INTRODUCTION
Renal cell carcinoma (RCC) is the most common renal tumor, and the most lethal of all malignant urinary tract tumors, comprising more than 90% of them and accounting for 2-3% of malignant adult tumors [1]. Due to the widespread use of abdominal imaging, 60% of RCC patients are diagnosed at an early stage and have a low-risk (<10%) of cancer-related death [2]. Large proportions (up to 30%) of patients with RCC have distant metastases at diagnosis, and this represents a sign of poor prognosis [3]. Studying the molecular genetics of RCC metastasis helps to know the prognosis and detect a new therapeutic target to decrease its incidence and subsequently improve the prognosis of such patients [4]. Epithelial-mesenchymal transition (EMT) is a transdifferentiation process in which cells change between epithelial and mesenchymal states; this leads to gain features, e.g. mobility, invasiveness, and resistance to apoptosis [5]. Through such process, carcinoma cells may invade the surrounding tissues and metastasize [6]. EMT is regulated by several transcription factors. Detection of EMT is essential for proper diagnosis and enhanced chemotherapy treatment [7].

Prognostic implication of MYb-like, swirm and Mpn domain-containing protein-1 and Twist-1 in renal cell carcinoma

Ola Harb¹, Abdelmonem Hegazy², Maged Ali³, Rasha Haggag⁴

ABSTRACT
Objective: The data available regarding the epithelial-mesenchymal transition (EMT) markers are little. This study aimed to evaluate the immunohistochemical expression of EMT markers; MYb-like, Swirm and Mpn domain-containing protein-1 (MYSM-1) and Twist-1 in renal cell carcinoma (RCC), in a trial to find their role in the prognosis of such type of cancer. Methods: ImmunoeXpressions of MYb-like, Swirm and Mpn domain-containing protein-1 (MYSM-1) and Twist-1 were assessed in tissue specimens of 50 patients with RCC, and correlated with other clinicopathological features. The findings and their correlations were statistically determined. Results: Low expression of MYSM-1 was negatively correlated with size of the tumor, primary tumor (pT) stage, Fuhrman grade (P < 0.001), age (P = 0.002), and lymph node metastases (P = 0.010). ImmunoeXpression of MYSM-1 was significantly correlated with distant metastases (P = 0.004), overall survival (OS) (P < 0.001), and local recurrence (P = 0.001). High expression of Twist-1 was positively correlated with pT stage, Fuhrman grade, lymph node, distant metastases, age (P < 0.001), clinical stage (P = 0.002), the presence of sarcomatous change (P = 0.004), and tumor size (P = 0.008). ImmunoeXpression of Twist-1 was significantly correlated with distant metastases (P = 0.009), OS (P < 0.001), and local recurrence (P = 0.003). Conclusion: Overexpression of Twist-1 is an indication of poor prognosis in patients with RCC, whereas that of MYSM-1 is a marker of good prognosis.

KEY WORDS: Immunohistochemical expression, MYb-like, Swirm and Mpn domain-containing protein-1, prognosis, renal cell carcinoma, Twist-1
MYb-like, Swirm and Mpn domain-containing protein-1 (MYSM-1) is a metalloprotease that deubiquitinates the K119 - monoubiquitinated form of histone 2A, a chromatin marker associated with silencing of gene transcription, required in human hematopoiesis [8], and lymphocyte differentiation [9]. However, a few significant reports have been available regarding MYSM-1 in cancers until now. On the other hand, Twist-1 is a highly conserved basic helix-loop-helix transcription factor, which can promote EMT [10]. It has been reported that Twist-1 represents a master regulator of embryonic morphogenesis [11]. It also controls the cell migration and differentiation at various physiological conditions [12]. In addition, it promotes EMT under some pathological conditions including cancer [13]. Moreover, it has been suggested that this marker plays an important role in tumor growth, cell invasion, and metastasis through regulation of cancer-related functions, such as angiogenesis, and the degradation of extracellular matrix in various malignancies [14,15].

