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RESEARCH

Survivin Expression in Medulloblastoma: A Possible Marker for Survival

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Abstract Medulloblastomas are highly invasive tumors which are generally disseminated at the time of diagnosis. High and continued morbidity and mortality have prompted the search for new biologic markers that might be used for targeted therapy to minimise treatment related side effects. In this work, we studied the positive expression of survivin in medulloblastoma and investigated its relation to clinical, pathologic data and survival. Tumor tissue specimens from 47 patients with medulloblastoma who underwent primary surgical treatment from June 2002 to June 2006 at the Mansoura university hospital, Egypt were collected. Paraffin sections of all samples were submitted for immunohistochemistry using anti-survivin antibody. The relation between the percentage of positive survivin cells with clinical, pathological and survival data was evaluated Results: In 47 cancer tissue specimens, one case large-cell-anaplastic (1.12 %), tweleve cases desmoplastic (25.53 %) and 34 cases classic medulloblastomas (72.34 %). The immunohistochemical expression of survivin was nulear with moderate intensity. It does not correlate with either age or sex. There was a significant negative correlation of survivin expression with survival ($p<0.001$), where negative survivin immunostaining was associated with prolonged overall and disease free survival, while survivin expression was associated with shortened survival. Conclusion: Survivin expression correlate with the clinical outcome with poor prognosis and could be a potential predictive factor for recurrence or metastasis.

Keywords Medulloblastoma-survivin-survival-antiapoptosis

Condesed abstract Survivin is a negative prognostic marker in medulloblastoma.

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Introduction

Medulloblastomas are the most common malignant CNS tumor of childhood [1]. They are neuroepithelial tumors arising from the cerebellum and account for about 20 % of all intracranial tumors in children, and for 40 % of all childhood posterior fossa tumors. Although medulloblastoma peaks at 8 years of age, about 30 % of medulloblastomas occur in adults [2].

Survivin, a member of the inhibitor of apoptosis protein family, has been reported to regulate cellular proliferation as well as apoptosis by binding to caspases-3 and −7. Survivin contributes to cell cycle regulation through stabilization of microtubules and facilitating mitotic progression, and it is degraded by an ubiquitin-proteasome pathway in a cell cycle dependent manner. Human survivin, negatively regulated by wild-type p53, also participates in p53-dependent apoptosis [3, 4].

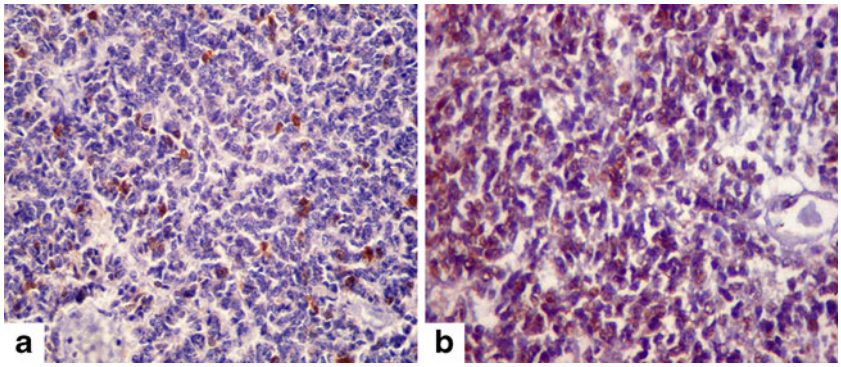

Fig. 1 Classic medulloblastoma with scattered nuclear survivin expression in about 20 % of tumor cells (**a**), 40 % of tumor cells (**b**). (Immunoperoxidase DAB X400)

There have been many advances in the treatment of medulloblastoma including improved surgical resection, radiation techniques, and chemotherapy. These changes have currently improved the 5-year survival rate to 55 % to 76 % for high-risk patients and 70 % to 80 % for standard-risk patients [2].

Despite these improvements in overall survival rate, a small but substantial number of patients will have recurrent or progressive disease. Unfortunately, attempts to further reduce the morbidity and mortality associated with medulloblastoma have been restricted by the toxicity of conventional treatments and the infiltrative nature of disease [5].

