EZH2-Regulated DAB2IP Is a Medulloblastoma Tumor Suppressor and a Positive Marker for Survival

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Abstract

Purpose: Medulloblastoma is the most common malignant brain tumor in children. Despite recent improvements, the molecular mechanisms driving medulloblastoma are not fully understood and further elucidation could provide cues to improve outcome prediction and therapeutic approaches.

Experimental Design: Here, we conducted a meta-analysis of mouse and human medulloblastoma gene expression data sets, to identify potential medulloblastoma tumor suppressor genes.

Results: We identified DAB2IP, a member of the RAS-GTPase–activating protein family (RAS GAP), and showed that DAB2IP expression is repressed in medulloblastoma by EZH2-induced trimethylation. Moreover, we observed that reduced DAB2IP expression correlates significantly with a poor overall survival of patients with medulloblastoma, independent of metastatic stage. Finally, we showed that ectopic DAB2IP expression enhances stress-induced apoptosis in medulloblastoma cells and that reduced expression of DAB2IP in medulloblastoma cells conveys resistance to irradiation-induced cell death.

Conclusion: These results suggest that repression of DAB2IP may at least partly protect medulloblastoma cells from apoptotic cell death. Moreover, DAB2IP may represent a molecular marker to distinguish patients with medulloblastoma at high risk from those with a longer survival prognosis. Clin Cancer Res; 18(15); 4048–58. ©2012 AACR.

Introduction

Brain tumors are the most common form of solid tumors in children of which medulloblastoma is the most frequent malignant variant, accounting for 20% of cases (1). Treatment modalities consist of surgery, radiotherapy, and chemotherapy and result in a 5-year survival rate of 40% in high-risk patients and 80% to 90% in low-risk patients (2). Approximately 30% of patients however remain incurable and current intensive treatment protocols cause significant adverse long-term effects (3). Medulloblastomas comprise 4 subtypes: WNT, SHH, group 3, and group 4, which differ regarding histology and clinical outcome (4) and are believed to derive from the deregulation of various signaling pathways in brain development, such as the WNT-pathway and sonic hedgehog (SHH) signaling pathways. Overactivation of these pathways leads to a loss of cell-cycle control and a dysfunctional apoptosis program, allowing for continued growth and tumorigenesis, predominantly in the cerebellum (5).

DAB2IP—disabled homolog 2–interacting protein, located at chromosome 9q33.1-q33.3—is a member of the RAS-GTPase activating protein family (RAS GAP) that inactivates RAS by promoting conversion of GTP into GDP (6). DAB2IP acts as a putative tumor suppressor gene and is downregulated by epigenetic modification in multiple aggressive cancers. In prostate cancer, DAB2IP expression was shown to be repressed by promoter methylation and histone modification (7), whereas in breast cancer (8), lung cancer (9), and gastrointestinal tumors (10), aberrant promoter hypermethylation was shown to downregulate DAB2IP. Moreover, it was shown in prostate cancer that downregulation of DAB2IP expression results in resistance to ionizing radiation (11). It initiates epithelial-to-mesenchymal transition (12) and promotes tumor growth and metastasis (13). In addition, DAB2IP is involved in TNFα-induced apoptosis in prostate cancer cells by suppressing the ASK1-JNK and PI3-AKT pathway (14), and in endothelial cells via the ASK1-JNK pathway (15).
**DAB2IP Is a Marker for Medulloblastoma Survival**

**Translational Relevance**
Medulloblastoma is the most common malignant pediatric brain tumor. Current treatment modalities result in a 5-year survival rate of 40% in high-risk patients and 80% to 90% in standard risk patients. Approximately 30% of patients however remain incurable and current intensive treatment protocols cause significant adverse long-term effects such as impaired neurologic function and endocrine dysfunction. Currently, the staging for medulloblastoma between high-risk and standard-risk disease is based on clinical parameters and histologic subtypes. However, it is suggested that including molecular markers in the risk stratification could improve survival and decrease treatment-related toxicity. Here we show that high expression of DAB2IP in medulloblastoma is associated with a favorable prognosis, independent of metastatic stage. This suggests DAB2IP expression inhibits tumor growth and further research in its use as a potentially important prognostic factor and/or therapeutic target may contribute to improvements in the future treatment of patients with medulloblastoma.