There is little information regarding the pathological roles and clinical significance of MYSM-1 and Twist-1 expressions in human RCC tissues. The few previous studies about MYSM-1 and Twist-1 in RCC make a real need for further studies. Therefore, we hypothesized that MYSM-1 may play a tumor-suppressing role in RCC; and Twist-1 is correlated with the potential of malignancy, metastases, and survival in patients with RCC. Because of the vague mechanism underlying RCC metastases, there has been urgent need to study these molecular markers and others to prove their possible use in RCC.

The current study aimed to evaluate the immunohistochemical expression of the EMT markers; MYSM-1 and Twist-1 in RCC in a trial to find their role in the prognosis of such type of cancers.

**MATERIALS AND METHODS**

**Patients and Tissue Specimens**

In this retrospective cohort study, formalin-fixed, paraffin-embedded tissue specimens from 50 patients of RCC were collected from the archives of the Pathology Department, Faculty of Medicine, Zagazig University between December 2012 and December 2015. Biopsies were obtained by radical nephrectomy and pelvic lymphadenectomy done in Urology Department, Zagazig University hospitals. Sex, age, tumor size, primary tumor (pT) stage, histological grade, clinical stage, lymph node, and distant metastasis for each case were recorded. Each tumor was re-evaluated histopathologically; 38 cases of conventional, 7 cases papillary and 5 cases chromophobe RCC were included. The TNM classification was used for pathologic staging of RCC [16], and Fuhrman histologic classification system was used for histologic grading [17,18]. The study complied with the guidelines of the Local Ethics Committee.

Imaging in the form of abdominal and pelvic ultrasonography and computed tomography scan with contrast of the abdomen and pelvis in selected cases were assessed. Overall survival (OS) was defined as the time from first surgery to death or to the last known alive data. Most patients had follow-up records for 3 years in Medical Oncology Department, Faculty of Medicine, Zagazig University. The follow-up deadline was November 2015.

**Immunohistochemical Staining**

Immunohistochemical staining was carried out using the streptavidin-biotin immunoperoxidase technique [19]. From each case, representative tissue blocks were selected for immunohistochemical investigation. They were cut in serial sections of 3 mm thickness, mounted on positively charged slides, deparaffinized in xylene, and then hydrated in a graded series of ethanol. Antigen retrieval was done by boiling in 0.01 mol/l sodium citrate buffer (pH 6.0) in a microwave oven for 10 min. Blocking the endogenous peroxidase activity with 0.3% hydrogen and the nonspecific protein binding with 1.5% normal goat serum. The slides were then incubated overnight with mouse monoclonal anti-MYSM-1 antibody (1:100, ab180570, Abcam) and Rabbit polyclonal anti-Twist antibody (1:50, ab50581; Abcam, Cambridge, UK). Incubation with a secondary antibody and product visualization were performed (Lab Vision Corporation, Fremont, USA), with diaminobenzidine substrate as chromogen. The slides were visualized with a labeled streptavidin-biotin method using 3, 3′-diaminobenzidine as a chromogen (Dako Canada Inc., Mississauga, Ontario, Canada) and counterstained with hematoxylin (BioGenex Laboratories, San Ramon, California, USA). Gastric cancer tissues were used as positive control for MYSM-1 [20], and thyroid cancer used as positive control for Twist-1 [21]. Negative controls were done by omitting the primary antibody, incubating the slides with PBS, and replacing the primary antibody with normal serum.

**Evaluation of Immunostaining Intensity of MYSM-1**

The degree of immunostaining was scored based on the intensity of staining and the percentage of MYSM-1 immunoreactive cells as follows: Negative, weak (<15% of cells with positive staining), medium (more than 15% but <30%), and strong (more than 30% of cells with positive immunostaining); negative and weak were categorized into low expression, whereas medium and strong was categorized into high expression [20].

