Molecular Risk Stratification of Medulloblastoma Patients Based on Immunohistochemical Analysis of MYC, LDHB, and CCNB1 Expression

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Abstract

Purpose: Medulloblastoma is the most common malignant embryonal brain tumor in children. The current clinical risk stratification to select treatment modalities is not optimal because it does not identify the standard-risk patients with resistant disease or the unknown number of high-risk patients who might be overtreated with current protocols. The aim of this study is to improve the risk stratification of medulloblastoma patients by using the expression of multiple prognostic markers in combination with current clinical parameters.

Experimental Design: Candidate prognostic markers were selected from literature or from medulloblastoma expression data. Selected genes were immunohistochemically analyzed for their prognostic value using medulloblastoma tissue arrays containing 124 well-characterized patient samples.

Results: Protein expression analyses showed that the combined expression of three genes was able to predict survival in medulloblastoma patients. Low MYC expression identified medulloblastoma patients with a very good outcome. In contrast, concomitant expression of LDHB and CCNB1 characterized patients with a very poor outcome. Multivariate analyses showed that both expression of MYC and the LDHB/CCNB1 gene signature were strong prognostic markers independent of the clinical parameters metastasis and residual disease. Combined analysis of clinical and molecular markers enabled greater resolution of disease risk than clinical factors alone.

Conclusions: A molecular risk stratification model for medulloblastoma patients is proposed based on the signature of MYC, LDHB, and CCNB1 expression. Combined with clinical variables, the model may provide a more accurate basis for targeting therapy in children with this disease.

Among children, medulloblastomas are the most common malignant embryonal brain tumors, with 5-year survival rates of up to 70% reported (1–3). Current therapy, consisting of a combination of surgery, craniospinal radiation, and chemotherapy, is responsible for serious neurocognitive and endocrine side effects among survivors. Medulloblastoma patients are stratified into two clinical groups. High-risk patients are <3 years of age at diagnosis, have more than 1.5 cm^2 of residual disease after surgery, and/or with evidence of metastasis at diagnosis. Patients not fulfilling these criteria are stratified as standard-risk patients. This clinical stratification does not identify the standard-risk patients with resistant disease or the unknown number of high-risk patients who might be overtreated with current protocols (4–6). A better understanding of the biology and genetics of this heterogeneous disease will be essential to improve the current risk stratification. Histologically, medulloblastomas are divided into four categories according to the WHO classification of tumors of the nervous system based on their morphology: classic, nodular/desmoplastic, medulloblastomas with extensive nodularity, and large cell anaplastic medulloblastomas (7, 8). For a long time and despite several studies, it remained unclear whether there was a difference in prognosis between the two major variants classic and nodular/desmoplastic medulloblastoma (9). Most likely, this was due to controversial criteria of morphologic subclassification. However, two recent studies suggest that the nodular/desmoplastic variant has a better prognosis than classic medulloblastomas (10, 11). The other two subtypes are much less frequent. Medulloblastomas with extensive nodularity, mainly occurring in young children, are clearly associated with a more favorable prognosis (11, 12), whereas patients with large cell anaplastic medulloblastomas generally have a poor outcome (11, 13). However, several studies also showed that an increasing degree of anaplasia in any medulloblastoma subtype is associated with a worse clinical

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outcome (13–16). Therefore, the sometimes confusing morphologic subtyping of medulloblastomas seems not to be the best way for the most optimal risk stratification of medulloblastoma patients.

Gene expression analyses revealed several marker genes, such as *MYC*, *ERBB2*, and *NTRK3* (*TRKC*), for which the expression could predict the outcome of medulloblastoma patients (6, 17–22). *MYC* amplification, occurring in 5% to 10% of the cases, and *MYC* mRNA expression have both been associated with a poor prognosis (23–26). In addition, elevated expression of *ERBB2* has been associated with an unfavorable outcome (6, 17, 18), whereas expression of *NTRK3* has been shown in some studies to be a marker for a favorable outcome (20, 21, 27). Common for all these studies is that only single marker genes were investigated for correlations with prognosis.

The aim of this study was to design a better risk stratification model for medulloblastoma patients using a set of immunohistochemical prognostic markers in combination with clinical parameters. We therefore studied the protein expression of several previously reported prognostic markers. All markers were analyzed on tissue arrays of 124 clinically well-characterized medulloblastomas. We identified a gene signature based on the expression of three genes (*MYC*, LDHB, and CCNB1), which predicts outcome independently of current clinical risk parameters. With this gene signature, a molecular risk stratification is proposed, which, in combination with clinical variables, may guide the therapeutic strategy.