Apoptosis is a programmed variant of cell death common to all human cells. Defects in the apoptosis program result in an imbalance in the rate of cell proliferation and the rate of cell death thereby contributing to tumor growth and treatment resistance. Essential steps in the apoptotic mechanism are inactivated in medulloblastoma cells, resulting in resistance to apoptosis. A recent *in vivo* study showed that cerebellar stem cells can give rise to medulloblastomas when having acquired an impaired apoptosis mechanism (16). Consequently, many of the apoptosis mediators are generally considered tumor suppressors (17).

Here, we describe a comprehensive meta-analysis of gene expression studies of mouse and human medulloblastoma (18–23) identifying multiple medulloblastoma tumor suppressor candidates, including DAB2IP. We found DAB2IP expression to be strongly downregulated in human medulloblastoma cells and in primary human medulloblastoma tissues. We show that DAB2IP downregulation is—at least partially—caused by EZH2-mediated repression through histone methylation, conveying apoptosis resistance in immortalized neural precursor and medulloblastoma cells. Furthermore, we show that DAB2IP expression correlates significantly with the overall survival of patients with medulloblastoma, independent of metastatic stage.

**Materials and Methods**

Detailed protocols are in the Supplementary Data.

**Biologic samples**
Original data on tumor samples from 2 retrospective studies (23, 24) were used for this study. Survival analysis was based on 108 cases for which expression and survival data were available. Patient and tumor characteristics are presented in Table 1. In brief, all samples were snap frozen in the institutional pathology departments immediately upon arrival. All samples were reviewed by experienced neuropathologists and examined for tumor content. Total RNA was extracted using TRIzol (Invitrogen). Gene expression profiles were obtained by Affymetrix HG-U133 Plus 2.0 arrays. Gene expression data were normalized using the GCRMA procedure. Informed consent and detailed methods are described elsewhere (23, 24).

**Immunohistochemistry** was conducted on a largely independent medulloblastoma tissue microarray (TMA) cohort with tumors from 87 patients obtained from the files of the Department of Neuropathology of the Academic Medical Center (University of Amsterdam). Subgroup information was obtained by immunohistochemistry using antibodies for the subgroup-specific protein markers β-catenin (WNT), DKK1 (WNT), SFRP1 (SHH), NPR3 (Group 3), and KCNA1 (Group 4). Information on gender, age at diagnosis, histology, metastatic stage at diagnosis, and survival are presented in Table 2. The mean follow-up time of survivors in the TMA cohort was 6.2 years (range 0.1–19.4 years). Informed consent was obtained for the use of brain tissue and for access to medical records for research purposes. MB1 and MB2 primary human medulloblastoma tissues were obtained from surgical specimens after informed consent and approval by the Medical Ethical Committee of the VU University Medical Center.

**Survival analysis**
Overall survival was calculated from the time of diagnosis to the patient’s last follow-up or death. Survival of patients was analyzed using Kaplan–Meier survival curves, and the log-rank test was used to examine the statistical significance. *P*-values < 0.05 were considered significant. Prognostic impact of covariates on survival was evaluated on the basis of hazard ratios from Cox’s proportional hazards regression model. Multivariate Cox’s proportional hazards regression models were used to estimate effects of additional the covariates age, metastatic stage, and histology.

**Cells**
Human D283-med, D556-med (Dr Darrell Bigner, Duke University, NC, USA), Daoy (ATCC, Manassas, VA) and C17.2 murine cerebellar progenitor cells immortalized by *v-my* (25) were cultured in DMEM containing 10% FBS and antibiotics.

**Acumen proliferation assay**
Cells were plated in 96-well plates (Greiner), fixed at 24, 48, 72, and 96 hours after plating using formaldehyde, stained with DAPI, and subsequently signal intensity was measured using an Acumen eX3 apparatus (TTP LabTech).

**Apoptosis assay**
Cells were plated in white opaque 96-well plates (Greiner) and treated with TNFα (Invitrogen). After 6 hours of
treatment, caspase activity was measured using the Caspase-Glo 3/7 assay (Promega) according to the manufacturer’s instructions. Fluorescence and luminescence read-out was conducted using a Tecan Infinite F200 Microplate Reader (Tecan Trading AG).

