Research Article

Immunohistochemical Expression of Survivin and B7- H1 in Renal Cell Carcinoma

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Abstract
Kidney cancer is the ninth most common cancer in developed countries. According to last registries of Egyptian National Cancer Institute, renal cell carcinoma represents 56.78% of all malignant renal tumors. Survivin is a member of the inhibitor of apoptosis protein family that inhibits apoptosis and play a critical role in regulating mitosis and microtubule stability. B7-H1 (also known as PD-L1) is a ligand that inhibits T cell – mediated immunity and has been implicated as a potent negative regulator of antitumor immunity. The aim of the current study was to investigate the immunohistochemical expression and the relevant clinicopathological significance of survivin and B7-H1 and to study the relationship between the two markers in one hundred cases of RCC tumors including histologically confirmed 70 case of clear renal cell carcinoma, 10 cases of chromophobe renal cell carcinoma, 10 cases of papillary renal cell carcinoma, 2 cases of mixed renal cell carcinoma, 3 cases of granular renal cell carcinoma and 5 cases of sarcomatoid renal cell carcinoma. A significant association was found between nuclear survivin expression and different clinicopathological features including (primary tumor classification, regional lymph involvement, advanced tumor stage, perinephric fat invasion, lymphocytic infiltrate, SSIGN score, MR score (p<0.001 for each), tumor size (p=0.001), nuclear grading (p=0.002) and coagulative tumor necrosis (p=0.002). However, no significant association between cytoplasmic survivin expression and any of clinic-pathological features. Regarding B7-H1expression, the present study showed positive B7-H1expression in 29% of RCC tumors. A significant association was found between B7-H1 expression and different clinicopathological features including, primary tumor classification, regional lymph involvement, advanced tumor stage, nuclear grading, coagulative tumor necrosis, perinephric fat invasion, lymphocytic infiltrate, SSIGN score, MR score (p<0.001) and tumor size (p=0.001). Combined expression patterns of both markers revealed 4 immunophenotypes, including 57 (57%) survivin^{Low}/B7-H1^{-} tumors, 14 (14%) survivin^{Hi}/B7-H1^{-} tumors, 9 (9%) survivin^{Low}/B7-H1^{+} tumors, and 20 (20%) survivin^{Hi}/B7-H1^{+} tumors. Among them, the survivin^{Hi}/B7-H1^{+} immunoprofile showed a strong significant association with the more aggressive clinicopathological features including advanced primary tumor classification, regional lymph involvement, advanced tumor stage, coagulative tumor necrosis, perinephric fat invasion, lymphocytic infiltrate, higher SSIGN score and MR score (p<0.001) and higher nuclear grading (p =0.002). On studying the differential expression of both markers in primary RCC tumors and their corresponding LN metastasis, no significant differences were noticed between primary tumors and their corresponding LN metastasis with high concordance rates were found between both locations. Taken together, it can be speculated that dual expression of survivin and B7-H1can be used to predict RCC tumor aggressiveness.

Key words: Survivin – B7-H1 – RCC – Immunohistochemistry

Introduction
Kidney cancer is the ninth most common cancer in developed countries (Lipworth et al., 2009). It constitutes about 3% of all solid neoplasms and ranks 10th as the leading cause of cancer mortality (Jemal et al., 2012). In Egypt, renal cell carcinoma represents nearly 0.68 % of total adult malignancies and accounts for 56.78% of all malignant renal tumors (Mokhtar et al., 2007).

Eighty percent of renal cell cancers are clear cell adenocarcinomas, the remainder being papillary (15%), chromophobe (5%), and collecting duct carcinomas (<1%) (Lipworth et al., 2009).

There are different factors influencing RCC prognosis which include anatomical, histological, prognostic nomograms and molecular factors (Delahunt, 2009; Ljungberg et al., 2012).

Regarding the anatomical factors, they include tumor size, venous invasion, renal capsule invasion, adrenal involvement, lymph node, and distant metastasis. These factors are commonly gathered together in the universally used TNM staging classification system (Delahunt, 2009; Sobin et al., 2009; Wagner et al., 2009; Ljungberg et al., 2012) while the histo-logical factors include Fuhrman grade, RCC subtype, sarcomatoid features and tumor necrosis and lymphocytic infiltate (Webster et al., 2006; Sengupta et al., 2005; Ljungberg et al. 2012; Volpe and Patard, 2010).

Several clinicopathologic scoring systems (also referred to as nomograms or algorithms) have been reported to predict outcomes for surgically treated RCC patients. Such algorithms include the 2002 American Joint Committee on Cancer (AJCC) TNM stage groupings, the (UCLA) University of California Los Angeles Integrated Scoring System (UISS), nomograms from Memorial Sloan-Kettering Cancer Center, and the Mayo Clinic stage, size, grade, and necrosis (SSIGN) score (Ficarra et al., 2007). These scoring systems alone do not fully account for the varied outcomes associated with RCC, and fail to reveal the molecular basis for RCC aggressiveness or rational targets for therapy (Sorbellini et al., 2005). As a result, there is considerable interest in the identification of tumor-associated biomarkers that might enhance RCC prognostication and guide development of new therapies (Ficarra et al., 2006).

Survivin is a member of the inhibitors of apoptosis (IAP) family of antiapoptotic proteins (Caldas et al., 2005). Survivin has attracted attention as a unique member of the IAP gene family with a potential dual role in apoptosis inhibition and regulation of mitosis (Altieri, 2003). In fact, although survivin is undetectable in most adult tissues, it is demonstrated a strong survivin expression in most human solid tumor types as lung, colon, breast, pancreas, liver cancer, as well as in hematologic malignancies. They also showed that high levels of the protein were predictive of tumor progression in terms of either disease-free or overall survival (Zhu et al., 2005; Invernizz et al., 2006; Bhanot et al., 2006; Hinnis et al., 2007; Wu et al., 2009). Several studies suggests that those RCC patients who present with tumors that express high levels of survivin are at increased risk of cancer progression and RCC death (Li et al., 2005; Hinnis et al., 2007; Zamparese et al., 2008 , Parker et al., 2009; Emaetig et al., 2013).

B7 homolog 1 (B7-H1) also known as Programmed cell death ligand 1 (PD-L1) or cluster of differentiation (CD274), is a protein is encoded by the CD274 gene in humans which belongs to group II B7 family (Carreno and Collins, 2002).

B7-H1 on tumor cells promotes immune suppression by binding to PD-1 on activated T cells, thereby sustaining tumor growth inhibit tumor-specific T cell–mediated immunity, through binding to the T-cell PD-1 (or a putative non–PD-1) receptor, inducing T cell apoptosis, impairing cytokine production, and diminishing the cytotoxicity of activated T cells (Seliger et al., 2008). Many studies revealed that B7-H1 is highly expressed in most human solid cancers including breast, colon, esophageal, gastric, head and neck squamous cell, kidney, liver, lung, ovarian, pancreatic, salivary and urothelial carcinomas, as well as in glioblastoma, wilms' tumor and melanoma (Tsushima et al., 2006; Inman et al., 2007; Geng et al., 2008; Routh et al., 2008). In RCC many studies showed that RCC patients harboring tumors expressing B7-H1 are at significantly increased risk for progression and mortality (Thompson et al., 2004; Thompson et al., 2006; Zamparese et al., 2008).

Both survivin and B7-H1 may promote RCC tumor progression: the former by promoting tumor cell immortalization and the latter through evasion of the immune system. Because both of these molecules act via very different mechanisms to preserve tumor cell viability, one might anticipate that RCC tumors expressing both of these molecules might behave significantly more aggressively than RCC tumors that express either marker alone. Alternatively, one might just as easily predict that these two molecules are randomly produced by increasingly dysplastic cells, overlapping as prognostic variables and acting as surrogate biomarkers for one another. Hence, we examined the clinical effect of combined survivin and B7-H1 expression in RCC tumors obtained from one hundreds surgically treated patients.

