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MARKERS OF EPITHELIAL-MESENCHYMAL TRANSITION IN RENAL CELL CARCINOMA

Y.V. Dumanskiy[1], A.G. Kudriashov[1,], I.V. Vasilenko[2], R.B. Kondratyuk[2], Y.K. Gulkov[2], R.S. Chystiakov[3]*
[1]Department of Oncology, Maksim Gorky National Medical University, Donetsk 83092, Ukraine
[2]Department of Pathology, Maksim Gorky National Medical University, Donetsk 83003, Ukraine
[3]Donetsk Regional Anticancer Center, Donetsk 83092, Ukraine

Aim: **To study the influence of markers of epithelial-mesenchymal transition (EMT) on a renal cell carcinoma (RCC) prognosis.** ***Methods:*** **The surgical material of 47 RCC patients who underwent nephrectomy was studied. RCC patients were distributed in two groups: a short-term survival group (3–6 months) and a long-term survival group (17–24 months). EMT markers expression was assessed by immunohistochemical methods.** ***Results:*** **It was determined that the rate of spindle-cell EMT was 96.4% in a short-term survival group and 42.1% in a long-term survival group. High rate Furhman's nuclear atypia, i.e. degree 3–4 occurred in 100% of cases in a short-term survival group versus 68.4% in a long-term survival group.** ***Conclusion:*** **The rate of spindle-cell EMT in RCC may serve a more sensible prognostic factor than the degree of Furhman's nuclear atypia.**
Key Words**: renal cell carcinoma, epithelial-mesenchymal transition, the degree of Furhman's nuclear atypia.**

The occurrence of renal cell carcinoma (RCC) has been increasing for the last 20 years worldwide [1]. For the period of 1975–2005 the RCC incidence doubled in Europe [2]. In Russia the morbidity rate increased by 15.7% in males and by 17.3% in females for 5 years (2003–2008) [3]. RCC is a heterogeneous group of histologic tumor subtypes of which clear cell carcinoma is the most frequent making up more than 70% of all cases; papillary renal cancer makes up 10–15%, chromophobic — 5% [4]. RCC prognosis varies depending on the occurrence and aggressiveness of the tumor [5]. In 2012 in Ukraine the renal carcinoma morbidity rate made up 15.2 per 100 000 of male population and 9.3 per 100 000 of female population. The death rate was 7.2 per 100 000 in males and 3.6 per 100 000 in females. Development of new diagnostic markers and approaches in renal cancer will allow to establish a number of new factors influencing the prognosis of this pathology.

MATERIAL AND METHODS

The nephrectomy material of 47 RCC patients was studied. By follow-up data (survival time after surgical treatment), the RCC cases were distributed as follows: 1) n = 11 (survival period < 3 months); 2) n = 17 (6 months); 3) n = 5 (> 17 months); 4) n = 14 (> 24 months). These cases were subdivided into two groups: the short-term survival group (3–6 months, n = 28) and the long-term survival group (> 17–24 months, n = 19). The long-term survival group patients received no adjuvant therapy, and the short-term survival group patient received standard immunotherapy. Informed consents were obtained from the patients according to the Ethical Commission requirements of the Donetsk Regional Anticancer Center.

The degree of RCC differentiation was assessed according to Furhman S.A. (1982) depending on the expression of cell atypia and nuclear atypia in standardly prepared 5 μm slides of paraffine-embedded tumor samples. Expression of epithelial markers (pancytokeratin, cytokeratin 18, E-cadherin) and mesenchimal markers (vimentin, α-smooth muscle actin) was determined using immunohistochemical analysis using monoclonal antibodies and visualization system from DAKO Cytomation (Denmark) (monoclonal antibodies against pancytokeratin AE1/AE3 (clone AE1/AE3), cytokeratin 18 (clone DC10), E-cadherin; vimentin (clone VY) and α-smooth muscle actin (clone 1A4)).

RESULTS AND DISCUSSION

From 19 long-term survival RCC cases, papillary structures were observed in 6 cases (31.6%). It should be noted that in each case there were foci of various degree of differentiation (high (HD), moderate (MD) and low (LD)). High differentiated RCC was found in 14 cases (73.7%), in 7 cases it was combined with the foci of MD or LD carcinoma. Moderately differentiated carcinoma was found in 12 cases, one of which was only MD, while in 7 patients it was associated with foci of HD carcinoma and in 4 patients — with foci of HD and LD carcinoma. In 8 cases (42.1%) it was LD carcinoma — not combined with other factors in 4 patients, and in 4 cases it was combined with foci of MD and HD.

