Immunohistochemical Detection of Cyclin A in Wilms Tumor

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Introduction

Cyclins displays oscillatory expression during the cell cycle. They regulate the activity of CDKs, and, together with CDKs, they form holoenzymes that phosphorylate regulatory substrates like retinoblastoma proteins (pRb) and p107 (1,2). Thus far, 14 different mammalian cyclins are known, named cyclins A-J (3). Cyclins are usually grouped into G1 cyclins, such as Cyclin E, which controls the G1/S transition, and mitotic cyclins, such as Cyclin B, which is required for entry into mitosis (4).

Cyclin A is the only cyclin known to play essential role not only in mitosis, but also in DNA replication (5). This, 60 kd protein, appears to be rate-limiting for initiation of DNA replication and is specifically localized to nuclear replication foci (6, 7).

Wilms tumor, one of the most common solid malignancies in childhood, 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).

Abnormalities of the cell cycle are important in the process of carcinogenesis. Immunohistochemical determination of the expression of various cyclins and CDKs in tumor cells has recently been applied to evaluate cancer growth (9,10,11). There are few reports on Cyclin A as a marker of proliferative cell fraction in cancer (12, 13, 14, 15, 16, 17, 18).

The aim of this study was to investigate the expression of Cyclin A protein in normal kidneys as well as in Wilms tumor by immunohistochemistry and to correlate the results with tumor stage, histological type and prognostic group.

Patients and Methods

Tumor specimens used in this study were obtained from 28 patients undergoing surgery for Wilms' tumor (F:M ratio 36:20; age 7-132 months), 2 metastases from Wilms' tumor found in lungs and 5 normal kidney specimens.

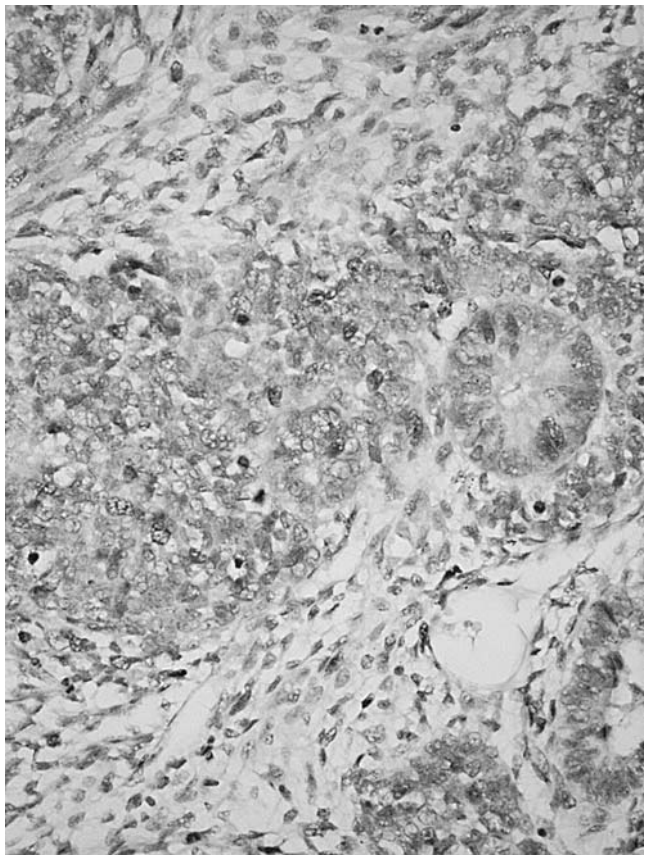
For immunohistochemistry, 5 μm-thick sections were cut from three blocks per case and following the procedure incubated with the primary polyclonal antibody against Cyclin A (H-432, Santa Cruz Biotechnology, USA). A standard peroxidase-conjugated streptavidin-biotin labeling (DAKO LSAB+ kit) was used for visualization, with 3,3diaminobenzidine as chromogen.

The results of immunohistochemical staining were scored by semiquatitive technique. Fisher's test, Mann-Whitney's and Student's T- test were used to do the statistic analysis, considering $P < 0.05$ as a significant finding.

