



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

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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:** Immunoeexpressions 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 ($P = 0.010$). Immunoeexpression 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 ($P = 0.008$). Immunoeexpression 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

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].

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, Ferment, 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 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 Immunoeexpression 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 immunoeexpression 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 Immunoeexpression 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 immunoeexpression 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.

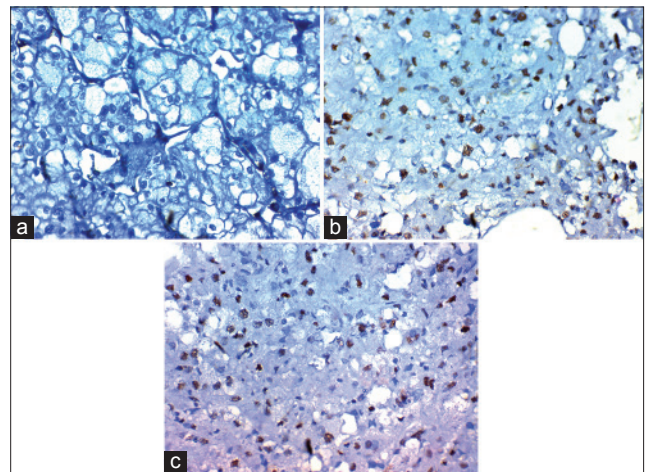


Figure 2: Immunohistochemical staining of MYb-like, Swirn and Mpn domain-containing protein-1 in renal cell carcinoma (RCC): (a) Negative immunohistochemical expression in the nucleus of clear cell RCC Grade 3, x400; (b and c) Low immunohistochemical expression in the nucleus of clear RCC Grade 3, x400

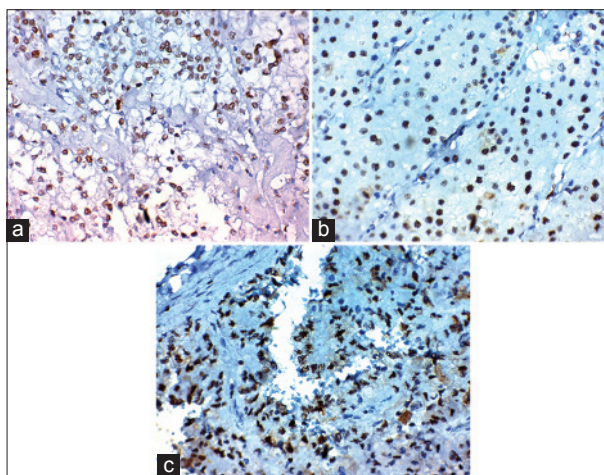


Figure 1: Immunohistochemical staining of MYb-like, Swirn and Mpn domain-containing protein-1 in renal cell carcinoma (RCC): (a) High immunohistochemical expression in the nucleus of clear cell RCC Grade 1, x400; (b) High immunohistochemical expression in the nucleus of chromophobe RCC Grade 1, x400; (c) High immunohistochemical expression in the nucleus of papillary RCC Grade 1, x400

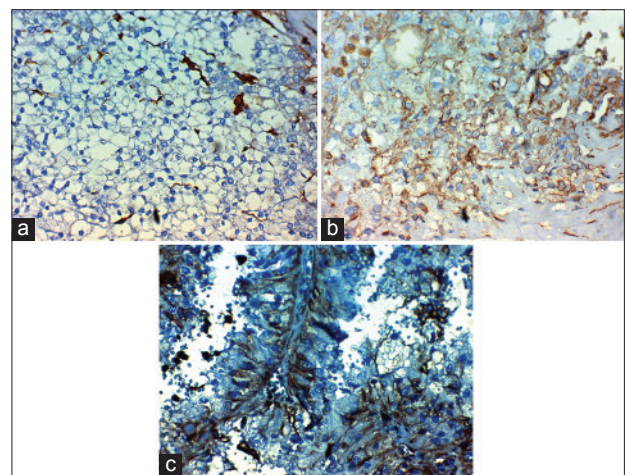


Figure 3: Immunohistochemical staining of Twist-1 in renal cell carcinoma (RCC): (a) Negative immunohistochemical expression in the cytoplasm of clear cell RCC Grade 1, x400; (b) Low immunohistochemical expression in the cytoplasm of clear cell RCC Grade 2, x400; (c) Low immunohistochemical expression in the cytoplasm of papillary RCC Grade 2, x400

