Immunohistochemical Detection of Cyclin A in Wilms Tumor
Institute of pathology, University of Belgrade, Belgrade

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 3,3-diaminobenzidine as chromogen.

The results of immunohistochemical staining were scored by semiquantitative 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-
Cyclin A expression was detected in all histologic types of Wilms tumor but without statistical 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). Analyzed 2 Wilms tumor metastases showed diffuse Cyclin A expression, as well as one case of bilateral Wilms tumor.

Discussion
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 prominent in blastemal than in epithelial component (46.43% : 32.14%), that was not of 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 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 more sensitive to chemotherapy.

<table>
<thead>
<tr>
<th>Level of expression Cyclin A</th>
<th>Stage of Wilms tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I/II</td>
</tr>
<tr>
<td>- (absent)</td>
<td>2</td>
</tr>
<tr>
<td>+ (focal)</td>
<td>26</td>
</tr>
<tr>
<td>++ (moderate)</td>
<td>14</td>
</tr>
<tr>
<td>+++ (diffuse)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>III/IV/V</td>
</tr>
<tr>
<td>- (absent)</td>
<td>0</td>
</tr>
<tr>
<td>+ (focal)</td>
<td>4</td>
</tr>
<tr>
<td>++ (moderate)</td>
<td>6</td>
</tr>
<tr>
<td>+++ (diffuse)</td>
<td>4</td>
</tr>
</tbody>
</table>

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.

References


