Pathol. Oncol. Res. (2011) 17:899–908
DOI 10.1007/s12253-011-9401-z

RESEARCH

Lack of Prognostic Significance of Survivin in Pediatric Medulloblastoma

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Received: 6 January 2011 / Accepted: 6 April 2011 / Published online: 17 June 2011

Abstract Medulloblastoma (MDB) is the most common malignant cerebellar tumor in children. Because of the significant rate of mortality and treatment-related morbidity, the identification of prognostic factors could lead to a more accurate selection of patients who can benefit from a less aggressive therapy and improve risk stratification. Survivin is an inhibitor of apoptosis protein (IAP), the expression of which has been associated with worse prognosis in MDB. However, both of its subcellular localizations may contribute to tumor progression, and ultimately, survivin subcellular localization prognostic value depends on tumor type biological features. The goal of this study was to analyze these survivin features in the pediatric MDB tumor samples and its impact on clinical outcome. Survivin expression and subcellular localization were accessed by immunohistochemistry in a series of 41 tumor samples. Kaplan-Meier survival curves were compared using the log-rank test. Survivin expression ranged from completely absent to fully present in a notably higher pattern of nuclear localization than cytoplasmic (19 of 41 versus 4 of 41, respectively). However, survivin expression and subcellular localization were not associated with five-year overall survival or metastasis status at diagnosis, which was the only statistically significant prognostic factor in our series ($p=0.008$). Taken together, our results suggest that survivin expression should be further studied in large, multicenter series to determine its accurate impact on prognosis and pathobiology of pediatric MDB.

Keywords Survivin · Childhood · Medulloblastoma · Prognostic factor · Immunohistochemistry

Introduction

Medulloblastoma (MDB) is the most common malignant cerebellar tumor in children, accounting for approximately

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20% of all pediatric central nervous system (CNS) cancers and 40% of pediatric posterior fossa tumors [1]. Median age of occurrence is around 8 years, and it is infrequent after 15 years, with adults accounting for only 30% of all MDB cases [1, 2]. In children, the tumor usually grows through the cerebellar vermis, enters the fourth ventricle, and frequently invades the ependyma in the direction of the brainstem [1, 3].

Pediatric MDB treatment is based on surgical resection followed by chemotherapy and local and cerebral spine irradiation, which renders major neurocognitive impairment as well as hormonal and neurological deficits, obesity and increased risk of developing secondary tumors, especially in young children [1–5]. Currently, adjuvant chemotherapy also is being adopted as an alternative to decrease radiotherapy dosage and its damaging effects [1–4]. Despite this new approach for pediatric MDB treatment, survival rates stabilize after 5 years at around 60% [1–4]. Because of the significant rate of mortality and treatment-related morbidity, the identification of clinical and biological factors that help to predict treatment response can lead to a more accurate selection of patients who may benefit from a more or less aggressive therapy as well as improve risk stratification [1, 6, 7].

Recently, inhibitor of apoptosis proteins (IAPs) have emerged as a promising prognostic factor in several tumor types [8–12]. In the past few years IAPs overexpression has been correlated to treatment resistance in different types of neoplasias [8–12]. Survivin, in particular, has been pointed as a promising prognostic factor and an interesting therapeutic target because of its rare expression in adult differentiated tissues [13–16]. Moreover, survivin expression has been detected not only in the cytoplasm, where it plays its antiapoptotic function [16, 17], but also in the nuclei of tumor cells, where it is crucial to promote accurate mitosis progression [18]. Depending on the tumor type, a wide variety of combinations of its subcellular localizations may determine treatment response. For instance, nuclear predominance ultimately has been reported to result in a favorable prognosis, whereas cytoplasmic predominance results in an unfavorable prognosis in a variety of tumor types, such as colorectal cancer, breast cancer, ovary tumors, and pancreatic cancer, whereas the opposite, for example, the association of nuclear survivin with poor survival, also has been reported in gastroenteropancreatic neuroendocrine tumors [19] and glioblastoma [20]. Nevertheless, localization of survivin in both nucleus and cytoplasm also has been associated with a worse prognosis [21]. Therefore, survivin subcellular localization prognostic value is likely to depend on tumor type biological features. So, pediatric MDB-specific survivin subcellular localization pattern and prognostic value must be elucidated.

