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THE FEATURES OF EXPRESSION AND PROGNOSTIC SIGNIFICANCE OF THE PEPTIDE MOLECULES CONNECTED WITH THE KEY PROPERTIES OF RENAL CELL CARCINOMA

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Relevance. The modern approach to prognostication of the course of renal carcinoma requires identification of the biological markers, which determine the key properties of the tumor in particular cases. *The purpose of this study* is to clarify the nature of expression of the molecules connected with the phenotype of the cancer stem cell (CD133, N-cadherin), TGF-α growth factor for tubular epithelium, and carboanhydrase IX (CA-IX) – one of the key molecules connected with VHL by the molecular way of carcinogenesis in cases of renal cancer. ***Materials and methods.*** We studied 61 observations of renal cell carcinoma (RCC). The immunohystochemical examination was based on a panel of antibodies, which included antibodies to TGF-α (Abcam), N-cadgerin (M3613 clone; Dako), CD133 (Prominin-1, Biorbyt), carboanhydrase IX (CAIX; NCL-L-CAIX, Leica Biosystems). The degree of the markers' expression was evaluated by a semiquantitative method depending on the content of the positive-reaction cells in the tumor. The differences between the compared groups were identified through the use of the Mann-Whitney *U* test and the chi-squared test. ***Results.*** Expression of TGF-α was found in 36 observations (59%) of RCC. TGF-α was most frequently present in the papillary (100%) and the clear-cell (55.9%) versions, and only rarely in chromophobic carcinoma (23.1%). A connection was found between the expression level of this marker and the neoplastic differentiation degree ($p < 0.05$). Expression of N-cadgerin was met in 38 observations (62.3%), CD133 occurred in 37 observations of renal carcinoma (60.6%). N-cadgerin and CD133 were found in the clear-cell and the papillary versions of RCC. There was a connection between the expression of N-cadgerin in the tumor and its invasive potential ($p < 0.05$). A higher occurrence rate of CD133 expression was recorded in the patients with metastatic forms of RCC, and also in cases of sarcomatoid transformation of the tumor ($p < 0.05$). Expression of carboanhydrase IX (CA-IX) occurred in 35 observations (57.4%). It was found in the clear-cell version of RCC. In cases of symptomatic RCC, expression of CA-IX was more frequently met ($p < 0.05$). There was a consistent association between the expression of CA-IX and the tumor size, stage and invasive potential.

Keywords: renal cell carcinoma; prognostication; biomarkers; CD133; N-cadgerin; TGF-alpha; carboanhydrase IX.

ОСОБЕННОСТИ ЭКСПРЕССИИ И ПРОГНОСТИЧЕСКОЕ ЗНАЧЕНИЕ ПЕПТИДНЫХ МОЛЕКУЛ, СВЯЗАННЫХ С КЛЮЧЕВЫМИ СВОЙСТВАМИ ПОЧЕЧНО-КЛЕТОЧНОГО РАКА

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Актуальность. Современный подход к прогнозированию течения рака почки требует поиска биологических маркеров, определяющих ключевые свойства опухоли у конкретного пациента. ***Цель исследования*** — оценить особенности экспрессии молекул, связанных с фенотипом раковой стволовой клетки (CD133, N-кадгерин), TGF-α — фактора роста для эпителия канальцев и карбоангидразы IX (CA-IX) — одной из ключевых молекул,

связанных с VHL-молекулярным путем канцерогенеза при раке почки. ***Материалы и методы.*** Проанализировано 61 наблюдение почечно-клеточного рака (ПКР). Для иммуногистохимического исследования использовали панель антител, включающую антитела к TGF-α (Abcam), N-кадгерину (клон M3613; Dako), CD133 (Prominin-1, Biorbyt), карбоангидразе IX (CAIX; NCL-L-CAIX, Leica Biosystems). Выраженность экспрессии маркеров оценивалась полуколичественным методом в зависимости от содержания клеток с позитивной реакцией в опухоли. Для выявления различий между сравниваемыми группами использовали непараметрический критерий Манна – Уитни и Хи-квадрат. ***Результаты.*** Экспрессия TGF-α встретилась в 36 наблюдениях (59 %) ПКР. Наиболее часто TGF-α выявляли при папиллярном (100 %) и светлоклеточном вариантах (55,9 %), редко при хромофобном раке (23,1 %). Установлена связь между уровнем экспрессии этого маркера и степенью дифференцировки новообразования ($p < 0,05$). Экспрессия N-кадгерина имела место в 38 наблюдениях (62,3 %), CD133 — в 37 наблюдениях рака почки (60,6 %). Преимущественно N-кадгерин и CD133 выявляли при светлоклеточном и папиллярном вариантах ПКР. Обнаружена связь между экспрессией в опухоли N-кадгерина и ее инвазивным потенциалом ($p < 0,05$). Более высокая частота экспрессии CD133 отмечена у больных с метастатическими формами ПКР, а также при саркоматоидной трансформации опухоли ($p < 0,05$). Экспрессия карбоангидразы IX (CA-IX) отмечена в 35 наблюдениях (57,4 %), при светлоклеточном варианте ПКР. При симптомном ПКР достоверно чаще встречалась экспрессия CA-IX ($p < 0,05$). Выявлена достоверная ассоциация между экспрессией CA-IX и размером опухоли, ее стадией и инвазивным потенциалом.

Ключевые слова: почечно-клеточный рак; прогноз; биомаркеры; CD133; N-кадгерин; TGF-альфа, карбоангидраза IX.