Clonogenic assay
Daoy cells were plated in 6-well plates at a density of 500 to 1,000 cells per well depending on the used dose of irradiation or TNFα concentration. Subsequently, cells were treated with increasing doses of irradiation or TNFα (10 ng/mL). After 10 to 14 days of culturing to allow colony formation, the colonies were fixed with 3.7% formaldehyde in PBS and stained with Giemsa solution. Groups consisting of 50 cells or more were defined as a colony. The colony counts using light microscopy were conducted independently by at least 2 investigators.

Results
Meta-analysis of candidate tumor suppressor genes in medulloblastoma
To identify candidate tumor suppressor genes in medulloblastoma, we determined transcripts that were downregulated in medulloblastoma as compared with normal cerebellar precursor cells in 7 data sets comparing gene expression of normal cerebellar precursor cells and medulloblastoma in mice. We used mouse studies because that allowed for a comparison between medulloblastoma cells and proliferating progenitor cells. All data sets used mouse models that mimic the SHH-medulloblastoma subgroup (Supplementary Table S1A, GSE9299, GSE2426, GSE7212, GSE11859, GSE6463; refs. 18–22). This analysis yielded 56 genes that were significantly downregulated (>2-fold) in at least 5 of 7 mouse medulloblastoma data sets. Subsequently, to increase the relevance of our screen for human medulloblastoma, we compared the expression of the significantly downregulated mouse genes to a human medulloblastoma gene expression dataset (GSE10327; ref. 23) and an independent gene expression data set of normal human cerebellum (GSE3526; ref. 26). We established that 37 genes of our set of 56 genes were significantly downregulated in both mouse and human medulloblastoma (Supplementary Table S2A). In addition, we compared genes that were significantly downregulated in both mouse and human medulloblastoma to a composed list of designated tumor suppressor genes. This resulted in 2 candidate gene sets, one set of 37 highly deregulated genes that have not before been designated as tumor suppressors (Fig. 1A and Supplementary Table S2A), and one set of 26 genes that have been reported as a tumor suppressor before—mostly in cancers other than medulloblastoma (Fig. 1B and Supplementary Table S2B).

DAB2IP is downregulated in medulloblastoma and is associated with poor clinical outcome
We focused on the downregulated medulloblastoma genes with designated tumor suppressor function (Fig. 1B) and analyzed by literature search to which of these genes a proapoptotic function could be attributed. This

Table 1. Patient/tumor characteristics of medulloblastoma series used for survival analysis

<table>
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<tr>
<th>DAB2IP low, M0</th>
<th>DAB2IP low, M+</th>
<th>DAB2IP high, M0</th>
<th>DAB2IP high, M+</th>
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<tr>
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</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Age at diagnosis</td>
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<td></td>
<td></td>
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<tr>
<td>Average age</td>
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<td>7.3</td>
<td>8.4</td>
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<td>7.6</td>
<td>6.6</td>
</tr>
<tr>
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<td>2.0–16.6</td>
<td>0.8–25.6</td>
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<td></td>
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<tr>
<td>Infants (&lt;4)</td>
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<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Children (4–16)</td>
<td>37</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
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<td>0</td>
<td>1</td>
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<tr>
<td>Histology</td>
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<tr>
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<td>21</td>
<td>16</td>
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<tr>
<td>Desmoplastic</td>
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<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Large cell/anaplastic</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Nodular/desmoplastic</td>
<td>3</td>
<td>0</td>
<td>0</td>
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<tr>
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<tr>
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<tr>
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<td>1</td>
</tr>
<tr>
<td>Group 3</td>
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<tr>
<td>Group 4</td>
<td>20</td>
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Table 2. Patient/tumor characteristics of medulloblastoma series on TMA

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<td>Female</td>
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<td>Age at diagnosis</td>
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<tr>
<td>Average age</td>
<td>14.2</td>
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<tr>
<td>Median age</td>
<td>7.0</td>
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<tr>
<td>Age range</td>
<td>1–53</td>
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<tr>
<td>Age groups</td>
<td></td>
</tr>
<tr>
<td>Infants (&lt;4)</td>
<td>7</td>
</tr>
<tr>
<td>Children (4–16)</td>
<td>19</td>
</tr>
<tr>
<td>Adults (&gt;16)</td>
<td>15</td>
</tr>
<tr>
<td>Unknown</td>
<td>46</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Classic</td>
<td>23</td>
</tr>
<tr>
<td>Large cell/anaplastic</td>
<td>3</td>
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<tr>
<td>Nodular/desmoplastic</td>
<td>8</td>
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<tr>
<td>Unknown</td>
<td>53</td>
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<tr>
<td>Molecular subgroups</td>
<td></td>
</tr>
<tr>
<td>WNT</td>
<td>7</td>
</tr>
<tr>
<td>SHH</td>
<td>18</td>
</tr>
<tr>
<td>Group 3</td>
<td>14</td>
</tr>
<tr>
<td>Group 4</td>
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<tr>
<td>Unknown</td>
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<tr>
<td>Metastatic stage</td>
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<tr>
<td>MO</td>
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<tr>
<td>M–</td>
<td>6</td>
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<tr>
<td>Unknown</td>
<td>46</td>
</tr>
<tr>
<td>Survival</td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>61</td>
</tr>
<tr>
<td>Diseased</td>
<td>26</td>
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</table>