Materials and Methods

Tumor specimens and clinical data. We collected medulloblastoma samples from 124 patients diagnosed with medulloblastoma between 1985 and 2003. Specimens were fixed in 10% buffered formalin for 24 h, embedded in paraffin, and stored at room temperature. All diagnoses were confirmed by histologic assessment of the tumor specimens by the local neuropathologist and by central review (D.E.) according to the WHO 2000 classification of tumors of the central nervous system. The median age at diagnosis was 9 y (range, 1-53 y). Metastatic disease was assessed by magnetic resonance imaging and/or lumbar cerebrospinal fluid sampling in 121 (98%) patients. Part of the patients were treated according to randomized trials. All other patients had rule-based treatments. All patients under the age of 3 y received chemotherapy. Two of these 11 infants did not receive radiotherapy. The end of the follow-up period was December 2005 and the mean follow-up time was 54 mo (median, 36 mo). The 5- and 10-y overall survival rates for the entire group were 66% and 58%, respectively. Center-based survival was not statistically different. The clinical characteristics of the study population are summarized in Table 1.

Immunohistochemistry. Medulloblastoma tissue arrays were constructed after defining representative tumor regions on H&E-stained sections of each specimen. Sections were deparaffinized according to standard procedures and blocked in 0.03% H_2O_2 in methanol at room temperature for 20 min followed by incubation in Tris-EDTA for 10 min at 100°C for antigen retrieval. Antibodies against the following antigens were used: cMYC Ab-2, clone 9E10.3 (1:100, room temperature for 30 min; NeoMarkers); LDH H-subunit specific, clone no. HH-17 (1:200, room temperature for 60 min; Sigma-Aldrich); CCNB1, clone V152 (1:1,000, room temperature for 30 min; Biosource Europe); STMN1 #3352 (1:50, room temperature for 60 min; Cell Signaling Technology). Blocking serum and horseradish peroxidase detection systems were selected depending on the species the antibody was raised in. All slides were washed again and stained with 0.03% H_2O_2/0.05% 3,3′-diaminobenzidine tetrachloride (Sigma) in 0.05 mol/L Tris-HCl buffer (pH 7.5). The reaction was terminated after 8 min by rinsing the sections with distilled water. Counterstaining was done with hematoxylin. Sections were washed, dehydrated, and coverslipped in Pertex. Sections incubated without the primary antibody served as control specimens and were essentially blank. All antibodies were tested for their specificity by Western blot analysis of extracts from five established medulloblastoma cell lines (data not shown).

Evaluation of tissue staining. The staining intensity of tissue slides was evaluated independently by two observers who were blinded toward the patient's characteristics and survival. Cases with disagreement were discussed using a multiheaded microscope until agreement was achieved. To assess differences in staining intensity, an immunoreactivity scoring system was applied. MYC was scored positive when >50% of cells in the tumor specimen showed either nuclear and/or cytoplasmic expression of this protein. LDHB was scored positive when >50% of cells in the tumor specimen showed cytoplasmic expression. CCNB1 was scored positive when >50% of cells in the tumor specimen showed either perinuclear or diffuse cytoplasmic expression. The immunostaining pattern of STMN1 was scored positive when >50% of the tumor cells showed perinuclear and or diffuse cytoplasmic staining. All samples were stained more than once and the results were highly reproducible.

Statistical analysis. Overall survival time was calculated from the date of diagnosis until death or the last follow-up date. Survival distribution was estimated with the Kaplan-Meier method and compared by the log-rank test (SPSS 12.0.1). A multivariate Cox

Table 1. Clinical characteristics of study population (n = 124)

Characteristic	No. patients (%)
Age (y)	
<3	11 (9)
3-18	92 (74)
≥18	21 (17)
Gender	
Male	81 (65)
Female	38 (31)
Unknown	5 (4)
Histology	
Classic	91 (73)
Desmoplastic	22 (18)
Large cell anaplastic	11 (9)
Metastatic stage	
M0	77 (62)
≥M1	44 (36)
Unknown	3 (2)
Residual disease after surgery	
Yes	51 (41)
No	47 (38)
Unknown	26 (21)
Chemotherapy	
Yes	93 (75)
No	29 (23)
Unknown	2 (2)
Radiotherapy	
Yes	134 (91)
No	9 (7)
Unknown	2 (2)
Clinical risk stratification	
Standard risk	26 (21)
High risk	82 (66)
Unknown	16 (13)

proportional hazards regression model, with overall survival as the dependent variable, was used to assess the effect of our multiple gene models on the three individual components of the current risk stratification model (metastasis, residual disease, and age <3 y). Two-sided $P < 0.05$ using 95% confidence interval was considered to indicate statistical significance.

Results

Selection of genes for validation on tissue microarray. Previous microarray studies in medulloblastoma identified 100 genes for which the mRNA expression was associated with clinical outcome (28). We used the expression of these 100 genes to cluster medulloblastoma samples for which complete mRNA expression profiles were generated by Boon et al. (29) using SAGE. Comparison of the two identified clusters resulted in the selection of 350 genes as most differentially expressed, including many genes not found with the microarrays (data not shown). From this list of genes, we selected several genes (CCNB1, *CXCR4*, *NPM1*, *NCL*, LDHB, and *STMN1*) for which good antibodies were available to test their prognostic value in a large series of medulloblastomas embedded in paraffin. Some of these genes were also reported by others as prognostic markers (28, 30). We also included *MYC* because several studies have shown that expression of *MYC* mRNA is a marker for poor prognosis (22, 24, 27, 31), but this has never been confirmed at the protein level.

