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ORIGINAL ARTICLE

Prognostic value of survivin expression in Wilms tumor

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Summary

Purpose: *To determine survivin expression patterns in Wilms tumor (WT) and compare it with the expression in normal renal tissue. Also, to analyse cytoplasmic and nuclear survivin expression in relation to histological type, prognostic group and tumor stage.*

Methods: *Immunohistochemical expression of survivin was analysed in 59 cases of primary WT and in 10 normal kidney specimens, taken from the same patients, but distant from the tumor.*

Results: *51 out of 59 cases of WT (86.44%) showed decreased cytoplasmic survivin expression and 4 out of 59 cases of WT (6.78%) showed nuclear overexpression of survivin. There was statistically significant difference in the frequency of decreased cytoplasmic expression of survivin in individual components of WT (p=0.005). Decreased cytoplasmic expression of survivin in epithelial, blastemal and stromal component was found significantly more often in low stage WT compared to high stage WT (Fisher exact test, p=0.0002, p=0.002, p=0.002, respectively). There was no statistically significant difference in the frequency of survivin nuclear overexpression between different stages of WT (Fisher exact test, p=0.564), histological types (Fisher exact test, p=0.915), or between different prognostic groups (Fisher exact test, p=1).*

Conclusion: *Decreased survivin cytoplasmic expression or nuclear overexpression may be related to favorable prognosis of WT.*

Key words: apoptosis, expression, prognostic value, survivin, Wilms tumor

Introduction

Apoptosis has an essential role in the normal development of tissues and is frequently impaired in human malignancies. Survivin, a bifunctional protein that regulates cell division and suppresses apoptosis, may play an important role in tumorigenesis. Survivin is expressed in cell cycle-regulated manner, with a peak in the G2/M phase of the cell cycle, when it is associated with the microtubules of the mitotic spindles, and a rapid downregulation in the G1 phase [1]. Polymorphism of the survivin gene (17q25,-31G/C) seems to be associated with overexpression of survivin at both mRNA and protein levels. Mutation of the survivin gene leads to changes in cell cycle-dependent transcription through the functional disruption of binding at the CDE (cell cycle dependent elements)/CHR (cell cycle homology regions) repressor motifs in a number of cancer cell lines [2].

Survivin is primarily expressed in embryonic cells and only to very low extent in differentiated normal adult cells of any organ [3]. However, it is highly expressed in a wide range of cancer tissues [4] and thus may represent one of few "universal" tumor antigens.

WT, an embryonic kidney-derived tumor, is one of the most common solid malignancies of childhood, usually presenting between the ages of 3 and 6 years old [5-7]. It is highly responsive to chemotherapy and affected children usually have a good prognosis, with a reported 5-year survival rate of more than 80% [8].

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The aim of this study was to determine survivin expression patterns in WT and to compare them with the expression in normal renal tissue. We also analysed the cytoplasmic and nuclear survivin expression in relation to histological type, prognostic group and tumor stage.

Methods

The study was approved by the Ethics Committee of Belgrade School of Medicine, and was carried on 59 children with WT. All tumor specimens investigated were obtained from the archives of the Institute of Pathology, School of Medicine, University of Belgrade. There were 35 (59%) female and 24 (41%) male patients, and the mean age at the time of surgery was 52.24 months (range 1-132). The revised SIOP working classification of renal tumors of childhood [9] was used to determine tumor stage, histological type, and prognostic group. Accordingly, 23 (39%) cases were classified as WT stage I, 21 (36%) as stage II, 12 (20%) as stage III and 3 (5%) as stage IV. Three cases (5%) of bilateral WT were also analysed. Four out of 59 cases (6.8%) had epithelial histological type, 12 had blastemal (20.3%), 8 stromal (13.6%), 20 mixed (33.9%), 9 regressive histological type (15.3%), 3 cases of WT had focal anaplasia (5.1%) and 3 cases of WT had diffuse anaplasia. Forty-three children (75%) were classified as intermediate risk (IR) group and 16 (25%) as high risk (HR) group.

