Medicinska istaživanja 2024; 57(1):33-40 | DOI: 10.5937/medi57-46396

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

The role of HE4 protein expression in relation to clinicopathological features of renal cell tumors

Jovan Jevtić[1], Ana Mioljević[1] Ljubica Simić[1], Milica Tubić[1], Voin Brković[2], Marko Baralić[2], Gorana Nikolić[1], Maja Životić[1]

[1]University of Belgrade, Faculty of Medicine, Institute of Pathology, Belgrade, Serbia

[2]University Clinical Center of Serbia, Department of Nephrology, Belgrade, Serbia

Recived: 11 September 2023

Revised: 06 October 2023

Accepted: 26 October 2023

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

Correspondence to:
Maja Životić

Institute of Pathology, Faculty of Medicine, University of Belgrade,

1, Dr Subotića Street, 11000 Belgrade, Serbia

Email: majajoker@gmail.com

Gorana Nikolić

Institute of Pathology, Faculty of Medicine, University of Belgrade

1, Dr Subotića Street, 11000 Belgrade, Serbia

Email: gorana.nikolic03@gmail.com

Summary

Introduction: Widespread routine ultrasound diagnostics enables early detection of kidney tumors. However, due to nonspecific symptomatology and signs that usually appear as the disease progresses, there are still patients whose diagnosis is made only in advanced stages of the disease.

Aim: Our aim was to investigate the correlation of human epididymis protein 4 (HE4) expression, including the intensity and localization of HE4 positivity, with the clinical and pathohistological characteristics of kidney tumors.

Material and Methods: The study included 96 kidney tumors diagnosed between 2010 and 2013 at the Institute of Pathology in Belgrade. Anti-HE4 antibodies were used for immunohistochemical analysis. Demographic, clinical, and pathohistological characteristics were examined in relation to HE4 expression.

Results: No correlation was observed between HE4 expression in kidney tumors and patients' gender and the nuclear grade of tumors. However, HE4 expression was significantly more frequent in larger tumors, specifically in T3 and T4 tumors, compared to T1 and T2 tumors (p=0.009; p=0.006, respectively). No correlation was observed between HE4 expression and the pathohistological type of kidney tumors, but it is important to emphasize that membrane expression of HE4, unlike most renal cell carcinomas, was not observed in oncocytomas.

Conclusion: It's possible that HE4 plays a role in progression of kidney tumor growth. Membrane expression of HE4 could be used as a new parameter in differentiating renal cell carcinomas from oncocytomas.

Keywords: renal cell carcinoma, oncocytoma, human epididymis protein 4, HE4, pathohistological characteristics

Cite this article as: Jevtić J, Mioljević A, Simić Lj, Tubuć M, Brković V, Baralić M, Nikolić G, Životić M. The role of he4 protein expression in relation to clinicopathological features of renal cell tumors; Medicinska istraživanja 2024; 57(1):33-40 10.5937/medi57-46396

INTRODUCTION

According to their biological behavior, tumors of the kidney with renal cell origin can be either benign or malignant. Kidney cancers in adult population occur with a prevalence of about 2%, and they are more frequent in males (2:1) (1, 2). Renal cell carcinoma (RCC) is the most common type of kidney tumor and ranks eighth in terms of its incidence among adult malignancies (3,4). There are several histopathological subtypes of RCC, which occur with variable frequency, the most common being clear cell carcinoma (80-90%), followed by papillary (10-15%), chromophobe (4-5%), and carcinoma of the collecting ducts (1%) (2). These histopathological subtypes have different origins, genetics, morphology, and biological behavior (2). Oncocytoma is a benign tumor of renal cell origin which occurs with a frequency of 3-5% (5). Enhancing our comprehension of the molecular pathways involved in the development and progression of kidney tumors holds a potential to drive the formulation of innovative approaches for early detection and treatment. Human epididymis protein 4 (HE4) is encoded by the gene on chromosome 20q12-13.1 and it was first identified in the epithelium of the distal epididymis (6, 7). Initially, it was believed that HE4 played a role in maturation of the sperm and intrinsic immunity (7, 8). Clinical research in the past decade has shown that HE4 express in other organs, as well, including the female reproductive system, breast tissue, and kidneys, as well as in certain regions of the respiratory tract and nasopharynx (9-11). HE4 synthesis occurs within the endoplasmic reticulum and Golgi apparatus. Subsequently, the protein is secreted via exocytosis into the extracellular space, where it functions as a protease inhibitor (7). HE4 has been recently identified as a potential serum biomarker for ovarian carcinoma, either alone or in combination with CA125 (11-15), also elevated serum levels of HE4 protein have been reported in renal fibrosis (16,17). Interestingly, researchers have shown that systemic administration of HE4-neutralizing antibodies inhibits the progression of renal fibrosis in an animal model (16,17). Considering this, recent studies have shown that HE4 could be a diagnostic marker for ovarian tumors, lung adenocarcinoma, breast, urothelial and pancreatic carcinomas (18-23). Our aim was to investigate the correlation between the expression of HE4 protein, including the intensity and localization of HE4 positivity, with clinical and pathohistological characteristics of renal tumors.

