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Original Article

Expression and localization of estrogen receptors in human renal cell carcinoma and their clinical significance

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Abstract: This study aims to (1) evaluate the immunohistochemical expression of ERα, ERα36 and ERβ in combination in human renal cell carcinoma (RCC) and nearby non-tumorous tissue (2) correlate their expression pattern with the clinicopathological parameters and prognosis of the patients; this may provide a new insight into prediction of the disease outcome and understanding its progression. The three markers showed positive cytoplasmic (± membranous) staining pattern in tumor cells. The tubules in the nearby non-tumorous tissue showed either nuclear (± cytoplasmic) staining pattern (ERα and ERβ) or only cytoplasmic staining pattern (ERα36). The mean of cytoplasmic expression of ERα, ERα36 and ERβ was significantly higher in association with poor prognostic factors: larger tumor size (P<0.0001) for each, late clinical stage (P<0.0001) for each, higher nuclear grade (P = 0.003, P = 0.002 and P = 0.022) respectively, and presence of lymphovascular invasion (P<0.0001, P = 0.006 and P<0.0001) respectively. We have demonstrated for the first time that patients whose tumors express high cytoplasmic levels of ERα, ERα36 or ERβ experience shorter overall survival and disease-free survival. The independent role of ER subunits as markers of poor prognosis is proven only for ERβ and ERα36 but not ERα. In conclusion, our results indicate that the main staining pattern of ERα, ERα36 and ERβ in RCC is cytoplasmic with relation of this pattern to bad prognosis. So we can suggest the assessment of these receptors as markers of poor prognosis in RCC patients.

Keywords: Renal cell carcinoma, ERα, ERα36, ERβ

Introduction

Renal cell carcinoma (RCC) is the most common type of kidney cancer in adults accounting for more than 90% of such malignancy, with a high mortality rate approximating 100,000 individuals per year all over the world [1]. According to the International Agency for Research on Cancer GLOBOCAN 2012, the incidence rates of RCC in Egypt are 3.0/100,000 in men and 1.7/100,000 in women [2].

Surgery is considered the main line of treatment of RCC followed by chemotherapy and radiotherapy especially in advanced stage and metastatic disease. Despite these treatment modalities, the outcome of those patients is still poor due to the high recurrence rate, cancer dissemination, and resistance to chemotherapy [3]. This might be attributed to the molecular heterogeneity of RCC with different patient outcomes despite having the same clinical and pathologic characteristics [4]. So it is important to identify effective prognostic molecular markers that can provide adequate categorization and customization of patients for the proper line of treatment.

It is documented that estrogen and its receptors are variably expressed in different types of tissue, either reproductive or non- reproductive, including human kidney and implicated in the control of normal proliferation, differentiation and functions of these tissues [5, 6].

Estrogen receptors (ERs) are of two types; namely estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) with their genes located on chromosome 6q25.1 [6] and 14q respectively [7]. Although ERα and ERβ share some degree of structural homology, their biological functions are not the same [8]. While *ERα* gene is considered to act as oncogene; has proliferative activities by increasing tran-

scription of cell cycle genes, the opposite was found for *ERβ* gene which is postulated to act as tumor suppressor gene, has an anti-proliferative function, and induces apoptosis. Under normal conditions, these antagonist co-exist in a homeostatic balance and are postulated to be disrupted in certain tumor types [9].

There is strong evidence that these receptors promote the development and progression of many types of cancer [5, 10] with emerging proof speculating that human kidney may be one of these organs [11]. This is based on clinical observation such as significant sex difference in RCC with the incidence in men being twice as high as in women [11]. Other evidence includes the development of RCC in hamsters after diethylstilbestrol administration and its inhibition by hormone therapy [12], this led to hypothesis that some kidney cancer may be hormone-dependent.

Interestingly, a truncated variant of ERα was identified and called ERα36 with some studies suggesting its role in the progression and treatment resistance of certain carcinomas [13-15]. ERα36 differs from other ERα family members, which are located mainly in the nucleus, by its cytoplasmic and membranous location [16]. As a result, it transduces rapid, non-genomic, estrogen signaling cascades and affects transactivation activities of both ERα and ERβ [16].

The diverse actions of estrogens and their inhibitors in certain tumor types and the variation of ERα/ERβ ratio in these tumors, indicate that the ER subtypes have different functions in cancer biology and therapy [17, 18]. Improving patient outcome after selectively targeting or restoring ER levels in such cancer tissue is one of the current therapeutic strategies [19].