Table 1: Correlation between MYSM-1 and Twist-1 and clinicopathological features of patients with RCC

Characteristics	All (n=50)	n (%)		P value	n (%)		P value
		MYSM-1			Twist-1		
		Low (n=33)	High (n=17)		Low (n=32)	High (n=18)	
Age (years)							
Mean±SD	60.22±10.01	62.54±8.62	55.70±11.19	0.068 [‡]	57.28±10.99	65.44±4.81	0.029 [‡]
Median (range)	64 (40-77)	65 (45-77)	55 (40-75)		55 (40-77)	65 (50-74)	
≤60 years	20 (40)	8 (24.2)	12 (70.6)	0.002 [§]	19 (59.4)	1 (5.6)	<0.001 [§]
>60 years	30 (60)	25 (75.8)	5 (29.4)		13 (40.6)	17 (94.4)	
Sex							
Male	40 (80)	32 (97)	8 (47.1)	<0.001 [§]	22 (68.8)	18 (100)	0.009 [§]
Female	10 (20)	1 (3)	9 (52.9)		10 (31.3)	0 (0)	
Size (cm)							
Mean±SD	8.14±2.19	8.87±1.84	6.70±2.14	0.001 [‡]	7.31±2.05	9.61±1.61	<0.001 [‡]
Median (range)	8 (4-12)	9 (4-12)	6 (4-11)		8 (4-11)	10 (5-12)	
≤7 cm	14 (28)	4 (12.1)	10 (58.8)	0.001 [§]	13 (40.6)	1 (5.6)	0.008 [§]
>7 cm	36 (72)	29 (87.9)	7 (41.2)		19 (59.4)	17 (94.4)	
Type							
Conventional	38 (76)	22 (66.7)	16 (94.1)	0.076 [§]	21 (65.6)	17 (94.4)	0.064 [§]
Papillary	7 (14)	7 (21.2)	0 (0)		6 (18.8)	1 (5.6)	
Chromophobe	5 (10)	4 (12.1)	1 (5.9)		5 (15.6)	0 (0)	
Grade							
Grade 1	8 (16)	4 (12.1)	4 (23.5)	<0.001 [§]	7 (21.9)	1 (5.6)	<0.001 [§]
Grade 2	14 (28)	7 (21.2)	7 (41.2)		13 (40.6)	1 (5.6)	
Grade 3	23 (46)	22 (66.7)	1 (5.9)		7 (21.9)	16 (88.9)	
Grade 4	5 (10)	0 (0)	5 (29.4)		5 (15.6)	0 (0)	
Sarcomatous							
Absent	45 (90)	28 (84.8)	17 (100)	0.152 [§]	32 (100)	13 (72.2)	0.004 [§]
Present	5 (10)	5 (15.2)	0 (0)		0 (0)	5 (27.8)	
T							
T1	12 (24)	3 (9.1)	9 (52.9)	<0.001 [§]	11 (34.4)	1 (5.6)	<0.001 [§]
T2	8 (16)	3 (9.1)	5 (29.4)		8 (25)	0 (0)	
T3	15 (30)	13 (39.4)	2 (11.8)		10 (31.3)	5 (27.8)	
T4	15 (30)	14 (42.4)	1 (5.9)		3 (9.4)	12 (66.7)	
Lymph node							
Negative	20 (40)	9 (27.3)	11 (64.7)	0.010 [§]	19 (59.4)	1 (5.6)	<0.001 [§]
Positive	30 (60)	24 (72.7)	6 (35.3)		13 (40.6)	17 (94.4)	
Stages							
Stage I	11 (22)	5 (15.2)	6 (35.3)	0.079 [§]	10 (31.3)	1 (5.6)	0.002 [§]
Stage II	9 (18)	4 (12.1)	5 (29.4)		9 (28.1)	0 (0)	
Stage III	18 (36)	15 (45.5)	3 (17.6)		9 (28.1)	9 (50)	
Stage IV	12 (24)	9 (27.3)	3 (17.6)		4 (12.5)	8 (44.4)	
MYSM-1							
Mean±SD	21.44±26.85	5.81±5.84	51.76±25.67	<0.001 [‡]	32.43±27.97	1.88±4.36	<0.001 [‡]
Median (range)	11 (0-90)	8 (0-14)	50 (20-90)		20 (0-90)	0 (0-12)	
Low	33 (66)				15 (46.9)	18 (100)	<0.001 [§]
High	17 (34)				17 (53.1)	0 (0)	
Twist-1							
Mean±SD	28.32±34.17	42.90±33.80	0±0	<0.001 [‡]	3.59±7.32	72.27±8.46	<0.001 [‡]
Median (range)	5 (0-80)	60 (0-80)			0 (0-25)	75 (55-80)	
Low	32 (64)	15 (45.5)	17 (100)	<0.001 [§]			
High	18 (36)	18 (54.5)	0 (0)				