Renal cell carcinoma (RCC) remains a significant health burden, accounting for nearly 3% of all malignancies in the adult population. In recent decades, the incidence of RCC has been increasing by 3% to 4% per year. This growth is probably associated with the expansion of methods of intravital kidney imaging, including ultrasound, computed tomography, and magnetic resonance imaging [1]. Despite the high prognostic value of the existing diagnostic tools for assessing clinical and histological features of the tumor, its stage, and the degree of differentiation, they cannot accurately predict the course of the disease in a particular patient or the individual characteristics of RCC. Evaluating tumor prognosis at different stages is also challenging. The current approach to predicting the course of RCC involves searching for new biological markers that allow estimation of the most important characteristics of the tumor, such as proliferative activity, resistance to apoptosis, invasion and metastasis ability, and suppression of immune reactions [2].

The aim of this study was to evaluate the expression of molecules associated with cancer stem cell phenotype (CD133, N-cadherin): transforming growth factor (TGF)-α (growth factor for epithelial tubules) and carbonic anhydrase IX (CAIX), one of the key molecules involved in von Hippel–Lindau (VHL)-associated carcinogenesis in RCC.

The study included 61 patients with RCC (35 cases of clear cell RCC, 13 cases of type 1 papillary RCC, and 13 cases of chromophobe RCC). We performed morphological examination of tissue samples collected during radical nephrectomy. The specimens were fixed in 10% buffered neutral formalin and embedded in paraffin according to standard procedures. Histological slides were stained with hematoxylin and eosin. The following antibodies were used for immunohistochemical analysis: anti-TGF-α antibodies (TGF-α, dilution 1: 100; Abcam, Cambridge, United Kingdom), anti-N-cadherin antibodies (N-cadherin, клон M3613; dilution 1: 50; Dako, Glostrup, Denmark), anti-CD133 antibodies (prominin-1 dilution 1: 200; Biorbyt, Cambridge, United Kingdom), and anti-CAIX antibodies (CAIX; NCL–L-CAIX, dilution 1: 100; Leica Biosystems, Hamburg, Germany). A semiquantitative method was used to estimate the expression of immunohistochemical markers: 0–no expression (in 0% of tumor cells), 1–weak expression (in up to 10% of tumor cells), 2–moderate expression (in 11%–30% of tumor cells), and 3–strong expression (in over 30% of tumor cells). Additionally, we evaluated the location of positive staining as membranous, cytoplasmic, or nuclear. Statistical analysis was performed with the Statistica 6.1 software package. Fisher's exact test and Pearson's χ^2 test were used to assess differences between binary variables; continuous variables were compared by the Mann–Whitney U test.

RESULTS

TGF-α

TGF-α is a multifunctional regulatory peptide with a wide range of effects related to cell growth and differentiation. In the kidney, it stimulates proliferation

of proximal tubule epithelial cells. The effects of TGF-α are mediated through epidermal growth factor receptors [3, 4].

TGF-α expression was observed in 36 RCC patients (59%). A positive TGF-α reaction was more common in clear cell (55.9%) (Fig. 1–3) and type 1 papillary (100%) variants (Fig. 4), whereas it was less common in chromophobe RCC (23.1%) (Fig. 5). Expression of TGF-α was primarily detected in the tumor cell cytoplasm (59%), and less frequently along cell membranes (32.8%). Analyzing the association between TGF-α expression and clinical characteristics of RSS, we found that such factors as age, gender, body mass index, history of hypertension, RCC symptoms, and laboratory signs of chronic kidney disease had no effect on TGF-α expression. We found a correlation between the level of TGF-α production and the degree of tumor differentiation ($\chi^2 = 6.816$; $p < 0.05$) (Table 1).

N-cadherin

N-cadherin is a membrane protein of the cadherin superfamily that is involved in the formation of calcium-dependent cell–cell contacts. A high level of N-cadherin expression is typical of embryonic mesoderm. Recent studies demonstrated that N-cadherin can be used as a marker of the epithelial–mesenchymal transition, which has a crucial role in increasing tumor aggressiveness (invasion and metastasis) [5].

Expression of N-cadherin was observed in 38 patients (62.3%). It was detected significantly more frequently in clear cell RCC (80%) ($p < 0.0001$) (Fig. 6) and type 1 papillary RCC (76.9%) ($p < 0.0001$) (Fig. 7) than

Table 1

Correlation between TGF-α expression and morphological and prognostic characteristics of RCC

Таблица 1

Связь между экспрессией TGF-α, морфологическими и прогностическими характеристиками почечно-клеточного рака

Characteristic	Histological type						Total	χ^2
	Clear cell		Type 1 papillary		Chromophobe			
	No expression detected	Expression detected	No expression detected	Expression detected	No expression detected	Expression detected		
Size of the primary tumor								
<4 cm	2	3	0	8	3	1	17	
4–7 cm	6	9	0	3	3	2	23	
>7 cm	7	8	0	2	4	0	21	
Total	15	20	0	13	10	3	61	2.399 $p > 0.05$
Tumor stage (T)								
T1a	2	6	0	8	3	1	20	
T1b	1	1	0	2	1	1	6	
T2	0	0	0	0	1	0	1	
T3a	11	10	0	3	4	1	29	
T3b	1	3	0	0	1	0	5	
Total	15	20	0	13	10	3	61	5.083 $p > 0.05$
Invasion into the tissue and blood vessels of the renal sinus, renal vein, and its branches								
Yes	11	10	0	3	4	1	29	
No	4	10	0	10	6	2	32	
Total	15	20	0	13	10	3	61	2.637 $p > 0.05$
Presence of regional lymphogenous metastases								
Yes	1	0	0	0	0	0	1	
No	14	20	0	13	10	3	60	
Total	15	20	0	13	10	3	61	1.464 $p > 0.05$