MATERIALS AND METHODS

The study conducted at the Institute of Pathology, Faculty of Medicine, University of Belgrade, between 2010 and 2013, included 96 renal tumors. These comprised 66 clear cell RCCs, 12 papillary RCCs, 7 chromophobe RCCs, 4 multilocular cystic RCCs, 2 collecting duct carcinomas (Bellini), and 5 oncocytomas. Tissue microarray cylinders were obtained from paraffin-embedded renal tumor tissue samples. The sampling process involved triplicate collection from the region of interest in paraffin blocks of the tumor. A hollow medical needle with 0.6 mm diameter was used for this purpose. Taken tissue cylinders were subsequently inserted into the paraffin block and precisely arranged as a series. Using a microtome, the paraffin blocks of the tissue microarray were sliced to the thickness of 5 μm and placed on slides, for subsequent immunohistochemical analysis.

Immunohistochemistry

Immunohistochemistry was performed on tissue microarray plates. Having been deparaffinized in xylol and hydration, the plates were inserted in the citrate buffer (pH 6.0) and exposed to microwave irradiation at 400W for 20 min. Blockage of peroxidase activity was performed with 1% BSA (Bovine Serum Albumin). After the extraction of the antigen, incubation with the primary HE4 antibody (1:40, ab24480, Abcam, UK) was performed for 1 hour. EnVisionTM (DAKO, Denmark) was used to visualize the antigen-antibody reaction with a 3,3'-diaminobenzidine (DAB), and consequently contrasting with hematoxylin (Merc, USA). Negative controls were established by excluding the primary antibody during the immunohistochemistry procedure. As for the positive control, normal human epididymis tissue was used. The slides were examined using a BX53 light microscope with a DP12CCD camera (Olympus).

Statistical analysis

Statistical analysis was performed using IBM SPSS software, version 26.0. Demographic, clinical and pathological characteristics of renal tumors (patient's gender, tumor size, tumor type, nuclear grade and TNM stage of disease) were examined concerning the presence, intensity and localization of the expression of HE4 protein.

RESULTS

In the analysis of 96 cases of kidney tumors, no significant difference was observed in the distribution of HE4-expression in relation to patients' gender. However, we did observe variability in the expression of HE4 among different histopathological types of kidney tumors. The positivity of HE4 was detected in approximately 76% of clear cell RCC and 90% of papillary RCC type 2. In all other tumor subtypes, the expression of HE4 was present in 100% of cases. Upon statistical analysis, no significant correlation was found between the frequency of HE4 expression and nuclear grade (p=0.427).

Table 1. Pathohistological characteristics of kidney tumors and HE4 expression

Pathohistological characteristics		HE4 protein expression n (%)		p
		Absent	Present	
Tumor type	*Clear cell RCC*	16 (24.2%)	50 (75.8%)	#
	Papillary RCC, type 1	0 (0.0%)	2 (100.0%)	
	Papillary RCC, type 2	1 (10.0%)	9 (90.0%)	
	Multilocular cystic RCC	0 (0.0%)	4 (100.0%)	
	Chromophobe RCC	0 (0.0%)	7 (100.0%)	
	Carcinoma of the collecting ducts-Bellini	0 (0.0%)	2 (100.0%)	
	Oncocytoma	0 (0.0%)	5 (100.0%)	
Nuclear grade (NG)	*NG I, NG II*	8 (16.0%)	42 (84.0%)	χ^2=0.710 p=0.427
	NG III, NG IV	9 (23.1%)	30 (76.9%)	
T stage	*T1, T2*	12 (28.6%)	30 (71.4%)	χ^2=7.507 p=0.006*
	T3, T4	2 (5.3%)	36 (94.7%)	
N stage	*N0*	1 (11.1%)	8 (88.9%)	#
	N1	0 (0.0%)	2 (100.0%)	
M stage	*M0*	0 (0.0%)	1 (100.0%)	#
	M1	0 (0.0%)	2 (100.0%)	

In contrast to T1 and T2 tumors, where the expression of HE4 was positive in 71% of cases, higher stage tumors (T3 and T4) were significantly more likely to express HE4 (95%), with a p=0.006. **(Table 1)**.

Given the fact that lymph nodes were submitted only in 11 patients, an adequate statistical analysis was not possible. However, it was observed that tumors without regional lymph node metastases (N0) exhibited HE4 expression in 89% of cases, while all tumors with regional lymph node metastases (N1) showed HE4 positivity **(Table 1)**. Additionally, information regarding systemic metastases was available for a small number of patients (3 out of 96). All of these three patients had HE4 expression in their tumor tissue. Two out of three patients had systemic metastases (M1) at the time of diagnosis. One patient did not have any metastases (M0) during the examination of the surgical specimen, but they were later detected **(Table 1)**.

There is a trend of increasing HE4 expression intensity with an increase in the average tumor size. The average size of the tumors without HE4 expression was 5.3±1.8 cm, while the tumors with mild, moderate and strong expression of HE4 had the following average dimensions: 6.5±3.1 cm, 6.6±3.6 cm 7.2±5.0 cm, respectively.