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, Swirn and Mpn domain-containing protein-1, RCC: Renal cell carcinoma

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

Table 2: Association of clinicopathological features, MYSM-1, and Twist-1 with outcome of patients with RCC

Characteristics	All (n=50)	Local recurrence		P value	Distant metastasis		P value	Survival		P value
		No (n=30)	Yes (n=20)		No (n=38)	Yes (n=12)		Alive (n=35)	Died (n=15)	
Age (years)										
Mean±SD	60.22±10.01	56.23±9.76	66.20±7.07	0.001 [‡]	57.94±10.35	67.41±3.39	0.006 [‡]	58.14±10.63	65.06±6.34	0.078 [‡]
Median (range)	64 (40-77)	55 (40-77)	67 (40-75)		59 (40-77)	67 (63-75)		58 (40-77)	65 (45-74)	
≤60 years	20 (40)	19 (95)	1 (5)	<0.001 [§]	20 (100)	0 (0)	0.001 [§]	19 (95)	1 (5)	0.002 [§]
>60 years	30 (60)	11 (36.7)	19 (63.3)		18 (60)	12 (40)		16 (53.3)	14 (46.7)	
Sex										
Male	40 (80)	26 (65)	14 (35)	0.171 [§]	31 (77.5)	9 (22.5)	0.686 [§]	25 (62.5)	15 (37.5)	0.022 [§]
Female	10 (20)	4 (40)	6 (60)		7 (70)	3 (30)		10 (100)	0 (0)	
Size										
Mean±SD	8.14±2.19	7.10±1.98	9.70±1.45	<0.001 [‡]	7.50±1.96	10.16±1.58	<0.001 [‡]	7.34±2.05	10±1.13	<0.001 [‡]
Median (range)	8 (4-12)	8 (4-10)	10 (6-12)		8 (4-10)	10 (6-12)		8 (4-11)	10 (8-12)	
≤7 cm	14 (28)	13 (92.9)	1 (7.1)	0.003 [§]	13 (92.9)	1 (7.1)	0.140 [§]	14 (100)	0 (0)	0.004 [§]
>7 cm	36 (72)	17 (47.2)	19 (52.8)		25 (69.4)	11 (30.6)		21 (58.3)	15 (41.7)	
Type										
Conventional	38 (76)	19 (50)	19 (50)	0.029 [§]	27 (71.1)	11 (28.9)	0.251 [§]	25 (65.8)	13 (34.2)	0.291 [§]
Papillary	7 (14)	7 (100)	0 (0)		7 (100)	0 (0)		5 (71.4)	2 (28.6)	
Chromophobe	5 (10)	4 (80)	1 (20)		4 (80)	1 (20)		5 (100)	0 (0)	
Grade										
Grade 1	8 (16)	8 (100)	0 (0)	<0.001 [§]	8 (100)	0 (0)	0.004 [§]	8 (100)	0 (0)	0.002 [§]
Grade 2	14 (28)	14 (100)	0 (0)		14 (100)	0 (0)		12 (85.7)	2 (14.3)	
Grade 3	23 (46)	8 (34.8)	15 (65.2)		14 (60.9)	9 (39.1)		10 (43.5)	13 (56.5)	
Grade 4	5 (10)	0 (0)	5 (100)		2 (40)	3 (60)		5 (100)	0 (0)	
Sarcomatous										