**Evaluation of Immunostaining Intensity of Twist-1**

The staining intensity was graded semi-quantitatively as: 0, negative; 1, weak; 2, medium; and 3, strong. The staining extent was scored as: 0, 0%; 1, 1-25%; 2, 26-50%; 3, 51-75%; and 4, 76-100%. The final staining score (0-7) was calculated as the sum of the intensity score and extent score. Staining scores of 0-1 were considered to be negative, scores of 2-3 were considered as low expression, and a score of ≥3 were considered as high expression [22].

**Statistical Analysis**

The strength of correlations between MYSM-1, Twist-1, and clinicopathological features was determined by computing...
appropriate correlation coefficient (Spearman’s, Kendall tau, biserial, point biserial and rank biserial). Stratification of OS, local recurrence-free survival (LRFS), and distant metastases-free survival (DMFS) was done according to all clinicopathological features and immunohistochemical markers. These time-to-death distributions were estimated using the method of Kaplan–Meier plot and compared using two-sided exact log-rank test. A $P < 0.05$ was considered significant. All statistics were performed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium).

RESULTS

Fifty patients, 40 (80%) males and 10 (20%) females were included in our study, with the age ranged from 40 to 77 years (median: 64; mean: 60.22 ± 10.01) [Table 1].

MYSM-1 Immunoexpression and its Correlation with Clinicopathological Feature

MYSM-1 was nuclear in RCC cells and its low expression was negatively correlated with size of the tumor, pT stage, Fuhrman grade ($P < 0.001$), age ($P = 0.002$), lymph node ($P = 0.010$), and distant metastases ($P = 0.079$) [Table 1, Figures 1 and 2].

Regarding outcome of patients, positive immunoexpression of MYSM-1 was significantly correlated with distant metastases ($P = 0.004$), OS ($P < 0.001$), and local recurrence ($P = 0.001$) [Table 2].

Twist-1 Immunoexpression and its Correlation with Clinicopathological Features

Twist-1 was cytoplasmic in RCC cells and its high expression was significantly positively correlated with pT stage, Fuhrman grade, lymph node, distant metastases, age ($P < 0.001$), clinical stage ($P = 0.002$), the presence of sarcomatous change ($P = 0.004$), and tumor size ($P = 0.008$) [Table 1, Figures 3 and 4].

Regarding outcome of patients, positive immunoexpression of Twist-1 was significantly correlated with distant metastases ($P = 0.009$), OS ($P < 0.001$), and local recurrence ($P = 0.003$) [Table 2].

There was a significant inverse relationship between MYSM-1 and Twist-1 expression, $r = -0.838$ ($P < 0.001$).

The sensitivity and specificity of a combination of both markers as predictors for advanced stage RCC were 56.7% and 95%, respectively.
Survival Analysis

The 3-years OS rate of our patients was 67% in all cases, 100% and 53.8% in high, and low MYSM-1 expression, respectively; and 27.8% and 92% in high and low Twist-1, respectively. The 3 years OS is inversely related to low MYSM-1 immunoreactivity and high Twist-1 immunoreactivity ($P = 0.004$ and $<0.001$, respectively).

The 3-years DMFS rate of our cases was 75.9% in all cases, 72.7% and 81.9% in low and high MYSM-1 expression, respectively, and 87.4% and 55.6% in low and high Twist-1, respectively. There was an inverse relationship between 3-years DMFS rate and both low MYSM-1 expression and high Twist-1 expression ($P = 0.403$ and 0.006, respectively) [Table 3, Figures 5 and 6].

