>Eomesodermi</td>
<td>3p24.1</td>
<td>0.88</td>
</tr>
<tr>
<td>MPP3</td>
<td>Membrane protein, palmitoylated 3 (MAGUK p55 subfamily member 3)</td>
<td>17q21.31</td>
<td>0.95</td>
</tr>
<tr>
<td>FSTL5</td>
<td>Fallulatin-like 5</td>
<td>4q32.3</td>
<td>0.84</td>
</tr>
<tr>
<td>PGDFRA</td>
<td>Platelet-derived growth factor receptor, alpha polypeptide</td>
<td>4q12</td>
<td>0.96</td>
</tr>
<tr>
<td>OTX2</td>
<td>Orthodenticle homeobox 2</td>
<td>14q22.3</td>
<td>0.96</td>
</tr>
</tbody>
</table>

**NOTE:** AUC values for distinguishing WNT and SHH from the Group 3 and 4. Four-way ANOVA $P < 0.001$ for all genes in signature.
histology exhibited a distinct pattern of macrophage infiltration in the intermodal, poorly differentiated areas while sparing the more differentiated nodules (Fig. 3 and Supplementary Fig. S7). Interestingly, in the subset of SHH medulloblastomas with classic histology, CD163+ macrophages sometimes loosely recapitulated this lobular organization. We examined the extent and pattern of tumor cell proliferation as an increased proliferation index has been described in cells in the presence of macrophages (6). The areas of macrophage infiltration in the SHH tumors corresponded with areas of increased proliferation as evidenced by positive staining for Ki-67, a nuclear marker of cell proliferation, which was performed in a subset of 23 tumor samples (Fig. 4 and Supplementary Fig. S8).

Discussion

Treatment strategies aimed at improving survival of young children with medulloblastoma by avoiding radiotherapy and its neurocognitive sequelae require identification of novel subgroup-specific targets. Our study suggests for the first time that TAMs contribute to the microenvironment of a childhood brain tumor and demonstrates their prevalence in tumors of children with the SHH subgroup of medulloblastoma. We show that expression of inflammation-related genes, including TAM-related genes, CD163 and CSF1R, is higher in SHH as compared with the other medulloblastoma subgroups. The increased expression of CD163 and CSF1R suggests that the TAMs seen on IHC are of the M2 phenotype, and therefore associated with tumor progression (4, 6, 7, 10, 37). A 31-gene signature, inclusive of both inflammatory and tumor cell genes, enables proper identification of molecular subgroups with 98% accuracy. The 31-gene-expression scoring model has clinical applicability and could be of use for risk stratification, whereas identification of TAMs in SHH tumors uncovers a previously unrecognized potential target for therapy. CD163 expression was observed in the limited number WNT samples, suggesting that macrophages may also play a role in the WNT subgroup of medulloblastomas; however, given the lack of number of WNT samples (nine in HuEx, three in TLDA, and two IHC), we do not feel that we have enough sufficient evidence to draw a strong conclusion.

Although targeted therapy with SHH pathway inhibitors shows tremendous promise, it has become clear that novel treatments that overcome mechanisms of resistance to these inhibitors need to be identified to improve OS (38). In recent years, the concept of inflammatory cells in the tumor microenvironment as critical participants in tumor progression has gained acceptance. Large numbers of infiltrating TAMs are predictive of a poor prognosis in many adult cancers (39–41), and a 14-gene signature inclusive of five genes representing TAMs has been shown to predict PFS in patients with metastatic neuroblastoma (13). Our study also suggests a prognostic role for expression of CD163 among patients with SHH medulloblastoma, but validation with larger number of samples is needed. The tumor microenvironment also plays an important role in drug resistance mechanisms of tumors. Coculture of leukemia cells with stromal cells allows for environment-mediated drug resistance (EMDR) to tyrosine kinase inhibitors (42). This EMDR is associated with differential regulation of inflammation-related genes. TAMs produce cytokines that activate STAT3 and Hedgehog signals in colon and lung cancer stem cells rendering them resistant to chemotherapy (43). This suggests that combination therapy aimed at targeting the microenvironment in addition to the tumors cells may improve the response to chemotherapy and decrease the risk of development of EMDR.