Table 1 (continued) / Окончание табл. 1

Characteristic	Histological type						Total	χ^2
	Clear cell		Type 1 papillary		Chromophobe			
	No expression detected	Expression detected	No expression detected	Expression detected	No expression detected	Expression detected		
No	13	19	0	11	10	3	56	
Total	15	20	0	13	10	3	61	0.002 $p > 0.05$
Degree of differentiation (Fuhrman's nuclear grading)								
G_1 (grade 1–2)	1	6	0	6	1	1	15	
G_2 (grade 2–3)	11	11	0	4	8	2	36	
G_3 (grade 3–4)	3	3	0	3	1	0	10	
Total	15	20	0	13	10	3	61	6.816 $p < 0.05$
Presence of sarcomatoid and rhabdoid transformation								
Yes	3	3	0	2	1	0	9	
No	12	17	0	11	9	3	52	
Total	15	20	0	13	10	3	61	0.052 $p > 0.05$
Presence of coagulative necrosis foci in the tumor								
Yes	2	1	0	4	1	0	8	
No	13	19	0	9	9	3	53	
Total	15	20	0	13	10	3	61	0.024 $p > 0.05$

in the chromophobe variant, which had no expression of this marker. N-cadherin was primarily located in the cell membrane (75%), and sometimes in the cytoplasm (35.4%). Analyzing possible correlations between the expression of N-cadherin and clinical characteristics of RCC, we found that patients suffering from chronic kidney disease (glomerular filtration rate [GFR] < 60) were more likely to express N-cadherin ($\chi^2 = 5.67$; $p < 0.05$). We also analyzed the main morphological characteristics of RCC that have a prognostic value (Table 2) and found that N-cadherin production in a tumor was correlated with its invasive potential ($\chi^2 = 6.486$; $p < 0.05$).

CD133

CD133 (prominin-1) is a transmembrane glycoprotein expressed in hematopoietic stem cells [6]. This protein has recently been used as a marker of cancer stem cells. Hypoxia and activation of hypoxia-inducible factor (HIF)-1α can induce proliferation of CD133-positive cells. The level of CD133 production may vary depending on the condition of the surrounding cells [7].

CD133 was expressed in 37 patients (60.6%). CD133-positive cells were more frequently detected in clear cell RCC (80%) (Fig. 8 and 9) and papillary RCC (69.2%) (Fig. 10), whereas patients with chromophobe cancer had no CD133 expression. The marker was primarily located in the cytoplasm (60.6%), and less frequently in the cell membrane (44.3%).

We found an association between the patient`s age and CD133 expression ($\chi^2 = 17.467$; $p < 0.01$): strong expression was more frequent among young patients. The mean age of patients with strong CD133 expression was 56.3 ± 5.4 years, whereas patients with no CD133 expression were significantly older (mean age

Table 2

Correlation between N-cadherin expression and morphological and prognostic characteristics of RCC

Таблица 2

Связь между экспрессией N-кадгерина, морфологическими и прогностическими характеристиками почечно-клеточного рака

Characteristic	Histological type						Total	χ^2
	Clear cell		Type 1 papillary		Chromophobe			
	No expression detected	Expression detected	No expression detected	Expression detected	No expression detected	Expression detected		
Size of the primary tumor								
<4 cm	1	3	3	6	4	0	17	
4–7 cm	3	11	0	3	6	0	23	
>7 cm	3	14	0	1	3	0	21	
Total	7	28	3	10	13	0	61	3.52 $p > 0.05$
Tumor stage (T)								
T1a	1	7	3	5	4	0	20	
T1b	0	1	0	2	1	0	4	
T2	0	0	0	0	0	0	0	
T3a	5	17	0	3	6	0	31	
T3b	1	3	0	0	2	0	6	
Total	7	28	3	10	13	0	61	1.777 $p > 0.05$
Invasion into the tissue and blood vessels of the renal sinus, renal vein, and its branches								
Yes	1	12	0	7	3	0	23	
No	6	16	3	3	10	0	38	
Total	7	28	0	10	13	0	61	6.486 $p < 0.05$
Presence of regional lymphogenous metastases								
Yes	0	1	0	0	0	0	1	
No	7	27	3	10	13	0	60	
Total	7	28	3	10	13	0	61	0.496 $p > 0.05$
Presence of distant lymphogenous and hematogenous metastases								
Yes	1	3	0	2	0	0	6	
No	6	25	3	8	13	0	55	
Total	7	28	3	10	13	0	61	0.224 $p > 0.05$
Degree of differentiation (Fuhrman's nuclear grading)								
G_1 (grade 1–2)	1	7	2	4	1	0	15	
G_2 (grade 2–3)	6	19	1	3	11	0	40	
G_3 (grade 3–4)	0	2	0	3	1	0	6	
Total	7	28	3	10	13	0	61	2.815 $p > 0.05$
Presence of sarcomatoid and rhabdoid transformation								
Yes	2	4	0	2	1	0	9	
No	5	24	3	8	12	0	52	
Total	7	28	3	10	13	0	61	0.086 $p > 0.05$
Presence of coagulative necrosis foci in the tumor								
Yes	1	2	0	4	1	0	9	
No	6	26	3	6	12	0	52	
Total	7	28	3	10	13	0	61	0.086 $p > 0.05$

64.8 ± 2.21 years, $p = 0.014$). The correlation was observed for both membranous and cytoplasmic expression. A significant association was found between the level of CD133 production and the risk of decreased GFR in the early postoperative period ($\chi^2 = 4.155$; $p < 0.05$). The frequency of CD133 expression was higher in individuals with metastatic forms of RCC. Moreover, we found a significant correlation between CD133 expression in tumor cells and the probability of sarcomatoid transformation ($\chi^2 = 6.480$; $p < 0.05$) (Table 3).