Absent	45 (90)	30 (66.7)	15 (33.3)	0.007 [§]	38 (84.4)	7 (15.6)	<0.001 [§]	35 (77.8)	10 (22.2)	0.001 [§]
Present	5 (10)	0 (0)	5 (100)		0 (0)	5 (100)		0 (0)	5 (100)	
T										
T1	12 (24)	11 (91.7)	1 (8.3)	0.004 [§]	11 (91.7)	1 (8.3)	0.002 [§]	12 (100)	0 (0)	<0.001 [§]
T2	8 (16)	4 (50)	4 (50)		7 (87.5)	1 (12.5)		8 (100)	0 (0)	
T3	15 (30)	11 (73.3)	4 (26.7)		14 (93.3)	1 (6.7)		11 (73.3)	4 (26.7)	
T4	15 (30)	4 (26.7)	11 (73.3)		6 (40)	9 (60)		4 (26.7)	11 (73.3)	
Lymph node										
Negative	20 (40)	20 (100)	0 (0)	<0.001 [§]	20 (100)	0 (0)	0.001 [§]	18 (90)	2 (10)	0.012 [§]
Positive	30 (60)	10 (33.3)	20 (66.7)		18 (60)	12 (40)		17 (56.7)	13 (43.3)	
Stage										
Stage I	11 (22)	11 (100)	0 (0)	<0.001 [§]	11 (100)	0 (0)	<0.001 [§]	11 (100)	0 (0)	0.005 [§]
Stage II	9 (18)	9 (100)	0 (0)		9 (100)	0 (0)		7 (77.8)	2 (22.2)	
Stage III	18 (36)	10 (55.6)	8 (44.4)		18 (100)	0 (0)		13 (72.2)	5 (27.8)	
Stage IV	12 (24)	0 (0)	12 (100)		0 (0)	12 (100)		4 (33.3)	8 (66.7)	
MYSM-1										
Mean±SD	21.44±26.85	31.23±29.92	6.75±10.67	0.001 [‡]	26.63±28.55	5±9.04	0.004 [‡]	29.91±28.01	1.66±4.40	<0.001 [‡]
Median (range)	11 (0-90)	12.50 (0-90)	0 (0-25)		12 (0-90)	0 (0-20)		14 (0-90)	0 (0-13)	
Low	33 (66)	19 (57.6)	14 (42.4)	0.626 [§]	24 (72.7)	9 (27.3)	0.450 [§]	18 (54.5)	15 (45.5)	0.001 [§]
High	17 (34)	11 (64.7)	6 (35.3)		14 (82.4)	3 (17.6)		17 (100)	0 (0)	
Twist-1										
Mean±SD	28.32±34.17	14.66±24.31	48.80±37.06	0.003 [‡]	20.78±29.35	52.16±38.57	0.009 [‡]	12.57±23.05	65.06±27.01	<0.001 [‡]
Median (range)	5 (0-80)	0 (0-80)	70 (0-80)		0 (0-80)	75.50 (0-80)		0 (0-80)	75 (0-80)	
Low	32 (64)	25 (78.1)	7 (21.9)	<0.001 [§]	28 (87.5)	4 (12.5)	0.011 [§]	30 (93.8)	2 (6.3)	<0.001 [§]
High	18 (36)	5 (27.8)	13 (72.2)		10 (55.6)	8 (44.4)		5 (27.8)	13 (72.2)	

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, Swirn and Mpn domain-containing protein-1, RCC: Renal cell carcinoma

Twist-1. The results showed the decreased immunoexpression 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 3: Association of clinicopathological features, MYSM-1, and TWIST-1 with survival of patients with RCC