Material and Methods

Cases Selection

The present study comprised one hundred case randomly selected from formalin – fixed paraffin embedded cases of renal cell carcinomas which were chosen from the archive of histopathological laboratories of Minia University Hospital and National Cancer Institute (NCI) of Cairo (In the period between 2005 and 2011). The cases included; 70 case of clear renal cell carcinoma, 10 cases of chromophobe renal cell carcinoma, 10 cases of papillary renal cell carcinoma, 2 cases of mixed renal cell carcinoma, 3 cases of granular renal cell carcinoma and 5 cases of sarcomatoid renal cell carcinoma.

Clinical and pathological features

The available clinicopathological data were obtained from the pathology reports of the cases. This data includes patients' age and sex, tumor localization, tumor size, tumor necrosis, perinephric fat invasion, sarcomatoid differentiation, tumor type (tumor classification was performed according to the WHO criteria) (Eble et al., 2004), nuclear grade was revised according to Fuhrman nuclear grading system and subdivided into 4 grades; 1, 2, 3 and 4 respectively (Sun et al., 2009), tumor stage and lymph node metastasis was estimated according to TNM staging classification system (Sobin et al., 2009). The key clinicopathological data of the patients are summarized in Table (1).

Regarding clear cell type, assessment of lymphocytic infiltrate was done. T lymphocytes cell infiltration is then categorized according to the density as: grade 0, absent; grade 1, focal infiltration (scattered lymphoid aggregates); grade 2, moderate infiltration; grade 3, marked infiltration (Webster et al., 2006). SSIGN score and Metastatic Risk score were also estimated according to the data obtained from the pathology reports in each case.
All primary tumors and 17 / 23 (available blocks) of metastatic malignant lymph nodes were prepared and stained with haematoxylin and eosin stain to revise the histological findings of all the cases.

Immunohistochemistry for survivin and B7-H1.

Immunohistochemistry was carried out using the avidin–biotin peroxidase complex method. Two, 3-µm sections thickness from representative paraffin-embedded tissue blocks were sectioned for each case of the cohort and its available corresponding lymph node. One slide was stained with anti-survivin (Monoclonal mouse antibody, clone12 C4, 0.2 ml concentrated, Dako ; 1: 50 dilution, incubated for one hour) using standard techniques. The second slide was stained with 5H1, a mouse anti-human monoclonal antibody specific for B7-H1 (Polycolonal rabbit antibody, 0.1ml concentrated, US Biological; 1:700 dilution, incubated overnight).

Positive and negative control

Each staining batch included both positive and negative control sections. One negative control tissue was processed for each run by omitting the specific primary antibody from the staining procedure and replaced with PBS. Regarding survivin, the positive control was sections of prostate adenocarcinoma, while sections of human tonsillar tissue were used as positive control for B7-H1 expression.

Scoring system

Survivin Expression

- To assess positive staining for survivin, the entire tissue section was screened for positive tumour cells, defined as cells with

nuclear and /or cytoplasmic staining. Survivin nuclear and cytoplasmic staining was evaluated separately for each case.

- Nuclear survivin expression was evaluated as the percentage of tumor cells stained positive by counting numbers of survivin-positive (versus total) tumor cells in five representative high-powered fields (X 400 magnification). Cases were then stratified into low expression ($Survivin^{low}$) and high expression ($Survivin^{Hi}$**)** corresponding to <15 positive cell per mm^2 and ≥ 15positive cells per mm^2 according to (Parker et al., 2009).

- Cytoplasmic survivin expression was evaluated with each slide as the percentage of positively stained cells in five high power fields (X 400 magnification). Cases were considered +ve when ≥10% of tumor cells showed cytoplasmic survivin expression according to (Byun et al., 2007).

B7-H1 Expression

Tissue section was screened for positive tumor cells, defined as cells with memberanous and/or cytoplasmic staining. Cases were considered +ve when ≥10% of tumor cells showed memberanous and/or cytoplasmic B7-H1 expression according to **(Thompson et al., 2007)**.

Statistical analysis

All statistical analysis was done using stastical package of social science (SPSS® Release 16) (SPSS, Inc.) soft ware. Association between immunoreactivity and different clinicopathological data were done by Chi-square test. Spearman's rho coefficient was used for continuous variables to assess the correlation between the two markers. Mc Nemer test was used to compare expression of survivin and B7-H1 in primary tumors and their corresponding LN metastasis. Statistical significance was determined at p value of ≤0.05.

Results

On studying the expression of survivin, we found that both nuclear and cytoplasmic survivin expressions were widely expressed in tumor cells. In contrast, it was undetectable in normal renal tublar cells.

For clear RCC cases, the association between lymphocytic infiltrate and SSIGN score was summarized in Table (2). A significant association was observed between lymphocytic infiltrate and SSIGN score (p<0.001).The frequency of marked lymphoctic infiltrate was much higher in cases with high SSIGN score (0-47% 5y SR) reaching up to 90% compared to only one case with marked lymphocytic infiltrate was observed in whom with low SSIGN score (100% 5y SR).

The association between nuclear survivin expression and different clinicopathological features was summarized in table (3). A significant positive association was observed with different clinicopathological data including primary tumor classification, regional lymph involvement, advanced tumor stage, perinephric fat invasion, lymphocytic infiltrate, SSIGN score, MR score (p<0.001 for each), tumor size (p=0.001), nuclear grading (fig. 2-A,B)(p=0.002) and coagulative tumor necrosis (p=0.002).

In the current study, we noticed that survivin was expressed in all histologic RCC types with a higher survivin immune-staining scores in SRCC type (fig. 2-C) and GRCC type as compared to clear cell type, papillary type and chromopobe type, Moreover, we demonstrated a significant association between survivin nuclear expression and lymphocytic infiltrate in ccRCC cases.

On the other hand, no significant association was noticed between cyto-plasmic survivin expression and any of clinicopathological features as shown in table (4).

Seventeen pairs of primary RCC and their corresponding LN metastasis were compared for nuclear and cytoplasmic survivin expression, which was summarized in table (5). Regarding both nuclear and cytoplasmic survivin, no significant difference were found between primary RCC and their corresponding LN metastasis (p=1.000).

With respect to B7-H1expression, we reported a positive B7-H1expression in 29% of RCC tumors. The association

between B7-H1 expression and different clinicopathological features was summarized in table (6). A significant positive association was observed with different clinicopathological data including, primary tumor classification, regional lymph involvement, advanced tumor stage, nuclear grading, coagulative tumor necrosis, perinephric fat invasion, lymphocytic infiltrate, SSIGN score, MR score (p<0.001 for each) and tumor size (p=0.001).

Here in, we noticed that B7-H1expressed in all histologic RCC types (fig.3-A,B,C). No significant difference was noticed among different histological subtypes of RCC, although a higher immunostaining scores were noticed more frequently in SRCC type and GRCC type as compared to other histological subtypes. Our results showed a significant association between positive B7-H1 expression and lymphocytic infiltrate.

Primary RCC tumors and their corresponding LN metastasis were compared for B7-H1 expression, which was summarized in table (7). No significant difference between primary RCC and their corresponding LN metastasis (p=.625).

As the cytoplasmic expression of survivin showed no significant association with any of clinicopathological features as compared to its nuclear expression that showed a significant association with many of clinicopathological features, so we assessed the combined expression between B7-H1 and the nuclear type of survivin expression.

According to the combined expression patterns of both markers in patients with RCCs, 4 immunopheno types were identified, including 57 (57%) survivin^{Low}/B7-H1^{-} tumors, 14 (14%) survivin^{Hi}/B7-H1^{-} tumors, 9 (9%) survivin^{Low}/B7-H1^{+} tumors, and 20 (20%) survivin^{Hi}/B7-H1^{+} tumors. The association of theses immunoprofiles with different clinicopathological variables was shown in table (8).

In this study, our findings demonstrated a strong association of survivin^{Hi}/B7-H1^{+} immunoprofile and the more aggressive clinicopathological including advanced primary tumor classification, regional lymph involvement, advanced tumor stage, coagulative tumor necrosis, perinephric fat invasion, lymphocytic infiltrate, higher SSIGN score and MR score (p<0.001 for each) and higher nuclear grading (p =0.002). We also observed a high level of lymphocytic infiltrate within ccRCC cases with survivin^{Hi}/B7-H1^{+} expression.