CAIX

CAIX is an HIF-1α-regulated transmembrane protein that is associated with tumor growth, aggressive tumor phenotype, and poor prognosis in clear cell RCC [8]. CAIX is believed to be involved in the regulation of intracellular and extracellular pH levels in response to hypoxia in tumor tissue.

CAIX expression was observed in 35 patients (57.4%), primarily in individuals with clear cell RCC (97.1%) (Fig. 11 and 12). One patient with chromophobe RCC had a weak focal reaction with anti-CAIX antibodies; patients with the papillary type of tumor had no CAIX expression. Patients with symptomatic RCC were more likely to have CAIX expression ($\chi^2 = 6.213$; $p < 0.05$). We also found a significant association between the level of CAIX expression and tumor size ($\chi^2 = 7.710$; $p < 0.05$), stage ($\chi^2 = 8.535$; $p < 0.05$), and invasive potential ($\chi^2 = 5.111$; $p < 0.05$). No correlation between the degree of differentiation and the level of CAIX production was observed (Table 4).

Table 3

Correlation between CD133 expression and morphological and prognostic characteristics of RCC

Таблица 3

Связь между экспрессией CD133, морфологическими и прогностическими характеристиками почечно-клеточного рака

Characteristic	Histological type						Total	χ^2
	Clear cell		Type 1 papillary		Chromophobe			
	No expression detected	Expression detected	No expression detected	Expression detected	No expression detected	Expression detected		
Size of the primary tumor								
<4 cm	1	4	3	5	4	0	17	
4–7 cm	5	10	0	3	5	0	23	
>7 cm	1	14	1	1	4	0	21	
Total	7	28	4	9	13	0	61	5.810 $p > 0.05$
Tumor stage (T)								
T1a	1	7	3	5	4	0	20	
T1b	0	2	0	2	2	0	6	
T2	0	0	0	0	1	0	1	
T3a	5	16	1	2	6	0	30	
T3b	1	3	0	0	0	0	4	
Total	7	28	4	9	13	0	61	0.390 $p > 0.05$
Invasion into the tissue and blood vessels of the renal sinus, renal vein, and its branches								
Yes	5	16	1	2	5	0	29	
No	2	12	3	7	8	0	32	
Total	7	28	4	9	13	0	61	0.046 $p > 0.05$
Presence of regional lymphogenous metastases								
Yes	1	0	0	0	0	0	1	
No	6	28	4	9	13	0	60	
Total	7	28	4	9	13	0	61	1.567 $p > 0.05$

Table 3 (continued) / Окончание табл. 3

Characteristic	Histological type						Total	χ^2
	Clear cell		Type 1 papillary		Chromophobe			
	No expression detected	Expression detected	No expression detected	Expression detected	No expression detected	Expression detected		
No	5	28	2	9	13	0	57	
Total	7	28	4	9	13	0	61	6.599 $P < 0.05$
Degree of differentiation (Fuhrman's nuclear grading)								
G_1 (grade 1–2)	1	7	1	5	1	0	15	
G_2 (grade 2–3)	5	16	2	2	11	0	36	
G_3 (grade 3–4)	1	5	1	2	1	0	10	
Total	7	28	4	9	13	0	61	4.431 $p > 0.05$
Presence of sarcomatoid and rhabdoid transformation								
Yes	1	5	1	1	1	0	9	
No	6	23	3	8	12	0	52	
Total	7	28	4	9	13	0	61	6.480 $p < 0.05$
Presence of coagulative necrosis foci in the tumor								
Yes	1	2	2	2	1	0	8	
No	6	26	2	7	12	0	53	
Total	7	28	4	9	13	0	61	0.438 $p > 0.05$

DISCUSSION

Our study demonstrated that the levels of TGF-α, N-cadherin, CD133, and CAIX expression differ among various RCC histological subtypes, which reflects the different molecular pathways underlying their development. The CAIX protein was detected only in clear cell tumors, whereas N-cadherin and CD133 were expressed in both clear cell and type 1 papillary RCC and were absent in chromophobe tumors. Our results are in agreement with previous investigations. Knebelmann et al. (2009) found that TGF-α expression can be suppressed by the VHL protein. A number of authors demonstrated increased TGF-α production in clear cell and papillary RCC compared with normal tissue [9, 10]. They also showed that this protein is likely to be among the factors inducing cancer development in the early stages. The absence of TGF-α in the chromophobe variant of RCC is probably responsible for the low malignant potential of these tumors and their poor vascularization [11]. Expression of N-cadherin in clear cell RCC was studied by Markovic-Lipkovski et al. (2001), Shimazui et al. (2006), and Behnes et al. (2012) [12–14].