Through the analysis of the HE4 protein expression intensity, it was observed that the majority of clear cell RCC (36%), papillary RCC type 2 (40%), and multilocular cystic carcinoma (50%) exhibited a mild expression of HE4. On the other hand, oncocytoma (60%), chromophobe RCC (57%), and collecting duct carcinoma

Table 2. Pathohistological characteristics of kidney tumors and intensity of HE4 expression

Pathohistological characteristics		HE4 protein expression n (%)				p
		Absent	mild	moderate	strong	
Tumor type	*Clear cell RCC*	16 (24.2%)	24 (36.4%)	19 (28.8%)	7 (10.6%)	#
	Papillary RCC, type 1	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	
	Papillary RCC, type 2	1 (10.0%)	4 (40.0%)	3 (30.0%)	2 (20.0%)	
	Multilocular cystic renal cell neoplasm	0 (0.0%)	2 (50.0%)	1 (25.0%)	1 (25.0%)	
	Chromophobe RCC	0 (0.0%)	1 (14.3%)	4 (57.1%)	2 (28.6%)	
	Carcinoma of the collecting ducts-Bellini	0 (0.0%)	0 (0.0%)	2 (100.0%)	0 (0.0%)	
	Oncocytoma	0 (0.0%)	2 (40.0%)	3 (60.0%)	0 (0.0%)	
Nuclear grade (NG)	*NG I, NG II*	8 (16.0%)	18 (36.0%)	17 (34.0%)	7 (14.0%)	χ^2=0.712 p=0.870
	NG III, NG IV	9 (23.1%)	13 (33.3%)	12 (30.8%)	5 (12.8%)	
T staging	*T1, T2*	12 (28.6%)	11 (26.2%)	11 (26.2%)	8 (19.0%)	χ^2=10.203 p=0.017*
	T3, T4	2 (5.3%)	17 (44.7%)	15 (39.5%)	4 (10.5%)	
N staging	*N0*	1 (11.1%)	4 (44.4%)	3 (33.3%)	1 (11.1%)	#
	N1	0 (0.0%)	0 (0.0%)	1 (50.0%)	1 (50.0%)	
M staging	*M0*	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	#
	M1	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	

*- statistically significant results; #- Due to high occurrence of lower expected frequencies, statistical analysis could not be performed; n- number of cases; N0 – regional lymph node involvement; N1- regional lymph node involvement; M0 – without metastases; M1 – present metastases.

Table 3. Pathohistological characteristics of kidney tumors in relation to the localization of HE4 expression

Pathohistological characteristics		Membranous HE4 expression n (%)		p
		Present	Absent	
Tumor type	*Clear cell RCC*	44 (88.0%)	6 (12.0%)	#
	Papillary RCC, type 1	1 (50.0%)	1 (50.0%)	
	Papillary RCC, type 2	4 (44.4%)	5 (55.6%)	
	Multilocular cystic renal cell neoplasm	4 (100.0%)	0 (0.0%)	
	Chromophobe RCC	4 (57.1%)	3 (42.9%)	
	Carcinoma of the collecting ducts-Bellini	1 (50.0%)	1 (50.0%)	
	Oncocytoma	0 (0.0%)	5 (100.0%)	
Nuclear grade (NG)	*NG I, NG II*	36 (85.7%)	6 (14.3%)	χ^2=1.713 p=0.191
	NG III, NG IV	22 (73.3%)	8 (26.7%)	
T staging	*T1, T2*	26 (86.7%)	4 (13.3%)	χ^2=3.654 p=0.059
	T3, T4	24 (66.7%)	12 (33.3%)	
N staging	*N0*	5 (62.5%)	3 (37.5%)	#
	N1	2 (100.0%)	0 (0.0%)	
M staging	*M0*	1 (100.0%)	0 (0.0%)	#
	M1	2 (100.0%)	0 (0.0%)	

(100%) more frequently demonstrated a moderate intensity of HE4 expression. Half of the cases of type 1 papillary RCC demonstrated a weak expression of HE4, while the remaining half showed a moderate expression of HE4 **(Table 2)**.

The expression intensity of HE4 showed no statistically significant difference (p = 0.870) in relation to the nuclear grade of tumors, the majority of tumors with both lower nuclear grades (NG I and II) and higher nuclear grades (NG III and IV) exhibited a weak expression of HE4 (Table 2).

The highest percentage of tumors in the lower T stages of the disease (T1 and T2) exhibited no expression of the HE4 protein. In contrast, in the higher stages (T3 and T4), the expression of HE4 was more frequent, with lower and moderate intensity, and this difference was statistically significant, p = 0.017, **(Table 2)**.

Tumors without regional and systemic metastases typically displayed weak expression of the HE4 protein, whereas tumors with regional and systemic metastases exhibited more intense expression of the HE4 protein **(Table 2)**.

When analyzing the correlation between tumor size and the localization of HE4 expression, it was observed that kidney tumors without membrane expression of HE4 tended to be slightly smaller in size (6.7 ± 4.0) compared to tumors with membrane HE4 positivity (average of 7.1 ± 4.1).

Out of 79 kidney tumors that expressed the HE4 protein, 58 tumors (75%) exhibited a membrane localization of HE4 either alone or in combination with cytoplasmic localization. On the other hand, 21 tumors (25%) showed exclusive cytoplasmic localization of HE4. Notably, all cases of oncocytoma demonstrated exclusive cytoplasmic expression of HE4, whereas the multilocular cystic neoplasm showed exclusive membrane positivity. **(Table 3, Figure 1)**.

The frequency of membrane expression of HE4 was not significantly different between tumors with lower and higher nuclear grade (p=0.191; **Table 3, Figure 1**) or between tumors with lower and higher T stage (p=0.059; **Table 3, Figure 1**). However, it was observed that with an increase in nuclear grade, clear cell RCC demonstrated not only membrane localization of HE4 but it also exhibited cytoplasmic expression, as illustrated in **Figure 1**. In all cases of tumors with regional and systemic metastases, the presence of membrane expression of the HE4 protein was observed. **(Table 3)**.