Finally, the present study identified a moderate positive significant correlation between nuclear survivin and B7-H1 expression levels (Sperman's rank correlation p<0.001, r=0.73) was identified (Fig. 1).

Table1: Clinicopathological features for patients with RCC (n=100)

Clinicopathological features	No. (%)
Age at Surgery, y	
<65	76 (76%)
≥65	24 (24%)
Sex	
Male	53 (53%)
Female	47 (47%)
Localization	
Right	50 (50%)
Left	50 (50%)
Histological subtypes	
Clear RCC	70 (70%)
Papillary RCC	10 (10%)
Chromophobe RCC	10 (10%)
Granular RCC	3 (3%)
Mixed RCC	2 (2%)
Sarcomatoid RCC	5 (5%)

Primary tumor classification	
T1a	15 (15%)
T1b	18 (18%)
T2a	13 (13%)
T2b	24 (24%)
T3a	25 (25%)
T3b	2 (2%)
T4	3 (3%)
Regional lymph node involvement	
N0	77 (77%)
N1	21 (21%)
N2	2 (2%)
TNM stage groupings	
I	32 (32%)
II	33 (33%)
III	30 (30%)
IV	5 (5%)
Tumor size, cm	
<5	17 (17%)
5 to< 7	22 (22%)
7 to< 10	20 (20%)
≥10	41 (41%)
Nuclear grade	
1	19 (19%)
2	43 (43%)
3	28 (28%)
4	10 (10%)
Coagulative tumor necrosis	
-ve	48 (48%)
+ve	52 (52%)
Perinephric fat invasion	
-ve	73 (73%)
+ve	27 (27%)
Sarcomatoid differentiation	
-ve	92 (92%)
+ve	8 (8%)
Lymphocytic infiltrate*	
-ve	39 (55.7%)
Focal	14 (20%)
Moderate	11 (15.7%)
marked	6 (8.6%)
SSIGN score*	
5y survival rate 100%	22 (31.4%)
5y survival rate 90%	17 (24.3%)
5y survival rate 64%	15 (21.4%)
5y survival rate 47%	13 (18.6%)
5y survival rate 0%	3 (4.3%)
MR score*	
Low risk	21 (30%)
Moderate risk	33 (47.1%)
High risk	16 (22.9%)

***** variable specific only for clear cell type group **(**n=70)
SSIGN: Mayo Clinic's Stage, Size, Grade and Necrosis scoring system
MR *:* Mayo scoring system for metastatic risk

Table (2): Association of lymphocytic infiltrate and SSIGN score for patients with clear RCC (n=70)

SSIGN	Total No. 70	Lymphocytic infiltration				P value
		Absent No.39	Focal No.14	Moderate No.11	Marked No.6	
5y. survival R.100%	22	15(68.2%)	6(27.3%)	0(0%)	1(4.5%)	<0.001*
5y. survival R.90%	17	13(76.5%)	3(17.6%)	1(5.9%)	0(0%)	
5y. survival R.64%	15	8(53.3%)	3(20%)	4(26.7%)	0(0%)	
5y. survival R.47%	13	2(15.4%)	2(15.4%)	6(46.2%)	3(23.1%)	
5y. survival R.0%	3	1(33.3%)	0(0%)	0(0%)	2(66.7%)	

Test of significance: Chi- square- test, * P - value ≤ 0.05 are considered statistically significant

Low SSIGN: 5y. survival R.100% , **Moderate SSIGN**: 5y. survival R. 64- 90%

High SSIGN: 5y. survival R.0-47%

Table (3): Association of nuclear survivin expression and clinicopathological features for patients with RCC (n=100)

Clinicopathological features	*Total* 100 (100%)	Nuclear survivin		P value
		<15 Low expression No. (%) 66(66%)	≥15 High expression No. (%) 34(34%)	
Age at Surgery, y				
<65	76(76%)	50(65.8%)	26(34.2%)	0.9
≥65	24(24%)	16(66.7%)	8(33.3%)	
Sex				
Male	53(53%)	36(67.9%)	17(32.1%)	0.6
Female	47(47%)	30(63.7%)	17(36.2%)	
Localization				
Right	50(50%)	33(66%)	17(34%)	1.00
Left	50(50%)	33(66%)	17(34%)	
Histological type				
Clear RCC	70(70%)	47(67.1%)	23(32.9%)	
Papillary RCC	10(10%)	7(70%)	3 (30%)	0.2
Chromophobe RCC	10(10%)	8(80%)	2(20%)	
Granular RCC	3(3%)	1(33.3%)	2(66.7%)	
Mixed RCC	2(2%)	0(0%)	2(100%)	
Sarcomatoid RCC	5(5%)	3(60%)	2(40%)	
Primary tumor classification				
T1a	15(15%)	15(100%)	0(0%)	
T1b	18(18%)	17(94.4%)	1(5.6%)	
T2a	13(13%)	13(100%)	0(0%)	<0.001*
T2b	24(24%)	15(62.5%)	9(37.5%)	
T3a	25(25%)	4(16%)	21(84%)	
T3b	2(2%)	1(50%)	1(50%)	
T4	3(3%)	1(33.3%)	2(66.7%)	
Regional lymph node involvement	77(77%)	64(83.1%)	13(16.9%)	<0.001*
N0	21(21%)	2(9.5%)	19(90.5%)	
N1	2(2%)	0(0%)	2(100%)	
N2				
TNM stage groupings				
I	32(32%)	32(100%)	0(0%)	
II	33(33%)	27(81.8%)	6(18.2%)	<0.001*
III	30(30%)	6(20%)	24(80%)	
IV	5(5%)	1(20%)	4(80%)	

Tumor size, cm				
<5	17(17%)	16(94.1%)	1(5.9%)	<0.001*
5 to < 7	22(22%)	18(81.1%)	4(18.2%)	
7 to < 10	20(20%)	15(75%)	5(25%)	
≥10	41(41%)	17(41.5%)	24(58.5%)	
Nuclear grade				
G1	19(19%)	17(89.5%)	2(10.5%)	0.002*
G2	43(43%)	32(74.4%)	11(25.6%)	
G3	28(28%)	14(50%)	14(50%)	
G4	10(10%)	3(30%)	7(70%)	
Coagulative tumor necrosis				
-ve	48(48%)	42(87.5%)	6(12.5%)	<0.001*
+ve	52(52%)	24(46.2%)	28(53.8%)	
Perinephric fat invasion				
-ve	73(73%)	61(83.6%)	12(16.4%)	<0.001*
+ve	27(27%)	5(18.5%)	22(81.5%)	
Sarcomatoid differentiation				
-ve	92(92%)	63(68.5%)	29(31.5%)	0.07
+ve	8(8%)	3(37.5%)	5(62.5%)	
Lymphocytic infiltrate*				
-ve	39 (55.7%)	32 (82.1%)	7 (17.9%)	<0.001*
Focal	14 (20%)	11 (78.6%)	3 (21.4%)	
Moderate	11(15.7%)	2 (18.2%)	9 (81.8%)	
marked	6 (8.6%)	2 (33.3%)	4 (66.7%)	
SSIGN score*				
5y SR 100%	22 (31.4%)	22 (100%)	0 (0%)	
5y SR 90%	17 (24.3%)	14 (82.4%)	3 (17.6%)	<0.001*
5y SR 64%	15 (21.4%)	10 (66.7%)	5 (33.3%)	
5y SR 47%	13 (18.6%)	1 (7.7%)	12 (92.3%)	
5y SR 0%	3 (4.3%)	0 (0%)	3 (100%)	
MR score*				
Low risk	21(30%)	21 (100%)	0 (0%)	
Moderate risk	33(47.1%)	25 (75.8%)	8 (24.2%)	<0.001*
High risk	16 (22.9%)	1 (6.2%)	15 (93.8%)	