Immunohistochemistry

Rabbit polyclonal antibody to survivin (RB-9245-P1), purchased from Lab Vision (Lövåsen, Sweden), was used. Sections (5-μ thick) from formalin-fixed, paraffin-embedded tissue samples were deparaffinized and treated with 3% hydrogen peroxide for 15 min to block endogenous peroxidase activity. For the heat-induced antigen retrieval, tissue sections were immersed in 0.01 mol/L citrate buffer (pH=6.0) and treated in a microwave oven for 20 min at 620 W. After cooling off for 30 min at room temperature, blocking peptide (DAKO, Glostrup, Denmark) was utilized to block the non-specific staining and primary antibody, diluted 1:20, was applied overnight at 4° C. Streptavidin-biotin technique using DAKO's LSAB+kit (DAKO Cytomation, Glostrup, Denmark) was applied, with diaminobenzidine (DAB) as the chromogen solution and Mayer's hematoxylin for the counterstain. Prostatic carcinoma tissue was included in every staining procedure as a positive control for survivin, whereas incubation with the pure antibody dilutent (without the primary antibody) served as a negative control. The results of immunohistochemical staining were scored by semiquantitative technique for positive staining: absence of staining in all tumor cells (negative staining: –); positive staining involving less than 10% of cells (focal expression: +), 10-50% positive cells (moderate expression: ++), and more than 50% positive cells (diffuse expression: +++). For statistical analyses, cases with no or focal expression and those with moderate or diffuse expression were grouped together in 2 groups. The cases with moderate and diffuse expression were regarded to have survivin overexpression. According to tumor stage, all cases were divided into 2 groups: cases of WT stage I and II as one group (low-stage tumors) and cases of WT stage III and IV as another one (high-stage tumors).

Statistical analysis

For data processing the statistical package R (version 2.8.1 [2008-12-22] Copyright (C) 2008, The R Foundation for Statistical Computing ISBN 3-900051-07-0) was used. For testing the differences between parameters, Pearson x^2 test, Fisher exact test and Wilcoxon rank sum test were used, and a p value <0.05 was considered statistically significant.

Results

In normal renal parenchyma adjacent to the tumor, diffuse cytoplasmic expression of survivin was found in epithelial cells of proximal and distal convoluted tubules (Figure 1A). For that reason, negative and focal expression in WT was considered as decreased expression. Nuclear survivin expression was found in endothelial cells of capillary loops of the glomeruli (Figure 1A). For that reason, moderate and diffuse expression in WT was considered as overexpression. Cytoplasmic survivin expression in WT is presented in Table 1, and nuclear expression in Table 2.

Total expression (in all components of WT) of survivin

Cytoplasmic expression

Fifty-one out of 59 cases (86.44%) of WT showed decreased cytoplasmic survivin expression. In 40/44 (90.91%) cases of low-stage WT and in 11/15 (73.33%) cases of high-stage WT we detected decreased expression of survivin, but without statistical significance (Fisher exact test, p=0.184). Decreased survivin expression was found more often in mixed WT (Figure 1B), but the difference between this and other histological types was not statistically significant (Fisher exact test, p=0.593). In 39/43 (90.7%) cases of IR tumors and in 12/16 (70%) cases of HR tumors we detected decreased surviving expression, but without significant difference (Fisher exact test, p=0.194).

Nuclear expression

Four out of 59 cases (6.78%) of WT showed nuclear overexpression of survivin. These 4 cases were classified as low-stage WT (Figure 1C), while none of the high-stage WT showed survivin nuclear overexpression (Fisher exact test, p=0.564). There was no statistically significant difference in the frequency of survivin nuclear overexpression between different histological types of WT (Fisher exact test, p=0.915) or between different prognostic groups (Fisher exact test, p=1).

