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**Original Research**

# Survivin Expression in Renal Epithelial Tumors: It's Usage in the Differential Diagnosis of Eosinophilic Renal Epithelial Tumors

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**Abstract**

**Objective:** The differential diagnosis of renal tumors can be problematic due to overlapping morphologic features. The purpose of this study was to assess the potential contribution of survivin expression in the differential diagnosis and determination of therapy modalities of these tumors.

**Methods:** This study consisted of 15 chromophobe (ChRCC), 15 clear cell (CCRCC) and 9 papillary (PRCC) renal cell carcinomas, and 13 oncocytomas. Sections were stained against survivin antibody.

**Results:** PRCCs and CCRCCs showed diffuse and strong survivin expression. Survivin expression was strikingly prominent in type1 PRCCs and cystic CCRCCs. In CCRCCs, survivin expression was more pronounced in low grade areas than high grade and sarcomatoid areas. In ChRCC, survivin expression was more limited and weaker than that of oncocytomas and other malignant renal tumors. In non-neoplastic renal tissue, survivin expression was more pronounced in podocytes and atrophic tubules than other nephron parts.

**Conclusions:** Our results suggested that survivin may helpful in the differential diagnosis of renal tumors despite limited number of our cases. Experimental studies have revealed that inhibition of survivin induces apoptosis and enhance radiosensitivity of RCC cells. Taken together, to be known the proportion of survivin expression in subtypes of renal tumors may contribute to determine new therapeutic strategies for RCCs. This needs to be proven in more wide series.



## INTRODUCTION

The differential diagnosis of renal epithelial tumors, especially those with eosinophilic cytoplasm, can be problematic due to overlapping morphologic features. This problem is magnified in the context of small biopsy specimens and metastatic disease. Immunohistochemical and sometimes electron microscopic examinations may therefore require. Immunohistochemical markers may be critical for the diagnosis of renal neoplasms in several instances including differentiation of renal cell from non-renal cell neoplasms, histologic subtyping of renal neoplasms, accurate diagnosis of renal neoplasm in small biopsy samples, and diagnosing metastatic renal cell carcinoma (RCC). Several markers are available for these diagnostic goals including the Renal Cell Carcinoma Marker antigen (RCCM), kidney-specific cadherin (KSC), cytokeratin subtypes, anti-mitochondrial antibody (AMA) CD10, CD 138, vimentin, alpha-methylacyl coenzyme A racemase, parvalbumin, carbonic anhydrase IX, PAX2, and PAX8 [1-17]. Sometimes traditional markers are, however, limited, since they are often expressed not only by renal neoplasms but also by tumors of other organs, or they are seen in variable percentages of only some specific subtypes of renal neoplasms.

Survivin, a member of the family of inhibitor apoptosis proteins, is a bifunctional protein that suppresses apoptosis and regulates cell division [18]. Survivin has been found to play an important role in the initiation and progression of human malignancies [19-24]. Several studies demonstrated that the overexpression of survivin determined in some human malignancies, such

as hepatocellular carcinoma, colorectal cancer, lung cancer, pancreatic cancer, osteosarcoma and renal cell carcinoma [19-24]. Some studies have been suggested that the overexpression of survivin in neoplastic cells has been found to be correlated with radioresistance of rectal, pancreatic and renal cancers [25-27]. As a result, survivin might play important role in malignant transformation. Survivin may help us to develop more effective therapeutic strategies. The purpose of the present study was to assess the potential contribution of survivin expression in the differential diagnosis and the determining new therapeutic strategies of this group of tumors.

## MATERIALS AND METHODS

The case series consisted of 15 chromophobe (ChRCC), 15 clear cell (CCRCC) and 9 papillary (PRCC) renal cell carcinomas, and 13 oncocytomas (Table 1 and 2). There were prominent cytoplasmic eosinophilic changes in 5 cases of 15 CCRCCs and most of them were high grade CRCC. Two cases of CRCCs had multilocular cystic features. PRCCs were consisted of 7 cases type 1 and 2 cases type 2 PRCCs. Sections from the selected blocks were stained with the survivin antibody (Neomarkers, Labvision, Ab-2, clone 4F7, 1:100, avidin-biotin-peroxidase) on an automatic device (Ventana, Benchmark XT). The staining intensity was graded and recorded semiquantitatively as 0 (no staining), 1+ (weak), 2+ (moderate) and 3+ (strong). The nephron parts of the non-neoplastic kidney neighboring the tumor were used as positive control. An intensity score of $\geq$2 was used to categorize tumor s with high survivin expression group, and <2 intensity score was used to categorize tumor s with low survivin expression group (Table 2) [26].

