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Examination of Expression of WT1 Gene Product and CD44 Adhesive Molecule in Nephroblastoma Histologic Types

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In spite of success of modern pediatric oncology, cases in which we are not able to reach the prospective affirmative effect of performed therapy are still observed. The purpose of our study was to examine the expression of WT1 gene product and CD44 adhesive molecule in nephroblastoma histologic types - one of the currently used prognostic marker for this group of tumor. We found correlations between CD44 expression and histologic type of tumor. We suppose that high CD44 expression in nephroblastoma group of tumors may confirm their high malignant potential. Expression of the WT1 gene product we found in all the investigated tumor tissue samples. However we did not found statistically significant correlations between WT1 expression and histologic type of the tumor and there was no correlation between CD44 and WT1 expression in blastemal nor of epithelial component of nephroblastoma in our study. Lack of this correlation also permits to suppose that the high activity is an integral feature of all Wilms tumor cells and is not only characteristic for anaplastic and blastemal nephroblastomas.

Introduction

A group of solid tumors which embraces about 50% of childhood malignancies deserves special attention. There is observed that the frequency of solid tumors in children increases (0,9% per year). From all the solid tumors - this so numerous and various group, we chose nephroblastoma (Wilms' tumor) for our study. Due to many recognized biological factors included in the pathogenesis of nephroblastoma and with the regard of widely known molecular analysis of this tumor, we found nephroblastoma the base of studies on malignant neoplasms of childhood. Current histologic nephroblastoma classification is of the essential practical value and at present histologic type of the tumor is the main prognostic factor. Seeking new prognostic markers we investigated the expression of WT1 gene product and CD44 adhesive particle in nephroblastoma. According the literature those two markers play different part in the pathogenesis of human neoplasms. WT1 is the protein expressed in malignancies and either during development of many organs, and in numerous normal tissues [4, 10, 13, 25]. CD44 is one of adhesive molecules which expression first of all is connected with the ability of dissemination of tumoral cells and progression of neoplastic disease. At the beginning of seventieth the first molecular model of nephroblastoma was proposed. Research let to identify loci of numerous genes of involved into the pathogenesis of this neoplasm [19, 21, 22]. Nowadays it is widely accepted that four genes play the special part in the pathogenesis of Wilms' tumor: the WT1 gene (11p13), the WT2 gene (11p15) and the two connected genes with the development of family form of nephroblastoma FWT1 (17q12-q21) and FWT2 (19q13) [23]. WT1 gene became cloned and its mutations observed in 10-15% of Wilms' tumors were described. Essential part in the development of nephroblastoma play suppressor genes localized on chromosomes: 1, 16 and 17. The WT1 gene is one of the suppressor genes and it is composed of 10 of exons and embraces 50 kb. WT1 expression is observed during the development of kidney, spleen and of

mesodermal components of gonads and in mesothelial cells. Product of WT1 gene is also found in many adult tissues and organs eg: pleura, spleen, heart, endometrium. Except normal tissues WT1 protein is observed in many neoplastic tissues (nephroblastoma, mesothelioma, desmoplastic round blue cell tumors, prostate cancer and leukemia). In Wilms' tumors the strong expression WT1 is observed in cells of epithelial and blastemal component and week in stroma [1, 3, 5]. The CD44 expression is observed on the surface of various cells, mostly monocytes, granulocytes, erythrocytes, lymphocytes B and mature lymphocytes T, but epithelial cells, fibroblasts and oncocytes, either. CD44 protein plays an essential part in interactions between cells, and also between cells and intercellular matrix. The most important CD44 ligand is hyaluronic acid (the main component of the extra cellular matter), but the small CD44 part interacts with fibronektyn and collagen either. CD44 is included in the process of hematopoesis, activation of lymphocytes, embryonic development, intercellular transfer of signals, progressions of neoplasms and inflammations [9, 11, 14, 15, 27]. The CD44 gene consists of 21 of exons from which usually only 10 is subject the expression. The process of the alternative RNA splicing and modifications of CD44 protein lead to formation of variants with different adhesive proprieties. Till now 30 CD44 isoforms were identified [26].