High morbidity and continued mortality have prompted the search for new treatment strategies as well as for biologic markers that might be used for targeted therapy to minimise current treatment-related side effects. Survivin is an attractive target gene to evaluate for these purposes, as it has been shown to be a significant marker of tumour aggression in a number of malignancies [6].

In the current work, our objective was to study survivin expression in medulloblastoma and to test the prognostic significance of its expression.

Material and Methods

Forty seven medulloblastoma specimens were examined in this retrospective study in pathology department from patients who were treated between June 2002 June 2006 at neurosurgery department Mansoura University, Egypt, by surgical resection. The degree of tumor resection was determined by the postoperative images. Patients were then referred to the clinical oncology and nuclear medicine department, where postoperative imaging modalities included CT scan and/or MRI of the brain and spine and CSF (cerebrospinal fluid) aspiration for cytologic examination were performed for all the patients whereas only 23 patients underwent bone marrow biopsy. The patients age ranged from 3 to 37 years. Thirty patients were children ranging from 3 to18years with mean age 8.07±3.67, while 17 patients were adult ranging from 19 to 37 years with mean age 26.41±5.71. Out of the 47 patients, 24 were males and 23 were females. Fifteen (31.91 %) patients underwent near total resection, while 32 (68.09 %) patients underwent subtotal resection. The patients were stratified into high risk patients (age < 3 years, >1.5 cm2 residual, or M+), 14 patients (29.79 %) their age ranged from 3 to 27 years with mean age 11.5±7.39 and standard risk patients (age > 3 years, <1.5 cm2 residual and M0), 33 patients (70.21 %) their age ranged from 7 to 37 years with mean age 15.79±10.66. where metastasis(M) is based on Chang staging system [7].

All patients received radiotherapy (RT). Postoperative RT was conducted as craniospinal irradiation(CSI) and a subsequent boost to the posterior fossa in all patients. CSI was delivered with a total dose of 36 Gy, and the boost to the posterior fossa was applied up to a total dose of 54 Gy. Forty one patients received 54 Gy, while six patients received a total dose ranging from 43.2 Gy to 50.4 Gy. Imaging studies were

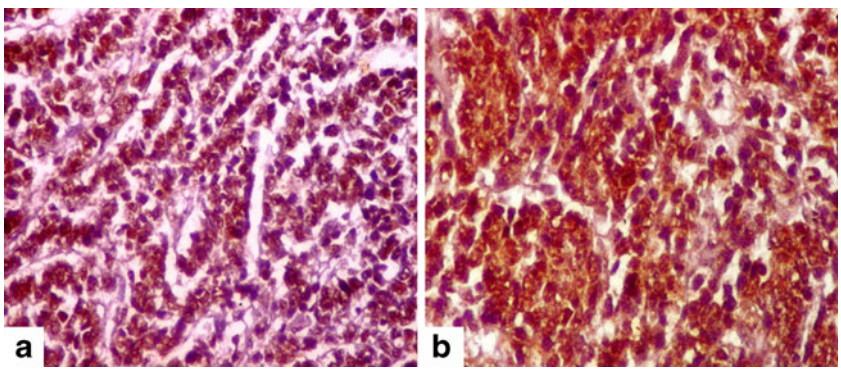

Fig. 2 Classic medulloblastoma with nuclear survivin expression in more than 50 % of tumor cells (**a**), Large cell medulloblastoma with strong nuclear survivin expression in about 65 % of tumor cells (**b**). (Immunoperoxidase DAB X400)