Because the expression pattern of different ERs in human RCC has not been fully investigated, we hypothesized that ERα, ERβ and ERα36 may be altered in RCC and this alteration might affect the prognosis and outcome of such patients. To the best of our knowledge, this is the first study of immunohistochemical expression of these markers together in human RCC and correlation of their expression patterns with the patient's prognosis. This may provide a new insight into cancer outcome and progression.

Materials and methods

Specimens

This is a retrospective study that included 70 formalin-fixed paraffin embedded blocks of RCC and their nearby non-tumor tissue. Tissue specimens were obtained from the archive of the Surgical Pathology Laboratory Assiut University Hospital, Faculty of Medicine (between years 2004 to 2014). The study was approved by the Medical Ethical Committee at Faculty of Medicine, Assuit University on 21/9/2016. The cliniopathological features were extracted from the hospital medical records, including patient age, gender, tumor site, tumor size, type of operation, clinical stage, and survival data (median follow-up, 35 months; range, 5-36 months).

Primary tumors were examined histopathologically for identification of the following features: histologic type (according to the World Health Organization histologic classification 2016) [20], nuclear grade (according to International Society of Urological Pathology "ISUP" grading scheme: grade 1 to grade 4, 2014) [21], tumor stage (according to AJCC Cancer Staging Handbook of the American Joint Committee on Cancer) [22], presence or absence of tumor necrosis, presence or absence of lymphovascular emboli (LVI), intensity of the host immune response, and the presence of infiltration of the adjacent tissue (capsule, perinephric fat and renal sinus).

Immunohistochemical staining

Tissue sections of 4 μm thickness of formalin-fixed paraffin-embedded specimens were taken from tissue blocks. Sections were deparaffinized in xylene and rehydrated in a descending graded ethanol series. The endogenous peroxidase was blocked with 6% hydrogen peroxide for 7 min. For epitope retrieval, sections were microwaved in citrate buffer, pH 6 for a total 20 min. Sections were incubated overnight at 4°C with the primary antibodies. The antibodies used was ERα (clone SP1, Thermo Scientific, diluted at 1/100), ERβ (clone ERb455, Scy teck laboratories, diluted at 1/100) and ERα36 (antibody against the last 20 amino acids as custom service by Alpha Diagnostic International, San Antonio, diluted 1/50). Secondary staining kits were used

Table 1. Clinicopathological parameters of studied cases (n = 70)

Clinicopathological features	Number	Percentage
Total	70	100%
Age (years)		
Median (range)	56 (38-75)	
Gender		
Male	44	62.9%
Female	26	37.1%
Tumor size (cm)		
Median (range)	10 (3-21)	
≤ 10 cm	38	54.3%
> 10 cm	32	45.7%
Site		
Right	37	52.9%
Left	33	47.1%
Bilateral	0	0%
Histopathological type		
Clear cell RCC	45	64.3%
Papillary RCC	15	21.4%
Chromophobe RCC	10	14.3%
Grade of clear cell and papillary RCC		
G1	9	15%
G2	34	56.7%
G3	16	26.7%
G4	1	1.6%
Grade of clear cell and papillary RCC (grouped)		
G1-2	43	71.7%
G3-4	17	28.3%
Clinical stage		
I	12	17.1%
II	13	18.6%
III	26	37.1%
IV	19	27.1%
Clinical stage (grouped)		
I-II	25	35.7%
III-IV	45	64.3%
T stage		
T1	20	28.6%
T2	21	30%
T3	26	37.1%
T4	3	4.3%
N stage		
N0	25	35.7%
N1	14	20%
Unreported	31	44.3%
M stage		
M0	51	72.9%
M1	19	27.1%
Lymphovascular invasion		
Positive	37	52.9%
Negative	33	47.1%
Tumor necrosis		
Positive	50	71.4%
Negative	20	28.6%

according to the manufacturer's instructions (Thermo Scientific, Fremont, CA, USA). Counterstaining was done with hematoxylin and examined by light microscopy.