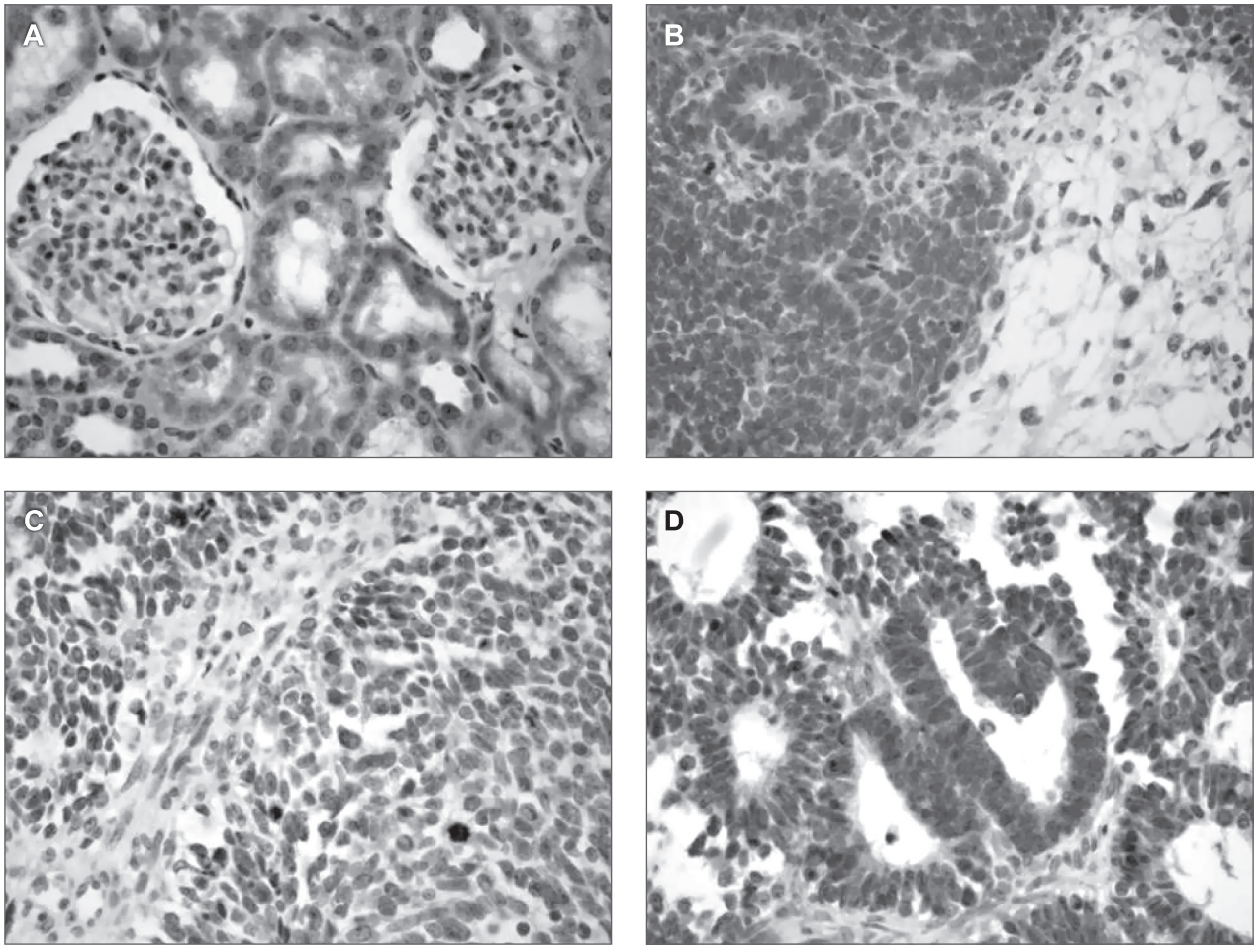

Figure 1. A: Diffuse positive cytoplasmic staining in proximal and distal tubules of normal kidney tissue. Focal positive nuclei in endothelial cells of capillary loops of the glomeruli. **B:** Moderate survivin cytoplasmic expression in epithelial and blastemal components of mixed type WT. **C:** Moderate survivin nuclear expression in blastemal component. **D:** Focal cytoplasmic staining in epithelial component (streptavidin-biotin; original magnification A, B, C, D ×400).

Table 1. Clinico-morphological features of Wilms tumor and decreased survivin cytoplasmic expression (total expression, as well as expression in individual components of Wilms tumor)

		Survivin expression							
	N	*TE* *N (%)*	*p-value*	*EE* *N (%)*	*p-value*	*EB* *N (%)*	*p-value*	*ES* *N (%)*	*p-value*
Tumor stage									
I/II	44	40/44 (90.91)	0.184	31/44 (70.45)	0.0002	27/44 (61.36)	0.002	39/44 (88.64)	0.002
III/IV	15	11/15 (73.33)		3/15 (20)		2/15 (13.33)		8/15 (53.33)	
Prognostic group									
Intermediate risk	43	39/43 (90.7)	0.194	29/43 (67.44)	0.026	22/43 (51.16)	0.557	36/43 (83.72)	0.089
High risk	16	12/16 (75)		5/16 (31.25)		7/16 (43.75)		11/16 (68.75)	
Histological type									
Blastemal	12	9/12 (75)		4/12 (33.33)		5/12 (8.47)		8/12 (66.67)	
Diffuse anaplasia	3	2/3 (66.67)		0/3 (0)		0/3 (0)		1/3 (33.33)	
Epithelial	4	4/4 (100)	0.593	3/4 (75)	0.063	3/4 (3.08)	0.291	3/4 (75)	0.218
Focal anaplasia	3	3/3 (100)		3/3 (100)		2/3 (3.39)		3/3 (100)	
Mixed	20	17/20 (85)		14/20 (70)		9/20 (15.25)		17/20 (85)	
Regressive	9	9/9 (100)		4/9 (44.44)		6/9 (10.17)		8/9 (88.89)	
Stromal	8	7/8 (87.5)		6/8 (75)		4/8 (6.78)		7/8 (87.5)	