Survivin expression has been reported in MDB, and a negative association with survival was observed [22–27]. However, validation of these results by independent groups is required to verify if survivin expression can be a clinically useful prognostic parameter. So far, there are no reports on that subject on the Brazilian population. A detailed analysis of its expression in the tumor and its subcellular localization is still lacking. With this in mind, we addressed these matters in a group of pediatric MDB patients receiving our institution's standard treatment protocol, aiming to answer these unsolved questions. As a result, we found the following: (1) survivin subcellular localization greatly varies between nuclear, cytoplasmic, both nuclear and cytoplasmic, or no expression; (2) survivin expressing cells within the tumor seems to account for a low percentage of total tumor cells yet to display a disperse organization across the tumor area; and (3) both overall expression and its stratification in subcellular localization failed to have an impact on the overall survival of our group of children, which reinforces the need of a translation research effort toward the elucidation of survivin role in pediatric MDB pathobiology and prognosis.

Materials and Methods

Patient Characteristics and Treatment

A total of 74 patients with pediatric MDB were registered at the National Cancer Institute (Brazil) between 1998 and 2007. From those, 41 received complete treatment at our institution, had adequate tumor samples and complete follow-up information, and were therefore eligible for entering this study. Demographic, clinical, and treatment response data were collected from patient records.

Patients were stratified into the average or the high-risk group. The average-risk group is composed of children older than 3 years, with gross-total or near-gross total resected tumor (<1.5 cm^2 of residual disease) and with nondisseminated disease. The high-risk group is composed of children 3 years old or younger and/or with disseminated disease and/or with subtotal resected tumor (>1.5 cm^2 of residual disease).

Treatment included surgical resection followed by postoperative, reduced-dose (23.4 Gy) craniospinal radiation therapy and 55.8 Gy of local radiation therapy for patients on the average risk and conventional-dose (36 Gy) craniospinal irradiation supplemented with 18 to 20 Gy of local irradiation (total dose of 54 to 56 Gy) for those on high-risk group and standard chemotherapy that included the drugs vincristine, lomustine, and cisplatin [28]. Overall survival (OS) was defined as the time between the first

medical attendance at our hospital and the last clinical follow-up.

Tumor samples from all included patients represented surgically resected tumors at the first manifestation of the disease. All included patients did not receive previous treatment. Tumor slides were revised, and the cases were classified into the five categories defined by the fourth edition of the WHO classification of tumors of the central nervous system [29]. The local institutional ethics committee approved this study which was conducted in accordance with the recommendations of the Declaration of Helsinki.

Immunohistochemistry Assay

For this analysis, selection of tumor samples was based on the quality of the material. Immunohistochemistry was performed in 4-μm formalin-fixed paraffin-embedded tumor sections. The protocol was adapted from a previous work from our group [30]. Briefly, after paraffin removal and rehydration, antigenic retrieval was performed with citrate buffer pH 6.0 in a steamer for 30 min at 98°C. Endogenous peroxidase activity and antibody nonspecific binding were blocked with hydrogen peroxide and a blocking solution, respectively. Tumor slides were then incubated overnight at 4°C with a polyclonal antisurvivin antibody (Sigma-Aldrich catalog number S8191). As for the detection system, a labeled streptavidin biotin method with a coupled HRP peroxidase (LSAB-Dako) was used. After 3.3′ diaminobenzidine tetrahydrochloride (DAB) staining, Harris hematoxylin was used for a slight counterstaining.

Results were visualized and registered in an Eclipse E200 Nikon microscope connected to a Digital Sight System by two different analyzers independently. From the observations, a scoring system was developed in which the absence or rare staining was considered as negative and the positive staining was measured as the percentage of tumor cells that presented immunoreactivity for the antibody (brown staining). Hence, survivin positivity was stratified into four categories: (1) positive tumor cells of 5-15%, (2) 20-35%, (3) 40-65%, and (4) 70-100%. At least ten fields in a magnification of×40 were analyzed. Subcellular localization of survivin was accounted as nuclear and/or cytoplasmic in all positive samples.

Statistical Methods

Survival rates were plotted using the Kaplan-Meier method. The event was defined as disease-related death of the patient. The remaining cases were censored. Associations between survival or survivin expression and the analyzed parameters were evaluated by applying the log-rank test or the Pearson's chi-square test, respectively. For a 95% confidence interval, differences between the analyzed groups were considered significant when $p<0.05$. Statistical analysis was performed in the SPSS 17.0 software.