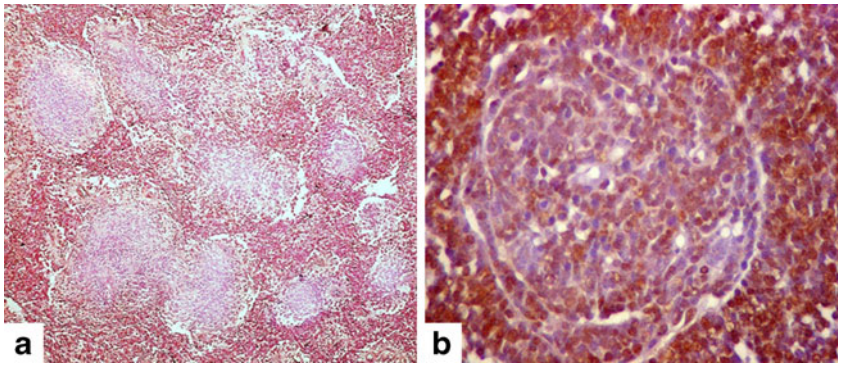

Fig. 3 Desmoplastic medulloblastoma; the pattern of survivin expression higher percentages of survivin staining in internodular areas (**a**), the nodular highly differentiated region showing low percentages of survivin staining (**b**). (Immunoperoxidase DAB X40, X400)

repeated 4–8 weeks post radiotherapy to assess treatment response and was repeated at 3–6 months interval. Complete clinicopathological information was obtained including the complete information of the patients' follow up visits to evaluate the response and prognosis.

Histopathological Analysis The formalin fixed paraffin embedded blocks were retrieved in pathology department and 4 μm thickness sections were prepared for routine H&E. Other sections were prepared on charged slides for immunohistochemistry. Examination of the slides was done by two examiners for histopathologic diagnosis and subtyping according to the recent WHO classification 2007 [8]. Examination were done on an Olympus CX31 light microscope. Pictures were obtained by a PC-driven digital camera (Olympus E-620). The computer software (Cell*, Olympus Soft Imaging Solution GmbH) allowed scorring to be performed. Histopathologically, the medulloblastoma tumors were classified into the following subtypes: thirty-four classic medulloblastomas (72.34 %), tweleve desmoplastic (25.53 %) and one large-cell-anaplastic (1.12 %).

Immunohistochemistry Deparaffinised sections of all cases were incubated for 30 min with 0.3 % hydrogen peroxide in methanol and microwave heated for 30 min in EDTA buffer solution ph8.0. Subsequently, an indirect immunoperoxidase technique was applied, using primary antibodies directed against survivin (Thermo Scientific Clone RB-9245-R7). It is performed using ImmunoPure Ultra-Sensitive ABC Peroxidase (Thermo Scientific Cat. # 32052), using (DAB) as chromogen. Positive control is pancreatic tissue as indicated by the procedures.

Immunohistochemical Scoring Both nuclear and cytoplasmic survivin immunohistochemical expression occurs in medulloblastoma cells. Evaluation of the studied cases was

Table 1 Clinicopathologic features in relation to survivin expression

Variable	No	Percentage	Survivin expression				*P*
			–	+ ≤25 %	+(25–50)	+ > 50 %	
Age							
≤18 y	30	63.83 %	15	7	3	5	0.328
>18 y	17	36.17 %	6	2	4	5	
Gender							
Males	24	51.06 %	11	4	2	7	0.388
Females	23	48.94 %	10	5	5	3	
Extent of tumor resection							
Subtotal	32	68.09 %	12	9	6	5	0.430
Total	15	31.91 %	9	2	3	1	
Risk Stratification							
Standard risk	33	70.21 %	19	8	4	2	0.005*
High risk	14	29.79 %	2	2	7	3	
Histopathologic subtypes							
Classic	33	70.21 %	15	5	5	8	0.068
Desmoblastic	12	25.53 %	6	4	2	–	
Large cell	2	4.26 %	–	–	–	2	