The difference in epithelial expression (EE), blastemal expression (EB), and stromal expression (ES) between low and high tumor stage was significant at $p<0.01$; TE: expression in all components of Wilms tumor

Table 2. Clinico-morphological features of Wilms tumor and nuclear survivin overexpression (total expression, as well as expression in individual components of Wilms tumor)

		Survivin expression							
	N	*TE* *N (%)*	*p-value*	*EE* *N (%)*	*p-value*	*EB* *N (%)*	*p-value*	*ES* *N (%)*	*p-value*
Tumor stage									
I/II	44	4/44 (9.09)	0.564	1/44 (2.27)	1.000	4 /44 (9.09)	1.000	2/44 (4.55)	1.000
III/IV	15	0/15 (0)		0/15 (0)		1/15 (6.67)		0/15 (0)	
Prognostic group									
Intermediate risk	43	3/43 (6.98)	1.000	1/43 (2.33)	1.000	3/43 (6.98)	0.609	1/43 (2.33)	0.486
High risk	16	1/16 (6.25)		0/16 (0)		2/16 (12.5)		1/16 (6.25)	
Histological type									
Blastemal	12	1/12 (8.33)		0/12 (0)		1/12 (8.337)		1/12 (8.33)	
Diffuse anaplasia	3	0/3 (0)		0/3 (0)		1/3 (33.33)		0/3 (0)	
Epithelial	4	0/4 (0)		0/4 (0)		0/4 (0)		0/4 (0)	
Focal anaplasia	3	0/3 (0)	0.915	0/3 (0)	0.453	0/3 (0)	0.650	0/3 (0)	0.650
Mixed	20	1/20 (5)		0/20 (0)		1/20 (5)		1/20 (5)	
Regressive	9	1/9 (11.11)		1/9 (11.11)		1/9 (11.11)		0/9 (0)	
Stromal	8	1/8 (12.5)		0/8 (0)		1/8 (12.5)		0/8 (0)	

For abbreviations see footnote of Table 1

Survivin expression in individual components of WT

Cytoplasmic expression of survivin

There was statistically significant difference in the frequency of decreased cytoplasmic expression of survivin in individual components of WT (p=0.005). Decreased cytoplasmic expression of survivin in epithelial, blastemal and stromal component was found significantly more often in low-stage WT (Figure 1D) compared to high-stage WT (Fisher exact test, p=0.0002, p=0.002, p=0.002, respectively). Mixed WT more often showed decreased survivin expression in all individual components compared to other histological types, but without statistical significance (Fisher exact test, p=0.063, p=0.291, p=0.218, respectively). The epithelial component of IR tumors showed significantly more often decreased survivin expression compared to the epithelial component of HR tumors (67.44 vs. 31.25%; Pearson x^2 test: p= 0.026). Also, we found more often decreased survivin expression in blastemal and stromal component of IR WT compared with the same components of HR WT, but this difference did not reach statistical significance (51.16 vs. 43.75%; Pearson x^2 test: p= 0.557; 83.72 vs. 68.75%; Pearson x^2 test: p= 0.089).

Nuclear expression of survivin

We did not observe significant difference in the frequency of survivin nuclear overexpression among the analysed components of WT. Epithelial, blastemal and stromal component in low-stage WT (2.27, 9.09 and 4.55%, respectively) more often overexpressed survivin compared to high-stage WT (0, 6.67 and 0%, respectively), but without statistical significance (Fisher exact test, p=1, p=1 and p=1, respectively). Regressive WT more often showed nuclear overexpression of survivin in the epithelial component (1/9; 11.11%) compared to other histological types of WT, but without statistical significance (Fisher exact test, p=0.453). We observed that all histological types showed nuclear overexpression of survivin in the blastemal and stromal component with similar frequency (Fisher exact test, p=0.65 and p=0.65, respectively).