DAB2IP analysis shows that low DAB2IP mRNA expression correlates significantly with a poor prognosis, as measured by a lower overall survival probability ($P = 0.010$). Multivariate Cox proportional-hazards analysis shows that DAB2IP expression can predict prognosis (HR, 3.0; 95% CI, 1.1–8.6, $P = 0.036$), independently of clinical variables such as age, metastatic stage, and histology. Medulloblastoma is known to comprise 4 subtypes: WNT, SHH, group 3, and group 4, which differ regarding histology, molecular biology, genetics, and clinical outcome (4). However, we did not find a significant correlation between DAB2IP expression and any specific subtype in our patient series (Supplementary Fig. S1). We also investigated the relation between DAB2IP expression and metastatic stage in our patient group. However, we did not find a significant correlation (Fig. 2D). Finally, to further analyze the effects of DAB2IP expression and metastatic stage on prognosis, patients were stratified on the basis of metastatic stage (Fig. 2D and Supplementary Fig. S2A and S2B). Again Kaplan–Meier analysis showed that low DAB2IP expression correlated with a poor prognosis ($P = 0.055$) both in the metastatic patient and nonmetastatic patient groups.

**DAB2IP and EZH2 are inversely expressed in medulloblastoma**

Previously it was described that DAB2IP expression is epigenetically suppressed by EZH2, a member of the polycomb complex and a histone-methylating enzyme (28). Therefore, we determined the EZH2 expression levels in medulloblastoma cells and tissues and compared these to DAB2IP expression. First, DAB2IP and EZH2 expression levels were evaluated in the mRNA expression data set of 62 human medulloblastoma (9 WNT, 15 SHH, 11 group 3, and 27 group 4; ref. 23) and 9 normal cerebellum samples (26). As expected, the DAB2IP mRNA levels were significantly downregulated in the medulloblastoma samples as compared with normal human cerebellum (Fig. 3A). In contrast, EZH2 mRNA levels were significantly upregulated in the medulloblastoma samples as compared with normal human cerebellum (Fig. 3A). There was no significant difference in the expression of DAB2IP between the 4 subgroups of medulloblastoma, whereas the expression of EZH2 showed an increasing trend from the WNT and SHH subgroups to groups 3 and 4 (Fig. 3A). In addition, we compared DAB2IP and EZH2 expression in individual samples and found a negative correlation between DAB2IP and EZH2 mRNA expression (Supplementary Fig. S3A). This negative correlation was also found on protein level (Supplementary Fig. S3B). In parallel to the DAB2IP expression analysis in medulloblastoma cell lines and primary tissues (Fig. 2A), EZH2 expression analysis was conducted using the same samples. This showed an increased EZH2 expression in medulloblastoma cells as compared with normal cerebellum (Fig. 3B), again correlating inversely with DAB2IP expression in these samples. Finally, immunohistochemical analysis on TMA, composed of 276 pediatric medulloblastoma tissues from 87 patients, showed that EZH2 expression was significantly overexpressed in 30
Epigenetic modulation of DAB2IP expression in medulloblastoma