Statistical analyses were performed by using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). Chi-square test ($\chi 2$ test) was used for all comparisons. A p-value <0.05 was considered statistically significant for overall comparisons.

## RESULTS

Survivin was successfully detected in routinely processed tissue with appropriate positive and negative controls. The staining was almost exclusively nuclear, with no or negligible cytoplasmic expression in all cases except type 2 PRCCs, which had strikingly focal weak nuclear with prominent cytoplasmic staining. The staining results according to extent (percentage of positive tumor cell nuclei) and intensity of tumor cells is presented in Table 1.

PRCCs and CCRCCs showed diffuse (in 60-100% of tumor cells) and strong (2+/3+) nuclear survivin expression (Figures 1 a-f). Especially, nuclear survivin expression was strikingly prominent (in %100 of tumor cells and 3+) in type1 PRCCs (Figure 1e) and cystic CCRCCs (Figure 1d). In type 2 PRCCs, diffuse and strong cytoplasmic survivin staining was striking while it's nuclear staining was focal and weak (Figure 1f). In CCRCCs, nuclear survivin expression was more pronounced in low grade areas than high grade areas and sarcomatoid areas (Figures 1a and 1c). CRCCs with prominent eosinophilic cytoplasm, which most of them were high grade CRCCs, demonstrated similar nuclear survivin staining, but weaker (2+) than that of CCRCCs (Figure 1b). The survivin staining in ChRCCs was focal (in 10-60% of tumor cells) with palisading accentuation (2+) at periphery of the tumor nests and weaker (1+) than other renal epithelial tumors (Figure 1g). Oncocytomas with diffuse (in 65-95% of tumor cells) and strong (2+/3+) survivin staining differ from ChRCC (Figure 1h).

In addition, survivin expression was more prominent in podocytes and epithelial cells of atrophic tubules rather than in epithelial cells of distal and proximal tubules, and collecting ducts in non-neoplastic renal tissues adjacent to tumor (Figure 1i).

The relationships between survivin expression and histologic variants of RCC, and oncocytoma summarized in Table 2. Survivin expressions according to degree of its intensity was statistically meaningful among histologic variants of RCCs and also among all renal epithelial tumors ($p<0.001$, $p=0.002$, respectively).

**Table 1.** Survivin expression in renal epithelial tumors

| | % of Cells Stained (Mean ± SD) | Staining Intensity (Mean ±SD) |
|---|---|---|
| **CCRCC s (n=15)** | 75 (±17.6) | 2.3 (±0.7) |
| **PRCCs (n=9)** | 83.3 (±28.7) | 2.4 (±0.7) |
| **ChRCCs (n=15)** | 42.1 (±30.0) | 1.1 (±0.2) |
| **Oncocytomas (n=13)** | 85.8 (±11.2) | 2.0 (±0.5) |

**CCRCC:** Clear Cell Renal Cell Carcinoma; **PRCC:** Papillary Renal Cell Carcinoma; **ChRCC:** Chromophobe Renal Cell Carcinoma

**Table 2.** The staining intensity of survivin expression in renal epithelial tumors

| | Staining Intensity | | | |
|---|---|---|---|---|
| | Low (<2) N (%) | High (≥2) N (%) | *p* | *p* |
| **CCRCCs (n=15)** | 3 (20) | 12 (80) | | |
| **PRCCs (n=9)** | 1 (11) | 8 (89) | 0.002* | |
| **ChRCCs (n=15)** | 11 (73) | 4 (27) | | 0.001# |
| **Oncocytomas (n=13)** | 1 (8) | 12 (92) | | |

**CCRCC:** Clear Cell Renal Cell Carcinoma; **PRCC:** Papillary Renal Cell Carcinoma; **ChRCC:** Chromophobe Renal Cell Carcinoma
(*) This value is only for histologic variants of RCCs.
(#) This value is all renal epithelial tumors including oncocytomas.