Table 4

Correlation between CAIX expression and morphological and prognostic characteristics of RCC

Таблица 4

Связь между экспрессией CA-IX, морфологическими и прогностическими характеристиками почечно-клеточного рака

Characteristic	Histological type						Total	χ^2
	Clear cell		Type 1 papillary		Chromophobe			
	No expression detected	Expression detected	No expression detected	Expression detected	No expression detected	Expression detected		
Size of the primary tumor								
<4 cm	0	5	4	0	8	0	17	
4–7 cm	1	14	3	0	4	1	23	
>7 cm	0	15	6	0	0	0	21	
Total	1	34	13	0	12	1	61	7.710 $p < 0.05$

Table 4 (continued) / Окончание табл. 4

Characteristic	Histological type						Total	χ^2
	Clear cell		Type 1 papillary		Chromophobe			
	No expression detected	Expression detected	No expression detected	Expression detected	No expression detected	Expression detected		
Tumor stage (T)								
T1b	0	2	2	0	2	0	6	
T2	0	0	0	0	1	0	1	
T3a	0	22	3	0	5	1	31	
T3b	1	2	0	0	0	0	3	
Total	1	34	13	0	12	1	61	8.535 $p < 0.05$
Invasion into the tissue and blood vessels of the renal sinus, renal vein, and its branches								
Yes	0	21	3	0	5	0	29	
No	1	13	10	0	7	1	32	
Total	1	34	13	0	12	1	61	5.111 $p < 0.05$
Presence of regional lymphogenous metastases								
Yes	0	1	0	0	0	0	1	
No	1	33	13	0	12	1	60	
Total	1	34	13	0	12	1	61	0.755 $p > 0.05$
Presence of distant lymphogenous and hematogenous metastases								
Yes	0	3	2	0	0	0	5	
No	1	31	11	0	12	1	56	
Total	1	34	13	0	12	1	61	0.755 $p > 0.05$
Degree of differentiation (Fuhrman's nuclear grading)								
G_1 (grade 1–2)	0	8	6	0	1	0	15	
G_2 (grade 2–3)	1	20	4	0	10	1	36	
G_3 (grade 3–4)	0	6	3	0	1	0	10	
Total	1	34	13	0	12	1	61	0.142 $p > 0.05$
Presence of sarcomatoid and rhabdoid transformation								
Yes	0	6	2	0	1	0	9	
No	1	28	11	0	11	1	52	
Total	1	34	13	0	12	0	61	0.373 $p > 0.05$
Presence of coagulative necrosis foci in the tumor								
Yes	0	3	4	0	1	0	8	
No	1	31	9	0	11	1	53	
Total	1	34	13	0	12	1	61	1.488 $p > 0.05$

Histogenetic correlations between these markers and various forms of RCC allow them to be used in morphological differential diagnosis. Furthermore, detection of these proteins in biological fluids (blood, urine) can provide valuable information for screening and monitoring of RCC.

The involvement of the molecules assessed in this study in various molecular pathways of RCC deve-

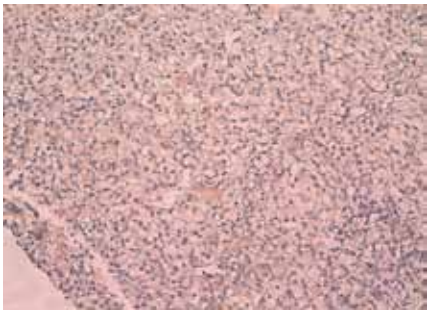

Fig. 1. Moderately differentiated renal cell carcinoma (RCC) (clear cell variant), nuclear grade 2. Pronounced focal positive cytoplasmic reaction with anti-transforming growth factor (TGF)-α antibodies

Рис. 1. Умеренно дифференцированный почечно-клеточный рак (светлоклеточный вариант), ядерный индекс 2. Выраженная очаговая позитивная цитоплазматическая реакция с анти-TGF-α-антителом

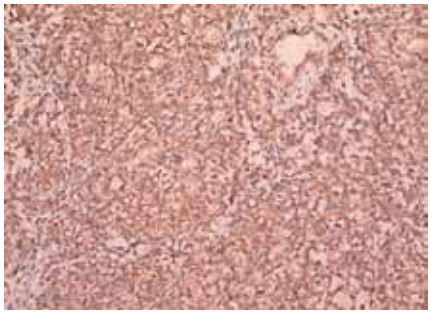

Fig. 2. Moderately differentiated renal cell carcinoma (RCC) (clear cell variant), nuclear grade 2–3. Pronounced diffuse positive cytoplasmic reaction with anti-transforming growth factor (TGF)-α antibodies

Рис. 2. Умеренно дифференцированный почечно-клеточный рак (светлоклеточный вариант), ядерный индекс 2–3. Выраженная диффузная позитивная цитоплазматическая реакция с анти-TGF-α-антителом

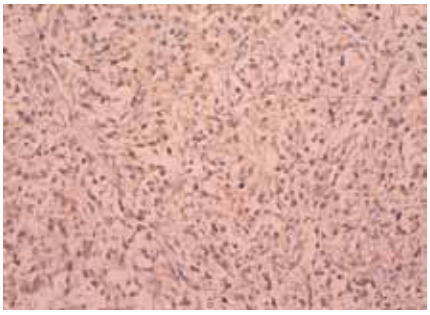

Fig. 3. Poorly differentiated renal cell carcinoma (RCC) (clear cell variant) with sarcomatoid transformation. Pronounced diffuse positive cytoplasmic reaction anti-transforming growth factor (TGF)-α antibodies

Рис. 3. Низкодифференцированный почечно-клеточный рак (светлоклеточный вариант) с саркоматоидной трансформацией. Выраженная диффузная позитивная цитоплазматическая реакция с анти-TGF-α-антителом

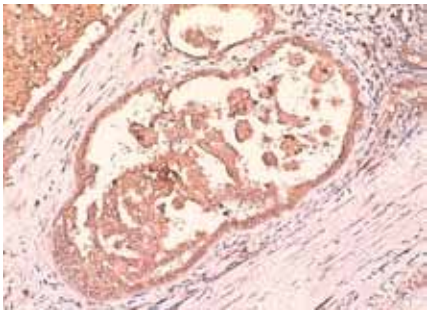

Fig. 4. Highly differentiated renal cell carcinoma (RCC) (type 1 papillary variant). Diffuse positive cytoplasmic and membranous reaction with anti-transforming growth factor (TGF)-α antibodies