DISCUSSION

Over the past three decades, there has been an increasing incidence of renal tumors in Europe, the United States (US), and Australia (3, 4). Approximately 270,000 new cases of renal cell carcinoma (RCC) are diagnosed worldwide each year, and approximately 116,000 patients die from the disease (3, 4). Due to its characteristic rarity of early signs, diverse clinical manifestations, and high resistance to conventional treatments such as radiotherapy and chemotherapy, renal cell carcinoma (RCC) presents a significant challenge in terms of early diagnosis and effective treatment. Consequently, numerous clinical and pathological studies have been conducted to identify potential biomarkers that can aid in early diagnosis and facilitate targeted immunomodulatory interventions to inhibit tumor growth. The aim of such research is to improve patient outcomes and develop more personalized approaches to RCC management. Recognition of HE4 as a biomarker of ovarian cancer, as well as the study of Galgano who examined the expression of HE4 in a variety of normal and malignant human tissues, and who expressed the need for further investigation of this protein (11), led us to further examine the expression of HE4 in different histology types of kidney tumors.

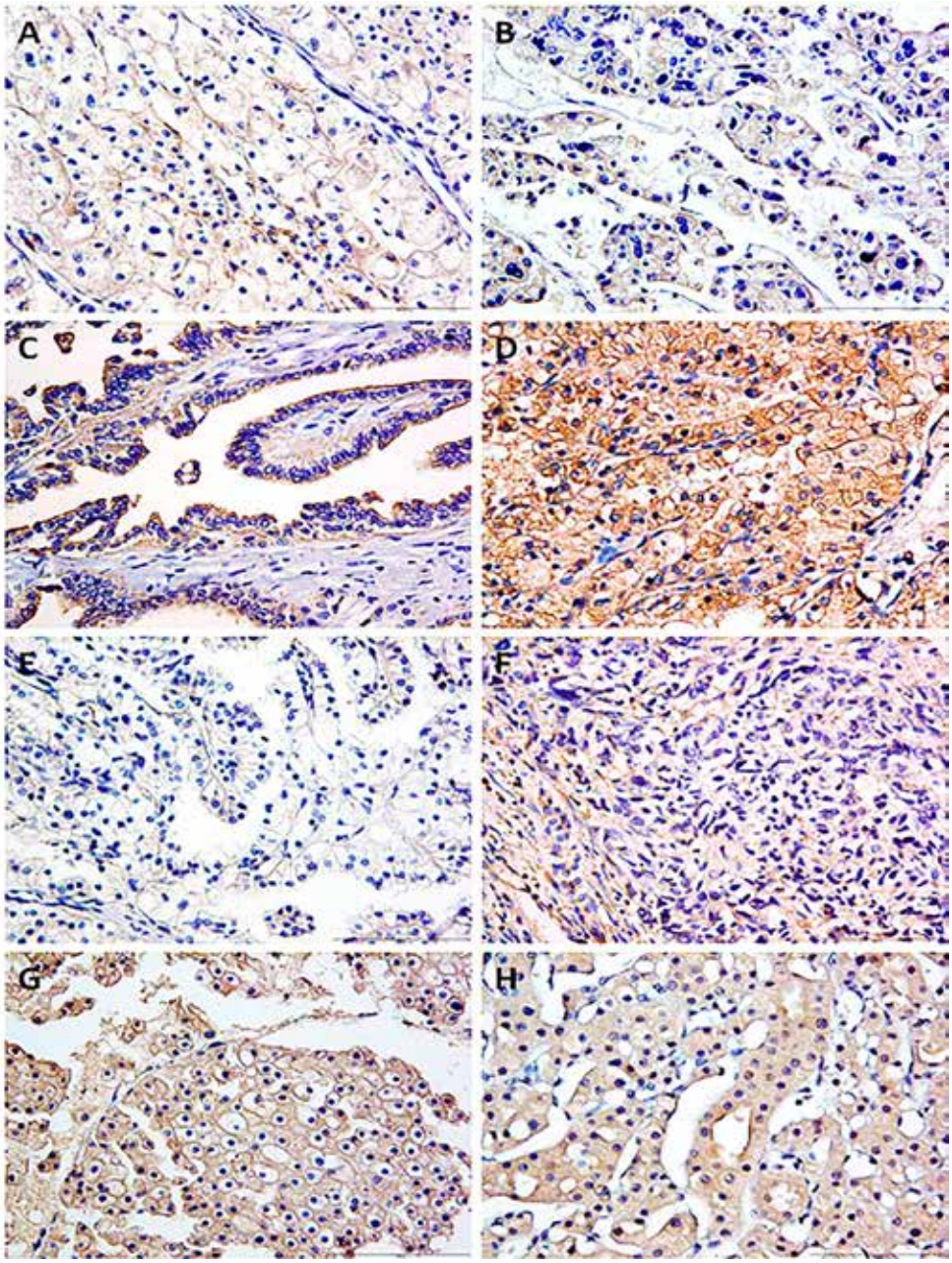

Figure 1. Different patterns of HE4 protein expression in relation to the histopathological tumor type, nuclear grade of the tumor, and biological behavior. **A)** Low nuclear grade clear cell carcinoma - membrane expression of HE4. **B)** High nuclear grade clear cell carcinoma – cytoplasmic and membrane expression of HE4. **C)** Papillary carcinoma type 1 - moderate cytoplasmic and membrane expression of HE4. **D)** Papillary carcinoma type 2 - moderate to strong cytoplasmic and membrane expression of HE4. **E)** Clear cell papillary carcinoma - very weak membrane expression of HE4. **F)** Bellini's collecting duct carcinoma - moderate cytoplasmic and membrane expression of HE4. **G)** Chromophobe carcinoma - moderate cytoplasmic and membrane expression of HE4. **F)** Oncocytoma - moderate expression of HE4 exclusively in the cytoplasm.