Protein expression of selected genes on medulloblastoma tissue microarrays. Antibodies were tested for their gene specificity, reproducibility of the staining pattern, and uniformity in the evaluation of the labeling. Antibodies against NPM1 and NCL detected a strong nuclear staining pattern in all medulloblastomas and were therefore excluded from further analyses. Antibodies against CXCR4 were excluded because of a diffuse, strong, nonspecific background signal in the neuropil environment of the paraffin-embedded medulloblastomas. Only protein expression patterns of MYC, LDHB, CCNB1, and STMN1 were found to be specific and reproducible and, thus, suitable for further analyses (Fig. 1). A positive staining for MYC was found in 80%, LDHB in 58%, CCNB1 in 33%, and STMN1 in 85% of the samples.

Survival analysis. Table 1 summarizes the clinical characteristics of the patient population. For the clinical parameters, significant correlations with overall survival were found for the degree of tumor resection ($P = 0.0021$) and metastatic disease at diagnosis ($P < 0.00005$; Table 2). This confirms the well-established prognostic significance of these clinical parameters in medulloblastoma. For the molecular markers analyzed, only for the expression of MYC a clear significant difference in overall survival was found ($P = 0.005$; Fig. 2A; Table 2). The 10-year overall survival of MYC-negative patients was much better than that of MYC-positive patients (91% versus 48%, respectively). For LDHB and CCNB1, patients with expression tended to do worse ($P = 0.07$ and $P = 0.11$, respectively), but the differences were not significant (Table 2). No difference in overall survival was found for patients with or without expression of STMN1 ($P = 0.66$; Table 2). Multivariate analysis showed that expression of MYC was independent of metastatic stage and the presence of residual disease (Table 3).

We also analyzed whether any combination of the immunohistochemical data for the three genes (*MYC*, LDHB, and CCNB1) would be a better prognostic marker. Patients were divided into two groups: a "signature group" and a "no-signature group." To be included in the signature group, the patient must have all genes in the model expressed. All other gene expression patterns were included in the no-signature group. In contrast to the absence of a significant relationship between expression of LDHB and/or CCNB1 alone and survival, we found a highly significant correlation when the combined expression pattern of LDHB and CCNB1 was analyzed ($P < 0.00005$; Table 2; Fig. 2B). Patients with both LDHB and CCNB1 expression had a 5-year overall survival of