Test of significance: Chi- square- test * P - value ≤ 0.05 are considered statistically significant

***** variable specific only for clear cell type group **(**n=70)

Table (4): Association of cytoplasmic survivin expression and clinicopathological features for patients with RCC (n=100)

Clinicopathological features	*Total* 100(100%)	Cytoplasmic survivin		P value
		(-ve) <10 No. (%) 53(53%)	(+ve) ≥10 No. (%) 47(47%)	
Age at Surgery, y				
<65	76(76%)	42(55.3%)	34(44.7%)	0.4
≥65	24(24%)	11(45.8%)	13(54.2%)	
Sex				
Male	53(53%)	32(60.4%)	21(39.6%)	0.1
Female	47(47%)	21(44.7%)	26(55.3%)	
Localization				
Right	50(50%)	25(50%)	25(50%)	0.5
Left	50(50%)	28(56%)	22(44%)	
Histological type				
Clear RCC	70(70%)	37(52.9%)	33(47.1%)	
Papillary RCC	10(10%)	5(50%)	5(50%)	0.9
Chromophobe RCC	10(10%)	5(50%)	5(50%)	
Granular RCC	3(3%)	2(66.7%)	1(33.3%)	
Mixed RCC	2(2%)	1(50%)	1(50%)	
Sarcomatoid RCC	5(5%)	3(60%)	2(40%)	
Primary tumor classification				
T1a	15(15%)	5(33.3%)	10(66.7%)	
T1b	18(18%)	11(61.1%)	7(38.9%)	0.06
T2a	13(13%)	10(76.9%)	3(23.1%)	
T2b	24(24%)	16(66.7%)	8(33.3%)	
T3a	25(25%)	10(40%)	15(60%)	
T3b	2(2%)	1(50%)	1(50%)	
T4	3(3%)	0(0%)	3(100%)	
Regional lymph node involvement				
N0	77(77%)	41(53.2%)	36(46.8%)	
N1	21(21%)	11(52.4%)	10(47.6%)	0.9
N2	2(2%)	1(50%)	1(50%)	
TNM stage groupings				
I	32(32%)	15(46.9%)	17(53.1%)	
II	33(33%)	22(66.7%)	11(33.3%)	0.1
III	30(30%)	15(50%)	15(50%)	
IV	5(5%)	1(20%)	4(80%)	
Tumor size, cm				
<5	17(17%)	7(41.2%)	10(58.8%)	
5 to< 7	22(22%)	12(54.5%)	10(45.5%)	0.5
7 to < 10	20(20%)	13(65%)	7(35%)	
≥10	41(41%)	21(51.2%)	20(48.8%)	
Nuclear grade				
G1	19(19%)	12(63.2%)	7(36.8%)	
G2	43(43%)	21(48.8%)	22(51.2%)	0.7
G3	28(28%)	14(50%)	14(50%)	
G4	10(10%)	6(60%)	4(40%)	

Coagulative tumor necrosis				
-ve	48(48%)	27(56.2%)	21(43.8%)	0.5
+ve	52(52%)	26(50%)	26(50%)	
Perinephric fat invasion				
-ve	73(73%)	44(60.3%)	29(39.7%)	0.07
+ve	27(27%)	9(33.3%)	18(66.7%)	
Sarcomatoid differentiation				
-ve	92(92%)	49(53.3%)	43(46.7%)	0.8
+ve	8(8%)	4(50%)	4(50%)	
Lymphocytic infiltrate*				
-ve	39 (55.7%)	21 (53.8%)	18 (46.2%)	
Focal	14 (20%)	6 (42.9%)	8 (57.1%)	0.3
Moderate	11 (15.7%)	5 (45.5%)	6 (54.5%)	
Marked	6 (8.6%)	5 (83.3%)	1 (16.7%)	
SSIGN score*				
5y SR 100%	22 (31.4%)	9 (40.9%)	13 (59.1%)	
5y SR 90%	17 (24.3%)	12 (70.6%)	5 (29.4%)	0.4
5y SR 64%	15 (21.4%)	7 (46.7%)	8 (53.3%)	
5y SR 47%	13 (18.6%)	7 (53.8%)	6 (46.2%)	
5y SR 0%	3 (4.3%)	2 (66.7%)	1 (33.3%)	
MR score*				
Low risk	21 (30%)	9 (42.9%)	12(57.1%)	
Moderate risk	33 (47.1%)	19 (57.6%)	14 (42.4%)	0.5
High risk	16(22.9%)	9 (56.2%)	7 (43.8%)	

Test of significance: Chi- square- test. P - value ≤ 0.05 are considered statistically significant
***** variable specific only for clear cell type group (n=70)

Table (5): Comparsion for nuclear and cytoplasmic survivin expression among 17 pairs of primary RCC and their corresponding LN metastasis.

	Positive Expression Rate		**Change in Expression Pattern**			
	Primary(n=17)	**Metastasis(n=17)**	**P = M**	**P > M**	**M > P**	**P value**
Nuclear Survivin	15(88.2%)	14(82.4%)	14(82.4%)	2(11.8%)	1(5.9%)	1.000
Cytoplasmic Survivin	7(41.2%)	6(35.3%)	16(94.1%)	1(5.9%)	0(0%)	1.000

P: primary renal cell carcinoma tumor M: metastatic malignant LN
Test of significance: Mc Nemar Test

Table (6): Association of B7-H1 expression and clinicopathological features for patients with RCC (n=100)

Clinicopathological features	***Total*** **100 (100%)**	**B7-H1 expression**		**P value**
		-ve (<10) No. (%) 71(71%)	**+ve (≥10) No. (%) 29(29%)**	
Age at Surgery, y				
<65	76(76%)	56(73.7%)	20(26.3%)	0.7
≥65	24(24%)	15(62.5%)	9(37.5%)	
Sex				
Male	53(53%)	37(69.8%)	16(30.2%)	0.4
Female	47(47%)	34(72.3%)	13(27.7%)	

Localization				
Right	50(50%)	35(70%)	15(30%)	0.8
Left	50(50%)	36(72%)	14(28%)	
Histological type				
Clear RCC	70(70%)	50(71.4%)	20(28.6%)	
Papillary RCC	10(10%)	8(80%)	2(20%)	0.3
Chromophobe RCC	10(10%)	8(80%)	2(20%)	
Granular RCC	3(3%)	2(66.7%)	1(33.3%)	
Mixed RCC	2(2%)	0(0%)	2(100%)	
Sarcomatoid RCC	5(5%)	3(60%)	2(40%)	
Primary tumor classification				
T1a	15(15%)	15(100%)	0(0%)	
T1b	18(18%)	17(94.9%)	1 (5.6%)	<0.001*
T2a	13(13%)	12(92.3%)	1(7.7%)	
T2b	24(24%)	18(75%)	6(25%)	
T3a	25(25%)	9(36%)	16(64%)	
T3b	2(2%)	0(0%)	2(100%)	
T4	3(3%)	0(0%)	3(100%)	
Regional lymph node involvement				<0.001*
N0	77(78.6%)	66(85.7%)	11(14.3%)	
N1	21(20%)	5(23.8%)	16(76.2%)	
N2	2(1.4%)	0(0%)	2(100%)	
TNM stage groupings				
I	32(32%)	32(100%)	0(0%)	
II	33(33%)	28(84.8%)	5(15.2%)	<0.001*
III	30(30%)	11(36.7%)	19(63.3%)	
IV	5(5%)	0(0%)	5(100%)	
Tumor size, cm				
<5	17(17%)	15(88.2%)	2(11.8%)	0.001*
5 to < 7	22(22%)	20(90.9%)	2(9.1%)	
7 to< 10	20(20%)	16(80%)	4(20%)	
≥10	41(41%)	20(48.8%)	21(51.2%)	
Nuclear grade				
G1	19(19%)	16(84.5%)	3(15.3%)	<0.001*
G2	43(43%)	37(86%)	6(14%)	
G3	28(28%)	15(53.6%)	13(46.4%)	
G4	10(10%)	3(30%)	7(70%)	
Coagulative tumor necrosis				<0.001*
-ve	48(48%)	43(89.6%)	5(10.4%)	
+ve	52(52%)	28(53.8%)	24(46.2%)	
Perinephric fat invasion				<0.001*
-ve	73(73%)	63(86.3%)	10(13.7%)	
+ve	27(27%)	8(29.6%)	19(70.4%)	
Sarcomatoid differentiation				0.1
-ve	92(92%)	67(72.8%)	25(27.2%)	
+ve	8(8%)	4(50%)	4(50%)	
Lymphocytic infiltrate*				
-ve	39(55.7%)	35(89.7%)	4(10.3%)	
Focal	14(20%)	11(78.6%)	3(21.4%)	<0.001*