Results

Cyclin A focal expression was found in epithelial cells of distal convoluted tubules in normal, unchanged kidneys.

Figure. 1 Diffuse expression of Cyclin A in anaplastic type of Wilms tumor.



In our group of 28 Wilms tumor cases we have detected Cyclin A expression in 12 cases (42.9%). Expression of Cyclin A was more frequent in blastemal then in epithelial component (46.43% : 32.14 %). This correlation showed no statistic significance (p=0.218). Expression of Cyclin A was more frequent in stage III and IV (71.42%) than in stage I and II (33.33%), showing significant statistic corre-

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lation (p=0.045). Intermediate risks group of Wilms tumor showed more frequent Cyclin A expression (50%) in comparison with high risks cases (30%), showing no statistic significance (p=0.434).

Table 1. Correlation between level of Cyclin A expression and stage of Wilms tumor

Level of expression Cyclin A	Stage of Wilms tumor	
	I/II	III/IV/V
- (absent)	2	0
+ (focal)	26	4
++ (moderate)	14	6
+++ (diffuse)	0	4

Cyclin A expression was detected in all histologic types of Wilms tumor but without statistic significance in intensity and distribution of expression. (p= 0.698). From four cases of diffuse anaplasia, two cases showed Cyclin A expression, but there was no statistically significant correlation (p= 0.386). Analysed 2 Wilms tumor metastases showed diffuse Cyclin A expression, as well as one case of bilateral Wilms tumor.

Discusion

We have observed that normal kidney tissue shows week and focal Cyclin A expression. This finding confirms well known fact that normal renal tissue has a low cell proliferative ability (20). In 42.9% of Wilms tumor cases we have found Cyclin A expression which was more prominant in blastemal than in epithelial component (46.43% : 32.14%), that was not of statistic signifficance (p=0.218). Expression of Cyclin A was more frequent in stage III and IV (71.42%) than in stage I and II (33.33%), showing significant statistic correlation (p=0.045). Correlation of tumor stage and Cyclin A expression corresponded to some findings published in other different examined tumors (21, 22, 23). However, in literature we have also found oposite findings (24,25,26). It is known that staging in Wilms tumor cases is accepted as a prognostic marker (the higher stage, the worse prognoses). Therefore, we have assumed that higher Cyclin A expression, detected in Wilms tumor cases, could be used as a prognostic marker of unfavourable prognosis.

In all examined cases of Wilms tumor metastases we have detected diffuse Cyclin A expression that corresponded to Cyclin A expression in primary tumor. Knowing this, we asume that increased Cyclin A expression in Wilms tumor could be a predictiv parametar for its metatsatic ability (the stronger expression, the higher risk of metastases). Such connection was demonstrated in cases of pancreatic and oral squamous cell carcinomas and their metastases (22,23).

Our finding that Cyclin A expression did not correlate to histologic type of Wilms tumor (p=0.698), corresponded to literature data denying the conection of Cyclin A expression and histologic type of renal cell carcinoma. (21).

One of the most important principles in chemotherapy is the fact that cancer cells in different phases of the cell cycle have different sensitivities to treatment procedures. By determining the different cyclins, the fractions of cells in this phases can be described. Cyclin A determines the fraction of cells in the S phase-G2 phase (27). The results of our studies showed that increased Cyclin A expression confirms that tumor cells are in S-G2 phase, and as such, they are more sensitive to chemotherapy.

Some clinical studies have shown the connection between the intensive Cyclin A expression and a good response to therapy of lung and oesophagus cancer (16,27). In our cases of Wilms tumor, there were not signifficant differences in Cyclin A expression of treated and nontreated patients (p=0.208).

Investigating Cyclin A expression in different prognostic groups of Wilms tumor, we have found that there was no statisticaly signifficant correlation between Cyclin A expression and prognostic group (p=0.434), altough we have noticed that intermediate tumorus showed more prominant Cyclin A expression in comparison to high risk group.

In conclusion, the results of our study suggest that Cyclin A may contribute to the progression of Wilms tumor: there was a significant statistic correlation between Cyclin A expression and tumor stage, as well as diffuse and strong expression in Wilms tumor metastases.

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