Only one out of 43 cases (2.33%) of the IR group of WT showed nuclear overexpression of survivin in the epithelial component, and none of 16 HR cases (Fisher exact test, p=1). High risk WT more often showed nuclear overexpression of survivin in the blastemal and stromal component (2/16; 12.5%; 1/16; 6.25%, respectively) compared to IR tumors (3/43; 6.98%; 1/43; 2.33%, respectively), but without statistical significance (Fisher exact test, p=0.609 and p=0.486 respectively).

Discussion

Survivin upregulation in a number of cancers suggests that apoptosis-related genes play an important role in tumor formation or progression [10]. The molecular and cellular mechanisms that explain survivin deregulation in cancer have been intensely investigated. These include the amplification of the survivin locus at 17q25-17q in neuroblastoma [11], the demethylation of survivin exon 1 in ovarian cancer [12], the transcriptional

repression of survivin by p53 [13] and the deregulation of the WNT/TCF signaling pathway [14]. In WT, survivin mRNA was present at markedly greater levels in tumors than in normal renal tissue, but without association with tumor stage or risk for tumor recurrence [15]. Radojevic-Skodric et al. showed strong association between the survivin gene promoter -31G/C polymorphism and risk for development of WT (submitted for publication).

Normal renal tissue analysed in the present study showed diffuse cytoplasmic and focal nuclear survivin expression, which is in agreement with the well known fact that all immature tissues, including kidney, express high levels of survivin. In our study, 51 out of 59 WT (86.44%) showed decreased survivin cytoplasmic expression, defined as decreased level of survivin compared to the normal renal tissue. Also, 4 out of 59 cases of WT (6.78%) showed nuclear overexpression of survivin, defined as increased level of survivin compared to the normal renal tissue. Survivin aberrant expression is found in a wide variety of human tumors, such as hepatocellular carcinoma [16], esophageal squamous cell carcinoma [17], ovarian serous cancer [18], gastric carcinoma [19], bladder mucosa and transitional cell carcinoma [20,21], pediatric ependymomas and choroid plexus tumors [22], endometrial carcinoma [23] etc.

Decreased survivin cytoplasmic expression was found more frequently in low-stage WT compared to high-stage WT, but without significance (p=0.184). However, when analysing separately each component of the tumor, we detected statistically significant difference in the frequency of decreased cytoplasmic expression between low and high-stages WT in epithelial, stromal and blastemal component (p=0.005). Previous studies reported no association between survivin cytoplasmic expression and stages of urothelial bladder cancer [20] and gastric carcinoma [19]. Nuclear overexpression of survivin was increased in low-stage (p=0.564) of WT. The stage of WT is a well known prognostic factor, therefore correlation between the stage of WT and survivin expression suggests that the decreased survivin cytoplasmic expression or nuclear overexpression may be related to favorable prognosis of WT.

There was no statistically significant difference in survivin expression (total expression as well as expression in individual components of WT) between the analysed histological types of WT. However, decreased survivin cytoplasmic expression (total expression as well as expression in individual components of WT) was observed most often in mixed WT, although without statistical significance. Regressive WT more often showed nuclear overexpression of survivin in the epithelial component compared to other histological types of WT, but without statistical significance (Fisher exact test, p=0.453). The anaplastic type of WT is rare, and in comparison to the classical, tricomponent type, it gives metastases more often, is resistant to therapy, and has a very bad prognosis. We detected absence of survivin nuclear expression in all samples of anaplastic WT. This result is in agreement with the results of other studies, which showed correlation between nuclear survivin expression and well differentiated tumors [19,22]. Also, in ovarian tumors, nuclear localization of survivin is more common in benign or borderline tumors than in malignant serous tumors of the ovary [18]. In the studies of pediatric ependymomas and choroid plexus tumors, it was shown that a strikingly high level of survivin expression was present within normal ependyma and choroid plexus. Analysis of the corresponding neoplastic tissue in pediatric ependymomas and choroid plexus tumors showed that nuclear expression of survivin correlated with morphologic (low) tumor grade, and loss of nuclear expression of survivin was associated with more anaplasia [22].

In our study we found decreased cytoplasmic survivin expression or nuclear overexpression more often in IR prognostic group than in HR (blastemal type of WT after receiving chemotherapy and WT with diffuse anaplasia) prognostic group, but the observed difference was not statistically significant. These results suggest that decreased cytoplasmic survivin expression or nuclear overexpression was associated with favorable prognosis in WT. Other studies also reported nuclear survivin overexpression in different tumors with favorable prognosis, such as gastric carcinoma [19] or pediatric ependymomas and choroid plexus tumors [22].

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