Moderate	11(15.7%)	2(18.2%)	9(81.8%)	
marked	6(8.6%)	2(33.3%)	4(66.7%)	
SSIGN score*				
5y SR 100%	22(31.4%)	22(100%)	0(0%)	
5y SR 90%	17(24.3%)	16(94.1%)	1(5.9%)	<0.001*
5y SR 64%	15(21.4%)	9(60%)	6(40%)	
5y SR 47%	13(18.6%)	3 (23.1%)	10(76.9%)	
5y SR 0%	3(4.3%)	0(0%)	3(100%)	
MR score*				
Low risk	21(30%)	21(100%)	0(0%)	<0.001*
Moderate risk	33(47.1%)	26(78.8%)	7(21.2%)	
High risk	16(22.9%)	3 (18.8%)	13(81.2%)	

Test of significance: Chi- square- test * P - value ≤ 0.05 are considered statistically significant.
***** Variables specific only for clear cell type group (n=70)

Table (7): Comparsion for B7-H1 expression among 17 pairs of primary RCC and their corresponding LN metastasis.

	Positive Expression Rate		Change in Expression Pattern			
	Primary (n=17)	**Metastasis (n=17)**	**P = M**	**P > M**	**M > P**	**P value**
B7-H1 Expression	15(88.2%)	13(76.5%)	13(76.5%)	3(17.6%)	1(5.9%)	.625

P: primary renal cell carcinoma tumor M: metastatic malignant LN
Test of significance: Mc Nemar Test

Table (8): Association of combination of nuclear survivin expression &B7-H1 expression and clinicopathological features for patients with RCC (n=100)

Clinicopathological features	*Total* 100(100%)	Combination of Nuclear survivin & B7-H1 expression				P value
		Low S/ -ve B7-H1 No. (%) 57(57%)	High S/ -ve B7-H1 No. (%) 14(14%)	Low S/ +ve B7-H1 No. (%) 9(9%)	High S/ +ve B7-H1 No. (%) 20(20%)	
Age at Surgery, y						
<65	76(76%)	4(57.9%)	2(15.8%)	6(7.9%)	4(18.4%)	0.6
≥65	24(24%)	13(54.2%)	2(8.3%)	3(12.5%)	6(25%)	
Sex						
Male	53(53%)	31(58.5%)	6(11.3%)	5(9.4%)	11(20.8%)	0.8
Female	47(47%)	26(55.3%)	8(17%)	4(8.5%)	9(19.1%)	
Localization						
Right	50(50%)	29(58%)	6(12%)	4(8%)	11(22%)	0.8
Left	50(50%)	28(56%)	8(16%)	5(10%)	9(18%)	
Histological type						
Clear RCC	70(70%)	40(57.1%)	0(14.3%)	7(10%)	13(18.6%)	
Papillary RCC	10(10%)	7(70%)	1(10%)	0(0%)	2(20%)	
Chromophobe RCC	10(10%)	7(70%)	1(10%)	1(10%)	1(10%)	0.5
Granular RCC	3(3%)	1(33.3%)	1(33.3%)	0(0%)	1(33.3%)	
Mixed RCC	2(2%)	0(0%)	0(0%)	0(0%)	(100%)	
Sarcomatoid RCC	5(5%)	2(40%)	1(20%)	1(20%)	1(20%)	
Primary tumor classification						
T1a	15(15%)	15(100%)	0(0%)	0(0%)	0(0%)	
T1b	18(18%)	17(94.4%)	0(0%)	0(0%)	1(5.6%)	

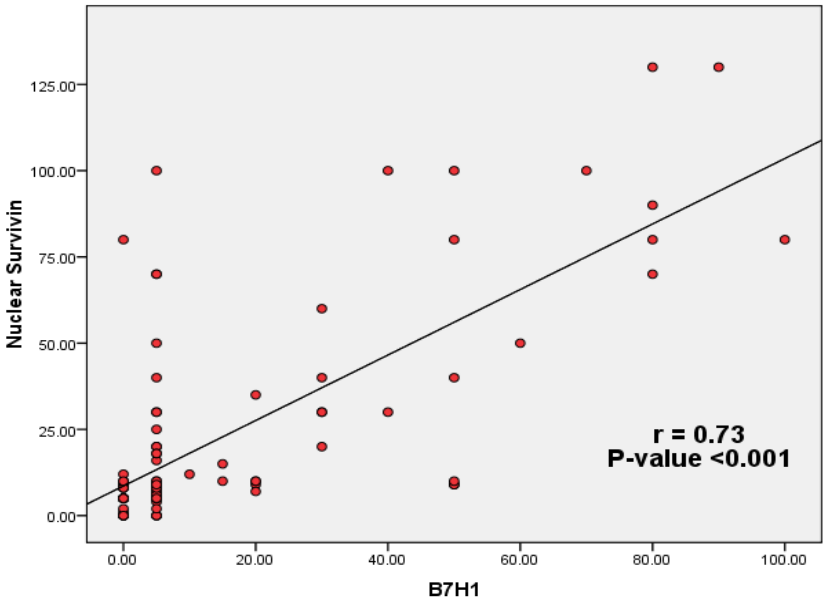
T2a	13(13%)	12(92.3%)	0(0%)	1(7.7%)	0(0%)	<0.001*
T2b	24(24%)	12(50%)	6(25%)	3(12.5%)	3(12.5%)	
T3a	25(25%)	1(4%)	8(32%)	3(12%)	13(52%)	
T3b	2(2%)	0(0%)	0(0%)	1(50%)	1(50%)	
T4	3(3%)	0(0%)	0(0%)	1(33.3%)	2(66.7%)	
Regional lymph node involvement						
N0	77(77%)	55(71.4%)	11(14.3%)	9(11.7%)	2(2.6%)	
N1	21(21%)	2(9.5%)	3(14.3%)	0(0%)	16(76.2%)	<0.001*
N2	2(2%)	0(0%)	0(0%)	0(0%)	2(100%)	
TNM stage groupings						
I	32(32%)	32(100%)	0(0%)	0(0%)	0(0%)	
II	33(33%)	23(69.7%)	5(15.2%)	4(12.1%)	1(3%)	<0.001*
III	30(30%)	2(6.7%)	9(30%)	4(13.3%)	15(50%)	
IV	5(50%)	0(0%)	0(0%)	1(20%)	4(80%)	
Tumor size, cm						
<5	17(17%)	15(88.2%)	0(0%)	1(5.9%)	1(5.9%)	<0.001*
5 to< 7	22(22%)	17(77.3%)	3(13.6%)	1(4.5%)	1(4.5%)	
7 to<10	20(20%)	12(60%)	4(20%)	3(15%)	1(5%)	
≥10	41(41%)	13(31.7%)	7(17.1%)	4(9.8%)	17(41.5%)	
Nuclear grade						
G1	19(19%)	15(78.9%)	1(5.3%)	2(10.5%)	1(5.3%)	
G2	43(43%)	30(69.8%)	7(16.3%)	2(4.7%)	4(9.3%)	0.002*
G3	28(28%)	10(35.7%)	5(17.9%)	4(14.3%)	9(32.1%)	
G4	10(10%)	2(20%)	1(10%)	1(10%)	6(60%)	
Coagulative tumor necrosis						
-ve	48(48%)	39(81.2%)	4(8.3%)	3(6.2%)	2(4.2%)	<0.001*
+ve	52(52%)	18(34.6%)	10(19.2%)	6(11.5%)	18(34.6%)	
Perinephric fat invasion						
-ve	73(73%)	56(76.7%)	7(9.6%)	5(6.8%)	5(6.8%)	<0.001*
+ve	27(27%)	1(3.7%)	7(25.9%)	4(14.8%)	15(55.6%)	
Sarcomatoid differentiation						
-ve	92(92%)	55(59.8%)	12(13%)	8(8.7%)	17(18.5%)	0.2
+ve	8(8%)	2(25%)	2(25%)	1(12.5%)	3(37.5%)	
Lymphocytic infiltrate*						
-ve	39(55.7%)	30(76.9%)	5(12.8%)	2(5.1%)	2(5.1%)	<0.001*
Focal	14(20%)	9(64.3%)	2(14.3%)	2(14.3%)	1(7.1%)	
Moderate	11(15.7%)	0(0%)	2(18.2%)	2(18.2%)	7(63.6%)	
marked	6(8.6%)	1(16.7%)	1(16.7%)	1(16.7%)	3(50%)	
SSIGN score*						
5y SR 100%	22(31.4%)	22(100%)	0(0%)	0(0%)	0(0%)	
5y SR 90%	17(24.3%)	13(76.5%)	3(17.6%)	1(5.3%)	0(0%)	<0.001*
5y SR 64%	15(21.4%)	5(33.3%)	4(26.7%)	5(33.3%)	1(6.7%)	
5y SR 47%	13(18.6%)	0 (0%)	3(23.1%)	1 (7.7%)	9 (69.2%)	
5y SR 0%	3(4.3%)	0(0%)	0(0%)	0(0%)	3(100%)	
MR score*						
Low risk	21(30%)	21(100%)	0(0%)	0(0%)	0(0%)	
Moderate risk	33(47.1%)	19(57.6%)	7(21.2%)	6(18.2%)	1(3%)	<0.001*
High risk	16(22.9%)	0 (0%)	3(18.8%)	1 (6.2%)	12 (75%)	