Evaluation of ERα, ERβ and ERα36 expression

The scoring of ERα, ERβ and ERα36 was evaluated using a semiquantitative scoring system that reported previously [23, 24]. Briefly, the percentage of stained cells was categorized as follows: 0 = <5%, 1 = 5-25%, 2 = 26-50%, 3 = 51-75%, 4 = >75%. The intensity of staining was also evaluated and graded from 0 to 3, where 0 = negative, 1 = weak staining, 2 = moderate staining and 3 = strong staining. The two values obtained were multiplied to calculate a receptor score (maximum value 12). For survival analysis, the data of each marker were dichotomized into low and high expression patterns according to the median of each receptor-score value.

Statistical analysis

Mann-Whitney test and Kruskal Wallis (K-test) were used to compare the means of ERα, ERα36 and ER β expression in the studied cases in relation to different clinicopathological features. Spearman correlation coefficient was used to investigate the correlation between the three markers. The prognostic effect of the various parameters on clinical outcome was tested using the Kaplan-Meier method with the log-rank test was applied to compare survival curves. Multivariate analysis was done using the Cox regression model.

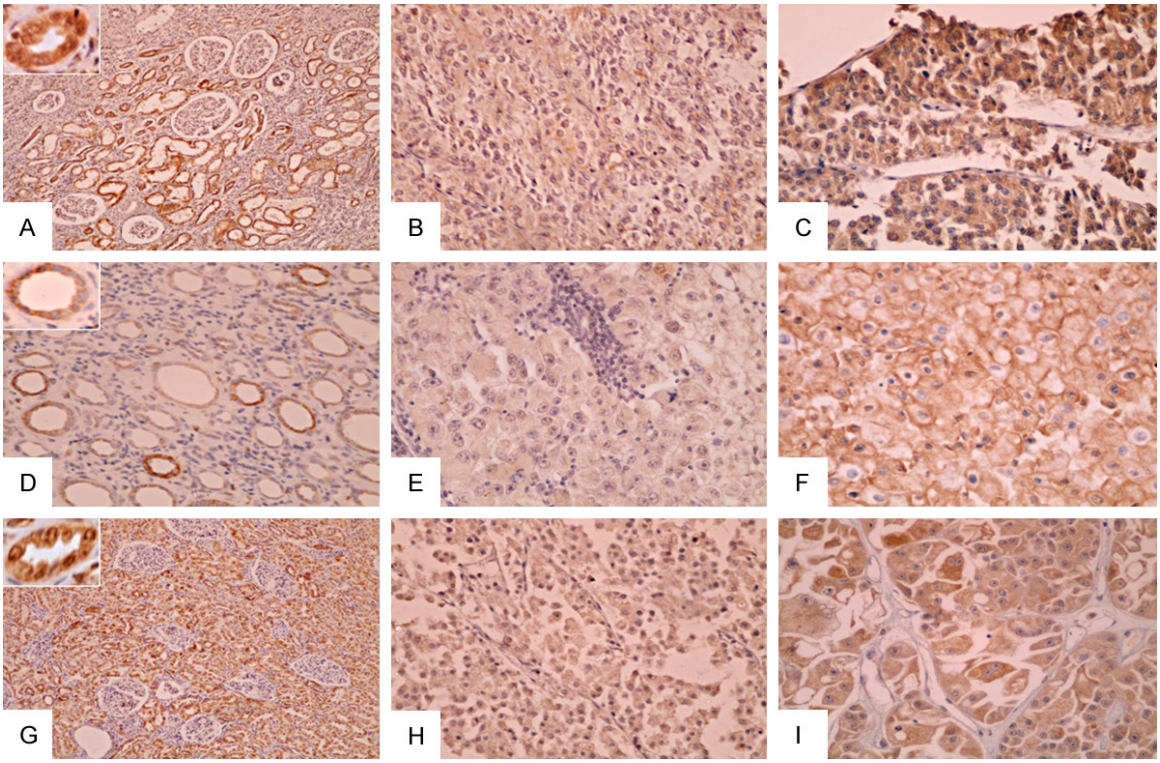

Figure 1. Expression of ERα, ERα36 and ERβ in RCC and nearby non-tumorous tissue. A. Positive high expression of ERα in renal tubules ×100 (inset shows positive nuclear and cytoplasmic staining pattern ×400). B. Low cytoplasmic expression of ERα in RCC ×400. C. High cytoplasmic expression of ERα in RCC ×400. D. Positive low expression of ERα36 in renal tubules ×100 (inset showed low positive cytoplasmic staining pattern ×400). E. Low cytoplasmic expression of ERα36 in RCC ×400. F. High cytoplasmic expression of ERα36 in RCC ×400. G. Positive high expression of ERβ in renal tubules ×100 (inset showed positive nuclear and cytoplasmic staining pattern ×400). H. Low cytoplasmic expression of ERβ in RCC ×400. I. High cytoplasmic expression of ERβ in RCC, ×400.