Materials and Methods

42 formalin-fixed and paraffin-embedded nephroblastoma tissue sections from the files of the Department of Pathology of the Age of Development and Department of Pathology Konopnicka Memorial Hospital Medical University of Lodz were selected for our study. From these tissues samples paraffin blocks about the thickness 3-4 of micrometers where prepared and stained with hematoxylin and eosin (HE). For the purpose of our study all of the previously diagnosed tumors became reclassified according current criteria for this group (based on SIOP Classification of Renal Tumors of Childhood).

We performed immunohistochemical stains with commercial mouse antibodies:

1. Anti-Human Wilms Tumor 1 (WT1) protein, 6F-H2 (DAKO)

2. CD44 (H-CAM) , F10-44-2 (Novocastra), and with use of immunoperoxidase reaction according to Hsu (EnVision+the System, Peroxidase - DAB (DAKO).

Positive reactions were accepted as:

In research of the expression CD44 we accepted cytoplasmic type of the reaction - the brown color of the cytoplasm of neoplastic cells and we rated CD44 expression as: weak (less than 10% of positive tumor cells), of intermediate degree (from 10% to 60%) or strong (more than 60%). In research of the expression WT1 - the nuclear type of the reaction - the brown color of the nucleus of tumor cells. The expression WT1 was counted as the index (% of positive for the investigated antigen tumor cells). In every case 1000 of tumor cells were counted. The average, median, and standard deviation were calculated.

The estimation of the expression of investigated proteins were examined with computer image analysis system (Multi Scan Base v. 8.08 - Computer Scanning System, Ltd.). All examined microscopic pictures (Nikon Microphot FXA) were transferred to the computer by camera (CC2OP).

For the analysis we used the statistical pack SYSTAT for Windows (Version 5.03, SYSTAT, Inc, Evaston, Illinois, USA , the license No: DA021594).

Results

According to SIOP 2001 Classification we diagnosed: 3 low risk, 19 intermediate risk and 20 high risk nephroblastomas. In two cases Wilms' tumors with diffuse anaplasia was diagnosed. The subgroups appeared as follow: mesoblastic nephroma - 1 case; cystic partially differentiated neproblastoma - 2 cases; nephroblastoma epithelial type - 13 cases; nephroblastoma stromal type - 2 cases; nephroblastoma mixed type – 4 cases; nephroblastoma blastemal type- 18 cases; nephroblastoma diffuse anaplasia - 2 cases. Details of the histologic and immunohistochemical examination represents Table 1.

Results of research of immunohistochemical expression of the WT1 gene product

Expression of the WT1 gene product we found in all investigated tumor tissue samples.

Index of WT1 expression WT1 in cells of blastemal component was from 32 to 97 (the average 78.52; standard deviation 18.46, median 88). In 13 cases indexes were equal or higher than 90. Expression limited to single cells was never observed. Index of WT1 expression in cells of epithelial component was from 2 to 95 (the average 73.21; standard deviation 31.06, median 85). In most of the cases -12 index was equal or higher than 70. In remaining 2 cases of this group we observed WT1 in single tumor.

The coexistence of blastemal and of epithelial component we noticed in 11 cases. We observed differences

TABLE 1
The histologic classification and the expression of examined protein in nephroblastoma

N°	SIOP 2001	WT1 expression (percentage of positive cells)		CD44 expression
		Blastemal component	Epithelial component	
1	IIIA	93	94	0
2	IIB	81	3	0
3	IIA	97	85	W
4	IIIB	89	89	W
5	IIIB	90	x	M
6	IIIA	95	95	M
7	IIIA	60	74	0
8	IIA	80	73	M
9	IB	69	x	M
10	IB	92	x	x
11	IIIA	90	x	0
12	IIIA	56	x	0
13	IIIA	95	x	S
14	IIIA	62	x	0
15	IIC	84	x	0
16	IIA	x	95	0
17	IIIA	92	x	0
18	IIIA	x	x	M
19	IIC	x	x	W
20	IIA	73	88	0
21	IIC	90	x	M
22	IIIA	45	x	x
23	IIA	91	x	0
24	IIC	82	x	0
25	IIIA	x	x	W
26	IIA	32	x	0
27	IIIA	x	85	0
28	IIA	x	80	0
29	IIA	34	x	W
30	IIIA	94	2	x
31	IIA	x	x	W
32	IIA	92	92	M
33	IIA	92	x	x
34	IIIA	88	x	0
35	IIB	x	x	0
36	IIIA	x	x	W
37	IIIA	x	x	M
38	IA	x	x	0
39	IIIA	70	x	0
40	IIA	69	70	0
41	IIA	x	x	M
42	IIIA	x	x	W