To determine whether DAB2IP expression in medulloblastoma is regulated by EZH2-mediated epigenetic histone modulation, we transfected medulloblastoma cells with siRNAs directed against EZH2 (siEZH2). At 96 hours after transfection, we observed significantly reduced EZH2 levels in the siEZH2 transfected medulloblastoma cells. We also analyzed lysates from medulloblastoma cells treated with the S-adenosylhomocysteine hydrolase inhibitor DZNep, a potent inhibitor of EZH2 histone methyltransferase activity (29–31). Again, a reduction in EZH2 protein levels was observed with a delayed increase in DAB2IP levels (Fig. 4B). Besides histone methylation, DAB2IP expression can be altered by histone acetylation (7) and promoter DNA hypermethylation (7–10). It was previously shown that in various cancer types DAB2IP expression could be restored by treatment with the DNA hypomethylation agent 5-aza-2′-deoxycytidine (DAC). In the medulloblastoma cells used here, we could not detect an increase in DAB2IP expression after DAC treatment (Fig. 4C). However, additional treatment with the histone deacetylase inhibitor trichostatin A (TSA) did significantly increase DAB2IP expression (Fig. 4D). This suggests that histone modifications may play a more significant role in suppressing DAB2IP expression in medulloblastoma, as was described also in prostate cancer (7).

DAB2IP promotes stress-induced apoptosis in medulloblastoma

To assess the functional role of DAB2IP in medulloblastoma, we examined the effects of DAB2IP modulation in human medulloblastoma cells and mouse neuronal precursor cells. Because DAB2IP was reported to enhance TNFα-induced apoptotic cell death in endothelial cells (15) and prostate cancer cells (14), we analyzed the effect of DAB2IP overexpression on TNFα-induced apoptotic cell death in medulloblastoma cells. Lentiviral vectors encoding DAB2IP or LacZ were used to stably transduce Daoy medulloblastoma cells. Treatment with TNFα (100 ng/mL) resulted in a 2-fold increase in caspase activity in Daoy cells overexpressing DAB2IP, as compared with LacZ control cells. This increase was neutralized by simultaneous treatment with the caspase inhibitor z-VAD (20 μmol/L; Fig. 5A). In addition, we measured the cell proliferation rate of Daoy-DAB2IP overexpressing DAB2IP, as compared with LacZ control. Daoy-DAB2IP cells showed a lower proliferation rate as compared with their controls. This result was confirmed in D283-med cursor cells. Because DAB2IP was reported to enhance suppressing DAB2IP expression in medulloblastoma, as was described also in prostate cancer (7).
overexpression also inhibited anchorage independent growth after treatment with a low dose of TNFα (10 ng/mL) in Daoy cells (Supplementary Fig. S4). Because ionizing radiation (IR) is an important treatment modality in medulloblastoma and downregulation of DAB2IP gene expression was related to resistance to IR in prostate cancer cells (11), we studied the effect of DAB2IP modulation on the clonogenic growth of medulloblastoma cells after IR. Daoy-DAB2IP and Daoy-LacZ cells were irradiated with doses of 0 to 5 Gy. DAB2IP overexpression showed an IR dose-dependent reduction in clonogenic survival as compared with LacZ control cells (Fig. 5C). Finally, to further study the effect of DAB2IP on TNFα-induced apoptosis, we used a shDAB2IP construct to transiently knockdown DAB2IP expression in C17.2 murine cerebellar progenitor cells immortalized by v-myc (25). Moderate knockdown of DAB2IP was confirmed by Western blot analysis. DAB2IP knockdown in these immortalized neural precursor cells significantly reduced TNFα-induced caspase activation (Fig. 5D), suggesting that DAB2IP has a proapoptotic function in these stressed neural precursor cells. In the absence of TNFα-induced stress, caspase activation was similar as was observed for DAB2IP knockdown and C17.2 shCTRL cells (Fig. 5D).

Discussion

We conducted a meta-analysis of publicly available medulloblastoma gene expression data sets to identify potential medulloblastoma tumor suppressor genes. We identified DAB2IP to be strongly downregulated in medulloblastoma cells and in primary medulloblastoma tissues. Reduced DAB2IP expression was shown to correlate significantly with poor overall survival of patients with medulloblastoma, independent of clinical variables such as age, metastatic stage, and histology. Moreover, DAB2IP was shown to be regulated by histone modifications, including histone acetylation, and histone methylation by the
polycomb group member EZH2. Finally, we showed that ectopic DAB2IP expression enhances stress-induced apoptosis in medulloblastoma cells, and that reduced expression of DAB2IP in medulloblastoma conveys resistance to irradiation-induced cell death.