DISCUSSION

In this study, we focused on the immunohistochemical expression of two of the molecular markers of EMT; MYSM-1 and Twist-1. The correlation between MYSM-1 and Twist-1 and clinicopathological features of patients with RCC is presented in Table 1.
Twist-1. The results showed the decreased immunoeexpression of MYSM-1 in RCC of larger size, higher grade, advanced stage that with lymph node and distant metastases in comparison to tumors with smaller size, low grade, early stage and that without nodal or distant metastases. Therefore, the higher expression of MYSM-1 was the more favorable the overall prognosis will be. The results of our study are similar to results of Zhou et al. [20] that proved that low expression of MYSM-1 was negatively associated with tumor stage, lymph node metastases, and poor prognosis. They attributed their findings to that MYSM-1 is able to suppress the proliferation, migration, invasion, and tumorigenic ability through inhibiting the EMT process. Also similar to our results, they found that MYSM-1 was decreased in RCC tissues with metastasis. Original finding of MYSM-1 as a deubiquitinase appears in the control of the development of B lymphocytes [23]; and control of hematopoietic stem cell differentiation by controlling the expression of certain important transcription factors [24]. Furthermore, MYSM-1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All (n=50)</th>
<th>Local recurrence</th>
<th>P value</th>
<th>Distant metastasis</th>
<th>P value</th>
<th>Survival</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.22±10.01</td>
<td>66.20±7.07</td>
<td>0.001</td>
<td>57.94±10.35</td>
<td>0.006</td>
<td>58.14±10.63</td>
<td>0.078</td>
</tr>
<tr>
<td>Sex</td>
<td>40 (80)</td>
<td>19 (95)</td>
<td>1 (5)</td>
<td>&lt;0.001</td>
<td>19 (95)</td>
<td>1 (5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Size</td>
<td>8.14±2.19</td>
<td>9.70±1.45</td>
<td>0.001</td>
<td>7.50±1.96</td>
<td>0.001</td>
<td>7.34±2.05</td>
<td>0.001</td>
</tr>
<tr>
<td>Grade</td>
<td>8 (16)</td>
<td>14 (28)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
<td>8 (16)</td>
<td>0 (0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Sarcomatous</td>
<td>45 (90)</td>
<td>15 (33.3)</td>
<td>0.007</td>
<td>38 (84.4)</td>
<td>0.001</td>
<td>35 (77.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>T</td>
<td>12 (24)</td>
<td>1 (8.3)</td>
<td>0.004</td>
<td>11 (91.7)</td>
<td>0.002</td>
<td>12 (100)</td>
<td>0.001</td>
</tr>
<tr>
<td>Lymph node</td>
<td>20 (40)</td>
<td>20 (100)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
<td>20 (100)</td>
<td>0 (0)</td>
<td>0.012</td>
</tr>
<tr>
<td>Stage I</td>
<td>11 (22)</td>
<td>11 (100)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
<td>11 (100)</td>
<td>0 (0)</td>
<td>0.005</td>
</tr>
<tr>
<td>Stage II</td>
<td>18 (36)</td>
<td>10 (55.6)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
<td>9 (100)</td>
<td>0 (0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Stage III</td>
<td>15 (30)</td>
<td>4 (26.7)</td>
<td>7 (46.7)</td>
<td>14 (93.3)</td>
<td>4.5 (27.8)</td>
<td>2 (13.3)</td>
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<tr>
<td>Stage IV</td>
<td>12 (24)</td>
<td>12 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>12 (100)</td>
<td>0 (0)</td>
<td>0.001</td>
</tr>
<tr>
<td>MYSM-1</td>
<td>21.44±26.85</td>
<td>26.63±25.58</td>
<td>0.004</td>
<td>29.91±28.01</td>
<td>0.001</td>
<td>1.66±5.40</td>
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<tr>
<td>Low</td>
<td>11 (22)</td>
<td>11 (100)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
<td>11 (100)</td>
<td>0 (0)</td>
<td>0.005</td>
</tr>
<tr>
<td>Stage I</td>
<td>33 (66)</td>
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<td>&lt;0.001</td>
<td>19 (56.7)</td>
<td>0 (0)</td>
<td>0.001</td>
</tr>
<tr>
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<td>12 (24)</td>
<td>10 (83.3)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
<td>10 (83.3)</td>
<td>0 (0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Stage III</td>
<td>18 (36)</td>
<td>10 (55.6)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
<td>10 (55.6)</td>
<td>0 (0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Stage IV</td>
<td>15 (30)</td>
<td>10 (66.7)</td>
<td>6 (40.0)</td>
<td>6 (40.0)</td>
<td>6 (40.0)</td>
<td>6 (40.0)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean±SD and median (range), Mann–Whitney U test; Chi-square test; P<0.05 is significant, SD: Standard deviation, MYSP-1: MYb-like, Swirm and Mpn domain-containing protein-1, RCC: Renal cell carcinoma