Results

Clinical, Demographic, and Histopathology Data

Among our 41-patients series, median age was 7 years (ranging from one to 17) with a prevalence of a nearly 2:1 ratio for the male gender (26 males versus 15 females). All histological variants were present in our series, with the classical MDB as the more common (24 of 41). For this study analysis, desmoplastic and extensive nodularity variants, as well as anaplastic and large cell variants, were grouped, following the trends of the latest WHO classification guidelines [29]. The majority of patients did not present metastatic disease (29/41), although this was not directly reflected in the distribution of the patients between risk groups (18 with intermediate risk versus 23 with high risk). For 25 of 41, gross-total tumor resection was achieved. Complete demographic, histopathological, and clinical data are shown in Table 1.

Survivin Expression and Subcellular Localization in Pediatric MDB

Survivin localized to either the nucleus or the cytoplasm of pediatric MDB cells. Survivin expression ranged from completely absent (12 cases) to fully present (29 cases) in a notably higher pattern of nuclear than cytoplasmic localization (19 versus four, respectively). Additionally, in six cases, survivin was detected both in the nucleus and in the cytoplasm of tumor cells. Overall, 29 cases were positive for survivin, either in the nucleus or the cytoplasm of tumor cells (Table 1). The remaining 12 cases were virtually negative for survivin expression. The observed patterns of survivin localization are shown in Fig. 1.

In terms of percentage of positive tumor cells, in 17 of the 29 survivin positive samples, there were 5-15% survivin positive tumor cells (14 cases with nuclear survivin, none with cytoplasmic survivin and three with both nuclear and cytoplasmic survivin). In 7 of the 29 samples, there were 20-35% of survivin positive tumor cells (four cases with nuclear survivin, two cases with cytoplasmic survivin and one with both nuclear and cytoplasmic survivin). In three of the 29 samples, there were 40-65% of survivin positive tumor cells (one case with nuclear survivin, one with cytoplasmic survivin and one with both nuclear and cytoplasmic survivin). In two cases of the 29 samples, there were 70-100% of survivin positive tumor cells (none

Table 1 Five-year probability of survival of childhood MDB according to demographic, histopathological and clinical data

Feature	Absolute e relative frequencies (%)	Five-year OS rate±SE (%)	p.
Age			
≤ 3 years	5 (12.2)	42.9±18.7	0.112
> 3 years	36 (87.8)	69±8.2	
Gender			
Male	26 (63.4)	58±9.7	0.224
Female	15 (36.6)	76.2±12.2	
Histological variant			
Classic	24 (58.5)	64.5±10.2	0.198
Desmoplastic + extensive nodularity	14 (34.1)	70.7±12.4%	
Anaplastic + large Cells	3 (7.3)	33,3±27.2	
Metastasis at diagnosis			
Yes	12 (29.3)	30±14.5	0.008*
No	29 (70.7)	76.6±7.8	
Risk group			
High	23 (56.1)	57.3±10.9	0.306
Intermediate	18 (43.9)	72.9±10.4	
Resection surgery extension			
Gross-total	26 (63.4)	67.8±9.5	0.486
Sub-total	15 (36.6)	59.3±12.9	
Survivin expression			
Negative	12 (29.3)	50±14.4	0.098
Positive	29 (70.7)	70±9	
Survivin nuclear localization			
Negative and cytoplasm positive cases	16 (39.0)	61.1±11.5	0.434
Nucleus and nucleus and cytoplasm positive cases	25 (61.0)	66.7±11.5	
Survivin subcellular localization			
Negative	12 (29.3)	50±14.4	0.430
Cytoplasm positive	4 (9.8)	75±21.7	
Nucleus and cytoplasm positive	6 (14.6)	60±21.9	
Nucleus positive	19 (46.3)	73±10.4	
Survivin compartmentalization			
Negative or both nucleus and cytoplasm positive	18 (43.9)	54.5±11.9	0.219
Nucleus or cytoplasm positive	23 (56.1)	73.4±9.3	
Survivin expression in over 20% of tumor cells			
No	29 (36.6)	57.9±9.3	0.134
Yes	12 (29.3)	81.5±11.9	

OS overall survival, *SE* standard error. Associations were analyzed through Kaplan-Meier method by the log-rank test. *$p<0.05$ was considered significant. *ND* not determined

with nuclear survivin, one with cytoplasmic survivin and one with both nuclear and cytoplasmic survivin). Percentage analysis of survivin expression is shown in Table 1.

