The Contribution of Histochemical and Immunohistochemical Techniques in Assessing the Proliferative Capacity and Prognosis for Renal Cell Carcinoma

DANIELA ANCA PUSCASIU[1#], GABRIELA TANASIE[2#], MARIUS GEORGESCU[2#], ROMULUS FABIAN TATU[3]*, CARMEN SOFICA TATU[2]

[1]Victor Babes University of Medicine and Pharmacy Timisoara, Microscopic Morphology Department, Cellular and Molecular Biology Discipline, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[2]Victor Babes University of Medicine and Pharmacy Timisoara, Functional Sciences Department, Physiology Discipline, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[3]Victor Babes University of Medicine and Pharmacy, Timisoara, Ortopaedics-Traumatology-Urology and Imagistic Department, Ortopaedics-Traumatology III Discipline, 2 Eftimie Muurgu Sq., 300041, Timisoara, Romania

The drive to prolong life and to improve life quality in patients suffering from cancer forms of any kind means that, aside from the necessary treatment, prognostic evaluations are needed. In our present study we included 63 patients with renal cell carcinoma. The histological samples obtained in surgery were studied through the classical histopathology methods, histochemical and immunohistochemical techniques. The histological degree of tumor differentiation was assessed for all cases, falling within all 4 classification grades. The immunohistochemical reaction for PCNA (proliferating cell nuclear antigen) was performed, utilizing the PC 10-LSAB antibody through visualization with the LSAB-DAB kit. This was the method used for assessing the process of tumor proliferation. Subsequently, the histological degree of differentiation was correlated with the PCNA score. We anticipate a reserved prognosis for patients with a positive expression on most tumor cells, which represents a high PCNA score.

Keywords: renal cell carcinoma, histochemical techniques, immunohistochemical staining, histopathological differentiation grade, PCNA score

Evaluating the prognosis for renal cell carcinoma is the aim of many present day research studies [1]. Kidney cancer accounts for 5 and 3% of all adult malignancies in men and women, respectively, thus representing the 7th most common cancer in men and the 10th most common cancer in women [2, 3, 12]. Multiple studies exist in literature, which propose different prognosis approaches to these situations [4-6]. Initially we used the classical techniques of processing [7]. By identifying the PCNA antigen using the monoclonal antibody PC10, the velocity of tumor proliferation can be investigated. When correlating PCNA score with the histopathological degree [8, 13] of differentiation through the LSAB technique, we can achieve prognostic evaluation of renal cell carcinoma.

Experimental part

Material and method

We included in our study 63 renal tumors, sampled in the Urology Clinic of the County Hospital of Timisoara, through partial and total nephrectomies. The material thus obtained was processed in the Morphopathology Laboratory of the Morphopatology Department of the University of Medicine and Pharmacy Victor Babes, Timisoara. The tissues were initially processed through the classical methods: paraffin inclusion, sectioning, hematoxylin-eosin staining, followed by histochemical methods (the PAS stain). Subsequently, immuno-histochemical methods were applied, which allowed us to assess the degree of malign proliferation by using the PCNA antigen. The stage of tumor development for our study material was evaluated using the PAS and hematoxylin-eosin stains. The renal cell carcinomas are divided into 4 differentiation grades [9], unlike the classification in 3 degrees used in the case of other types of tumor [10].

- Grade I – Uniform nuclei with a diameter of 10μ. Nucleoli are absent or their presence is uncertain.
- Grade II - 15μ nuclei with discrete contour variations. Uniform nucleoli.
- Grade III - 20μ nuclei with chromatic and contour variations. Nucleoli are evidenced at 100x magnification.
- Grade IV – nuclei larger than 20μ, pleomorphic, fusiform, chromatin is arranged as coarse granules