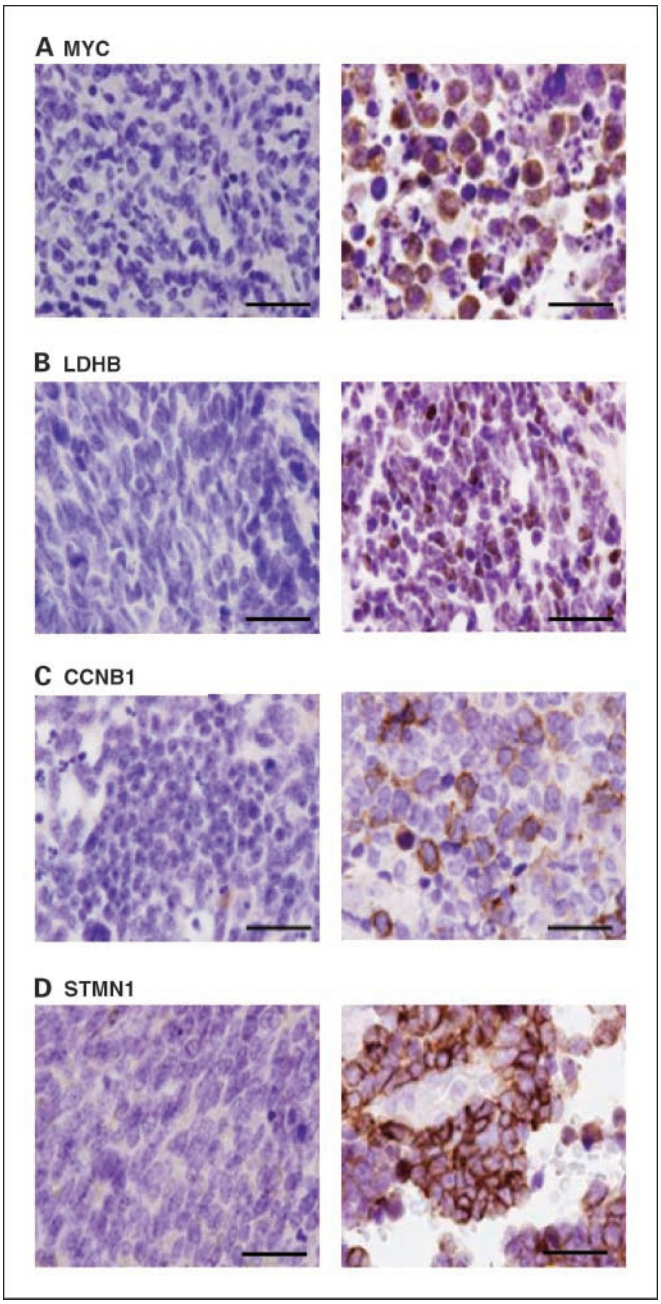

Fig. 1. Immunohistochemical staining of medulloblastoma tissue arrays with antibodies directed against MYC (*A*), LDHB (*B*), CCNB1 (*C*), and STMN1 (*D*). Left, an example of an antigen-negative medulloblastoma for each antibody. Bar, 100 μm.

only 16%, and the average survival time of the survivors in this group (9 of 19) was only 17 months (range, 1-52 months). In contrast, patients without the LDHB/CCNB1 signature had a 5-year overall survival of 74%, and the average survival time of the survivors in this group (69 of 87) was 72 months (range, 1-198 months). All patients with the LDHB/CCNB1 signature were also positive for MYC and, therefore, the same results were found for the MYC/LDHB/CCNB1 signature. The other gene signatures (MYC/CCNB1 and MYC/LDHB) were also significant ($P < 0.022$) but were less powerful. Multivariate survival analysis showed that the LDHB/CCNB1 signature was also independent of the clinical parameters metastatic disease and residual disease (Table 3).

Molecular risk stratification. Whereas absence or low MYC expression on its own was able to identify a group of patients ($n = 24$; 20% of all patients) with a high survival rate (5- and 10-year overall survival, 91%), the combined expression of LDHB and CCNB1 within the group of MYC-positive patients identified a group of patients ($n = 19$; 18% of all patients) with a very low survival rate (5-year overall survival, 16%; the maximal follow-up in this group was 52 months; Fig. 2C). Based on these results, medulloblastoma patients may be stratified into three molecular risk groups: patients with high molecular risk, who are MYC, LDHB, and CCNB1 positive; those with intermediate molecular risk, who are MYC positive but not positive for both LDHB and CCNB1; and those with low molecular risk, who are MYC negative regardless of the LDHB and CCNB1 expression (Fig. 2E). However, patients at low molecular risk were never positive for both LDHB and CCNB1. Similar and highly significant results were found when we included only children in different age groups or adults for the analyses. For infants (age 0-3 years) the molecular risk groups became too small to find significant differences in overall survival (Supplementary Fig. S1). Multivariate survival analysis done for the complete data set (all patients) shows that this molecular risk stratification is independent of the clinical variables metastasis and residual disease and that a greater resolution of disease risk is found than with the clinical variables alone (Table 3). No clear differences were observed for the distribution of histologic subtypes between the different molecular risk groups (see Supplementary Table S1). Moreover, when we analyzed each histologic subtype separately, we found similar distributions in overall survival between the three molecular risk groups (Supplementary Fig. S2). Because not all patients in our study received chemotherapy and/or radiotherapy (Table 1), we wondered whether the different molecular risk groups reflected a difference in therapy. However, the same molecular risk groups with similar survival rates were identified when all patients ($n = 76$), all children (age 0-18 years; $n = 68$), or all children older than 3 years of age ($n = 55$) who received both chemotherapy and radiotherapy were analyzed (Supplementary Fig. S3A-C). We also realized that patient samples were collected over a long period of time, during which treatment protocols have been changed and cure rates improved. But again, the same molecular risk groups with significant differences in survival rates were identified when we analyzed all patients or children treated in different periods (Supplementary Fig. S3D-I). However, for patients treated after 2000, the groups became too small and the follow-up was limited to find significant differences in survival rates, but the trend was the same.

Molecular risk model identifies low-risk patients in clinical high-risk group. The overall survival and the number of patients for the low molecular risk group are comparable with the overall survival and the number of patients that were clinically stratified as standard-risk (Fig. 2D). However, they were not the same patients. Fifteen of these 24 patients stratified as low molecular risk were originally stratified as clinical high-risk; 6 of them because of the presence of metastases at diagnosis, 8 because of residual disease after surgery, and 1 patient because of his age (1 year). This is also shown when the molecular risk stratification was applied only to those patients who were clinically stratified as high-risk. Figure 2F shows that the molecular risk stratification is able to identify those patients with a very good outcome within the group of clinical high-risk patients. This was also true when we included only children for analysis (Supplementary Fig. S4). When we applied the same molecular risk

Table 2. Log-rank univariate survival analysis of clinical and molecular risk stratification parameters in medulloblastoma study population