Demographic, Histopathological, and Clinical Factors' Impact on OS

Median follow-up time was 46 months, ranging from 3 to 109. Cohort five-year OS rate was 64.5%, standard error (SE)=7.7% (Fig. 2a). Age and gender were not statistically significant prognostic factors ($p=0.112$ and $p=0.224$, respectively), but infants accounted for only five patients. Concerning histology, variant classification was not able to predict prognosis ($p=0.191$). Although anaplastic and large cell MDB group exhibited a worse OS than the classical and desmoplastic and extensive nodularity subtypes, there were only three patients whose tumors were classified as the former.

Among clinical features, regardless of the *locus* (CNS, non-CNS, SCF, or multiple sites), patients presenting metastasis at diagnosis had a statistically significant poorer overall survival than patients with localized disease ($p=0.008$) (Fig. 2b). Notably, risk stratification was not associated with outcome in our series ($p=0.306$). However, there was a

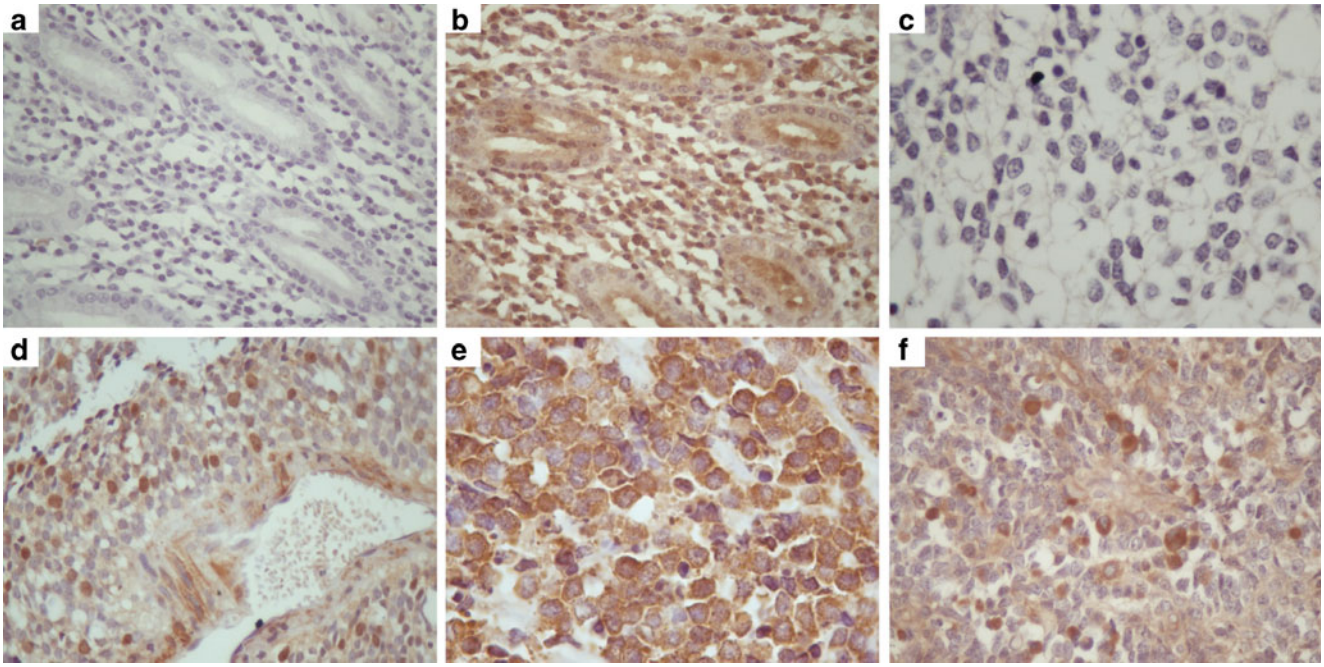

Fig. 1 Immunohistochemical detection of survivin expression and subcellular localization in medulloblastoma (MDB). Optic microscopy 40x (**a**, **b**, **d** and **f**) and 100x (**c** and **e**) magnification of survivin detection in stomach mucosa incubated with (**b**) and without (**a**) anti-survivin antibody (control) and 4 μm MDB tumor sections showing no survivin expression (**c**), nuclear survivin (**d**), cytoplasmic survivin (**e**) and both nuclear and cytoplasmic survivin (**f**)

tendency for intermediate-risk group to have a better survival than the high-risk group (OS=72.9%, SE=10.4%, versus OS=57.3%, SE=10.9%). Univariate analysis of the analyzed features' impact on five-year OS is shown in Table 1. Similar results were obtained when the eight-year OS association of these characteristics was considered (data not shown).