Our study yielded compelling results, indicating that 83% of renal tumors expressed the HE4 protein. This finding contrasts with the study conducted by Galgano et al., where a significantly lower percentage of renal tumors (37.5%) demonstrated HE4 immunoreactivity([11).

In our study, we found no association between the patients' gender and the expression of the HE4 protein in kidney tumors. Similarly, other studies measuring serum levels of HE4 concluded that gender did not impact its expression in patients with kidney tumors (15).

Tumor size is an important prognostic factor in RCC, which correlates with a poorer prognosis, higher incidence of metastases and higher mortality (24). US researchers have reported a significant association between serum levels of HE4 and tumor size as well as myometrial invasion in endometrial cancer (25). In our study, we made a novel discovery that larger renal tumors demonstrated a significant expression of HE4. Additionally, we observed that the membrane localization of HE4 positivity was considerably more prevalent in larger tumors compared to cytoplasmic localization. Moreover, we noticed a tendency for the intensity of HE4 expression to amplify as the tumor diameter increased.

The study conducted by Galgano (11) has provided partial examination of HE4 protein expression in different histopathological types of kidney tumors. The

localization of HE4 protein in relation to specific cell compartments has not been assessed in kidney tumors. HE4 is normally expressed within the reproductive system. However, Drapkin observed that ovarian carcinomas secreted this protein abundantly into the bloodstream and urine, allowing for easy detection of its presence and quantity. Consequently, HE4 emerged as a sensitive biomarker for ovarian cancer (10). In our study, we conducted further analysis to investigate the presence, intensity, and localization of the HE4 protein based on the histopathological subtype of renal cell carcinoma (RCC), nuclear grade, as well as the local, regional, and systemic spread of the disease, specifically in relation to the TNM stage.

Clear cell RCC is the most common kidney cancer. Extensive examination of gene expression in many proteins has been carried out (26). Most clear cell RCC expresses HE4 protein, mostly of mild intensity, with present membrane immunoreactivity. Weak expression of HE4 immunoreactivity was also noted by Galgano, but in a much smaller percentage of clear cell RCC (4%), compared to our study (11).

Papillary RCC is recognized as the second most prevalent kidney cancer (27). In our study, HE4 expression was universally present in type 1 clear cell RCC and was found in approximately 90% of type 2 cases, albeit often with mild intensity. This contrasts with the findings of Galgano, who reported HE4 expression in 46% of papillary RCC cases without specifying the subtype. Galgano also observed strong HE4 immunoreactivity in 38% of these papillary RCC cases (11). Interestingly, in our study, none of the type 1 papillary RCC cases exhibited strong HE4 expression, whereas 20% of type 2 cases did.

Multilocular cystic carcinoma is a rare subtype of RCC, which is now considered to be a "multilocular cystic renal cell neoplasia of low malignant potential" (28-30). Distinguishing renal cell carcinoma (RCC) from other histopathologic types, particularly clear cell RCC, is crucial due to the distinct therapeutic strategies involved. This differential diagnosis is essential for determining appropriate treatment approaches (30). Although the difference in HE4 expression compared to other cancers has not been fully understood, it is noteworthy that HE4 is consistently membranously present in all cases of this tumor, albeit with mild intensity.

Differentiating between chromophobe RCC and oncocytoma poses one of the most significant diagnostic challenges in kidney tumor pathology. These tumors have unique biological characteristics that necessitate different therapeutic strategies, making their accurate classification crucial for pathologists (31-34). It is known that histochemistry and the ultrastructure of these two tumors overlap, and the existence of a hybrid tumor which has histological characteristics of both, support the hypothesis of a common precursor of these two tumors (34). Although there are existing markers for the differential diagnosis of oncocytoma and chromophobe renal cell carcinoma (RCC), their sensitivity and specificity have been found to be less than satisfactory. Achieving an accurate diagnosis typically involves a combination of markers. Compared to American authors who have detected 67% oncocytoma and 69% chromophobe RCC with HE4 positivity (11), immunohistochemical analysis of our cases showed the presence of HE4 in all cases of oncocytoma and chromophobe RCC. In our study, for the first time, the localization of HE4 expression was analyzed and we observed a distinct pattern in both oncocytoma and chromophobe renal cell carcinoma (RCC). Specifically, we found that HE4 exhibited cytoplasmic positivity exclusively in oncocytoma, while in 57.1% of chromophobe RCC cases, membrane positivity was also observed. Considering this significant finding, it is conceivable that in the future, HE4 immunohistochemical analysis could be employed as a complementary tool to the routine panel of standard antibodies for the differential diagnosis of oncocytoma and kidney cancer. Since the membranous HE4 immunoreactivity could be an indirect indicator of the secretory activity of the tumor that express HE4, determining the serum level of HE4 might, in some instances, assist in the differentiation of oncocytoma and chromophobe cancer. It is necessary to carry out further testing to determine the correlation between the localization of HE4 positivity and serum values of HE4. The highest percentage of chromophobe RCC and oncocytoma were moderately expressing the HE4, while in his study Galgano observed strong expression of HE4 in both types of tumors (11).

Collecting duct carcinoma is a very rare and aggressive form of RCC (35), so we examined only two cases and noted that in both cases tumor tissue expressed HE4 with moderate intensity. There are multiple similarities between RCC and transitional cell carcinoma. Based on our results and Galgano's results we could point to one more regarding the expression of HE4 protein [36,11].