Рис. 4. Высокодифференцированный почечно-клеточный рак (папиллярный вариант первого типа). Диффузная позитивная цитоплазматическая и мембранная реакция с анти-TGF-α-антителом

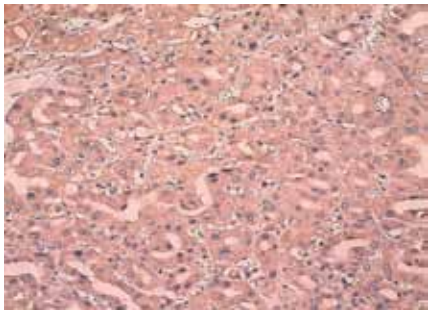

Fig. 5. Moderately differentiated renal cell carcinoma (RCC) (chromophobe variant). Focal positive cytoplasmic reaction with anti-transforming growth factor (TGF)-α antibodies

Рис. 5. Умеренно дифференцированный почечно-клеточный рак (хромофобный вариант). Очаговая позитивная цитоплазматическая реакция с анти-TGF-α-антителом

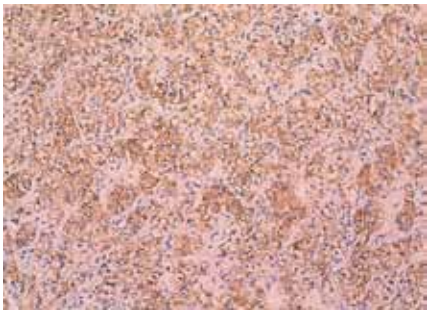

Fig. 6. Poorly differentiated renal cell carcinoma (RCC) (clear cell variant), nuclear grade 3. Pronounced diffuse positive membranous reaction with anti-N-cadherin antibodies

Рис. 6. Низко дифференцированный почечно-клеточный рак (светлоклеточный вариант), ядерный индекс 3. Выраженная диффузная позитивная мембранная реакция с анти-N-кадгерин-антителом

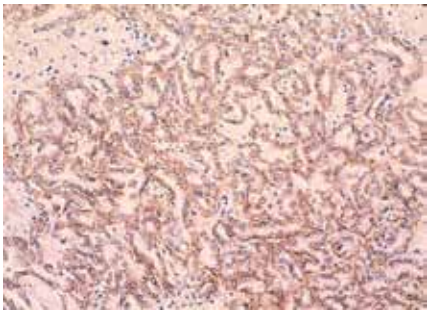

Fig. 7. Moderately differentiated renal cell carcinoma (RCC) (type 1 papillary variant), nuclear grade 2. Diffuse positive membranous reaction with anti-N-cadherin antibodies

Рис. 7. Умеренно дифференцированный почечно-клеточный рак (папиллярный вариант первого типа), ядерный индекс 2. Диффузная позитивная мембранная реакция с анти-N-кадгерин-антителом

Fig. 8. Moderately differentiated renal cell carcinoma (RCC) (clear cell variant), nuclear grade 2. Pronounced focal positive cytoplasmic reaction with anti-CD133-antibodies

Рис. 8. Умеренно дифференцированный почечно-клеточный рак (светлоклеточный вариант), ядерный индекс 2. Выраженная очаговая позитивная цитоплазматическая реакция с анти-CD133-антителом

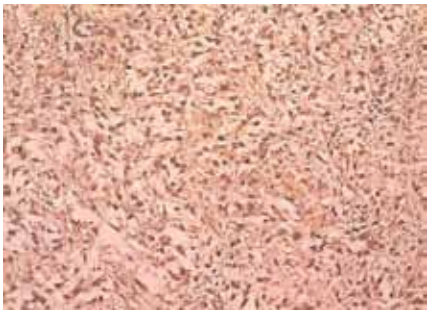

Fig. 9. Poorly differentiated renal cell carcinoma (RCC) (clear cell variant) with sarcomatoid transformation, nuclear grade 3. Pronounced positive cytoplasmic reaction with anti-CD133 antibodies

Рис. 9. Низко дифференцированный почечно-клеточный рак (светлоклеточный вариант) с саркоматоидной трансформацией, ядерный индекс 3. Выраженная позитивная цитоплазматическая реакция с анти-CD133-антителом

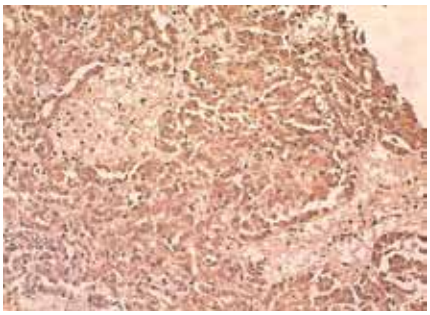

Fig. 10. Moderately differentiated renal cell carcinoma (RCC) (type 1 papillary variant), nuclear grade 2–3. Pronounced positive cytoplasmic reaction with anti-CD133 antibodies

Рис. 10. Умеренно дифференцированный почечно-клеточный рак (папиллярный вариант первого типа), ядерный индекс 2–3. Выраженная позитивная цитоплазматическая реакция с анти-CD133-антителом

Fig. 11. Moderately differentiated renal cell carcinoma (RCC) (clear cell variant, usual structure), nuclear grade 1–2. Pronounced positive membranous reaction with anti-carbonic anhydrase IX (CAIX) antibodies