Survivin Expression and Subcellular Localization Impact on OS

Survivin expression regardless of its subcellular localization did not correlate with OS (p=0.098) (Fig. 2c). Given that other groups considered only nuclear survivin in their analysis [22, 24], we tested if the cases with nuclear survivin (nuclear or nuclear plus cytoplasmic survivin) had a different OS in relation to cases without nuclear survivin (negative and cytoplasmic survivin). Nevertheless, there was no association between nuclear positivity and OS (p=0.434) (Fig. 2d).

On the other hand, being a multifunctional protein that plays different roles in each cellular compartment, survivin subcellular localization has been reported to determine OS in other neoplasias [19, 31]. Therefore, we hypothesized if survivin subcellular localization could influence OS of pediatric MDB patients. Hence, we tested if cases with nuclear, cytoplasmic, nuclear and cytoplasmic or with no survivin expression (negative cases) had different OS. However, there was no association between survivin subcellular localization and OS (p=0.430) (Fig. 2e).

Furthermore, because survivin requires subcellular localization dynamic motility to play its roles in cellular physiology, we hypothesized if localization exclusively in a given compartment (nucleus or cytoplasm) could reflect an impairment of normal survivin dynamics and therefore imply a more deregulated and aggressive cell behavior. Thus, we tested if cases with exclusive nuclear or cytoplasmic survivin (restricted) had a different OS than negative cases or with both nuclear and cytoplasmic survivin expression (dynamic). Again, there was no statistical difference in the OS of the two groups (p=0.219) (Fig. 2f).

In addition, other groups found correlation between survivin expression and OS when considering high and low survivin expression with a cutoff at the mean or median value [22, 24]. Because the distribution of survivin expression, in our study peaked in the group ranging from five to 15% of positive cells (17 of 41), we tested 20% of positive cells as a higher cutoff for our series and investigated if survivin expression in more than 20% of the tumor cells was associated with OS. Again, survivin expression (taking 20% of positive tumor cells as the cutoff) did not correlate with the OS of pediatric MDB patients (p=0.134). Correlation between survivin expression features and five-year OS is shown in Table 1.