To date, there is a lack of comprehensive studies investigating the expression of HE4 in kidney tumors and its relation with various clinical and pathological characteristics such as patients' gender, tumor size, histopathologic type, nuclear grade, and TNM tumor stage. However, our study, for the first time, revealed a potential association between HE4 expression in kidney tumors and tumor size.

Despite using the nuclear grade of the tumor as a prognostic indicator, we did not find a significant variation in the frequency and intensity of HE4 expression among tumors with different nuclear grades.

The TNM classification of a tumor is crucial, as it impacts both the therapeutic approach and the prognosis of the disease, while also providing valuable information regarding the possibility of metastasis (37). Our findings indicate that kidney tumors with higher T stages and larger dimensions exhibit a significantly higher frequency of HE4 positivity. Moreover, we observed a nota-

ble correlation between the intensity of HE4 expression and the progression of tumor growth. This might point to a stimulating role of HE4 in the progression of local growth of kidney cancer, as has already been observed in ovarian cancer (38,39).

Some studies show that HE4 can stimulate the invasion and metastasis of various cancers([40). We observed mainly moderate intensity and membrane localization of the HE4 expression in all cases of tumors with both regional and systemic metastases, which might speak in favor of a given hypothesis.

CONCLUSION

Our study showed that renal tumors with larger dimensions and higher stage, more often expressed the HE4 protein, which could play a role primarily in local tumor growth. In our study, a notable distinction was observed between benign tumors, specifically oncocytoma, and malignant tumors. Oncocytoma displayed exclusive cytoplasmic localization of HE4, whereas malignant tumors exhibited variable frequencies of membrane HE4 immunoreactivity. Therefore, the membrane HE4 positivity could be used as a new parameter in the differentiation of kidney cancer and oncocytoma.

Author contributions:

Conception and design: JJ, AM, MZ, GN
Data collection: MT, LS
Writing the article: JJ, AM, MZ, GN
Critical revision of the article: MB, VB
Final approval of the article: JJ, AM, LS, MT, MB, VB, GN, MZ

REFERENCES:

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T et al. Cancer statistics, 2008. CA Cancer J Clin 2008; 58(2):71-96. doi: 10.3322/CA.2007.0010. PMID: 18287387.
2. Rini BI, Campbell SC, Escudier B. Renal cell carcinoma. Lancet 2009; 28;373(9669):1119-32. doi: 10.1016/S0140-6736(09)60229-4. PMID: 19269025.
3. Levi F, Ferlay J, Galeone C, Lucchini F, Negri E, Boyle P et al. The changing pattern of kidney cancer incidence and mortality in Europe. BJU Int 2008; 101(8):949-58. doi: 10.1111/j.1464-410X.2008.07451.x. PMID: 18241251.
4. Lipworth L, Tarone RE, Lund L, McLaughlin JK. Epidemiologic characteristics and risk factors for renal cell cancer. Clin Epidemiol 2009; 9; 1:33-43. doi: 10.2147/clep.s4759. PMID: 20865085.
5. Trpkov K, Yilmaz A, Uzer D, Dishongh KM, Quick CM, Bismar TA et al. Renal oncocytoma revisited: a clinicopathological study of 109 cases with emphasis on problematic diagnostic features. Histopathology 2010; 57(6):893-906. doi: 10.1111/j.1365-2559.2010.03726.x. PMID: 21166703.
6. Clauss A, Lilja H, Lundwall A. A locus on human chromosome 20 contains several genes expressing protease inhibitor domains with homology to whey acidic protein. Biochem J 2002; 15;368(Pt 1):233-42. doi: 10.1042/BJ20020869. PMID: 12206714.
7. Kirchhoff C, Habben I, Ivell R, Krull N. A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. Biol Reprod 1991; 45(2):350-7. doi: 10.1095/biolreprod45.2.350. PMID: 1686187.
8. Kirchhoff C. Molecular characterization of epididymal proteins. Rev Reprod 1998; 3(2):86-95. doi: 10.1530/ror.0.0030086. PMID: 9685187.
9. Bingle L, Singleton V, Bingle CD. The putative ovarian tumour marker gene HE4 (WFDC2), is expressed in normal tissues and undergoes complex alternative splicing to yield multiple protein isoforms. Oncogene 2002; 21(17):2768-73. doi: 10.1038/sj.onc.1205363. PMID: 11965550.
10. Drapkin R, von Horsten HH, Lin Y, Mok SC, Crum CP, Welch WR et al. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. Cancer Res 2005; 65(6):2162-9. doi: 10.1158/0008-5472.CAN-04-3924. PMID: 15781627.
11. Galgano MT, Hampton GM, Frierson HF Jr. Comprehensive analysis of HE4 expression in normal and malignant human tissues. Mod Pathol 2006; 19(6):847-53. doi: 10.1038/modpathol.3800612. PMID: 16607372.
12. Hellström I, Raycraft J, Hayden-Ledbetter M, Ledbetter JA, Schummer M, McIntosh M et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res 2003; 63(13):3695-700. PMID: 12839961.
13. Lu KH, Patterson AP, Wang L, Marquez RT, Atkinson EN, Baggerly KA et al. Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. Clin Cancer Res 2004; 10(10):3291-300. doi: 10.1158/1078-0432.CCR-03-0409. PMID: 15161682.
14. Schummer M, Ng WV, Bumgarner RE, Nelson PS, Schummer B, Bednarski DW et al. Comparative hybridization of an array of 21,500 ovarian cDNAs for the discovery of genes overexpressed in ovarian carcinomas. Gene 1999; 238(2):375-85. doi: 10.1016/s0378-1119(99)00342-x. PMID: 10570965.
15. Hertlein L, Stieber P, Kirschenhofer A, Krocker K, Nagel D, Lenhard M et al. Human epididymis protein 4 (HE4) in benign and malignant diseases. Clin Chem Lab Med 2012; 50(12):2181-8. doi: 10.1515/cclm-2012-0097. PMID: 23093276.
16. Allison SJ. Fibrosis: HE4--a biomarker and target in renal fibrosis. Nat Rev Nephrol 2013; 9(3):124. doi: 10.1038/nrneph.2013.2. Epub 2013 Feb 12. PMID: 23399582.
17. LeBleu VS, Teng Y, O'Connell JT, Charytan D, Müller GA, Müller CA et al. Identification of human epididymis protein-4 as a fibroblast-derived mediator of fibrosis. Nat Med 2013; 19(2):227-31. doi: 10.1038/nm.2989. PMID: 23353556.
18. Kamei M, Yamashita S, Tokuishi K, Hashioto T, Moroga T, Suehiro S et al. HE4 expression can be associated with lymph node metastases and disease-free survival in breast cancer. Anticancer Res 2010; 30(11):4779-83. PMID: 21115941.
19. Moore RG, Brown AK, Miller MC, Badgwell D, Lu Z, Allard WJ et al. Utility of a novel serum tumor biomarker HE4 in patients with endometrioid adenocarcinoma of the uterus. Gynecol Oncol 2008; 110(2):196-201. doi: 10.1016/j.ygyno.2008.04.002. PMID: 18495222.
20. Yamashita S, Tokuishi K, Hashimoto T, Moroga T, Kamei M, Ono K et al. Prognostic significance of HE4 expression in pulmonary adenocarcinoma. Tumour Biol 2011; 32(2):265-71. doi: 10.1007/s13277-010-0118-5. PMID: 20953751.
21. Iwahori K, Suzuki H, Kishi Y, Fujii Y, Uehara R, Okamoto N et al. Serum HE4 as a diagnostic and prognostic marker for lung cancer. Tumour Biol 2012; 33(4):1141-9. doi: 10.1007/s13277-012-0356-9. PMID: 22373583.
22. Larramendy ML, Lushnikova T, Björkqvist AM, Wistuba II, Virmani AK, Shivapurkar N et al. Comparative genomic hybridization reveals complex genetic changes in primary breast cancer tumors and their cell lines. Cancer Genet Cytogenet 2000; 119(2):132-8. doi: 10.1016/s0165-4608(99)00226-5. PMID: 10867149.
23. O'Neal RL, Nam KT, LaFleur BJ, Barlow B, Nozaki K, Lee HJ et al. Human epididymis protein 4 is up-regulated in gastric and pancreatic adenocarcinomas. Hum Pathol 2013; 44(5):734-42. doi: 10.1016/j.humpath.2012.07.017. PMID: 23084584.