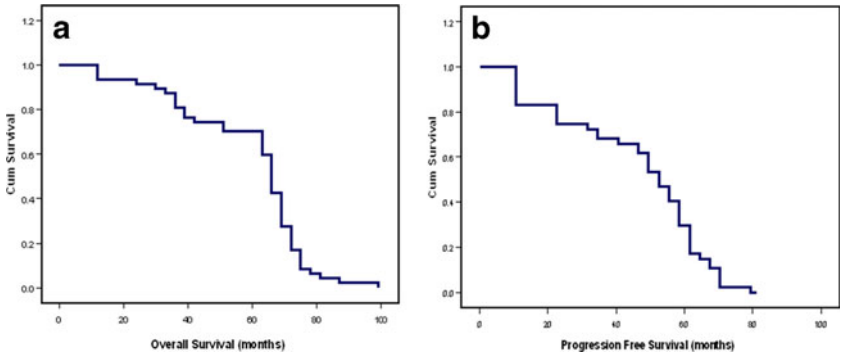

Fig. 4 Overall survival of all studied cases (**a**), Progression free survival of all studied cases (**b**)

semiquantitative depending on nuclear staining and were scored according to the percentage of positive nuclei to the total cells counted per10HPFs [3, 6].

Statistical Analysis Statistical analysis was done using Statistical Program for Social Sciences (SPSS 14.0). Qualitative data was presented as number and percent. Comparison between groups was done by Chi-square test. $P<0.05$ was considered significant difference. Overall survival time was counted (months) from the date of diagnosis to the date of death or last follow-up before study closure. Kaplan-Meier method was used to estimate the survival.

Results

Evaluation of the 47 medulloblastoma tumors by immunohistochemical staining showed negative survivin expression in 21 specimens whereas positive expression was detected in 26 specimens. Nuclear localisation was found in all tumour cells that expressed survivin while cytoplasmic expression was variable. The percentage of positive cells ranged from 5 % to a maximum of 65 % (Figs. 1 and 2a). The large-cell-anaplastic subtype showed the highest overall percentage of positive cells (Fig. 2b). The desmoplastic subtype showed a staining pattern where the internodular (poorly differentiated) areas had higher percentages of survivin staining when compared with the intranodular (highly differentiated) regions (Fig. 3). Although large cell subtype showed higher overall percentage of positive cells than the classic and the desmoplastic types (55, 65 %), no statistically significant relation was observed (Table 1).

Survivin expression had no significant association with age or sex, while it expressed a significant relation with risk stratification where, 14 out of 33 standard risk cases showed overexpression of survivin (42.42 %) while 12 out of 14 cases of high risk group overexpressed survivin (85.71 %).

The 5 –year overall and progression free survival for the whole studied cases were 70.21 % and 29.79 % respectively (Fig. 4).

Correlation of survivin expression to survival revealed 5 – year overall survival of 85.71 % and 57.69 % in survivin negative and positive cases respectively ,while the 5- year progression free survival were 42.85 % versus 19.23 % in survivin negative and positive cases respectively (Fig. 5).

Our study showed a significant correlation of survivin expression with survival, where negative expression was associated with prolonged overall and progression free survival, mean overall survival (OS) (65.71±14.77), mean progression free survival (PFS) (56.80±13.73), while positive expression was associated with shortened survival. The percentage of survivin positive cells affected significantly survival, where low positive expression ≤25 % expressed longer OS and PFS (66.12±3.86, 56.33±7.31 m)

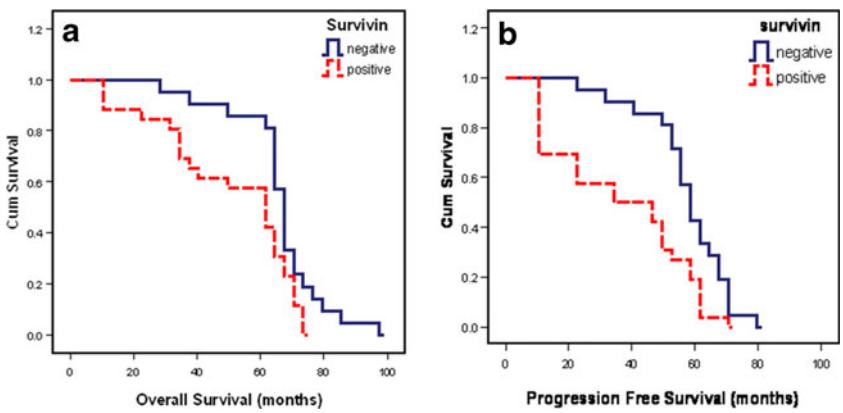

Fig. 5 Overall survival in relation to survivin expression (**a**), progression free survival in relation to survivin expression (**b**)