I – low risk nephroblastoma, II – intermediate risk, III – high risk; IA – mesoblastic nephroma, IB – cystic partially differentiated nephroblastoma, IIA – epithelial type, IIB – stromal type; IIC - mixed type; IIIA – blastemal type; IIB – nephroblastoma diffuse anaplasia, x - no tissue for examination, 0 – no expression in examined tissue sample, W – week, M – intermediate degree, S - strong

among values of WT1 indexes in two components of the same tumor from 1.06% to 97.87%. In 3 cases indexes were equal, in 4 cases higher in blastemal cells, in 4 cases higher in cells of epithelial component.

We introduced qualifications: index 'very high' (equal or higher than 90), 'high' (equal or higher than 80, but lower than 90), 'of intermediate degree' (equal or higher than 70, but lower than 80) and 'low' (lower than 70).

Results of research of the expression of the product of the gene WT1 in epithelial component

We observed WT1 expression in all the examined epithelial components.

We did not found statistically significant correlation between WT1 expression in epithelial component and degree of the histological risks. We observed very high WT1 indexes in epithelial component in two subtypes only: nephroblastoma epithelial type and nephroblastoma – blastemal type.

In the group of nephroblastoma with low indexes of the WT1 expression in epithelial component we found exclusively high or very high indexes of WT1 expression in blastemal component. In all of the cases with very high indexes of WT1 expression in epithelial component we found exclusively very high indexes WT1 expression WT1 in blastemal component. We did not found correlations between expression of the product of the gene WT1 in epithelial and blastemal components.

Results of research of the expression of the product of the gene WT1 in blastemal component

We observed WT1 expression WT1 in all the examined blastemal components.

There was no statistically significant correlation between WT1 expression and histological risk. All very high WT1 indexes in blastemal cells we observed in all histologic subtypes except mesoblastic nephroma.

Results of CD44 examination

CD44 expression was found in 18 cases (47.37%) of investigated nephroblastomas and classify as: strong - 1 case (2.63%), the intermediate degree into 9 cases (23.68%), and as weak in 8 (21.05%) (Fig. 1 and Fig. 2).

We found correlations between the expression CD44 and with the histologic type of tumor ($p=0.006$). We did not found statistically significant correlations between CD44 expression CD44 and expression WT1 in cells of blastemal nor of epithelial component of nephroblastoma.

Discussion

The treatment of nephroblastoma is based on type of the tumor and the stage of disease [2]. The retrospective analysis of prognostic factors made by SIOP showed histological type of tumor has higher prognostic value than the stage of disease in nephroblastoma group of tumor. Our research point that the estimation of WT1 and CD44 expression and its relationship with recognized prognostic factor as SIOP classification is, may show surprising results even in such well known tumors as nephroblastoma is. Among high risk nephroblastomas we observed exclusively high or very high indexes WT1 in blastemal component cells. We observed also intensification of the expression of WT1 protein in cells of tumors in the highest histological risk. We suppose that the height of WT1 expression may be a

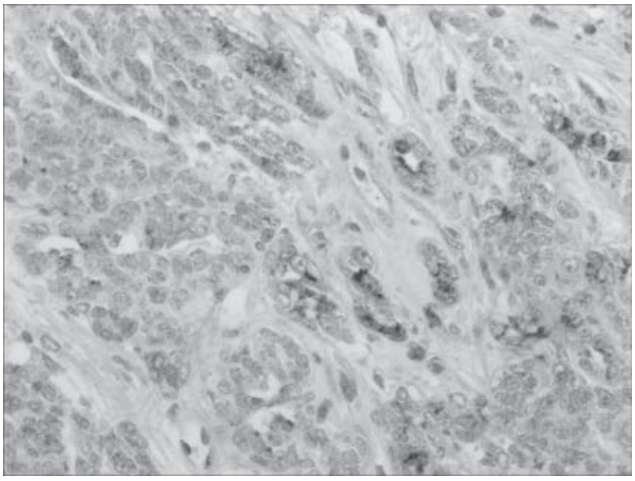

Fig. 1. CD44 expression in blastemal component of nephroblastoma, H&E. Oryg. magn. 400x.