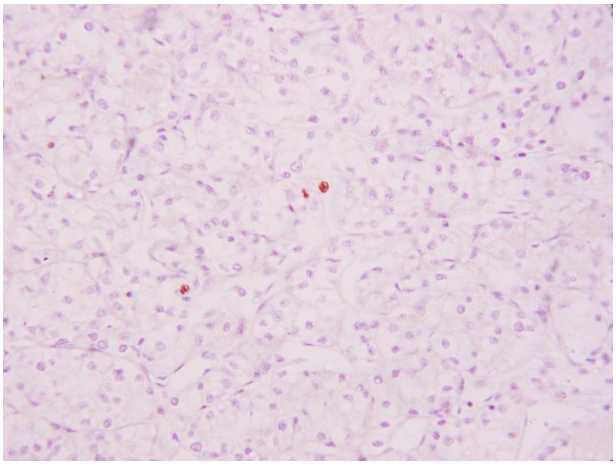
Test of significance: Chi- square- test

*P - value ≤ 0.05 are considered statistically significant

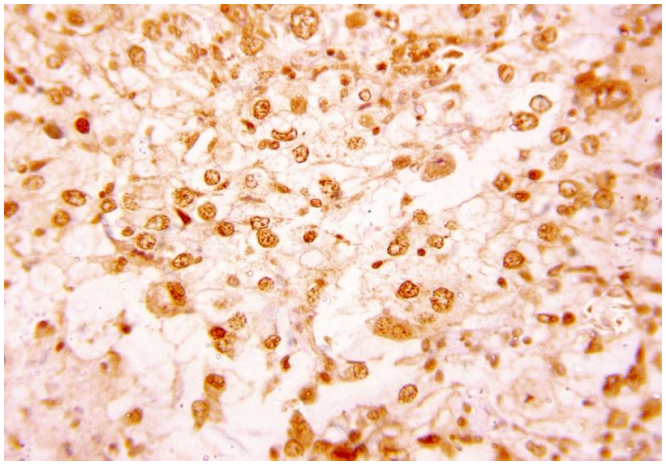
***** variables specific only for clear cell type group

Figure (1): Correlation of nuclear survivin and B7-H1 expression

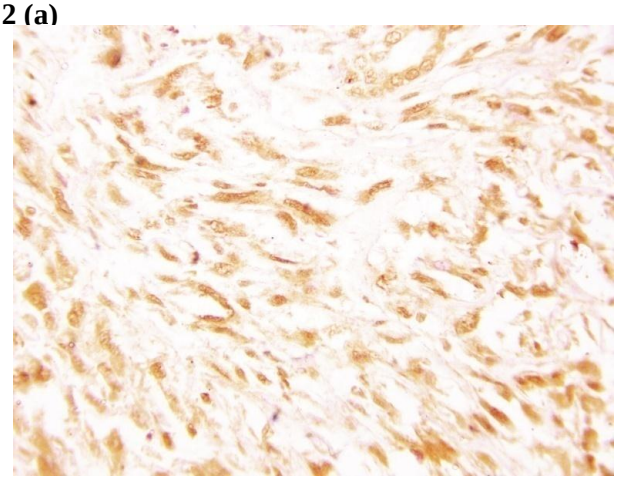




2 (a)

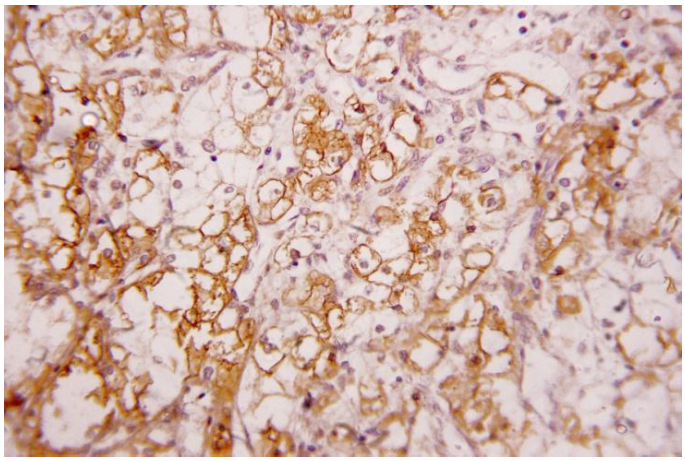


2(b)

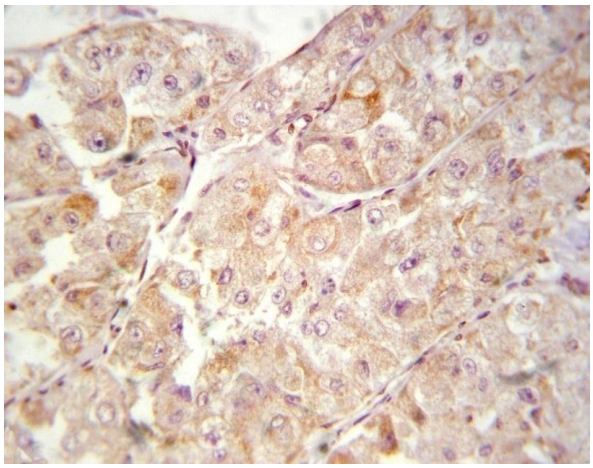


2(c)

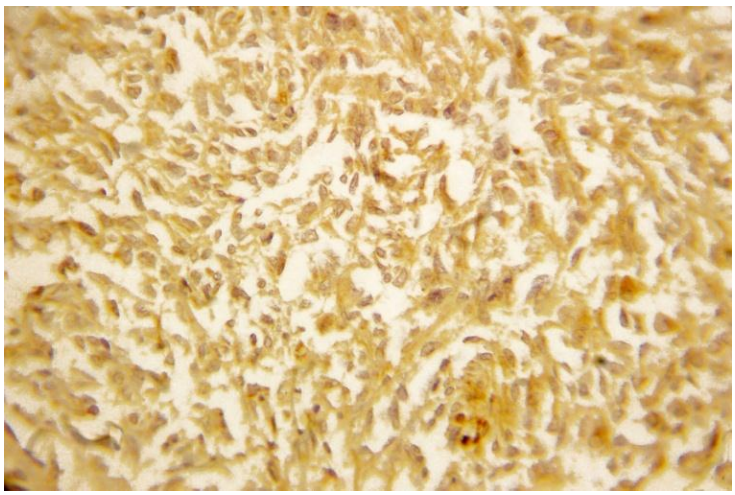
Fig. (2-A, B, C): Survivin immunostaining in representative sections of RCCs with (A) low nuclear survivin expression in grade 1 clear cell RCC, (B) high nuclear survivin expression in grade 3 clear cell RCC and (C) high nuclear and positive cytoplasmic survivin expression in sarcomatoid RCC(X400).