Table 2 Relation between Survivin expression and overall survival

	No	Overall Survival in months			*P*
		Mean	Median	Range	
Negative	21	65.71±14.77	66	60–84	0.002 ($P<0.05$)
Positive≤25 %	9	66.12±3.86	68	60–69	
Positive 25–50 %	7	62.71±7.43	62	50–65	
Positive≥50 %	10	26.30±11.70	32	10–39	
Total	47	56.31±17.38	64	10–84	

respectively in comparison to high positive expression > 50 %, which showed shorter OS and PFS (26.30±11.70, 13±15.03) respectively (Tables 2 and 3).

Discussion

Medulloblastomas correspond histologically to WHO grade IV. It is divided into four types according to WHO classification: - Desmoplastic/nodular medulloblastoma—Medulloblastoma with extensive nodularity—Anaplastic medulloblastoma—Large cell medulloblastoma [8]. Medulloblastoma poses a therapeutic challenge in adults. With current means of therapy, children with nondisseminated medulloblastoma have a high likelihood of long-term survival [9].

Medulloblastomas are highly invasive tumors, that display a strong tendency to spread through cerebrospinal pathways and form tumours of variable size along ventricular surfaces, in subarachnoid space, or along nerve roots. They sometimes grew plaque adjacent to brain or spinal cord. Distant metastases are rare, but when they occur, their most likely destination is the bone marrow [10]. Furthermore, conventional therapeutic approaches have not reduced the mortality associated with metastatic medulloblastoma. Thus, it is very important to find agents that are able to reduce tumoral invasiveness [11].

Medulloblastoma is the most common malignant childhood brain tumor and relatively uncommon in older patients. The overall mean age at diagnosis for all age groups is about 13 years with most patients (77.4 %) presenting before 19 years of age [12]. In adulthood, medulloblastomas arise within 20–40 years. It is rare in patients older than 50 years of age, and the oldest patient on record was 73 years old at the time of diagnosis [13]. In the present study, the age of the patients ranged from 3 to 37 years. Thirty patients were children ranging from 3 to 18 years with mean 8.07±3.67, while 17 patients were adult ranging from 19 to 37 years with mean 26.41±5.71. Out of the 47 patients, 24 were males and 23 were females; which agrees with other reports in the literature [14].

The survivin gene is located on chromosome 17q25. Its expression is highly cell cycle-regulated and is detectable in the nucleus selectively at the G2/M-phase [15]. Survivin, thus, appears to play a significant role in regulating apoptosis at cell cycle checkpoints. Survivin controls a checkpoint associated with chromosome segregation and cell division. Specifically, survivin inhibits apoptosis via its BIR domain by either directly or indirectly interfering with the function of the caspases, which are responsible for inducing apoptosis [16].

Survivin protein is over-expressed in a variety of human cancers, including but not limited to carcinomas of pancreas, lung, breast, colon, ovaries, liver, malignant melanoma, leukemia, and many CNS tumors such as malignant gliomas, medulloblastoma, neuroblastoma, and neurofibromas. The expression of survivin is found to be highly tumour specific, with only minimum expression detected in normal cells [3, 5, 15, 17].

Out of the 47 studied cases, immunohistochemical staining of survivin was positive in 26 tumors (55.32 %) as assessed by nuclear localisation with variable cytoplasmic expression. survivin-positive cells were either scattered or

Table 3 Relation between Survivin expression and progression free survival

	No	Progression free survival in months			*P*
		Mean	Median	Range	
Negative	21	56.80±13.73	58	60–80	<0.001 ($P<0.05$)
Positive≤25 %	9	56.33±7.31	57	60–66	
Positive 25–50 %	7	44.28±13.94	47	23–60	
Positive≥50 %	10	13±15.03	11	10–23	
Total	47	45.53±20.81	51	10–80	

clustered in small islands. Previous studies documented wide range variability in survivin positive medulloblastomas; Pizem et al. [18] analysed survivin expression in 56 medulloblastoma cases in different ages and different morphological subtypes, all their cases expressed survivin. on the other hand, more recent study done by Faccion et al. [19] on 41 tumor samples, survivin nuclear expression ranged from completely absent to fully present in 19 out of 41. The variability in survivin positivity in different studies could be explained by combination between the heterogeneity of medulloblastomas and the relatively small number of cases of each independent study.