Medulloblastomas are known to comprise 4 subtypes: WNT, SHH, group 3, and group 4, which differ regarding histology, molecular biology, genetics, and clinical outcome (4). The studies used in our meta-analysis all used mouse models that mimic SHH-subgroup medulloblastoma (Supplementary Table S1A). While this paper was being revised, the first MYC-based medulloblastoma mouse models that mimic group 3 were generated (32, 33). The medulloblastomas generated by one of these MYC models (32) show a reduced DAB2IP expression in line with our results (Supplementary Fig. S5A). However, the medulloblastomas generated by the other model (33) do not show differential DAB2IP expression compared with the control tissue used in that study (Supplementary Fig. S5B). Recently, a mouse model that mimics WNT subgroup medulloblastoma was also described (34). However, the small number (3) of samples and the strong variation in their DAB2IP expression preclude making any meaningful comments on DAB2IP expression in this model (Supplementary Fig. S5C). Finally, our human medulloblastoma data set shows that DAB2IP expression is significantly reduced in all subgroups (Fig. 3A and Supplementary Fig. S1).

To increase the likelihood of identifying tumor suppressors in medulloblastoma, we compared the deregulated mRNA transcripts with a composed list of tumor suppressor genes. This list was established by interrogating publicly available gene ontology databases that included transcripts that have been tied to a tumor suppressor function. A number of well-known tumor suppressors in the context of medulloblastoma such as PTCH1, SUFU, APC, AXIN and TP53 (35–41) are not included in our candidate gene sets. This is likely to be caused by our stringent threshold and the various medulloblastoma mouse models used to gather the data sets. For instance, PTCH1 is down-regulated >2-fold in 2 of 7 medulloblastoma mouse model datasets, thereby not reaching our cutoff of deregulation in at least 3 of 7 data sets. However, in 2 additional data sets PTCH1 is downregulated ~1.8-fold. Another well-known tumor suppressor, TP53, is downregulated >2-fold in 3 of 7 different mouse models.
datasets. However, TP53 is not differentially expressed between the human medulloblastoma and normal cerebellum data set, supporting reports that TP53 downregulating mutations in sporadic human medulloblastoma are not very common (37). In addition, transcripts can have inactivating mutations without being downregulated at mRNA level, and are therefore not detected using mRNA expression analysis. Similar observations have also been reported for PTCH1, AXIN2, and TP53 in various subgroups of medulloblastoma (23).

It was previously shown that DAB2IP expression can be silenced by promoter methylation and histone modification (7–10). Other reported mechanisms for DAB2IP inactivation include one case of a translocation that disrupts DAB2IP expression in acute myeloid leukemia (42), and a single nucleotide polymorphism in the DAB2IP gene in aggressive prostate cancer (43). LOH at DAB2IP was showed in 20% of cases in hepatocellular carcinoma (44). In addition, a common sequence variant within DAB2IP associates with the risk of abdominal aortic aneurysm (45) and recently a genetic variant of DAB2IP was also shown to be an independent risk factor for early onset of lung cancer (46). However, as far as we know in medulloblastoma no such mechanisms of DAB2IP deregulation have been reported yet. Here we show a significant negative correlation between overexpressed EZH2 and downregulated DAB2IP in human medulloblastoma samples. Moreover, we show that DAB2IP suppression in medulloblastoma cells can be at least partly reversed by EZH2 inhibition. Treatment with the DNA hypomethylation agent 5-aza-2'-deoxycytidine (DAC) did not affect DAB2IP expression in medulloblastoma cells. However, additional treatment with the histone deacetylase inhibitor trichostatin A (TSA) did significantly increase DAB2IP expression. This may suggest that histone modifications play a significant role in suppressing DAB2IP expression in medulloblastoma cells, however, we do not rule out that DAB2IP expression in medulloblastoma cells may also be impaired by additional mechanisms. Other transcripts in our list of potential medulloblastoma tumor suppressor genes may also be EZH2 targets, this is however only confirmed for CDH1 (47).

We found DAB2IP mRNA expression was reduced in medulloblastoma cell lines and primary tissues. DAB2IP protein levels were also reduced in medulloblastoma cell lines. Immunohistochemistry on the medulloblastoma TMA did not reveal DAB2IP expression in these samples. However, this does not necessarily mean that no protein is present at all. It may well be that small traces of DAB2IP are present at all. It may well be that small traces of DAB2IP are present in some of the medulloblastoma tissue samples, although undetectable by us in these experiments. Another explanation may be that posttranscriptional events account for the discrepancy between mRNA and protein levels.