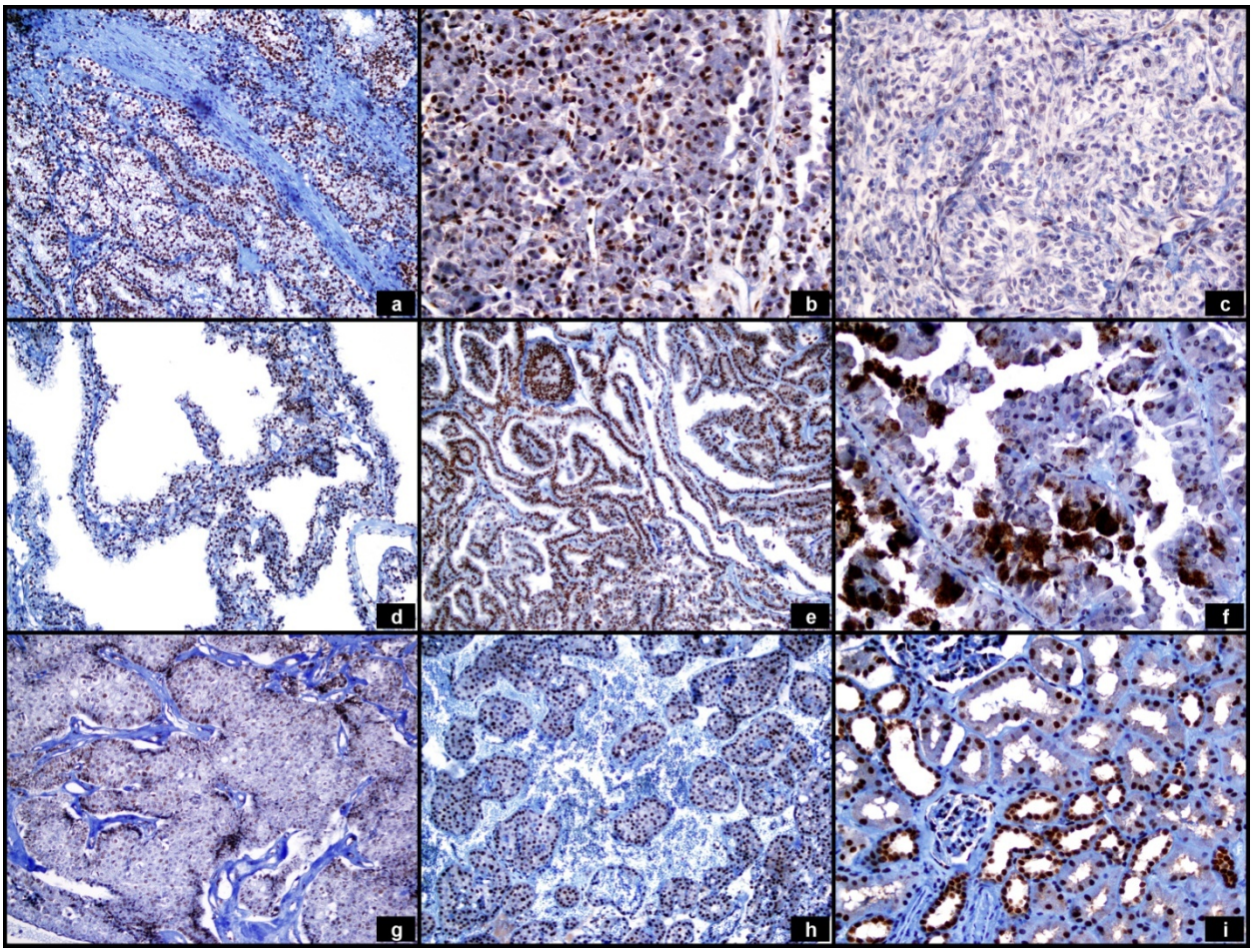


**Figure 1:** Diffuse and strong survivin expression was seen in low grade CCRCC **[a].** Focal and moderate or weak survivin expression with prominent eosinophilic cytoplasm was seen in high grade CCRCC **[b]** and sarcomatoid component **[c]**. There was no staining in other parts of these tumors. Diffuse and strong survivin expression was striking in cystic CCRCC **[d]** and type 1 PRCC **[e]**, while strong cytoplasmic and focal weak nuclear survivin expression was seen in type 2 PRCC **[f]**. Focal and weak survivin expression with peripheral accentuation was seen in ChRCC **[g]**, contrary diffuse and strong survivin expression was striking in oncocytoma **[h]**. Survivin expression was stronger in epithelial cells of atrophic tubules than in epithelial cells of distal and proximal tubules **[i]** (x400 for panel b, c, f and i; x200 for all other panels).

## DISCUSSION

Renal cell carcinoma (RCC) is the most common malignancy among primary renal tumors [1-17]. The classification of RCC is well established, enabling accurate diagnosis and distinction among different subtypes by routine histological studies in most cases. However, overlapping morphologic features can create differential diagnostic problems.

Immmunohistochemical markers may be critical for the diagnosis of renal tumors in several instances including differentiation of renal cell from non-renal cell tumors, histologic subtyping of renal tumors, accurate diagnosis of renal neoplasm in small biopsy samples, and diagnosing metastatic renal cell carcinoma. Sometimes, immunohistochemical markers for the differential diagnosis of RCC are limited and are not truly differentiate histologic subtypes of RCCs [1-17]. Therefore, it is crucial to use immunomarkers that can accurately represent biological features of RCCs and predict their outcomes, which will help us to determine appropriate therapy modality for RCC cases.

Many studies have documented the overexpression of anti-apoptotic factors such as the inhibitors of apoptosis proteins in a variety of solid tumors. Survivin is recently recognized novel immunomarker for RCC [24, 26]. It is a member of the family of inhibitor apoptosis proteins. Among these proteins, survivin is the smallest member. The overexpression of survivin has been found