P values of <0.05 were regarded as statistically significant.

Results

Clinicopathological characteristics

The clinical characteristics of the 70 RCC patients are presented in (**Table 1**). Briefly, the 70 evaluated cases of RCC include 45 clear cell renal cell carcinoma (CRCC), 15 papillary RCC and 10 cases chromophobe RCC. The age range of the patients at the time of diagnosis was (38-75) with a median of 56. Of the 60 clear and papillary RCC, according to the ISUP grading scheme, nuclear grade distribution was as follows: 9 cases were grade 1 (15%), 34 cases were grade 2 (56.7%), 16 cases were grade 3 (26.7%), and 1 case was grade 4 (1.6%).

Expression of ERα, ERα36 and ERβ

A total of 70 specimens of RCC were analyzed for ERα, ERα36 and ERβ with their nearby non-tumorous kidney tissue. The three markers showed positive cytoplasmic (± membranous) staining pattern in tumor cells without staining of the stroma or inflammatory cells. The nearby non-tumorous kidney tissue showed nuclear (± cytoplasmic) staining pattern (ERα and ERβ) and only cytoplasmic staining pattern (ERα36), with the expression detected in the renal tubules sparing the glomeruli (**Figure 1**). Positive staining of ERα, ERα36 and ERβ was detected in 51/70 (72.8%), 10/70 (14.2%), and 70/70 (100%) specimens in the nearby non-tumorous kidney tissue respectively and in 49/70 (70%), 65/70 (92.8%), and 67/70 (95.7%) of RCC specimens respectively (**Figure 1**). There was no significant difference in the mean ERα expression between non-tumorous kidney tissue and RCC (P = 0.754). Conversely, ERα36 and ERβ expression showed significant difference between RCC and nearby non-tumorous kidney tissue with ERα36 significantly higher in RCC while ERβ was significantly higher in nearby non-tumorous kidney tissue (P<0.0001) for both.

Table 2. Relationship between expression of ERα, ERα36 and ERβ and clinicopathological parameters

Clinicopathological factors	ERα		ERα36		ERβ	
	Mean ± SE	*P* value	Mean ± SE	*P value*	Mean ± SE	*P value*
Age						
≤ 56	5.03 ± 0.68	0.742	6.51 ± 0.58	*0.938*	6.46 ± 0.60	*0.887*
> 56	4.42 ± 0.68		6.33 ± 0.64		6.52 ± 0.60	
Gender						
Men	4.82 ± 0.59	0.701	6.09 ± 0.55	*0.288*	6.64 ± 0.55	*0.602*
Women	4.6 ± 0.84		7 ± 0.67		6.23 ± 0.66	
Tumor size (cm)						
≤ 10 cm	2.34 ± 0.47	*<0.0001*	4.16 ± 0.45	*<0.0001*	5.08 ± 0.55	*<0.0001*
> 10 cm	7.59 ± 0.58		9.13 ± 0.42		8.16 ± 0.52	
Site						
Right	4.68 ± 0.65	*0.924*	6.14 ± 0.64	*0.548*	6.05 ± 0.62	*0.251*
Left	4.82 ± 0.72		6.76 ± 0.56		6.97 ± 0.57	
Histopathological type						
Clear cell RCC	4.33 ± 0.57	*0.110*	5.98 ± 0.50	*0.110*	6.44 ± 0.50	*0.983*
Papillary RCC	5 ± 1.07		6.40 ± 0.96		6.67 ± 1.13	
Chromophobe RCC	6.2 ± 1.52		8.50 ± 1.21		6.40 ± 1.06	
Grade of clear cell and papillary RCC (grouped)						
G1-2	3.58 ± 0.56	*0.003*	5.3 ± 0.53	*0.002*	5.79 ± 0.52	*0.022*
G3-4	6.82 ± 0.82		8.06 ± 0.60		8.29 ± 0.83	
Clinical stage (grouped)						
I-II	1.48 ± 0.70	*<0.0001*	4.16 ± 0.70	*<0.0001*	2.44 ± 0.27	*<0.0001*
III-IV	6.56 ± 0.45		7.69 ± 0.44		8.73 ± 0.31	
Lymphvascular invasion						
Positive	6.59 ± 0.51	*<0.0001*	7.57 ± 0.51	*0.006*	8.65 ± 0.43	*<0.0001*
Negative	2.67 ± 0.67		5.15 ± 0.64		4.06 ± 0.49	
Tumor necrosis						
Positive	6.9 ± 0.99	*0.006*	9.2 ± 0.43	*<0.0001*	6.35 ± 0.52	*0.818*
Negative	3.88 ± 0.50		5.32 ± 0.74		6.54 ± 0.71	

