DIAGNOSTICS AND TREATMENT METHODS

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The Diagnostic and Prognostic Significance of Immunohistochemical Method of Investigation of Clear Cell Renal Cell Carcinomas of Low Differentiation

Aim. To analyze the cytokeratin expression profile (5\6, 7, 8, 10\13, 17, 18, 19, 20) at various degrees of differentiation of renal cell clear cell carcinomas. It will help diagnose and prognose the course of carcinomas of low differentiation.

Materials and methods. 358 cases of renal cell clear cell carcinomas have been examined. The analysis of morphological and immunohistochemical methods of investigation has been performed.

Results. The differentiation (G grade) of 358 cases of renal cell clear cell carcinomas by means of morphological and immunohistochemical methods of investigation has been established. Reaction table of cytokeratin antibodies (CK 5\6, 7, 8, 10\13, 17, 18, 19, 20) has been determined for each Grade of renal cell carcinoma with the control of normal renal tissue.

Conclusion. The use of additional immunohistochemical investigation especially at renal carcinomas of low differentiation has been considered to be significant and even required after the standard histological investigation.

Keywords: renal clear cell carcinoma, morphology, Grade, immunohistochemical method, cytokeratin antibodies.

INTRODUCTION

Kidney cancer is among the 10 most common epithelial neoplasms [8]. The fact that the renal cell carcinoma (RCC) incidence rates increase annually in European countries and the United States becomes troublesome for the researchers from the whole world. The estimate is about 2-3%. However in Russia the rise in RCC incidence rates accounts for 6-9% annually [3, 5, 6, 7, 8]. It is stated that the process of kidney cancer development is rather slow and durable. The kidney cancer is characterized by a solitary fibrous capsule formation without extending into the renal pelvis, kidney capsule and organ vessels. Although over some period of time this tumor begins to behave aggressively. It is manifested by the invasion into the kidney structures mentioned above causing metastasis [2, 5, 7, 8].

Table 1

Comparative characteristics of cytokeratin reaction due to different degrees of differentiation of clear renal cell

Antibodies / Material	CK 5\6	CK 7	CK 8	CK 10\13	CK 17	CK 18	CK 19	CK 20
N tissue (epithelium of tubules)	2+	0	1+	2+	2+	3+	3+	0
G1	2+	0	2+	2+	2+	3+	2+	2+
G2	1+	0	2+	2+	2+	3+	2+	2+
G3	0	0	2+	0	0	3+	2+	0
G4	0	0	2+	2+	3+	0	3+	3+

Note: 0 - no reaction, 1+ positive reaction, 2+ moderately positive reaction, 3+ strongly positive reaction.

RCC in a patient may not be detected clinically for a significant period of time. Tumors are usually revealed by the regular check-ups or during the examination in case of the presence of other diseases. When a patient starts to complain of pains in the area of kidneys and hematuria as a rule it points to the severe stage of cancer with the invasion into some kidney structures and the presence of metastases [2, 5, 7]. In such unfavorable oncological conditions it is of great importance to perform complete follow-up examination for early detection and adequate diagnostics of RCC.

The treatment scheme and prognosis of development of kidney cancers are firstly evaluated by the morphological characteristics of the tumor process [1, 2, 5, 8]. The histological method of examination allows determining the degree of tumor differentiation that is mandatory for a prognostic value of a disease course. The degree of differentiation for the commonest clear cell renal cell variant of RCC is identified by grading (G). The grading system of kidney tumor

differentiation ranges from 1-4 according to increasing malignancy: G1 - well differentiated; G2 – moderately differentiated; G3 – poorly differentiated; G4 – undifferentiated.

The introduced 2004 WHO classification of clear cell RCC is based on certain histological signs including the shape and size of nuclei of tumor cells, chromatin distribution, the shape and size of nucleoli and the presence of mitoses [3, 6, 7]. The tumors of higher differentiation grade (lower G – G1) are characterized by smaller and single typed nuclei (less than 10 μm), undifferentiated chromatin and nucleoli. The tumors of lower differentiation grade (G3, G4) are recognized by larger and more polymorphous nuclei, differentiated chromatin and nucleoli[6,7]. However the degree differentiation of malignant tumors of kidneys by histological method is considered to be of subjective character.

Nowadays the histochemical method of investigation is a key method for detection of tumor histogenesis and differentiation at a maximal degree [4]. This statement is proved by the fact that at RCC of lower differentiation degree epithelial cells lose their characteristic features and become the same as mesenchimal cells. Much attention is currently paid to the aspects mentioned above. We have not found out the researches devoted to the study of dependence between the degree of expression of cytokeratins and RCC differentiation degree. Though it should be of special significance as the differentiation degree determines the malignant potential of a tumor.

The ***aim*** of our research work is to analyze the cytokeratin expression profile (*5\6, 7, 8, 10\13, 17, 18, 19, 20)* at various degrees of differentiation of clear cell RCC. It will help diagnose and prognose the course of carcinomas of low differentiation.