Рис. 11. Умеренно дифференцированный почечно-клеточный рак (светлоклеточный вариант, классическое строение), ядерный индекс 1–2. Выраженная позитивная мембранная реакция с анти-карбоангидраза-IX-антителом

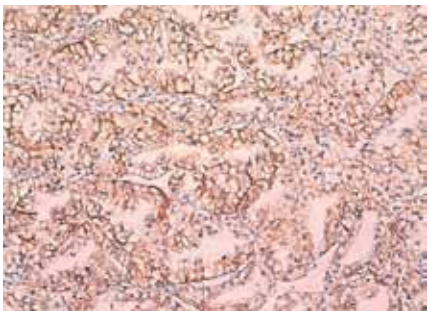

Fig. 12. Moderately differentiated renal cell carcinoma (RCC) (clear cell variant, tubulopapillary structure), nuclear grade 1–2. Positive membranous and cytoplasmic reaction with anti-carbonic anhydrase-IX (CAIX) antibodies

Рис. 12. Умеренно дифференцированный почечно-клеточный рак (светлоклеточный вариант, тубулопапиллярное строение), ядерный индекс 1–2. Позитивная мембранная и цитоплазматическая реакция с анти-карбоангидраза-IX-антителом

lopment and progression determines their prognostic value. N-cadherin was shown to be involved in transendothelial migration of cancer cells, which is an important step in metastasis [5]. Increased production of this marker is associated with a poor prognosis of RCC [12, 15, 16].

Costa et al. (2012) demonstrated that nearly 50% of RCCs have CD133 expression. Zhang et al. (2013) observed CD133 expression in 21% of RCC cases. Some authors suggest that high CD133 expression can be a favorable prognostic factor [16–19]. However, Feng et al. (2014) found that patients with an increased serum level of CD133 mRNA have a high risk of recurrence and metastasis [20]. CD133-positive cells in RCC are assumed to be a source of differentiated malignant cells and support tumor growth [21].

High CAIX expression is associated with favorable prognosis in localized and metastatic RCC [22]. The level of CAIX expression is inversely proportional to the risk of metastasis ($p = 0.036$). Patients with high CAIX expression had better survival, even after adjustment for such factors as T stage, Fuhrman grade, and overall patient condition ($p \leq 0.005$) [23]. Low CAIX expression (≤85%) was associated with unfavorable outcomes in patients with metastatic RCC (OR3.10; $p < 0.001$), even after adjustment for clinical and pathological factors (OR4.76; $p < 0.001$) [24]. Ingles et al. (2016) also demonstrated that tumors with low levels of CAIX expression have a worse prognosis (high risk of recurrence and poor cancer-specific and overall survival) [25].

Our findings suggest high prognostic values for TGF-α, N-cadherin, CD133, and CAIX. We found a correlation between TGF-α expression and the degree of tumor differentiation; expression of N-cadherin, CD133, and CAIX; and metastatic potential of the tumor. Thus, the markers can be used for individual prognosis of the disease course as well as for estimating the risk of cancer progression in each particular patient.

CONCLUSION

Expression of TGF-α is significantly more common in type 1 papillary and clear cell RCC than in chromophobe cancer. A statistically significant association was found between the level of TGF-α expression and the degree of tumor differentiation. N-cadherin was expressed in type 1 papillary and clear cell RCC, whereas the chromophobe variant had no expression of this marker. A correlation between N-cadherin production and stage 3 and 4 chronic kidney disease was established. Invasive forms of RCC demonstrated N-cadherin expression significantly more frequently. CD133 expression was detected in type 1 papillary and clear cell RCC, whereas the chromophobe variants lacked this marker. CD133-expressing tumor cells are more typical of younger patients. A significant association was found between the level of CD133 expression and the risk of decreased GFR in the early postoperative period. Metastatic forms of RCC are more likely to have CD133-positive tumor cells: there is a correlation between the positivity of the reaction and the probability of sarcomatoid transformation. Expression of CAIX is typical of clear cell RCC. The level of CAIX expression was significantly associated with tumor size, stage,

and invasive potential. Patients with symptomatic RCC were more likely to express CAIX than those without symptomatic RCC.