Relationship between ERα, ERα36, and ERβ expression and clinicopathological criteria

In RCC, the mean cytoplasmic expression of ERα, ERα36 and ERβ was significantly higher in association with adverse prognostic factors: larger tumor size with (P<0.0001) for each, late clinical stage with (P<0.0001) for each, higher nuclear grade (P = 0.003, P = 0.002 and P = 0.022) respectively, and presence of LVI (P<0.0001, P = 0.006 and P<0.0001) respectively. In addition, the mean cytoplasmic expression of both ERα and ERα36 was significantly higher in tumors that showed necrosis (P = 0.006 and P<0.0001) respectively. No statistically significant difference in the mean was detected between ERα, ERα36 and ERβ expression regarding patient age (P = 0.742, P = 0.938 and P = 0.887) respectively, gender (P = 0.701, P = 0.288 and P = 0.602) respectively, tumor site (P = 0.924, P = 0.548 and P = 0.251) respectively and histopathologic type of the tumor (P = 0.110, P = 0.110 and P = 0.983) respectively (**Table 2**).

Correlation between ERα, ERα36 and ERβ expression in RCC

A significant strong positive correlation was present between the expression of both ERα and ERα36 (r = 0.840, P<0.0001) and ERα and ERβ (r = 0.701, P<0.0001). On the other hand, a significant but moderate positive correlation was present between expression of ERα36 and ERβ (r = 0.578, P<0.0001) (**Table 3**).

Survival analysis

Survival analysis based on cytoplasmic ERα, ERα36 and ERβ expression was carried out fol-

Table 3. Spearman correlation coefficient

Spearman's rho		ERα	ERα36	ERβ
ERα	Correlation Coefficient	1.000	0.840	0.701
	Sig. (2-tailed)		*<0.0001*	*<0.0001*
	N	70	70	70
ERα36	Correlation Coefficient	0.840	1.000	0.578
	Sig. (2-tailed)	*<0.0001*		*<0.0001*
	N	70	70	70
ERβ	Correlation Coefficient	0.701	0.578	1.000
	Sig. (2-tailed)	*<0.0001*	*<0.0001*	
	N	70	70	70

lowing data dichotomization according to median receptor-score value; For ERα, the median expression score was 4 (low ≤ 4 and high > 4). For both ERα36 and ERβ, the median expression score was 6 (low ≤ 6 and high > 6).

The effect of different clinicopathological parameters and expression of each ER on 3-year survival and disease free survival (DFS) was investigated.

Univariate Kaplan-Meier-survival analysis demonstrated that high cytoplasmic expression of ERα, ERα36 and ERβ were unfavorable prognostic indicators as regards overall (OS) and disease-free survival (DFS). The difference achieved statistical significance (ERα; OS, P = 0.001 and DFS; P = 0.003; **Figure 2A**, **2D**), (ERα36; OS, P = 0.001 and DFS, P<0.0001; **Figure 2B**, **2E**) and (ERβ; OS, P<0.0001 and DFS, P<0.0001; **Figure 2C**, **2F**).

The univariate analysis of the other parameters examined showed that there was a progressive decline in both OS and DFS with increasing tumor size (OS, P = 0.033 and DFS, P = 0.034; **Figure 3A**, **3D**), with late clinical stage (OS, P = 0.002 and, DFS, P = 0.003; **Figure 3B**, **3E**) and also with presence of LVI (OS, P<0.0001 and, DFS, P = 0.001; **Figure 3C**, **3F**). The remaining clinicopathological parameters examined, namely: age, gender, tumor site, histopathological type and histologic grade, were found not to be associated significantly with either DSF or OS (P > 0.05).