Figure 3.
Evidence of TAMs across medulloblastoma subgroups. Representative CD163 IHC images in tumor samples from the SHH subgroup with desmoplastic histology (A), SHH with classic histology (B), the Group 3 subgroup (C), and the Group 4 subgroup (D). E, average CD163 IHC score is significantly higher in SHH tumors compared with Group 3 or Group 4 subgroups (P < 0.0001, respectively).
In this study, we show that the expression of inflammation-related genes, especially macrophage markers CD163 and CSF1R, is highest in the SHH subgroup of medulloblastomas, which was validated by the IHC analyses. The protumor effects of TAMs on tumor pathogenesis have been shown in de novo epithelial carcinogenesis in mice through production of cytokines such as IL6, IL10, and IL4 that stimulate tumor growth and angiogenesis. Coculture of neuroblastoma cells with peripheral blood monocytes or mesenchymal cells also increases tumor cell proliferation through IL6- and STAT3-dependent mechanisms (44, 45). In a prostate cancer model, macrophages induce CCL4 production that promotes tumorigenesis through STAT3 activation (46), whereas glioblastoma conditioned media protect TAM survival demonstrating the cross-talk between tumor cells and TAMs (47). The TAMs in SHH medulloblastoma are located near the proliferating tumor cells, as identified by Ki-67 marker, and points to their likely role in creating a progrowth tumor microenvironment.

Macrophages of the M2 phenotype have been shown to promote tumor progression via a variety of mechanisms, including immunosuppression and angiogenesis (4, 6, 7, 37, 40). CSF1R resides on the surface of tumor-promoting macrophages, and it has been demonstrated that inhibition of CSF1R can reduce the protumor effects of TAMs in the tumor microenvironment (37, 48). In a transgenic murine model of mammary adenocarcinoma, blockade of CSF1R signaling leads to a decrease in intratumoral TAMs resulting in increased sensitivity to chemotherapy. Administration of a CSF1R antagonist in combination with paclitaxel leads to a decrease in primary tumor progression as well as decreased rates of pulmonary metastasis and OS when compared with mice treated with paclitaxel alone (41). Inhibition of CSF1R in several preclinical models of proneural glioblastoma multiforme repolarizes TAMs from the M2 phenotype toward the M1 phenotype resulting in cessation of their tumorigenic functions (46). Our finding of TAMs with high expression of CSF1R in SHH medulloblastomas provides a novel therapeutic target. In the future, therapies aimed at blocking pathways mediating macrophage recruitment, polarization, and cross-talk with tumor cells could be combined with current or reduced intensity chemoradiation strategies.

Our study also defines a clinically applicable 31-gene-expression signature that identifies the four molecular subgroups of medulloblastomas with a 2% misclassification rate. Our group and others have demonstrated the clinical utility of these TLDA assays in childhood and adult clinical trials (34, 35). Although current techniques such as IHC, FISH, and cytogenetics could be used to identify only a portion of medulloblastomas subgroups (21, 23), our proposed 31-gene signature provides a rapid and highly accurate assay for determining these subgroups. Implementation of this assay could also be used in protocols aimed at avoiding or delaying radiotherapy in children with WNT or SHH tumors, or identification of children with Group 3 of Group 4 tumors for novel therapies. As the molecular subgroup becomes part of risk stratification, it will be important to have tools adept at making that determination.

In summary, our study reports the first evidence of the presence of TAMs in pediatric medulloblastoma and provides a 31-gene signature, inclusive of macrophage-associated genes, that accurately determines medulloblastoma subgroups. The increase in expression of macrophage markers can be used as a biomarker to identify subgroups of patients who may benefit from adjunctive treatments targeting TAMs and the tumor microenvironment. The success of therapies directed at reversing the suppressive role of immune cells in adult cancers (49) and the recent development of anti-CSF1R and other antibodies (48, 50) suggest opportunities for their application in pediatric SHH medulloblastoma.
Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: A.S. Margol, N.J. Robison, J.L. Finlay, M.D. Krieger, S. Asgharzadeh
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