3(a)



3(b)



3(c)

Fig. (3-A, B, C): B7−H1 immunostaining in representative sections of RCCs with (A) positive membranous B7−H1 expression in grade 1 clear cell RCC, (B) positive cytoplasmic B7−H1 expression in grade 3 clear cell RCC and (C) positive cytoplasmic B7−H1 expression in sarcomatoid RCC(X400).

Discussion

Renal cell carcinoma is recognized as a group of cancers that originate from the renal tubular epithelium and have distinct genetic and molecular backgrounds, unique morphological features and a characteristic clinical course (Lei et al., 2010).

The molecular mechanisms underlying the development of RCC are still poorly understood. Therefore, it is crucial to exploit markers that can accurately represent biological features of tumors and predict the outcome, which will help us to perform tailored therapy for individual cases.

The present work was conducted to study the immunohistochemical expression of survivin and B7-H1 in different types of RCC tumors, in order to evaluate their expression patterns and examine their association with various clinicopathological features and finally to investigate the presence of a possible relationship between both markers in RCC tumors.

In the current study, survivin expression was observed in both nuclei and cytoplasm of tumor cells, this was in line with Emaetig et al., 2013 who reported both nuclear and cytoplasmic localization of survivin. other studies reported survivin expression restricted to the nuclei of tumor cells (Parker et al., 2006; Krambeck et al., 2007;

Parker et al., 2008; Parker et al., 2009) while others demonstrated its cytoplasmic expression (Byun et al., 2007, Wang et al., 2009; Lei et al., 2010). These variations in survivin subcellular localization reported by different studies could be attributed to the different types of survivin antibody clones used which are specific for certain survivin localization. In contrast to RCC cells, survivin (nuclear and cytoplasmic) was undetectable in the adjacent normal renal tubular cell. Previous studies have shown the absence of survivin expression in normal renal tubular cells using immunohistochemical and reverse-transcription polymerase chain reaction assays (Byun et al., 2007; Parker et al., 2009; Lei et al., 2010; Emaetig et al., 2013). Therefore, one of the most significant features of survivin is its differential distribution in cancer compared with normal tissues. This sharp differential expression in cancer versus normal tissues is one the most intriguing features of survivin and sets it apart from other members of the IAP family; this can be helpful particularly in therapeutic intervention (Li et al., 2005).

The present study demonstrated a significant associations between nuclear survivin expression and different clinicopathological features including primary tumor classification, regional lymph involvement, advanced tumor stage, perinephric fat invasion, lymphocytic infiltrate, SSIGN score, MR score, tumor size ,nuclear grading and coagulative tumor necrosis .

In the current study, we noticed that survivin was expressed in all histologic RCC types with a higher survivin immunostaining scores in SRCC type and GRCC type as compared to clear cell type, papillary type and chromopobe type, suggesting the expected role of survivin in more advanced renal cell carcinoma types. Moreover, we demonstrated a significant association between survivin nuclear expression and lymphocytic infiltrate in ccRCC cases. The CTL recognition of survivin possibly contributes to the significant increase in lymphocytic infiltration that is observed within survivin^{Hi} tumors, a hypothesis that enforce that ccRCCs is regarded as an immunogenic malignancy.

On studying the association between the SSIGN score and MR score with nuclear survivin expression, a significant association became evident between the nuclear survivin expression and SSIGN score and MR category. These results are in accordance with previous studies confirmed a significant association with death from RCC in cases with survivin^{Hi} expression after adjusting for the Mayo Clinic SSIGN score in relation to metastatic risk category (Parker et al., 2006; Krambeck et al., 2007; Parker et al., 2008; Parker et al., 2009).

As regard the cytoplasmic expression of survivin, we noticed that the positive cytoplasmic survivin expression in 47% of RCC tumors. This was comparable with previous studies that reported expression rates ranged from 52.3% up to 79% (Byun et al., 2007; Lei et al., 2010). This wide difference in the expression rate could be related to biased case selection, different antibodies, scoring systems and different cutoff points for definition of positivity used by different studies. Our results were similar to the findings that reported by Wang et al., 2009 who confirmed a higher cytoplasmic survivin immunostaining score in SRCC and GRCC but not in ccRCC, suggesting an important role of survivin in more aggressive subtypes of renal cell carcinoma.

On studying the association of cytoplasmic survivin expression with different clinicopathological features, no significant association was observed between cytoplasmic survivin expression and any of clinicopathological features. Similarly, Wang et al., 2009 reported that the cytoplasmic immunostaining score of survivin in RCC tumors did not significantly correlate with clinicopathological features including nuclear grading and staging that explained by low expression rate. On the contrary, other studies reported that a high level of cytoplasmic survivin expression was significantly correlated with tumor pathological stage, grade, and lymph node

metastasis (Byun et al., 2007; Lei et al., 2010), but come in line with our results in the point of no significant association with other clinicopathological factors including age, sex, tumor size, histological type of RCC patients. The difference could be attributed to biased case selection, different scoring systems and different cutoff points for positivity used by different studies

On comparing the role of nuclear and cytoplasmic survivin expression in this cohort, nuclear survivin expression was significantly associated with aggressive tumor features, while no significant relations were noticed between cytoplasmic survivin expression and these features, although both nuclear and cytoplasmic survivin expression coexist in a considerable proportion of tumors.

Survivin seems to exist in 2 subcellular pools (cytoplasmic and nuclear) (Fortugno et al., 2002). This is consistent with its function in the regulation of both cell viability and cell division (Li, 2003). One possibility is that the nuclear pool of survivin is involved in promoting cell proliferation in most (if not all) cases, whereas the cytoplasmic pool of survivin may participate in controlling cell survival but not cell proliferation. Alternatively, survivin has a number of splicing variants, which may differ in their subcellular localization and functions with respect to cell survival and cell division (Badran et al., 2004). Survivin and survivin-2B are predominantly cytoplasmic, whereas survivin-ΔEx-3 is primarily nuclear. These different isoforms of survivin and their varied locations in the cell may represent a regulatory balance between apoptosis and inhibition of apoptosis (Noton et al., 2006).

As it is possible immunohistochemically to distinguish two intracellular pools of survivin, a nuclear and a cytosolic one, the prognostic significance of the protein has been analyzed in some studies as a function of its intracellular localization and inconsistent and sometimes contrasting results have been obtained regarding the prognostic value of nuclear vs. cytoplasmic survivin expression (Li et al., 2005). So the different prognostic value of survivin may reflect differential expression of survivin splice variants that exist.

In this study, a high concordance rate of survivin expression status was found between matched primary renal cell carcinoma and metastatic lymph node specimens. These results are in accordance with (Shariat et al., 2007) who reported survivin expression in both primary and metastatic lesions with no significant difference in between. These data along with our findings, suggest that survivin expression by tumor cells probably occurred before metastasis and that survivin bearing malignant cells have more ability to metastasize.

Our findings together with others suggests that nuclear survivin expression is an useful important biologic marker for aggressive RCCs, predicting prognosis in patients with RCC and for guiding the development of more effective methods for potential adjuvant therapy for high-risk patients.