Parameter	Total patients	No. patients positive (%)	5-y OS (%)	10-y OS (%)	No. patients negative (%)	5-y OS (%)	10-y OS (%)	*P*
Metastases	121	44 (36)	43	0	77 (64)	78	78	<0.00005
Age <3 y	124	11 (9)	67	67	113 (91)	63	57	0.17
Residual disease (>1.5 cm^2)	98	51 (52)	55	46	47 (48)	81	76	0.0021
LDHB	111	64 (58)	60	60	47 (42)	74	70	0.07
CCNB1	116	38 (33)	52	52	78 (67)	71	62	0.11
MYC	118	94 (80)	57	48	24 (20)	91	91	0.005
STMN1	103	88 (85)	68	65	15 (15)	78	65	0.66
LDHB + CCNB1	106	19 (18)	16	ND	87 (82)	74	74	<0.00005
MYC + CCNB1	113	32 (28)	40	40	81 (72)	72	68	0.005
MYC + LDHB	109	52 (48)	55	55	57 (52)	75	72	0.022
MYC + LDHB + CCNB1	106	19 (18)	16	ND	87 (82)	74	74	<0.00005

NOTE: *P* values were determined using 95% confidence intervals.
Abbreviations: OS, overall survival; ND, not determined.

stratification, but this time only for the clinical standard-risk patients, we found that only 1 of 23 standard-risk patients was classified as molecular high-risk. This patient was still alive at the time of analysis, but follow-up was limited (only 1 month). All other patients were classified as molecular low-risk (6 patients; follow-up of 24-144 months; all alive) or intermediate molecular risk (16 patients; follow-up of 16-180 months; 2 died). These data illustrate the ability of our gene expression model to refine disease risk attributed by clinical risk stratification alone.