24. Hafez KS, Fergany AF, Novick AC. Nephron sparing surgery for localized renal cell carcinoma: impact of tumor size on patient survival, tumor recurrence and TNM staging. J Urol 1999; 162(6):1930-3. doi: 10.1016/S0022-5347(05)68071-8. PMID: 10569540.
25. Kalogera E, Scholler N, Powless C, Weaver A, Drapkin R, Li J et al. Correlation of serum HE4 with tumor size and myometrial invasion in endometrial cancer. Gynecol Oncol 2012; 124(2):270-5. doi: 10.1016/j.ygyno.2011.10.025. PMID: 22037318.
26. Takahashi M, Rhodes DR, Furge KA, Kanayama H, Kagawa S, Haab BB et al. Gene expression profiling of clear cell renal cell carcinoma: gene identification and prognostic classification. Proc Natl Acad Sci USA 2001; 98(17):9754-9. doi: 10.1073/pnas.171209998. PMID: 11493696.
27. Delahunt B, Eble JN. Papillary renal cell carcinoma: a clinicopathologic and immunohistochemical study of 105 tumors. Mod Pathol 1997; 10(6):537-44. PMID: 9195569.
28. Bielsa O, Lloreta J, Gelabert-Mas A. Cystic renal cell carcinoma: pathological features, survival and implications for treatment. Br J Urol 1998; 82(1):16-20. doi:10.1046/j.1464-410x.1998.00689.x. PMID: 9698657.
29. Suzigan S, López-Beltrán A, Montironi R, Drut R, Romero A, Hayashi T et al. Multilocular cystic renal cell carcinoma : a report of 45 cases of a kidney tumor of low malignant potential. Am J Clin Pathol 2006; 125(2):217-22. doi: 10.1309/AH6F-C77P-YR2V-6YAY. PMID: 16393684.
30. Weiss SG 2nd, Hafez RG, Uehling DT. Multiocular cystic renal cell carcinoma: implications for nephron sparing surgery. Urology 1998; 51(4):635-7. doi: 10.1016/s0090-4295(97)00703-6. PMID: 9586620.
31. Ng KL, Rajandram R, Morais C, Yap NY, Samaratunga H, Gobe GC et al. Differentiation of oncocytoma from chromophobe renal cell carcinoma (RCC): can novel molecular biomarkers help solve an old problem? J Clin Pathol 2014; 67(2):97-104. doi: 10.1136/jclinpath-2013-201895. PMID: 24170213.
32. Liu L, Qian J, Singh H, Meiers I, Zhou X, Bostwick DG. Immunohistochemical analysis of chromophobe renal cell carcinoma, renal oncocytoma, and clear cell carcinoma: an optimal and practical panel for differential diagnosis. Arch Pathol Lab Med 2007; 131(8):1290-7. doi: 10.5858/2007-131-1290-IAOCRC. PMID: 17683191.
33. Schachter LR, Cookson MS, Chang SS, Smith JA Jr, Dietrich MS, Jayaram G et al. Second prize: frequency of benign renal cortical tumors and histologic subtypes based on size in a contemporary series: what to tell our patients. J Endourol 2007; 21(8):819-23. doi: 10.1089/end.2006.9937. PMID: 17867935.
34. Noguchi S, Nagashima Y, Shuin T, Kubota Y, Kitamura H, Yao M et al. Renal oncocytoma containing "chromophobe" cells. Int J Urol 1995; 2(4):279-80. doi: 10.1111/j.1442-2042.1995.tb00473.x. PMID: 8564750.
35. Kennedy SM, Merino MJ, Linehan WM, Roberts JR, Robertson CN, Neumann RD. Collecting duct carcinoma of the kidney. Hum Pathol 1990; 21(4):449-56. doi: 10.1016/0046-8177(90)90209-n. PMID: 2318486.
36. Okuda H, Tei N, Shimizu K, Imazu T, Yoshimura K, Kiyohara H et al. Case report: collecting (Bellini) duct carcinoma during the follow-up for bladder cancer. Hinyokika Kiyo 2008; 54(10):665-8. Japanese. PMID: 19048932.
37. Sobin LH, Fleming ID. TNM Classification of Malignant Tumors, fifth edition (1997). Union Internationale Contre le Cancer and the American Joint Committee on Cancer. Cancer 1997; 80(9):1803-4. doi: 10.1002/(sici)1097-0142(19971101)80:9<1803::aid-cncr16>3.0.co;2-9. PMID: 9351551.
38. Moore RG, Hill EK, Horan T, Yano N, Kim K, MacLaughlan S et al. HE4 (WFDC2) gene overexpression promotes ovarian tumor growth. Sci Rep 2014; 4:3574. doi: 10.1038/srep03574. PMID: 24389815.
39. Li J, Chen H, Mariani A, Chen D, Klatt E, Podratz K et al. HE4 (WFDC2) Promotes Tumor Growth in Endometrial Cancer Cell Lines. Int J Mol Sci 2013; 14(3):6026-43. doi: 10.3390/ijms14036026. PMID: 23502467.
40. Zhuang H, Tan M, Liu J, Hu Z, Liu D, Gao J et al. Human epididymis protein 4 in association with Annexin II promotes invasion and metastasis of ovarian cancer cells. Mol Cancer 2014; 13:243. doi: 10.1186/1476-4598-13-243. PMID: 25362534.