From 28 short-term survival cases, 13 cases (46.4%) were HD RCC, but in all these cases it was combined with foci of MD (5 cases) and in 8 cases — with foci of MD and LD. MD RCC was present in 23 cases, 5 cases of them were just MD, in 5 cases — combined with HD, in 5 cases — with LD, and in 8 cases — with the foci of HD and LD. LD RCC was present in 18 cases (64.3%), in 5 case — not combined with more differentiated forms, in 5 cases — combined with MD carcinoma and in 8 cases — combined with MD and HD carcinoma. Thus, HD RCC occurred more frequently (73.7%) in a long-term survival group in comparison with the short-term survival group (46.4%). LD RCC occurred more frequently in a short-

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*Correspondence: Fax: +38(062) 386 73 69
E-mail: agkoncology@rambler.ru
Abbreviations used: EMT – epithelial-mesenchymal transition; RCC – renal cell carcinoma.

term survival group (64.3%) compared with long-term survival group (42.1%).

We have determined the rate of a low (1–2) and high degree (3–4) Fuhrman's nuclear atypia in groups of different survival. Degree 1–2 of nuclear atypia occurred in 13 cases (46.4%) in a short-term survival group, degree 3–4 — in all the cases (100%). A low degree of nuclear atypia was more frequent a long-term survival group (17 of 19 cases (89.5%)) and was fairly higher than that in a short-term survival group. High degree nuclear atypia was observed in 12 cases (63.2%) in a long-term survival group 100% a the short-term survival group.

The tumor cells in RCC demonstrate a clear cytoplasm due to the loss of lipids and glycogen during the preparation of specimens. However the cells of a high degree atypia and poorly differentiated can acquire an eosinofilic and granular cytoplasm [6]. In our material it was observed in 21 (75%) cases of the short-term survival group and in 6 (55%) cases of the long-term survival group. A rhabdoid type is a particular type of cancer cell differentiation. It is characterized by a central eosinofilic inclusion in the cytoplasma, being positive to vimentin, and an eccentrically located nucleus [7, 8]. Such tumors characterized by aggressive course occur rarely (4.3%) according to the statistics of Leroy et al. [9]. In the current study such cell structure was detected in small sites in 6 cases (21.4%) from a short-term survival group and in 3 cases (15.8%) from a long-term survival group.

The foci of spindle-cell cancer were observed in 96.4% case from a short-term survival group versus 42.1% cases from long-term survival group. Spindle-cell or so called sarcomatoid carcinoma belongs to aggressive types, it is graded as degree 4 according to Fuhrman with a high stage and frequent metastases. Nowadays such RCC is considered to be an example of epithelial-mesenchymal transition (EMT) [10].

EMT is characterized by a number of special morphological features [11–13] that we have observed in our study as well, in particular, a loss of epithelial phenotype with the isolation of cancerous cells and a loss of polarity because of decreased expression of E-cadherin, a change of cell morphology into spindle-like form and acquired motility capacity due to decreased expression of cytokeratins (Fig. 1), acquirement the fibroblast phenotype (vimentin expression — Fig. 2) and myofibroblasts (expression of α-smooth muscular actin — Fig. 3). In our study such changes were observed as focal EMT more often at the border of the tumor on the surrounding tissue or on the greater part of the extensive tumor. However RCC can have the signs of the mesenchymal phenotype (Fig. 4).

The rate of spindle-cell EMT differed between the groups: 42.1% in long-term survival group versus 96.4% in short-term survival group. It is worth mentioning that there were differences even within each group, though the term-rate difference was relatively small. Thus, in the short-term survival group the differences were minor: in case of survival up to 3 months, EMT occurred in 90.8% of cases, in case of survival up to 6 months — 100%. In the long-term survival group > 17 month the EMT rate made up 80%, and > 24 months it made up 28.6%.

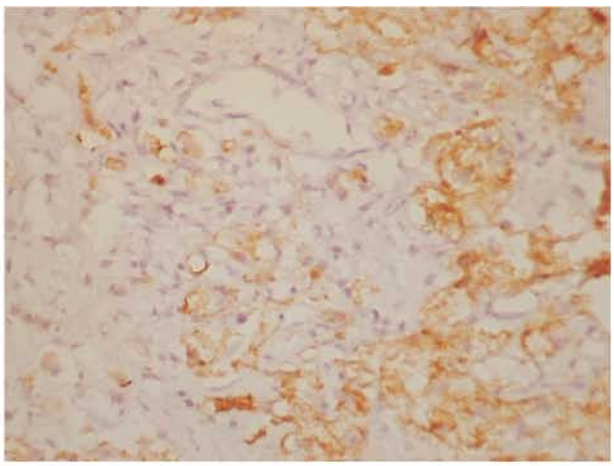

Fig. 1. Nidal reduction up to loosing AE1/AE3 pancytokeratine expression in tumor cells. Magnification ×400