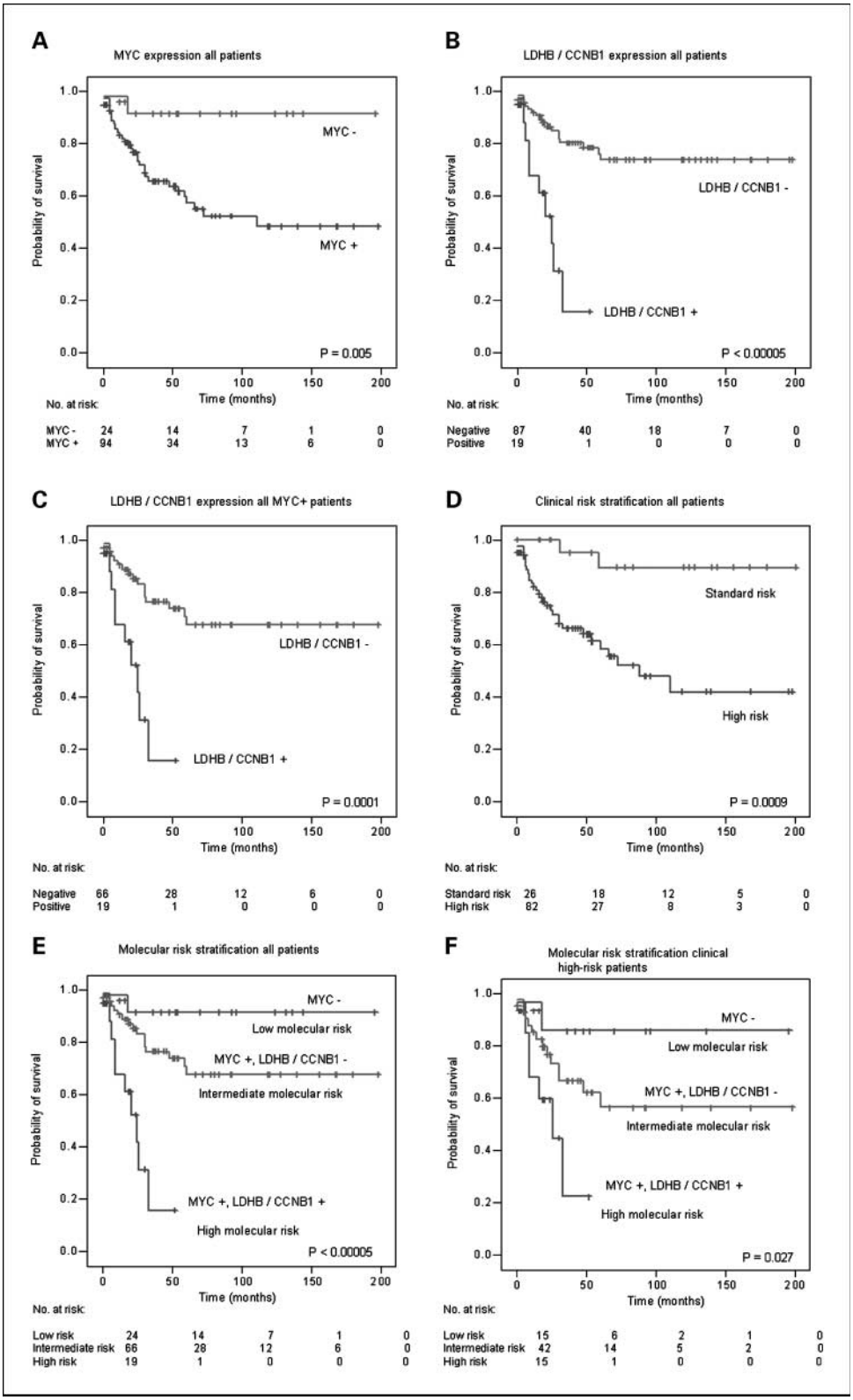

Fig. 2. Overall survival analyses of molecular markers and clinical risk. *A,* absence of MYC expression identifies a group of patients with a very good outcome. *B,* concomitant expression of LDHB and CCNB1 identifies a group of patients with a very poor outcome. *C,* LDHB/CCNB1 signature can be used to further stratify MYC-positive patients. *D,* clinical risk groups. Patients were stratified according to current clinical risk parameters: metastatic stage, residual disease, and age <3 y. *E,* molecular risk groups using all patients. Patients at low molecular risk are patients who are negative for MYC expression and do not have concomitant expression of LDHB and CCNB1. Patients at intermediate molecular risk are MYC positive and do not have concomitant expression of LDHB and CCNB1. High molecular risk patients are positive for MYC, LDHB, and CCNB1. *F,* molecular risk groups using only clinical high-risk patients. All *P* values were calculated with the log-rank test.

Table 3. Multivariate proportional hazard analysis on the prognostic role of the molecular and clinical parameters

Parameter	No. patients	*P*	HR (95% CI)
(A)			
Metastatic stage $\geq M_1$ at diagnosis	98	<0.001	6.2 (2.6-14.7)
Residual disease after surgical resection		0.035	2.6 (1.1-6.4)
Age <3 y at diagnosis		0.62	0.6 (0.1-4.6)
(B)			
Metastatic stage $\geq M_1$ at diagnosis	92	<0.001	5.2 (2.2-12.6)
Residual disease after surgical resection		0.034	2.8 (1.1-7.0)
MYC+		0.033	8.9 (1.2-66.0)
(C)			
Metastatic stage $\geq M_1$ at diagnosis	84	<0.001	6.7 (2.5-18.2)
Residual disease after surgical resection		0.027	3.4 (1.1-9.9)
LDHB/CCNB1 signature+		0.011	4.6 (1.4-14.7)
(D)			
Metastatic stage $\geq M_1$ at diagnosis	86	<0.001	6.2 (2.3-17.3)
Residual disease after surgical resection		0.030	3.3 (1.1-9.8)
High molecular risk vs low molecular risk		0.003	33.0 (3.6-323.6)
Intermediate molecular risk vs low molecular risk		0.041	8.6 (1.1-67.1)

NOTE: Multivariate Cox proportional hazard analysis of the risk to die of medulloblastoma in a multiparameter model shows the following: (A) When clinical parameters were tested alone, only metastasis and residual disease are significant and independent prognostic parameters. Age <3 y is not a prognostic factor in this study. (B) Expression of MYC is a significant prognostic factor and independent of the clinical parameters metastasis and residual disease. (C) The LDHB/CCNB1 signature is a significant prognostic factor and independent of the clinical parameters metastasis and residual disease. (D) All three molecular risk groups identify patients with a different outcome, independent of the clinical parameters metastasis and residual disease.
Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.

Discussion

The heterogeneous disease medulloblastoma requires a risk-adjusted approach to therapy. The clinical parameters (metastasis, residual disease, and age) currently used for risk stratification are unsatisfactory in predicting response to therapy. The aim of the present study was therefore to identify a set of molecular markers for improved risk stratification. Protein analysis of candidate prognostic markers was strongly limited by the availability of reliable antibodies. Based on our results for MYC, LDHB, and CCNB1, we developed a molecular classification model identifying patients at low, intermediate, or high risk to die of their disease. Overall survival in these groups of medulloblastoma patients can be predicted using the single expression of MYC in combination with a two-gene signature of LDHB and CCNB1. Absence of MYC expression identified a group of medulloblastoma patients with very good survival. In contrast, concomitant expression of LDHB and CCNB1 identified a group of high-risk medulloblastoma patients with an extremely poor response to current long-term and intensive therapy. Our results show that these molecular prognostic markers are independent of the current clinical markers metastasis and residual disease and thus provide additional information about the disease risk.