Recently, 2 publications have related the loss of DAB2IP expression to increased epithelial-to-mesenchymal transition (EMT) and metastasis in prostate cancer. Xie and colleagues show that DAB2IP knockdown increases nuclear β-catenin accumulation and trans-activation of target genes involved in EMT by inhibiting GSK3β, indicating an inhibitory function of DAB2IP in WNT/β-catenin signaling (12). In the context of medulloblastoma, this seems paradoxical as metastasis is uncommon in the subgroup of human medulloblastoma in which WNT signaling is active (4). In our patient series, average DAB2IP expression was slightly higher in the group of WNT-associated medulloblastomas (Fig. 3A), however this difference was nonsignificant. It was of interest to determine the role of
DAB2IP in WNT signaling in the context of medulloblastoma, because a major role in medulloblastoma oncogenesis has been attributed to WNT/β-catenin (48). Furthermore, it was reported that loss of DAB2IP expression induces the activation of Ras and NF-κB in prostate cancer, where Ras is presumed to play an essential role in primary tumor growth and NF-κB drives prostate cancer metastasis (13). Therefore, we investigated the relation between DAB2IP expression and metastatic stage. However, we were unable to show a significant correlation between DAB2IP expression and medulloblastoma metastases (Fig. 2E).

In a study of mammalian brain development, high levels of DAB2IP expression was found in the developing cerebellum, particularly in Purkinje cell precursors (49). Although there is no evidence that medulloblastoma arises directly from Purkinje cells, these cells play an important part in the development of the normal cerebellum. Purkinje cells generate SHH that projects on granule neuron precursor cells and stimulate their proliferation, before the granule neuron precursor cells migrate deeper into the forming cerebellum and differentiate further. A subgroup of medulloblastoma is believed to be derived from granule neuron precursor cells that fail to stop proliferating (3). We showed that DAB2IP knockdown in C17.2 neural precursor cells significantly reduced TNFα-induced caspase activation, suggesting that DAB2IP has a proapoptotic function in stressed neural precursor cells. However, because C17.2 cells are immortalized by overexpression of v-my c (25), the role of DAB2IP in altering apoptosis responses needs to be established in normal neural precursor cells.

We found a significant association between poor overall survival in patients with medulloblastoma and reduced DAB2IP expression (Fig. 5). DAB2IP promotes stress-induced apoptosis in medulloblastoma cells. A, DAB2IP overexpression increased caspase activation in Daoy cells treated with TNFα 6 hours after treatment. Overexpression of DAB2IP in Daoy cells was confirmed by Western blotting. B, Acumen proliferation assay of Daoy cells overexpressing DAB2IP after treatment with or without a low dose of TNFα. C, colony formation of DAB2IP overexpressing Daoy cells, after exposure to increasing doses of IR. D, DAB2IP knockdown decreased caspase activation in C17.2 neural precursor cells treated with TNFα 6 hours after treatment. Knockdown of DAB2IP in C17.2 cells was confirmed by Western blot analysis. Error bars indicate SD. *, P < 0.05; ***, P < 0.001; t test.
DAB2IP mRNA levels. Interestingly, no significant difference in DAB2IP expression was observed between the group of WNT associated medulloblastoma—which has the best prognosis—and group 3 medulloblastoma—which has the worse prognosis (4). This association was observed in metastatic as well as in nonmetastatic patients with medulloblastoma, albeit at near significant levels. These results show that DAB2IP expression is a prognostic marker for medulloblastoma outcome and suggest that patients with nonmetastatic medulloblastoma with low DAB2IP expression may benefit from more aggressive treatment strategies. Recent studies have shown that medulloblastoma is a heterogeneous disease with diverse treatment outcome (23, 50). Currently staging for treatment is based on clinical parameters such as age, extent of surgical resection, presence of metastases, and histologic classification (51, 52). Various studies have suggested that this risk stratification could be improved by including molecular determinants (23, 50, 53–57). However, it remains to be investigated to what extent DAB2IP could contribute to the subclassification of medulloblastoma and whether it can aid as a prognostic factor in clinical practice.

In conclusion, we identified DAB2IP as a potential antiapoptotic tumor suppressor in medulloblastoma. Further research in its use as a potentially important prognostic factor and/or therapeutic target may contribute to improvements in the future treatment of patients with medulloblastoma.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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EZH2-Regulated DAB2IP Is a Medulloblastoma Tumor Suppressor and a Positive Marker for Survival

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