Regarding B7-H1 expression, in the current study we demonstrated a combined membranous and cytoplasmic expression. Some studies detected B7-H1 cellular localization concentrated primarily within the cell membrane (Krambeck et al., 2007; Parker et al., 2009; Taube, 2012), while others mentioned that equivalent staining was seen either in the cytoplasm or in the membrane or even combined (Ghebeh et al., 2006; Thompson et al, 2007; Loos et al., 2011). It is worthwhile to mention that there are different antibodies that can identify different cellular compartments including cell surface and cytoplasmic. In our study, the antibody that used was known to identify both cellular compartments, membranous and cytoplasmic.

The current study demonstrated significant associations between B7-H1 expression and different clinicopathological features including, primary tumor classification, regional lymph involvement, advanced tumor stage, nuclear grading, coagulative tumor necrosis, perinephric fat invasion, lymphocytic infiltrate, SSIGN score, MR score and tumor size. Here in, we noticed

that B7-H1expressed in all histologic RCC types. No significant difference was noticed among different histological subtypes of RCC, although a higher immunostaining scores were noticed more frequently in SRCC type and GRCC type as compared to other histological subtypes, suggesting its role in these subtypes of renal cell carcinoma.

Our results showed a significant association between positive B7-H1 expression and lymphocytic infiltrate. This may be attributed to the fact that the positive B7-H1tumor cells might inhibit the function of tumor infiltrating T cells, either through the induction of apoptosis or anergy and contributes to the profile of immunosuppression observed in RCC patients that is responsible for immune-mediated tumor destruction for this treatment-refractory malignancy.

In this series, the expression rate of B7-H1 was much higher in cases with high SSIGN score compared to those with moderate SSIGN score, Keeping up with our findings, similar results were demonstrated by previous studies that confirmed a significant association with death from RCC in cases with positive B7-H1expression after adjusting for the Mayo Clinic SSIGN score in relation to metastatic risk category (Krambeck et al., 2007; Parker et al., 2009).

Our results showed a significant association between positive B7-H1 expression and lymphocytic infiltrate. A similar results was reported by Thompson et al., 2004; Krambeck et al., 2007; Thompson et al., 2007 which are supported by hypothesis that tumor express B7-H1, might further inhibit the function of tumor infiltrating T cells, either through the induction of apoptosis or anergy. This contributes to the profile of immunosuppression observed in RCC patients. As such, blockade of B7-H1 may theoretically permit immune-mediated tumor destruction for this treatment-refractory malignancy.

Based on its recognized ability to impair the function and survival of activated tumor-specific T cells, we infer that B7-H1 expressed by RCC tumor cells and associated increased infiltrating lymphocytes, may contribute to the profile of immunosuppresion that is observed in patients with RCC , so we further speculate that intratumoral B7-H1 functions as a critical host determinant of treatment responses in patients who receive immunotherapy for management of advanced RCC (i.e., IL-2, IFN vaccination, or T cell adoptive therapy).

Taken together, our previous findings suggest that positive B7-H1 expression is a useful important biologic marker for aggressive RCCs, predicting prognosis in patients with RCC.

In the current study a high concordance rate of B7-H1 expression status between matched primary renal cell carcinoma and metastatic lymph node specimens was found. This can be explained by the fact of positivity of tumor cells for B7-H1 occurs early in the process of tumorogenesis that can later on metastatize.

One of the new modalities in immunothrapy is the use of cell surface signaling molecules (CSSMs) which make ideal targets for mAb immunotherapy. Antagonist mAbs targeting inhibitory CSSMs such as PD-1 and B7-H1 promote immune activation against cancer may results in the generation of immune memory and, consequently, a durable response against cancer, which is of critical importance in immunotherapeutics (Weber, 2010).

Herein, studying the combined expression patterns of survivin and B7-H1 revealed a strong association of survivin^{Hi}/B7-H1^{+} immunoprofile and the more aggressive clinicopathological features. The frequency of survivin^{Hi}/B7-H1^{+} expression was higher in cases with T4 and T3 than those with both T2 and T1 and in cases positive for lymph node metastases than those negative for lymph node metastases.

Our results also demonstrated that the highest incidences of grade 4 and 3 tumors were more frequently noticed in survivin^{Hi}/B7-H1^{+} cases and the rate of

survivin^{Hi}/B7-H1^{+} expression in cases with perinephric fat invasion and coagulative tumor necrosis were much higher compared to those without perinephric fat invasion and coagulative tumor necrosis. Conversely, the survivin^{low}/ B7-H1^{-} phenotype was more frequently seen among stage I and stage II tumors with the highest incidences in grade 1 and 2 tumors as well as small sized tumors. All these findings were agree with that reported by Krambeck et al., 2007 who demonstrated that a combination of survivin^{Hi}/B7-H1^{+} expression was significantly associated with several adverse clinicopathological features and form the most aggressive phenotype.

Furthermore, the current work demonstrated a highly significant association between SSIGN score and MR score with the combined survivin/B7-H1 immunoprofile. The rate of survivin^{Hi}/B7-H1^{+} expression was higher in ccRCC cases with high SSIGN score compared to those with moderate SSIGN Score. None of tumors had low SSIGN score showed survivin^{Hi}/B7-H1^{+} expression. Also a significant positive association was evident in relation to immunoprofile with MR score in which the rate of survivin^{Hi}/B7-H1^{+} expression was higher in cases with high risk category (75 %) compared to those with intermediate (3 %) and low risk category risk categories (0 %).

Our findings showed that a combined survivin^{Hi}/B7-H1^{+} expression can provide a more significant degree of further stratification among each category of ccRCC risk patients based upon SSIGN score. So this further stratification within the same risk category can help to provide additional prognostic information that contributes in the therapeutic interventions. This was in line with a similar study was conducted by Parker et al., 2009. Owing to applicability of patient's follow up data records in their center, survival analysis could be conducted, so they could demonstrated that patients within the same risk category with combined expression are associated with poorer cancer-specific survival and the use of this combined expression can provide additional information to further stratify among patients initially predicted to be at intermediate risk and high risk by the SSIGN score with limited ability to be applied to low risk patients.

In our study, we observed high levels of lymphocytic infiltrate within survivin^{Hi}/B7-H1^{+} tumors relative to the survivin^{Low}/B7-H1^{-} tumors. So the combined effect of survivin^{Hi}/B7-H1^{+} on ccRCC tumor aggressiveness occurs at the cellular level could be explained as the tumor cell survivin can be recognized by CTLs that lead to significant increase in lymphocytic infiltration, this significant increase in lymphocytic infiltration produce IFN-ɤ, which cause tumor cell B7-H1 expression to be up-regulated. This up regulation inhibits the function of tumor-infiltrating T cells, either through the induction of apoptosis or anergy (Andersen et al., 2001).

The current study demonstrated a moderate positive correlation between nuclear survivin and B7-H1 expression. Also there was a distinctively positive correlation between combined nuclear survivin and B7-H1 and clinicopathological features related to tumor progression indicating that these two markers may act in concert to mediate a more aggressive tumor behavior and poor outcome. Given that both survivin and B7-H1 are widely expressed within human malignancies including RCC, we anticipate these observations will have broad implications for improving prognostication and treatment of RCC and other malignancies.

Conclusion and Recommendations

Our results can confirm that RCC patients whose tumors exhibit high levels of nuclear survivin expression based on immunohistochemical (IHC) analysis are at markedly increased risk of cancer progression and poor prognosis from RCC relative to patients whose tumors express low levels of survivin suggesting that this biomarker lends meaningful prognostic information beyond standard clinical and pathologic indices.

Our findings suggest that positive B7-H1 expression is a useful important biologic

marker for aggressive RCCs, predicting prognosis in patients with RCC, the basis for these associations may relate to the recognized ability of B7-H1 to inhibit antitumor T-cell–mediated immunity. As such, B7-H1 may represent a target for RCC immunotherapy and a potential biomarker to facilitate patient assignment to treatment, as well as aid in the determination of prognosis both before and after therapy.

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