MYC amplification has been reported in 5% to 10% of medulloblastomas and correlates with anaplasia and poor survival (15, 23–25, 32). *MYC* mRNA expression, present in more medulloblastomas, has also been correlated with a bad prognosis (22, 24, 27, 31). However, at the protein level, this correlation could never be confirmed (5, 19, 30). The reason for this discrepancy could be that in those studies, tumors were only scored as MYC positive when a nuclear staining was found. In our study, all sections were scored positive when >50% of the tumor cells were stained regardless of whether the protein was localized in the nucleus or the cytoplasm. This may also explain why in these previous studies the percentage of MYC-negative tumors (43-84%) was much higher than in our study (20%). In contrast to these previous studies, we show that MYC protein expression, just like *MYC* mRNA expression, is a marker for poor prognosis. Important for the clinic is the group of patients that has no or very low MYC expression. Our results show that the 5-year survival of these 24 patients is 91% and even 100% when only patients treated after 1995 ($n = 15$) were included (Supplementary Fig. S3E). This is particularly interesting because 6 of the 24 patients had metastases at diagnosis, but only one of them died shortly after diagnosis. The other five are still alive with an average follow-up of 53 months.

Expression of LDHB or CCNB1 alone was not able to predict a difference in overall survival, but concomitant expression of both LDHB and CCNB1 identified a group of patients with a significantly worse prognosis compared with other patients (Fig. 2B; $P < 0.00005$). Moreover, MYC-positive patients, showing a 5-year survival of 57%, were further stratified using this LDHB/CCNB1 signature (Fig. 2C). Interestingly, CCNB1 is one of the 11 genes of a gene signature that predicts outcome in several types of cancer, including medulloblastoma (33, 34). This gene signature predicts 5-year survival rates of 18% and 80% for the poor and good prognosis groups of medulloblastoma patients,

respectively (33). This is similar to what we find in our study with the LDHB/CCNB1 signature (Table 2). However, this 11-gene signature has not been tested yet at the protein level. CCNB1 was also identified by Neben et al. (30), using cDNA microarrays, as a gene that may predict the outcome for medulloblastoma patients. However, subsequent protein analysis using tissue arrays containing 180 medulloblastoma samples did not find significant differences in overall survival with CCNB1. These results for CCNB1 are in agreement with our results for CCNB1 when tested as a single gene.