MATERIALS AND METHODS

358 patients were operated in a Clinical Hospital n.a. R.V. Mirotvortzev of Saratov State Medical University n.a. V.I. Razumovsky over the period of 5 years and 5 months (from 2006 till 2011). The analysis of morphological data including the data of immunohistochemical investigation of cytokeratin profile *(*5\6, 7, 8, 10\13, 17, 18, 19, 20) at different differentiation degree (G1, G2, G3, G4) of clear cell RCC was carried out. The expression level of antibodies was determined by the summarized evaluation of expression of stained tumor cells and the general amount of positively stained cells. The case was estimated as positive if the staining intensity accounted over 10% of tumor cells. When the staining intensity accounted less than 10% of tumor cells the result was estimated as zero. The low reaction in more than 10% of tumor cells with the low staining intensity was estimated as «1+», with the mild staining intensity in more than 10% of tumor cells - as «2+»; with the expressed staining intensity in more than 10% of tumor cells -

as «3+». The cytokeratin expression profile was also determined in the normal kidney tissue (epithelium in the kidney tubules) and considered as a comparison group.

RESULTS AND DISCUSSION

The analysis of case histories of 487 patients with various kidney tumors received operative treatment during the period from 2006 till 2011 in the Clinical Hospital n.a. R.V. Mirotvortzev revealed that 459 patients suffered from malignant tumors (94% of all tumors) and the common clear cell RCC occurred in 358 patients (82,5% of RCCs).

Among 358 cases of clear cell RCC 257 patients had signs of invasion, 35 of them experienced the first signs of the beginning of metastasis – tumor emboli, infiltration into the renal hilum and 19 patients of them had metastasis into the lymph nodes or hematogenous metastasis.

According to 2004 WHO recommendations we determined the differentiation degree (grade - G) of all the clear cell RCCs by means of ocular micrometer. We have revealed the following data: G1 renal cell carcinoma has been found out in 53 patients; G2 renal cell carcinoma – in 199 patients; G3 renal cell carcinoma – in 75 patients; G4 renal cell carcinoma – in 32 patients. During the analysis of case histories it has been pointed out that well differentiated and moderately differentiated RCCs (G1 and G2) did not commonly have clinical manifestations and were detected during the regular check-ups or during the complex examination in case of presence of other diseases. In most cases RCC was not characterized by invasive growth and metastasis. However it should be noted that 17 cases with high differentiation degree (G1 and G2) of RCC showed the already presented signs of aggressive tumor behavior with the invasion into the renal capsule or renal pelvis that might give rise to disease progression and deep invasion into the kidney structures and probably metastasis.

All the cases of poorly differentiated and undifferentiated RCCs (G3 and G4) were characterized by the signs of invasion of different degree. In 12 patients the invasion into the renal capsule or pelvis was at the beginning stage or incomplete. The deep invasion, the infiltration into the adipose tissue, adrenal glands and renal hilum and the presence of regional and separate metastasis were observed in 95 cases out of 107.

According to the literature data the cytokeratin antibodies give the positive reaction on epithelial cells while conducting the immunohistochemical analysis. The clear cell RCC gives the positive reaction on CK of high values - CK18, CK19 – according to the foreign and the Russian researches [4,6,7].

While analyzing the immunohistochemical reaction on cytokeratins we have revealed that normal epithelial cells of renal tubules give moderate or expressed positive immunohistochemical reaction on cytokeratins of both low and high values (CK 5\6,10\13, 17, 18, 19), apart from CK7

and CK20. The expression of cytokeratins of low and high values at different grades (G) of clear cell RCCs showed the following tendency (table 1).

All the cytokeratins apart from CK7 were positive at well differentiated G1clear cell RCC with more expressed positive reaction of CK18. At moderately differentiated G2 clear cell RCC expressed positive reaction was observed with cytokeratins of high values, CK 18 was maximally positive, CK 7 was negative, CK5/6 was expressed by mild positive reaction. In the analysis of immunohistochemical reaction of poorly differentiated G3-4 clear cell RCC the following results were drawn: CK 17, 19, 20 were definitely positive, CK 5\6, 7,8,10\13 were slowly expressed or negative. It is worth while noting that at poorly differentiated RCC with sarcomatous element G4 CK18 was negative (table 1). This reaction might be determined by the functional and morphological atypism of poorly differentiated tumor cells. The only immunohistochemical investigation revealed the negative stromal tumor cells. While hematoxylin and eosin staining was not capable to differentiate sarcoma similar tumor elements and stroma. It should be pointed out that CK7 was negative in all cases of clear cell RCCs. However the use of CK7 is possible for differentiated diagnostics between clear cell variant and other variant of RCC (e.g. according to the literature data [4,6,7] CK7 is positive at chromophobe RCC).

The reaction of cytokeratins expression at differentiation grades (G) of clear cell RCCs is proved to be different. Therefore it is practically impossible to determine the grade of clear cell RCC on the basis of cytokeratin antibodies reaction. It is necessary to use the complex system of histological and immunohistochemical methods of investigation.

CONCLUSION

The search for the new methods of diagnostics and prognosis of development of RCC is an actual issue determined by the increased rates of tumor development, late diagnostics, aggressive course and unfavorable prognosis. The immunohistochemical method of investigation may serve as an additional method of diagnostics at poorly differentiated RCCs and as a standard method followed by histological examination. The immunohistochemical method with the use of cytokeratin antibodies allows performing the differentiated diagnostics of sarcomatous RCC with mesenchymal tumors, to determine the tumor variant and in some cases helps identify tumor grade (G). And consequently the method under the consideration helps administer the proper treatment and prognose the course of disease.

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