After multivariate analysis using Cox proportional hazard model, ERβ (P = 0.008; HR = 7.6; 95% CI, 1.684-34.88) and LVI (P = 0.043; HR = 0.37; 95% CI, 0.144-0.967) proved to be the only significant independent factor for OS while ERβ (P = 0.022; HR =3.002; 95% CI, 1.171-7.698) and ERα-36 (P = 0.002; HR = 5.19; 95% CI, 1.877-14.353) were the only significant independent factors for DFS (**Table 4**).

Discussion

Many lines of evidence suggest a relationship between the disturbance of estrogen signaling and cancer initiation and progression with variable response to treatment. In addition, the variation of ERα/ERβ ratio in these cancers as well as the different levels, functions, and subcellular localization of their splice variants seem to contribute to the complexity of ERs actions [17, 19]. Recent interest has been directed towards studying the different types of ERs and their splice variants in cancer.

As ERα and ERβ are classical nuclear receptors, so the positivity of ERs in different types of cancer using immunohistochemistry defined as those cancer cells that showed positive nuclear staining and any cytoplasmic and/or membranous staining were neglected by researchers. Recently, many studies on different cancer types showed that cytoplasmic and/or membranous staining of ERs have variable impacts on patient outcome and should not be ignored [11, 23, 25, 26].

In this study we observed that both ERα and ERβ have cytoplasmic (± membranous) staining pattern in the tumor cells while a nuclear (± cytoplasmic) staining pattern was observed in the nearby non-tumorous renal tubules. This is consistent with other studies on RCC, vulvar carcinoma, ovarian serous carcinoma and breast carcinoma [11, 14, 25, 26]. The mechanisms that account for this altered expression pattern between normal and tumor tissue remain an open question. Some authors have suggested that potential explanations are post-translational modifications, phosphorylation of a conserved serine residue in the DNA-binding domain, or fatty acylation of ERs [27]. Other authors proposed that ERs could be sequestered in the cytoplasm by a splice variant of metastatic tumor antigen-1 (MTA1s) as shown by Kumar et al. in breast cancer cells [28]. On