The immunohistochemical technique consisted of the use of the DAKO $LSAB_2$ HRP staining method which is based on a modified marking method (avidin – biotin, LAB), in which the secondary biotinylate antibody forms a complex with the peroxidase conjugated streptavidin molecules. In comparison with the usual avidin – biotin method, the LAB/LSAB method was 4 to 8 times more sensitive. The tumor proliferation process was evaluated for all cases, by performing the immunohistochemical reaction for PCNA, using the PC 10-LSAB antibody and by visualization with the LSAB-DAB kit (both reagents from the DAKO firm). The identification of the PCNA expression was done with the monoclonal mouse antibody anti-PCNA, PC 10 clone, isotope IgG_{2a} Kappa DAKO. The anti-PCNA antibody has potential in the analysis of the kinetics of tumor cells and plays a major role in assessing the proliferative process of the neoplasm, which makes detected PCNA useful as an operational marker for tumor growth and implicitly for prognosis. Subsequently to applying PCNA immunocoloration, all samples were examined at high magnification [14, 15]. From each tumor 1000 nuclei were studied, of which the positive ones were

* email: fabitatu@gmail.com; Phone: +40722202167

Authors contributed equally to the article

selected and their percentage determined. The PC 10 index was evaluated by reporting the number of cells with a certified positive nuclear expression, to the total number of cells examined.

Results and discussions

The biological material was sampled from 63 cases of renal cell carcinoma. Of the total 63 study patients, 44 were male and 19 female. The ages of patients were between 44 and 78 years old.

From a histopathological point of view, of the 63 renal cell tumors, 36 cases presented clear cells (making up 57.14%), 3 cases presented granular cells (making up 4.76%) and 24 mixed tumors with both clear and granular cells in varying proportions (making up 38.09%).

The distribution of cases depending on differentiation grades was the following: 3 cases were G_1 (4.76%), (fig. 1), most cases, 39 tumors, were G_2 (61.90%), (fig. 2), 19 cases G_3 (30.15%), (fig. 3) and only 2 cases G_4, 3.17% (table 1).

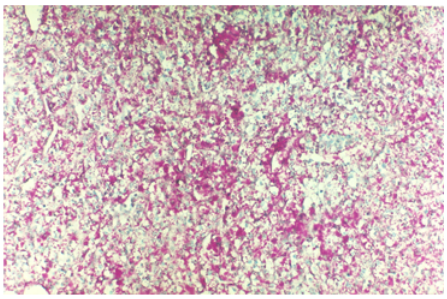

Fig. 1. Solid renal cell carcinoma with a high content of glycogen, grade 1, PAS, x200

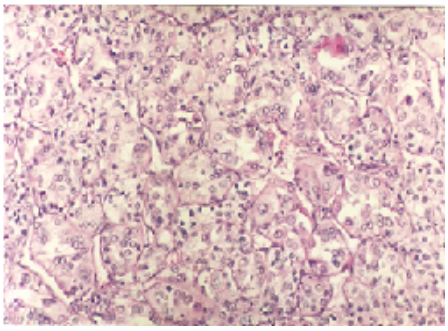

Fig. 2. Solid renal cell carcinoma, grade 2, with anisocoria and vesicular nuclei, HE, x200

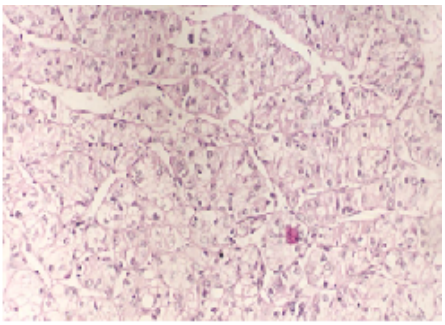

Fig. 3. Renal cell carcinoma showing trabecular pattern, grade 3, with spaces delineated by

PCNA immunoreactivity was divided into 3 scoring grades depending on the percentage of positively expressed nuclei, thus:

- the first group was considered to have low PCNA score. The growth expression fraction was low, with a maximum of 25% of tumor cells positive;
- the second group hadmedium values. In this category we classified tumors with between 25-75% positively expressed PCNA cells;
- the third group included tumors with over 75% positively expressed PCNA cells. These were evaluated as having a high score.

The results obtained by correlating histological differentiation degree with the PCNA score are presented in table 2.

The results in paranthesis marked with * represent the percentage of cells with a positive PCNA expression. By studying the correlation between the histological degree of differentiation and the PCNA score, we identified a low score expression in the G_1 and G_2 differentiated forms (fig. 4). The medium score was present in the 2 intermediate grades G_2 and G_3 (fig.5). The incidence of the high score was the most common, occurring in G_2, G_3, G_4. Thus, we can identify, in all of these differentiation grades, a high rate of growth (fig. 6).