Li et al. [4] mentioned in their results that the majority of the survivin-expressing medulloblastoma cells showed nuclear staining of medium intensity while Immuno-reactivities of cytoplasmic survivin were generally weak and varied from 5–10 % to 40–50 % of cells. Related to the current work the percentage of positive cells ranged from 5 % to a maximum of 65 %. Fangusaro et al. [6] reported nearby figures as the percentage of positive cells in their study ranged from 5 % to a maximum of 40 %.

In our study, the percentage of Survivin-positive cells within each sample had no significant association with age or sex of the patient. The same finding was observed in the study done by Fangusaro et al. [6].

The expression levels of survivin and its isoform were variable in different histopathological subtypes of medulloblastoma [7]. Fangusaro et al. [6] and Li et al. [7] observed that the percentage of survivin positive cells was higher in the large-cell-anaplastic subtype vs the classic subtype. In the current work, the large-cell-anaplastic subtype showed the highest overall percentage of positive cells (65 %). The studied desmoplastic subtype had a specific staining pattern similar to that described by Li et al. [7] where the internodular (poorly differentiated) areas had higher percentages of survivin staining when compared with the intra-nodular (highly differentiated) regions. However, the association between percentage of survivin positive cells and pathologic subtypes did not reach a statistically significant level in this study, which may be due to that most of the cases were of the classic type in relation to limited number of the other types. A significant relation of survivin expression with risk stratification (p=0.005) where 85.7 % of high risk group overexpressed survivin versus 42.4 % of standard risk cases.

Medulloblastoma is a malignant neuroepithelial with grim prognosis. The multidisciplinary management, together with the improvements in neurosurgical techniques and perioperative care and the use of neuroimaging, is responsible for the improved survival rate [19, 20]. Fangusaro et al. [6] detected 5 –year overall survival of 82 % for his 40 patients of medulloblastoma. Comparatively, in our study, the 5 –year overall survival of the whole studied cases was 70.21 %. we found a significant association of survivin expression with survival, where negative expression was associated with prolonged overall (OS) (p=0.002) and progression free survival (PFS) (p<0.001),mean OS (65.71±14.77), mean PFS (56.80±13.73), while positive expression was associated with shortened survival. In accordance, previous studies done on variable number of medulloblastoma cases reported that high survivin expression is related to unfavorable clinical outcome and overall survival [6, 18, 20, 21]. On the other hand, survivin expression and subcellular localization did not correlate with survival or metastasis status as studied by Faccion et al. in pediatric medulloblastoma [19].

The percentage of survivin–positive cells affected significantly survival of the studied cases, where low positive expression ≤25 % expressed longer OS and PFS (66.12± 3.86, 56.33±7.31 m) respectively in comparison to high positive expression > 50 %, which showed shorter OS and PFS (26.30±11.70, 13±15.03) respectively, which is similar to the previous study of Fangusaro et al. [6].

Survivin as expressed in medulloblastomas could be of further use in the grading of malignancy, but such grading will require the use of many more markers. Survivin serves as a prognostic marker that can possibly be incorporated in therapy options, which is indicative of its importance, thus, regarded as a useful tool in prognosis and in possible immunotherapy options [6, 22].

In conclusion, immunohistochemical staining of the medulloblastoma and its variants with survivin antibody is a very useful tool in differentiating the aggressive subtypes. Survivin positive cells correlate with the clinical outcome with poor prognosis and could be a potential predictive factor for recurrence or metastasis. suvivin may be a potential target for specific therapeutic interventions in the future.

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