ULOGA EKSPRESIJE HUMANOG EPIDIDIMISNOG PROTEINA 4 U ODNOSU NA KLINIČKO-PATOLOŠKE KARAKTERISTIKE TUMORA BUBREGA

Jovan Jevtić[1], Ana Mioljević[1], Ljubica Simić[1], Milica Tubuć[1], Voin Brković[2], Marko Baralić[2], Gorana Nikolić[1], Maja Životić[1]

Sažetak

Uvod: Široko rasprostranjena rutinska ultrazvučna dijagnostika omogućava otkrivanje tumora bubrega u početnim stadijumima. Međutim, zbog nespecifične simptomatologije i znakova, koji se javljaju tek kada bolest uznapreduje, još uvek postoje pacijenti kojima se dijagnoza postavlja tek u uznapredovalim fazama bolesti.

Cilj rada: Naš cilj bilo je ispitivanje povezanosti ekspresije humanog epididimisnog proteina 4 (HE4), uključujući intenzitet i lokalizaciju HE4 pozitivnosti, sa kliničkim i patohistološkim karakteristikama tumora bubrega.

Metode: Ispitivanje je uključilo 96 tumora dijagnostikovanih u periodu od 2010. do 2013. godine na Institutu za Patologiju u Beogradu. Korišćeno je anti-HE4 antitelo za imunohistohemijsku analizu. Ispitane su demografske, kliničke i patohistološke karakteristike u odnosu na HE4 ekspresiju.

Rezultati: Nije uočena povezanost HE4 ekspresije u tumorima bubrega sa polom pacijenata i nuklearnim gradusom tumora. Međutim, ekspresija HE4 je bila znatno češća kod većih tumora, odnosno kod tumora u stadijumima T3 i T4, u odnosu na tumore u stadijumima T1 i T2 (p=0,009; p=0,006; respektivno). Povezanost HE4 ekspresije i patohistološkog tipa tumora bubrega nije uočena, ali je u pogledu lokalizacije HE4 imunopozitivnosti najvažnije istaći da membranska ekspresija HE4, za razliku od većine karcinoma bubrega, nije viđena kod onkocitoma.

Zaključak: Moguće je da HE4 ima ulogu u progresiji rasta tumora bubrega. Membranska ekspresija HE4 bi se mogla koristiti kao novi parametar u diferencijaciji karcinoma bubrega i onkocitoma.

Ključne reči: karcinom bubrežnih ćelija, onkocitom, humani epididimisni protein 4, HE4, patohistološke karakteristike

Primljen: 11.09.2023. I **Revizija:** 06.10.2023. I **Prihvaćen:** 26.10.2023

Medicinska istaživanja 2024; 57(1):33-40