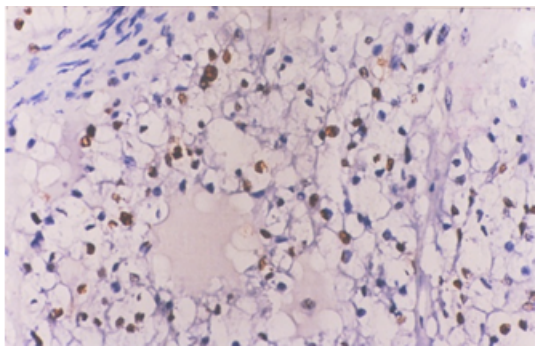

Fig. 4. Low PCNA score, in a differentiated form of renal cell carcinoma. PCNA antigenLSAB$_2$ DAB, x400

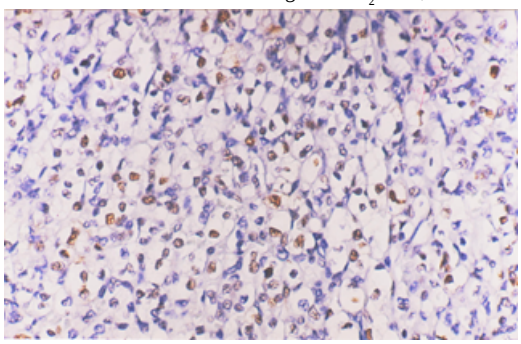

Fig. 5. PCNA expression with medium score in a grade 2 renal cell carcinoma. PCNA antigen LSAB2 DAB, x400

	G_1	G_2	G_3	G_4
Number of cases	3	39	19	2
Percentage	4.76%	61.90%	30.15%	3.17%

Table 1

PCNAScore	Number and % of cases	Histological grade			
		G_1	G_2	G_3	G_4
Low (0-25%)	8 (12.69%)	3 (9%)*	5 (17%)*		
Intermediate (25-75%)	36 (57.14%)		19 (31%)*	17 (53%)*	
High (75-100%)	19 (30.15%)		15 (75%)*	2 (78%)*	2 (97%)*
Total	63	3	39	19	2

Table 2

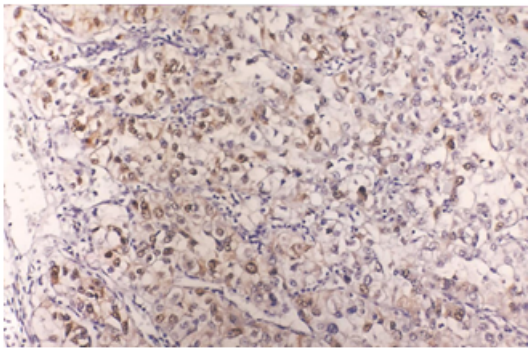

Fig. 6. PCNA antigen expression with high score, in a grade 3 renal cell carcinoma, $LSAB_2$ DAB method, x400

Using the DAKO, LSAB2, HRP system allowed us to highlight the PCNA score useful for evaluating the prognosis of clear and granulated cell carcinoma, PCNA being considered a marker of cell proliferation in the nuclei of cells on the verge of synthesizing DNA. Similar results have been obtained and other authors [11].

While the intensity of immunohistochemical staining in case of the anti-PCNA expression of nuclei presented large variations in our study group, all 63 studied tumors presented a positive expression.

Conclusions

Following this study, our conclusions are that a correlation exists between the PCNA score and the histological tumor differentiation grade, allowing a prognosis to be made on the value of this score. Thus, a low score was only present for low grade tumors (G_1 and G_2) with few positively expressed cells, at a maximum of 17%. The medium score was present for intermediate grade tumors G_2 and G_3, in which approximately half of the cells (53%) presented positive expression. A majority (97%) of cells being positively expressed was encountered in G_2 to G_4 tumors and determined a reserved prognosis.

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Manuscript received: 13.01.2017