in a variety of human malignancies, such as hepatocellular carcinoma, colorectal cancer, lung cancer, pancreatic cancer, osteosarcoma, and renal cell cancer [19-24]. Moreover, several studies suggested that the overexpression of survivin expression has been found to be correlated with radioresistance of some cancers such as pancreatic, rectal and renal cancers [25-

27]. Lei et al suggested that the status of survivin expression might be an independent prognostic factor for RCC patients [26]. Therefore, survivin might play important roles in malignant transformation and development.

Zamparese et al suggested that survivin expression in 49 CCRCCs was weak positivity in 18.4% of cases, and strong positivity in the remaining (81.6%) cases [24]. Lei et al demonstrated that survivin expression with low intensity was in 47% (25/53) of CCRCCs and 45% (10/22) of ChRCCs, while survivin expression with high intensity was in 52% (28/53) of CCRCCs and 54% (12/22) of ChRCCs [26]. They found that these results were not statistically meaningful. Contrary, in presented study, survivin expression in CCRCC, PRCC and oncocytoma was exclusively diffuse and strong, while ChRCCs had focal (mean 42.1±30.0) and weak (mean 1.1±0.2 with 73% low and 27% high intensity) staining for survivin (Table 1 and 2). Our results were statistically meaningful ($p<0.05$) and consisted with the results of Zamparese et al [24]. However, Zamparese et al were evaluated survivin expression in only CCRCC, not in other histologic variants of RCCs [24]. Our results corroborate and expand the results of previous studies.

Our findings suggest that survivin may helpful in the differential diagnosis of renal tumors because of the partially unique nuclear and cytoplasmic staining patterns in these problematic renal epithelial tumors, especially, with eosinophlic cytoplasm despite limited number of our cases. Experimental studies have revealed that inhibition of survivin induces apoptosis and enhance radiosensitivity of RCC cells. Taken together, to be known the proportion of survivin expression in subtypes of renal tumors may contribute to determine new therapeutic strategies for RCCs. This findings needs to be proven in more wide series.

## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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