Despite recent improvements in treatment, the prediction of disease outcome remains a major challenge for medulloblastoma patients. More patients survive from medulloblastoma, but the disease itself, surgery, radiotherapy, and chemotherapy all have a very negative effect on neurocognitive and endocrine functions in survivors. Therefore, a good risk stratification model is essential for the most effective treatment strategy with fewer side effects. The molecular risk stratification developed in this study might be useful for two groups of patients. The low molecular risk group with a very high chance to survive might be treated less aggressively thereby diminishing morbidity and reducing the negative side effects of the therapy. The high molecular risk group with a very poor prognosis may benefit from new therapeutic approaches. The proposed molecular risk classification in combination with current clinical variables may therefore be a first step for a useful guidance in treatment adjustment. However, more studies will be necessary to assess the value of these markers prospectively in uniformly treated patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Saran FH, Driever PH, Thilmann C, et al. Survival of very young children with medulloblastoma (primitive neuroectodermal tumor of the posterior fossa) treated with craniospinal irradiation. Int J Radiat Oncol Biol Phys 1998;42:959–67.
2. David KM, Casey AT, Hayward RD, et al. Medulloblastoma: is the 5-year survival rate improving? A review of 80 cases from a single institution. J Neurosurg 1997;86:13–21.
3. Gatta G, Capocaccia R, Coleman MP, et al. Childhood cancer survival in Europe and the United States. Cancer 2002;95:1767–72.
4. Pollack IF, Biegel J, Yates A, et al. Risk assignment in childhood brain tumors: the emerging role of molecular and biologic classification. Curr Oncol Rep 2002;4:114–22.
5. Korshunov A, Savostikova M, Ozerov S. Immunohistochemical markers for prognosis of average-risk pediatric medulloblastomas. The effect of apoptotic index, TrkC, and c-myc expression. J Neurooncol 2002;58:271–9.
6. Gilbertson R, Wickramasinghe C, Hernan R, et al. Clinical and molecular stratification of disease risk in medulloblastoma. Br J Cancer 2001;85:705–12.
7. Kleihues P, Louis DN, Scheithauer BW, et al. The WHO classification of tumors of the nervous system. J Neuropathol Exp Neurol 2002;61:215–25; discussion 226–9.
8. Ellison D. Classifying the medulloblastoma: insights from morphology and molecular genetics. Neuropathol Appl Neurobiol 2002;28:257–82.
9. Katsetos CD, Del Valle L, Legido A, et al. On the neuronal/neuroblastic nature of medulloblastomas: a tribute to Pio del Rio Hortega and Moises Polak. Acta Neuropathol 2003;105:1–13.
10. Rutkowski S, Bode U, Deinlein F, et al. Treatment of early childhood medulloblastoma by postoperative chemotherapy alone. N Engl J Med 2005;352:978–86.
11. McManamy CS, Pears J, Weston CL, et al. Nodule formation and desmoplasia in medulloblastomas—defining the nodular/desmoplastic variant and its biological behavior. Brain Pathol 2007;17:151–64.
12. Giangaspero F, Perilongo G, Fondelli MP, et al. Medulloblastoma with extensive nodularity: a variant with favorable prognosis. J Neurosurg 1999;91:971–7.
13. Giangaspero F, Wellek S, Masuoka J, et al. Stratification of medulloblastoma on the basis of histopathological grading. Acta Neuropathol 2006;112:5–12.
14. Eberhart CG, Kratz JE, Schuster A, et al. Comparative genomic hybridization detects an increased number of chromosomal alterations in large cell/anaplastic medulloblastomas. Brain Pathol 2002;12:36–44.
15. Lamont JM, McManamy CS, Pearson AD, et al. Combined histopathological and molecular cytogenetic stratification of medulloblastoma patients. Clin Cancer Res 2004;10:5482–93.
16. Min HS, Lee YJ, Park K, et al. Medulloblastoma: histopathologic and molecular markers of anaplasia and biologic behavior. Acta Neuropathol 2006;112:13–20.
17. Gilbertson RJ, Pearson AD, Perry RH, et al. Prognostic significance of the c-erbB-2 oncogene product in childhood medulloblastoma. Br J Cancer 1995;71:473–7.
18. Gajjar A, Hernan R, Kocak M, et al. Clinical, histopathologic, and molecular markers of prognosis: toward a new disease risk stratification system for medulloblastoma. J Clin Oncol 2004;22:984–93.
19. Ray A, Ho M, Ma J, et al. A clinicobiological model predicting survival in medulloblastoma. Clin Cancer Res 2004;10:7613–20.
20. Segal RA, Goumnerova LC, Kwon YK, et al. Expression of the neurotrophin receptor TrkC is linked to a favorable outcome in medulloblastoma. Proc Natl Acad Sci U S A 1994;91:12867–71.
21. Grotzer MA, Janss AJ, Phillips PC, et al. Neurotrophin receptor TrkC predicts good clinical outcome in medulloblastoma and other primitive neuroectodermal brain tumors. Klin Padiatr 2000;212:196–9.
22. Grotzer MA, Hogarty MD, Janss AJ, et al. MYC messenger RNA expression predicts survival outcome in childhood primitive neuroectodermal tumor/medulloblastoma. Clin Cancer Res 2001;7:2425–33.
23. Badiali M, Pession A, Basso G, et al. N-myc and c-myc oncogenes amplification in medulloblastomas. Evidence of particularly aggressive behavior of a tumor with c-myc amplification. Tumori 1991;77:118–21.
24. Herms J, Neidt I, Luscher B, et al. C-MYC expression in medulloblastoma and its prognostic value. Int J Cancer 2000;89:395–402.
25. Aldosari N, Bigner SH, Burger PC, et al. MYCC and MYCN oncogene amplification in medulloblastoma. A fluorescence *in situ* hybridization study on paraffin sections from the Children's Oncology Group. Arch Pathol Lab Med 2002;126:540–4.
26. Tong CY, Hui AB, Yin XL, et al. Detection of oncogene amplifications in medulloblastomas by comparative genomic hybridization and array-based comparative genomic hybridization. J Neurosurg 2004;100:187–93.
27. Rutkowski S, von Bueren A, von Hoff K, et al. Prognostic relevance of clinical and biological risk factors in childhood medulloblastoma: results of patients treated in the prospective multicenter trial HIT'91. Clin Cancer Res 2007;13:2651–7.
28. Pomeroy SL, Tamayo P, Gaasenbeek M, et al. Prediction of central nervous system embryonal tumour outcome based on gene expression. Nature 2002;415:436–42.
29. Boon K, Edwards JB, Siu IM, et al. Comparison of medulloblastoma and normal neural transcriptomes identifies a restricted set of activated genes. Oncogene 2003;22:7687–94.
30. Neben K, Korshunov A, Benner A, et al. Microarray-based screening for molecular markers in medulloblastoma revealed STK15 as independent predictor for survival. Cancer Res 2004;64:3103–11.
31. Eberhart CG, Kratz J, Wang Y, et al. Histopathological and molecular prognostic markers in medulloblastoma: c-myc, N-myc, TrkC, and anaplasia. J Neuropathol Exp Neurol 2004;63:441–9.
32. Leonard JR, Cai DX, Rivet DJ, et al. Large cell/anaplastic medulloblastomas and medullomyoblastomas: clinicopathological and genetic features. J Neurosurg 2001;95:82–8.
33. Glinsky GV, Berezovska O, Glinskii AB. Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer. J Clin Invest 2005;115:1503–21.
34. Lahad JP, Mills GB, Coombes KR. Stem cell-ness: a "magic marker" for cancer. J Clin Invest 2005;115:1463–7.