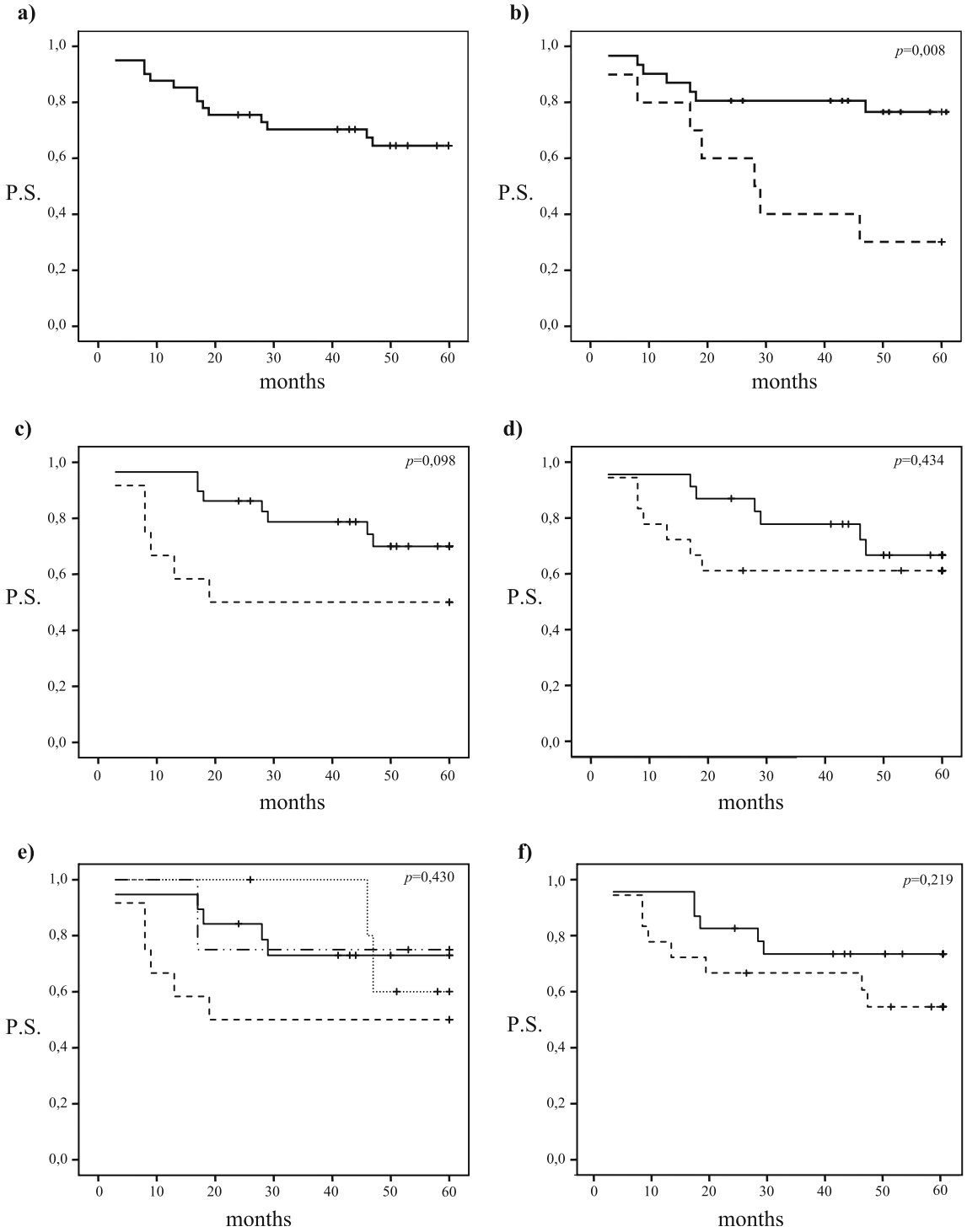

Fig. 2 Five-year overall survival (OS) curve of the 41 childhood medulloblastoma patients. Total five-year OS was 64.5% (**a**); OS of the non-metastatic disease group (n=31) was 76.6% SE=7.8% (straight curve) and the metastatic disease group (n=10) was 30% SE=14.5% (dashed curve) (p=0.008) (**b**); OS of the survivin positive group (n=29) was 70% SE=9% (straight curve) and the survivin negative group (n=12) was 50% SE=14.4% (dashed curve) (p=0.098) (**c**); OS of the nuclear survivin positive group (n=23) was 66.7% SE=11.5% (straight curve) and the nuclear survivin negative group (n=18) was 61.1% SE=11.5% (dashed curve) (p=0.434) (**d**); OS of the nuclear survivin group (n=19) was 73% SE=10.4% (straight curve), the cytoplasmic survivin group (n=4) was 75% SE=21.7% (dotted-dashed curve), the nuclear and cytoplasmic survivin group (n=6) was 60% SE=21.9% (dotted curve) and the survivin negative group (n=12) was 50% SE=14.4% (dashed curve), (p=0.430) (**e**) OS of the "restricted" survivin (only nuclear or only cytoplasmic) group (n=23) was 73.4% SE=9.3% (straight curve) and the "dynamic" survivin (negative or both nuclear and cytoplasmic) group (n=18) was 54.5% SE=11.9% (dashed curve) (p=0.219) (**f**). p values were obtained through the log-rank test. Differences were considered significant when $p<0.05$. P.S.: probability of survival

Next, as metastatic disease predicted a poorer outcome (p=0.008) (Fig. 2b), we tested if survivin expression correlated with this feature. However, survivin expression did not correlate with metastasis status at diagnosis (p=0.651). Because survivin is widely expressed during embryonic development and the central nervous system is known to continuously develop after birth throughout the first years of life, we also tested if survivin expression correlated with age, with cutoff being early childhood (3 years old). Again, there was no correlation (p=0.574) (Table 2). Similar results were obtained when the association of eight-year OS with these survivin features was considered (data not shown).