REFERENCES

1. Chow WH, Devesa SS, Warren JL, Fraumeni JFJr. Rising incidence of renal cell cancer in the United States. *JAMA*. 1999;281(17):1628-1631. doi: 10.1001/jama.281.17.1628.
2. Коган М.И., Ахохов З.М., Гусев А.А., Пасечник Д.Г. Молекулярно-биологические факторы прогнозирования течения почечно-клеточного рака (обзор литературы) // Онкоурология. – 2016. – № 3. – С. 45–50. [Kogan MI, Akhokhov ZM, Gusev AA, Pasechnik DG. Biomolecular prognostic factors in renal cell carcinoma: A literature review. *Onkourologiya*. 2016;12(3):45-50. (In Russ.)]. doi: 10.17650/1726-9776-2016-12-3-45-50.
3. de Paulsen N, Brychzy A, Fournier MC, et al. Role of transforming growth factor-alpha in von Hippel–Lindau (VHL)(-/-) clear cell renal carcinoma cell proliferation: a possible mechanism coupling VHL tumor suppressor inactivation and tumorigenesis. *Proc Natl Acad Sci USA*. 2001;98(4):1387-1392. doi: 10.1073/pnas.031587498.
4. Everitt JI, Walker CL, Goldsworthy TW, Wolf DC. Altered expression of transforming growth factor-alpha: an early event in renal cell carcinoma development. *Mol Carcinog.* 1997;19(3):213-19. doi: 10.1002/(sici)1098-2744(199707)19:3 < 213:: aid-mc9>3.0.co;2-e.
5. Ramis-Conde I, Chaplain MA, Anderson AR, Drasdo D. Multi-scale modelling of cancer cell intravasation: the role of cadherins in metastasis. *Phys Biol.* 2009;6(1):016008. doi: 10.1088/1478-3975/6/1/016008.
6. Mizrak D, Brittan M, Alison M. CD133: molecule of the moment. *J Pathol.* 2008;214(1):3-9. doi: 10.1002/path.2283.
7. Li Z. CD133: a stem cell biomarker and beyond. *Exp Hematol Oncol*. 2013;2(1):17. doi: 10.1186/2162-3619-2-17.
8. Tostain J, Li G, Gentil-Perret A, Gigante M. Carbonic anhydrase 9 in clear cell renal cell carcinoma: a marker for diagnosis, prognosis and treatment. *Eur J Cancer*. 2010;46(18):3141-3148. doi: 10.1016/j.ejca.2010.07.020.
9. Knebelmann B, Ananth S, Cohen HT, Sukhatme VP. Transforming growth factor alpha is a target for the von Hippel-Lindau tumor suppressor. *Cancer Res*. 1998;58(2):226-231.
10. Ramp U, Reinecke P, Gabbert HE, Gerharz CD. Differential response to transforming growth factor (TGF)-alpha and fibroblast growth factor (FGF) in human renal cell carcinomas of the clear cell and papillary types. *Eur J Cancer*. 2000;36(7):932-941. doi: 10.1016/s0959-8049(00)00030-7.
11. Chudek J, Schullerus D, Wilhelm M, Kovacs G. Lack of interleukin 6 (IL-6) and transforming growth factor alpha (TGF-alpha) expression in chromophobe renal cell carcinomas. *Br J Cancer*. 1998;78(9):1162-1164. doi: 10.1038/bjc.1998.647.
12. Shimazui T, Kojima T, Onozawa M, et al. Expression profile of N-cadherin differs from other classical cadherins as a prognostic marker in renal cell carcinoma. *Oncol Rep*. 2006;15(5):1181-4. doi: 10.3892/or.15.5.1181.
13. Markovic-Lipkovski J, Brasanac D, Müller GA, Müller CA. Cadherins and integrins in renal cell carcinoma: an immunohistochemical study. *Tumori*. 2001;87(3):173-178.
14. Behnes CL, Hemmerlein B, Strauss A, et al. N-cadherin is differentially expressed in histological subtypes of papillary renal cell carcinoma. *Diagn Pathol*. 2012;7:95. doi: 10.1186/1746-1596-7-95.
15. Zhou N, Lu F, Liu C, et al. IL-8 induces the epithelial-mesenchymal transition of renal cell carcinoma cells through the activation of AKT signaling. *Oncol Lett*. 2016;12(3):1915-1920. doi: 10.3892/ol.2016.4900.
16. Conant JL, Peng Z, Evans MF, et al. Sarcomatoid renal cell carcinoma is an example of epithelial-mesenchymal transition. *J Clin Pathol*. 2011;64(12):1088-1092. doi: 10.1136/jclinpath-2011-200216.
17. Costa WH, Rocha RM, Cunha IW, et al. CD133 immunohistochemical expression predicts progression and cancer-related death in renal cell carcinoma. *World J Urol*. 2012;30(4):553-558. doi: 10.1007/s00345-011-0769-x.
18. Cheng B, Yang G, Jiang R, et al. Cancer stem cell markers predict a poor prognosis in renal cell carcinoma: a meta-analysis. *Oncotarget*. 2016;7(40):65862-65875. doi: 10.18632/oncotarget.11672.
19. Matak D, Szymanski L, Szczylik C, et al. Biology of renal tumour cancer stem cells applied in medicine. *Contemporary Oncology*. 2015;19(1A): A44-A51. doi: 10.5114/wo.2014.47128.
20. Feng G, Jiang F, Pan C, et al. Quantification of peripheral blood CD133 mRNA in identifying metastasis and in predicting recurrence of patients with clear cell renal cell carcinoma. *Urol Oncol*. 2014;32(1):44.e9-14. doi: 10.1016/j.urolonc.2013.06.003.
21. Al-Lamki RS, Wang J, Yang J, et al. Tumor necrosis factor receptor 2-signaling in CD133-expressing cells in renal clear cell carcinoma. *Oncotarget*. 2016;7(17):24111-24124. doi: 10.18632/oncotarget.8125.
22. Zhang BY, Thompson RH, Lohse CM, et al. Carbonic anhydrase IX (CAIX) is not an independent predictor of outcome in patients with clear cell renal cell carcinoma (ccRCC) after long-term follow-up. *BJU Int*. 2013;111(7):1046-1053. doi: 10.1111/bju.12075.
23. Bui MH, Seligson D, Han KR, et al. Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: implications for prognosis and therapy. *Clin Cancer Res*. 2003;9(2):802-11.
24. Phuoc NB, Ehara H, Gotoh T, et al. Prognostic value of the co-expression of carbonic anhydrase IX and vascular endothelial growth

factor in patients with clear cell renal cell carcinoma. *Oncol Rep*. 2008;20(3):525-530. doi: 10.3892/or_00000037.

25. Ingels A, Hew M, Algaba F, et al. Vimentin over-expression and carbonic anhydrase IX under-expression are independent predictors of recurrence, specific and overall survival in non-metastatic clear-cell renal carcinoma: a validation study. *World J Urol*. 2017;35(1):81-87. doi: 10.1007/s00345-016-1854-y.

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