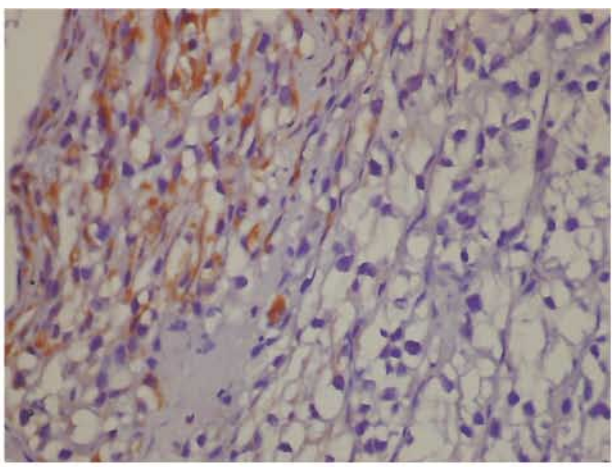

Fig. 2. Appearance of vimentin expression. Magnification ×400

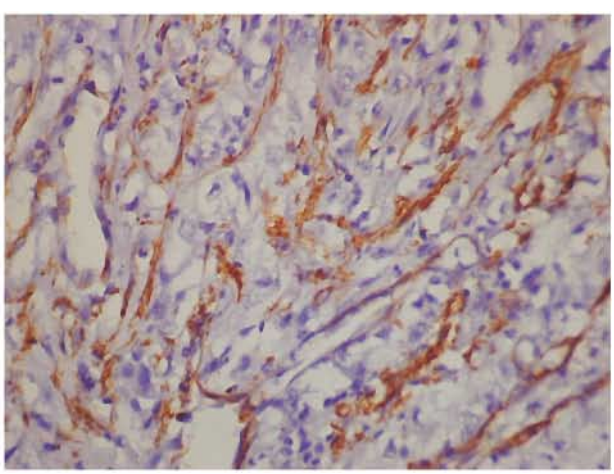

Fig. 3. Alpha smooth muscle actin expression in tumor cells. Magnification ×400

The relation of spindle-cell EMT development with a high degree of Fuhrman's nuclear atypia has been noted: a high degree (3–4) of nuclear atypia in the EMT foci and almost complete coincidence of their rate in the short-term survival group (96.4 and 100%) and relatively similar indices of rate in the long-term survival group (42.1 and 68.4%).

In our study such changes were seen in the form of focal EMT more often at the tumor boundary on the adjacent tissue (Fig. 5) or in the form of extensive EMT occupying the bulk of tumor.

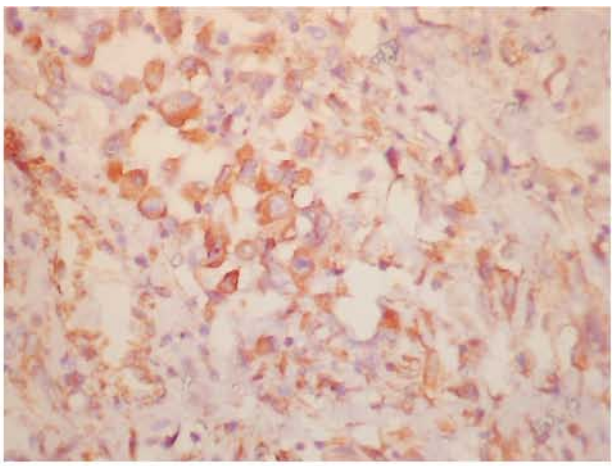

Fig. 4. Vimentin expression in renal clear cell carcinoma. Magnification ×400

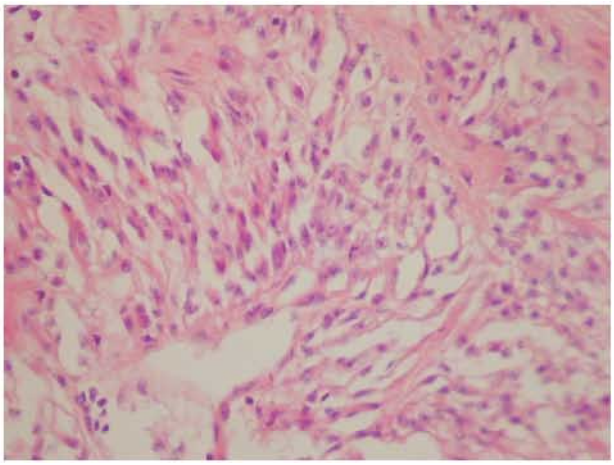

Fig. 5. Nidal EMT of elongated fibroblast-like tumor cells. Magnification ×400

According to the literature [14] EMT enhances the tumor invasiveness. Taking this fact into consideration we compared the rate of vessels invasion by the presence of tumor cells in their lumen in the histologic preparations (Fig. 6) and the lymph nodes with the rate of EMT. In the short-term survival group their rate made up 53.6%, and in the long-term survival group — only 21.1%. Thus, there is a relation between in the EMT rate and tumor invasiveness.