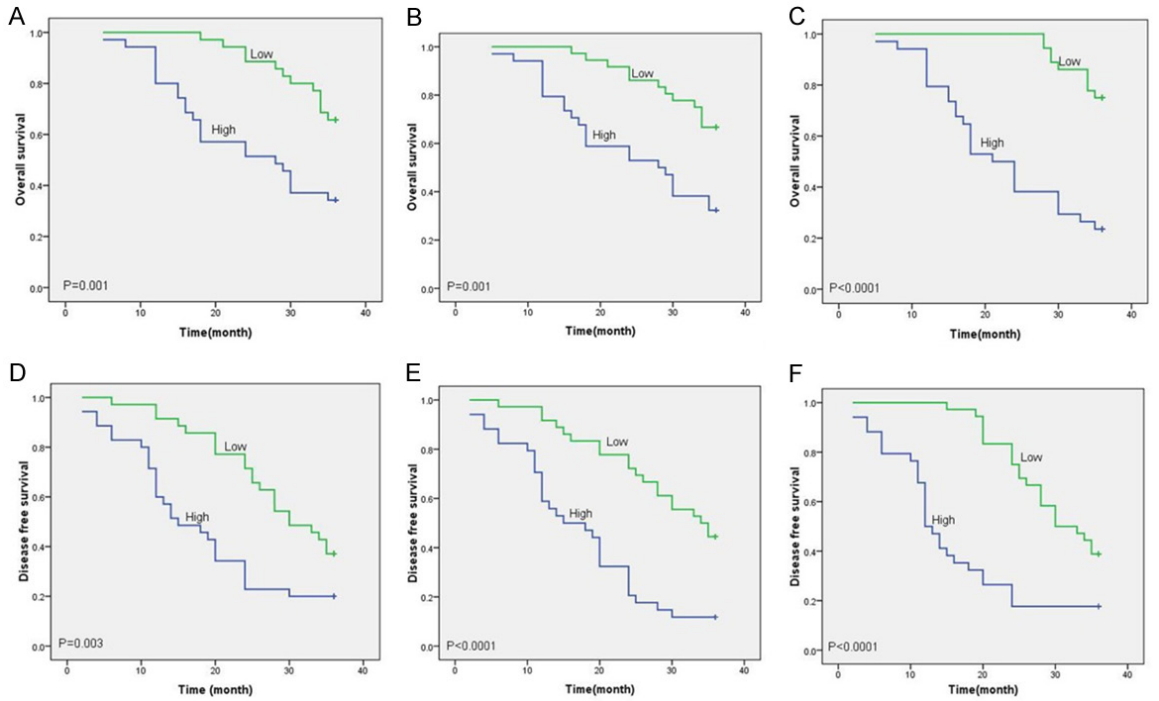

Figure 2. Kaplan-Meier survival curves with correlation between cytoplasmic expression of ERα, ERα36 and ERβ and RCC prognosis, assessed by univariate survival analysis. (A, D) High ERα expression is associated with poor prognosis; overall survival (A), and disease-free survival (D). (B, E) High ERα36 expression is associated with poor prognosis; overall survival (B) and disease-free survival (E). (C, F) High ERβ expression is associated with poor prognosis; overall survival (C), and disease-free survival (F).

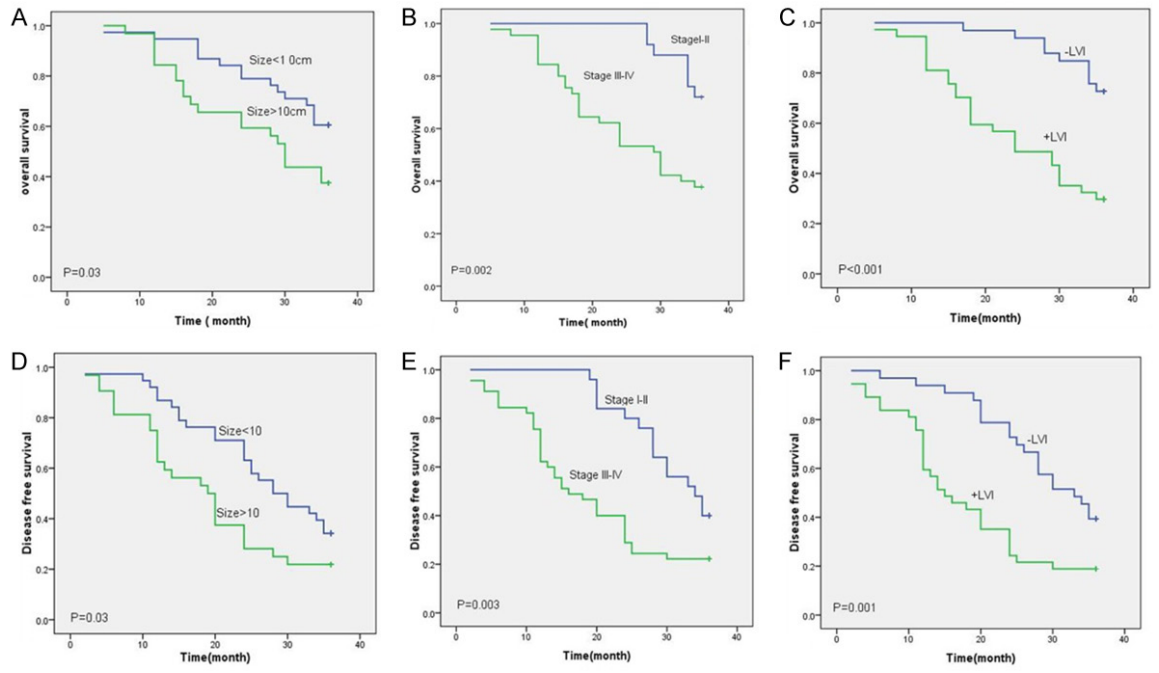

Figure 3. Kaplan-Meier survival curve: the correlation between clinicopathological factors and RCC prognosis, assessed by univariate survival analysis. (A, D) Larger tumor size is associated with poor prognosis; overall survival (A) and disease-free survival (D). (B, E) Advanced tumor stage is associated with poor prognosis; overall survival (B), and disease-free survival (E). (C, F) Lymphovascular emboli are associated with poor prognosis: overall survival (C) and disease-free survival (F).