The low immunohistochemical expression of MYSM-1 was found to be negatively associated with tumor stage, metastases, and poor prognosis of RCC. So, MYSM-1 could also suppress the proliferation and metastasis through inhibition of the EMT process.

Our results proved increased immunohistochemical expression of Twist-1 in RCC of larger tumor size, higher grade, stage, that with lymph node and distant metastases in comparison to smaller size, low grade, stage and that without nodal or distant metastases. So, the higher expression of Twist-1 was the worse the overall prognosis will be. These results are similar to that of Ohba et al. [27] stating that Twist expression was positively associated with grade, pT stage, and metastasis in patients with RCC.

The results of our study are in agree with the meta-analysis done by Wushou et al. [28], who concluded that expression of Twist-1, as measured by IHC, is associated with a worse prognosis in carcinoma, and they suggested that the development of strategies against this transcription factor could be a reasonable therapeutic approach. Results near ours were found by previous reports demonstrating that Twist-1 expression is significantly associated with poor prognosis in patients with RCC [29].

Other investigators have reported the higher expression of Twist-1 is associated with poor prognosis, high grade, invasive and metastases in other cancers, e.g. in thyroid [21], lung [30], stomach [31], bladder, prostate [32], and liver [33]. Twist-1 metastatic potential relies on its ability to induce an EMT, a process that converts joined and polarized epithelial cells into isolated and motile mesenchymal ones, able to bypass...
the basement membrane and infiltrate into the surrounding ECM [34]. The results of our research in Twist-1 may help to find a new therapeutic target to decrease RCC invasiveness and progression metastases.

As regard the correlation between the immunohistochemical expression of MYSM-1 and Twist-1, we found an inverse relation between both markers in RCC. The sensitivity and the specificity of combination of both markers as predictors for advanced stage RCC were 56.7% and 95%, respectively, as we proved that increased the immunoexpression of Twist-1 associated with increase the incidence of metastases and poor prognosis in RCC through stimulation of EMT; in contrast to, the increased expression of MYSM-1 that was associated with good prognosis in RCC.

It is concluded that Twist-1 overexpression is a marker of poor prognosis in patients with RCC, whereas MYSM-1 overexpression is a marker of good prognosis. The negative correlation between Twist-1 and MYSM-1 had a potential prognostic significance for RCC, as the combination of molecular inhibitors against

Figure 4: Immunohistochemical staining of Twist-1 in renal cell carcinoma (RCC): (a) High immunohistochemical expression in the cytoplasm of clear cell RCC Grade 3, ×400; (b) High immunohistochemical expression in the cytoplasm of papillary RCC Grade 3, ×400; (c) High immunohistochemical expression in the cytoplasm of RCC with sarcomatous changes, ×400

Figure 5: Kaplan-Meier plot of distant metastasis-free survival: (a) Stratified according to MYb-like, Swirm and Mpn domain-containing protein-1; (b) Stratified according to Twist-1

Figure 6: Kaplan–Meier plot of overall survival: (a) Stratified according to MYb-like, Swirm and Mpn domain-containing protein-1; (b) Stratified according to Twist-1
Twist-1 and molecular stimulators of MYSM-1 can be used as target therapy for inhibition of occurrence of metastases in RCC. Because of few studies that had been done regarding MYSM-1 immunohistochemical expression in different types of cancer, we recommend performing other studies with larger number of cases on its expression in kidney cancer and other types of cancers to prove its value on cancer molecular therapy.

REFERENCES