Discussion

Pediatric MDB is a rare but an aggressive WHO grade IV type of tumor [29] known by its rapid evolution and severe treatment sequelae [1–4]. Although recent multicenter clinical trials have demonstrated a stepwise improvement in the five-year survival rates, an important fraction of the patients still develop severe neurocognitive impairment because of radiotherapy, and approximately 40% die from the disease, [1–5] which also was observed in our patients. Currently, great efforts are being made to identify both clinical and biological factors that can help predict treatment response [2, 4]. A factor that can lead to more accurate risk stratification and can be used as a therapeutic target would help improve both the OS and the life quality of these children.

Survivin is an IAP family member that can act both in caspase inhibition when in the cytoplasm and in mitosis appropriate progression when in the nucleus [17, 18, 32]. Its expression and subcellular localization have been shown to predict prognosis for several types of tumors [14, 19, 31]. As for MDB, survivin expression was observed in tumor cells [23] and has been pointed as a possible prognostic marker for this disease [22, 24–26]. However, to our knowledge, there are no studies regarding these issues in the Brazilian population. In addition, survivin is widely expressed during fetal development but is virtually absent in specialized differentiated tissues [16], making it a promising therapeutic target. In fact, small molecule survivin inhibitors are currently being tested, and the first clinical trials have demonstrated its safety [33, 34]. Therefore, in the present work, we investigated the role of survivin in the prognosis of a Brazilian group of pediatric MDB patients.

Patients were selected by age, availability of adequate tumor histological samples, and complete follow-up information. Having set our study population, we wanted to know if our series features were in agreement with the observed in literature. Indeed, demographic, histopathological, and clinical data described in this study showed that our series is representative of the disease.

Next, we observed that survivin can localize either to the nucleus or the cytoplasm of pediatric MDB cells in different percentages of positive cells. The most common finding was a low percentage of positivity (between five and 15% of tumor cells), which also was observed by other groups [22–24, 26, 27]. This indicates that pediatric MDB cells probably do not overexpress survivin in terms of number of positive tumor cells but rather display a discrete but frequently found expression of this IAP. Moreover, we and other authors [26] have found that the positive cells are not grouped in large domains inside the tumor area but rather display a diffuse yet homogenous distribution throughout the slice, indicating that either survivin positive cells activated survivin expression independently from each other or their rise came from one survivin positive progenitor cell and they were able to spread through the tissue (maybe giving rise to others afterward). At this point, we do not know if survivin expression in these diffuse cell population could represent a transient response to the tumor/cerebellum microenvironment or if it is an intrinsic, persistent MDB cell feature.

Another interesting finding is that 12 of 41 cases of our series were virtually negative despite intense staining of the series positive control. Although Pizem et al. [24] and Haberler et al. [22] have reported all of their studied cases to be positive (56 and 82, respectively), Sasaki et al. [23] reported one tumor (of five) to be survivin negative in their study. This finding may be a reflection of the combination between the heterogeneity of these tumors and the relatively small number of cases of each independent study which highlights the need for large, multicenter studies to be undertaken to fully understand pediatric MDB biology.

Furthermore, while most groups reported nuclear survivin to be far more present than cytoplasmic [22, 24–26], Li

Table 2 Survivin expression has no correlation with metastasis status at diagnosis or age

	Survivin expression		*p.
	Positive cases (n)	Negative cases (n)	
Metastasis status at diagnosis			
Metastatic disease	9	3	0.651
Localized disease	19	9	
Age			
<3 years-old	3	2	0.574
≥3 years-old	26	10	

*Pearson chi-square test. Differences were considered significant when p<0.05

et al. [26] showed five positive cases of the five samples, all expressing both nuclear and cytoplasmic survivin, although cytoplasmic staining was more discrete than nuclear. Again, these discrepancies might be due to the small number of patients engaged *per* study because of the rarity of this disease, which ultimately results in limited but complementary observations by each group.

Proceeding with our analysis, we tested if any of the analyzed demographic, histological, and clinical features were significant as a prognostic factor for our series of pediatric MDB. From the analyzed clinical features, metastasis presenting at diagnosis was a strong OS predictor (p=0.008), which is in agreement with previous observations (reviewed in [35, 36]), meaning that metastasis is an indicator of the aggressiveness of the tumor which leads to the difficulty to achieve the cure in these cases with current treatment strategy. As for risk group, although it was not statistically significant (p=0.306), patients with intermediate risk have a better OS than patients in the high-risk group. This indicates that, in our study population, other factors included in the risk stratification system might be masking the importance of metastatic disease for failure risk.