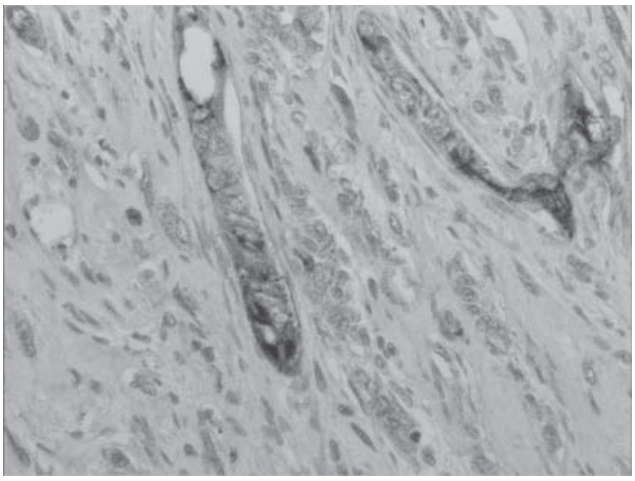

Fig. 2. CD44 expression in anaplastic Wilms' tumor, H&E. Oryg. magn. 400x.

sign of internal high activity and of the large proliferative potential of tumoral cells. Those results are also similar to other authors research in which high expression of connected with cell proliferation factors (Ki-57 and PCNA) were described in high risk nephroblastomas [18]. We believe that the expression of WT1 gene is typical for all neuroblastomas and it can be a sign of their high progression potential, however the final effect of treatment depends on other factors, e.g. resistance to chemotherapy of tumoral cells. We found also a report of the prognostic value of the expression WT1 in the literature. It was proved that the expression of this protein in cells in both component correlates with to stage of disease, but only WT1 expression in blastemal cells is an independent prognostic factor and is connected probably not with stage but with the progression of the disease. Estimation of expression of WT1 gene product seems to be promising factor, but proper interpretation of our results demands estimation of markers which are connected with the resistance of cells to performed chemotherapy. WT1 protein is only one of many elements of tumor cells biology. It regulates transcription of genes coding proliferating factors and their receptors (eg: IGF-I, IGF-II, TGFb, PDGF-I), and also takes part in modifications of their products after translation. WT1 gene product is also included into mechanisms of apoptosis, creates complexes with P53 protein, co works with p21, and one of its isoforms - WT1 (-KTS) induces apoptosis without P53 action [12, 16, 17, 24]. The presence of WT1 expression we observed in all of investigated tumors. The lack of correlation between WT1 expression in both components and the lack of correlation with the histologic subtype of tumor permits to suppose that the high activity is an integral feature of all Wilms' tumor cells and it is not only characteristic for anaplastic and blastemal nephroblastomas. Different results of treatment in nephroblastoma types in spite the same potential, can be explained by the differences in resistance of cells to chemotherapy, which is probably more important for the outcome than natural malignant potential of tumoral cells. It is necessary to remind that results of the treatment of Wilms' tumor before an introduction of chemotherapy were unsatisfactory, and clinical outcome was very poor and comparable with other the most malignant neoplasms. Our conclusions needs verification by research of other factors e.g. multidrug resistance protein as P -glycoprotein (Pgp) is. Maybe blastemal and anaplastic nephroblastoma types are composed of cells with higher chemo resistance.

Results of research of CD44 expression were also surprising according to current knowledge of this neoplasm. The presence of CD44 on the surface of tumoral cells is usually connected with the ability for dissemination. Enlarged CD44 expression was observed in many neoplasms, e.g. carcinomas of the skin, endometrium and collum of the uterus [20, 26, 28]. It was proved that CD44 expression permits tumoral cells to metastasize e.g, in stomach, colon and prostate cancers [6, 7, 8]. We found CD44 adhesion molecule in almost half of examined cases and mostly of strong or of the intermediate degree of expression. We found statistically significant correlation between CD44 expression and histologic type of tumor – the most important recognized prognostic factor in this group of tumors. We suppose that so high CD44 expression in nephroblastoma group of tumors may confirm their natural high malignant potential however demands clear interpretation and that the final estimation of the possible prognostic value of CD44 must be correlated with other prognostic factors. In spite of the interpretation of results, our research on WT1 gene product and CD44 adhesive molecule show that even well recognized tumors may surprise with unexpected aspects of their own biology.

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