One of the factors of the RCC prognosis is considered to be necrosis in tumor [9]. However it is not the presence of necrosis but its expansion that is thought to be important. A poor prognosis is associated with the presence of macronecroses [9]. That is why in our study we calculated separately the rate of micro and macronecroses. The rate of large necrosis areas in patients with a short-term survival was 64.3%, micronecroses — 35.4%. In the patients with a long-term survival, the rate of macronecroses was 42.1%, and micronecroses — 21%, i.e. they occurred in a considerably lesser rate than in the short-term survival group.

In conclusion, in our study the rate of spindle-cell (sarcomatoid) component development was connected with the frequency of Fuhrman's high nuclear degree and impacted on the survival duration of patients with RCC following nephrectomy. Detection of this form of EMT in RCC is of great importance as its presence influences the rate of vascular invasion (53.6% in the short-term survival group and 21.1% in the long-term survival group) and, therefore, prognosis of renal cancer.

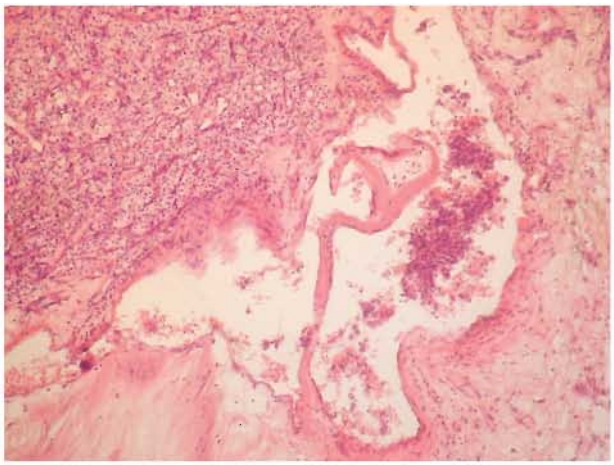

Fig. 6. Complex of tumor cells in vessel lumen. Hematoxylin and eosin staining. Magnification ×100

REFERENCES

1. **Moskvina LV, Andreev YuYu, Malkov PG.** Prognostic factors of renal cell carcinoma. Archiv Pathologii 2011; **73**: 47–52 (in Russian).
2. **Ferlay J, Autier P, Boniol M, *et al.*** Estimates of the cancer incidence and mortality in Europe. Ann Oncol 2006; **18**: 581–92.
3. **Tymopheev IV, Aksel EM.** Renal cell carcinoma in Russia in 2008. J Malignant Tumours 2011; **1**: 6–10 (in Russian).
4. **Chowdhury S, Choueiri TK.** Recent advances in the systemic treatment of metastatic papillary renal cancer. Expert Rev Anticancer Ther 2009; **9**: 373–9.
5. **Kallio J.** Prognostic factors in renal cell carcinoma. Academic dissertation. Acta Universitatis Tamperensis 2004; **5**: 43–51.
6. **Zhou M.** Molecular targets in renal tumors: pathological assessment. Renal cell carcinoma. Humana Press 2009; 35–48.
7. **Klatte T, Seligson DB, LaRochelle J, *et al.*** Molecular signatures of localized clear cell renal cell carcinoma to predict disease-free survival after nephrectomy. Cancer Epidemiol Biomarkers Prev 2009; **18**: 894–900.
8. **Salamanca J, Nevado M, Martínez-González MA, *et al.*** Undifferentiated carcinoma of the jejunum with extensive rhabdoid features. Case report and review of the literature. APMIS 2008; **116**: 941–6.
9. **Leroy X, Zini L, Buob D, *et al.*** Renal cell carcinoma with rhabdoid features: an aggressive neoplasm with overexpression of p53. Arch Pathol Lab Med 2007; **131**: 102–6.
10. **Conant JL, Peng Z, Evans MF, *et al.*** Sarcomatoid Renal cells carcinoma in an example of epithelial-mesenchymal transition. J Clin Pathol 2011; **20**: 8–16.
11. **Nieto MA.** Epithelial-Mesenchymal Transitions in development and disease: old views and new perspectives. Int J Dev Biol 2009; **53**: 1541–7.
12. **Radisky DC.** Epithelial-mesenchymal transition. J Cell Science 2005; **118**: 4325–6.
13. **Thierry JP, Acloque H, Huang RY, Nieto MA.** Epithelial-mesenchymal transition ns in development and disease. Cell 2009; **139**: 871–90.
14. **Weinberg RA.** Mechanisms of malignant progression. Carcinogenesis 2009; **29**: 1092–5.