We also investigated if the different features of survivin expression correlated with treatment response. We analyzed if (1) expression of survivin per se (regardless of its subcellular localization), (2) survivin subcellular localization (nuclear, cytoplasmic or both), (3) survivin nuclear localization (as opposed to absence or cytoplasmic localization), (4) survivin dynamics (absence or both localizations as opposed to nuclear or cytoplasm), and (5) survivin positivity with a 20% of positive cells cutoff had an impact on five-year OS. Because survivin expression was prognostically significant in none of these cases, we further investigated if it correlated with the prognostically significant clinical feature, which was metastasis presenting at diagnosis, and again, we found no correlation. This is different from the observations of Pizem et al. [24], Haberler et al. [22], and Fangusaro et al. [25] who observed that survivin (mainly nuclear) expression had a negative prognostic impact in their series. However, Pizem et al. [24] included both pediatric and adult patients in their analysis, which have different clinical and pathobiological characteristics [36–38], and Haberler et al. [22] included children treated with a variety of treatment protocols, which may have different prognostic factors. In addition, Fangusaro et al. [25] applied a different statistical approach which revealed that the range of positive cell percentage is quite large in the tumors from survivors and it merges with the range of positive cell percentage in the tumors from the deceased. This means that, although survivin was a negative prognostic factor in the analysis of the above-mentioned studies, children with high survivin did not necessarily have a poor outcome, which is in line with our findings. In fact, there are recent reports that show that despite the initial findings pointing survivin as a prognostic factor for several types of cancer, there are also many other types for which survivin is not [39–43].

As has been widely discussed in this study and those of others, it is important to keep in mind that being a rare disease with age- and risk-adapted treatment protocols that are continuously challenged to obtain less toxicity for the children who have this tumor, it is not easy for a single institution to assemble a large, homogeneous cohort to perform detailed and reproducible analysis regarding prognostic factors for pediatric MDB. This fact leads to a great number of issues. First, our results indicate that the prognostic impact of survivin expression in pediatric MDB must be further investigated in large, multicenter cohorts to be fully evaluated. Second, if larger groups of patients do prove survivin to be a negative prognostic factor, its clinical value is likely to be somewhat discreet because, in our series, survivin did not have an impact on OS and patients with survivin positive tumors exhibited better OS than patients with survivin negative tumors. Therefore, it is currently still unclear whether survivin expression is going to provide valuable information for clinical practice regarding pediatric MDB.

Currently, other biological factors also are being studied as potential prognostic markers for pediatric MDB [1, 3, 4, 44]. It might be possible that survivin expression or nuclear localization is partially associated with one of them, thus explaining the findings of the present work together with the findings from Pizem et al., Harbeler et al., and Li et al. [22, 24, 26]. For instance, common chromosomal abnormalities in MDB that impair p53 protein expression, such as partial loss of chromosome 17 where the *TP53* gene is localized at (17p13) (reviewed in [3, 44]), have negative prognostic value per se and might lead to altered survivin expression because p53 has been reported to inhibit survivin expression [45, 46]. However, because survivin expression also is regulated by other factors (insights in [47] and reviewed in [48]), it is possible that survivin expression does not necessarily associate with the prognosis of children with MDB.

Taken together, our results provide new information about survivin expression in pediatric MDB and its implications on the prognosis. These results argue as another piece of the puzzle of the discussion about whether survivin should really be considered as a pediatric MDB prognostic factor but, most importantly, disclose that there are still many questions that remain unanswered regarding the role of survivin in the pathobiology of pediatric MDB cells.

Acknowledgements This work was supported by grants from Programa de Oncobiologia (Universidade Federal do Rio de Janeiro & Fundação do Câncer) and Swissbridge Foundation. The authors would also like to thank MSc Mauricio G. S. Costa for the aid in the artwork design.

Conflict of interests The authors declare no conflicting interests.

Authors' contributions RSF carried out the immunohistochemistry assay, performed the statistical analysis and drafted the manuscript. RMF participated in the design of the study, performed the statistical analysis, revised part of the patients chart data and critically revised the manuscript. MFG revised part of the patients chart data. TCF contributed to the interpretation of the immunohistochemistry data. JAO contributed to the interpretation of the data. RCM conceived the study, and participated in its design and coordination and critically revised the manuscript. All authors read and approved the final manuscript.

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