Table 4. Cox regression analysis of factors affecting OS and DFS in RCC patients

	Overall survival (OS)			Disease free survival (DFS)		
Variable analysis	HR	95% CI	*P*	HR	95% CI	*P*
Size	1.37	0.534-3.533	*0.511*	1.77	0.743-4.232	*0.917*
Clinical stage	3.44	0.650-18.302	*0.146*	1.69	0.623-4.599	*0.303*
LVI	0.373	0.144-0.967	*0.043*	0.604	0.286-1.277	*0.187*
ERα	0.939	0.264-3.342	*0.923*	0.822	0.326-2.069	*0.677*
ERα36	2.60	0.690-9.823	*0.158*	5.19	1.877-14.353	*0.002*
ERβ	7.66	1.684-34.88	*0.008*	3.002	1.171-7.698	*0.022*

the other hand some authors proposed the possibility that ERs are targeted to other organelles rather than nucleus such as the mitochondria which was reported in other tumor types [29, 30].

In agreement with other studies on RCC and breast carcinoma [13, 31], we observed that ERα36 has cytoplasmic (± membranous) staining pattern in the tumor cells as well as in the nearby renal tubules with reduction in staining. This suggests that the presence of ERα36 may play a role in renal cell carcinoma initiation and progression.

A study by Li et al. on breast cancer found that 31/61 specimens of breast cancer showed positive cytoplasmic and (or) membranous staining pattern of ERα in tumor cells using IHC method. In addition they found by western blot that the ER protein that localized in cytoplasm and (or) membrane was mainly ERα36, in contrast to specimens that showed a nuclear staining pattern, where the main ER protein was ERα66 [14]. This may explain our findings where 100% of the tumor specimens that were positive for ERα were also positive for ERα36 with both having a cytoplasmic staining pattern. This finding was supported by the significant strong positive correlation that was found between the expression of both ERα and ERα36, which may indicate that the main ERα protein expressed in human RCC is ERα36.

In the current study, the mean cytoplasmic expression of ERs was significantly higher in association with bad prognostic factors in RCC including; larger tumor size, late clinical stage, higher nuclear grade, the presence of LVI and necrosis. This relation to bad prognostic parameters may rely on their involvement in non-genomic (rapid) signaling pathway of estrogen. This can be achieved by activation of different signaling molecules such as insulin-like growth factor-1 (IGF-I), epidermal growth factor (EGF) receptors, mitogen-activated protein kinase (MAPK), protein kinase B (Akt), and protein kinase C with release of calcium and nitric oxide [32-34], which may, in turn, promote rapid downstream signaling for tumor cell proliferation and survival [35].

The relation to bad prognostic parameters are consistent with others; a study on RCC found positive relation between high expression of ER α36 and larger tumor size, late clinical stage and presence of tumor necrosis [13]. A study by Li et al. found that ER α cytoplasmic/membranous positive breast cancer is associated with all clinicopathological parameters that correlated with poor prognosis [14]. In addition a study on vulvar squamous cell carcinoma found a significant relation between cytoplasmic expression of ERβ and higher tumor grade [25]. On the other hand, a discrepancy exists between our findings and results obtained by Chan et al. who found inverse relations between cytoplasmic expression of ERα and ERβ1 in relation to the stage and grade of ovarian cancer respectively [23]. This difference may be due to absence of standard scoring system for evaluation of ERs cytoplasmic expression.

In our study, we demonstrated for the first time that RCC patients whose tumors express high cytoplasmic levels of ERα, ERα36, or ERβ experience shorter OS and DFS. The independent role of high cytoplasmic expression of these markers as markers of poor prognosis is proven only for ERβ and ERα36 but not ERα. ERβ and ERα36 were proved to be an independent prognostic factors for DFS while only ERβ showed to be an independent prognostic factor to OS.

These findings are similar to that reported by a study on RCC that showed high ERα36 expression correlated with poor prognosis [13]. On the other hand our finding for ERβ is unlike

that reported by others. Some found that higher cytoplasmic expression of ERβ tissue resulted in better prognosis in RCC [11] and in ovarian carcinoma [36]. On the other hand, our result of ERβ as an independent prognostic factor for DFS and OS is similar to those in the literature but in different tumor types [25, 26].

We can conclude that the expression of ERs is altered in RCC with predominant cytoplasmic staining pattern in tumor cells and the relation of this pattern to bad prognostic parameters. Thus, the assessment of these receptors, especially ERα36 and ERβ, could be helpful to identify poor prognosis in patients with RCC. However, further genetic studies are required to support our results and to understand the role of different ERs, with their splice variants, in pathogenesis of RCC and their relation to bad prognosis.

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Disclosure of conflict of interest

None.

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