Characteristics	All (n=50)	LRFS		P value [§]	DMFS		P value [§]	OS		P value [§]
		Median LRFS (months)	3 years LRFS		Median DMFS (months)	3 years DMFS (%)		Median OS (months)	3 years OS (%)	
All patients		NR	60		NR	75.9		NR	67	
Age (years)										
≤60 years	20 (40)	NR	95	<0.001	NR	100	0.001	NR	94.7	<0.001
>60 years	30 (60)	20	36.7		NR	60		25	45.6	
Sex										
Male	40 (80)	NR	65	0.177	NR	77.5	0.713	NR	62	0.080
Female	10 (20)	18	40		NR	68.6		NR	100	
Size										
≤7 cm	14 (28)	NR	92.9	0.006	NR	92.9	0.088	NR	100	0.004
>7 cm	36 (72)	20	47.2		NR	69.3		NR	53.1	
Type										
Conventional	38 (76)	20	50	0.066	NR	71	0.300	NR	62.1	0.292
Papillary	7 (14)	NR	100		NR	100		NR	71.4	
Chromophobe	5 (10)	NR	80		NR	80		NR	100	
Grade										
Grade 1	8 (16)	NR	100	<0.001	NR	100	0.008	NR	100	0.002
Grade 2	14 (28)	NR	100		NR	100		NR	85.7	
Grade 3	23 (46)	20	34.8		NR	60.9		23	40.2	
Grade 4	5 (10)	15	0		16	40		NR	100	
Sarcomatous										
Absent	45 (90)	NR	66.7	<0.001	NR	84.4	<0.001	NR	74.8	<0.001
Present	5 (10)	10	0		11	0		15	0	
T										
T1	12 (24)	NR	91.7	0.001	NR	91.7	<0.001	NR	100	<0.001
T2	8 (16)	NR	50		NR	85.7		NR	100	
T3	15 (30)	NR	73.3		NR	93.3		NR	71.8	
T4	15 (30)	13	26.7		14	40		20	22.9	
Lymph node										
Negative	20 (40)	NR	100	<0.001	NR	100	0.001	NR	90	0.002
Positive	30 (60)	20	33.3		NR	59.8		24	48.6	
Stage										
Stage I	11 (22)	NR	100	<0.001	NR	100	<0.001	NR	100	<0.001
Stage II	9 (18)	NR	100		NR	100		NR	77.8	
Stage III	18 (36)	NR	55.6		NR	100		NR	67.2	
Stage IV	12 (24)	10.5	0		11.5	0		15	18.5	
MYSM-1										
Low	33 (66)	NR	57.6	0.580	NR	72.7	0.403	NR	53.8	0.004
High	17 (34)	NR	64.7		NR	81.9		NR	100	
Twist-1										
Low	33 (66)	NR	78.1	<0.001	NR	87.4	0.006	NR	92	<0.001
High	17 (34)	17	27.8		NR	55.6		20	27.8	

Categorical variables were expressed as number (percentage), NR denote not reached yet, [§]Log rank test, $P < 0.05$ is significant, LRFS: Local recurrence-free survival, DMFS: Distant metastases-free survival, OS: Overall survival, SD: Standard deviation, MYSP-1: MYb-like, Swirn and Mpn domain-containing protein-1, RCC: Renal cell carcinoma

controls the maturation of natural killer cells [25] and dendritic cells [26]. 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

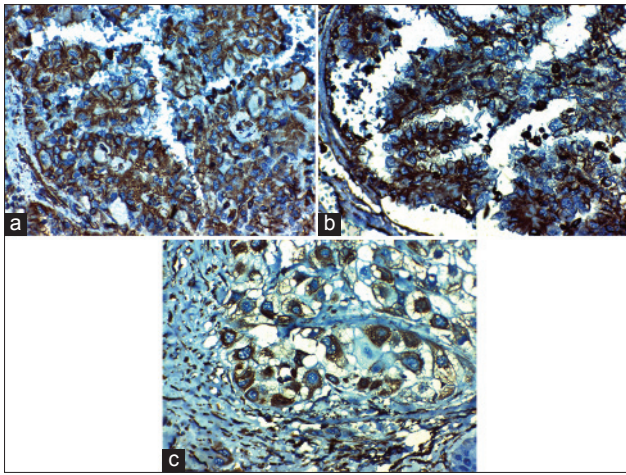


Figure 4: Immunohistochemical staining of Twist-1 in renal cell carcinoma (RCC): (a) High immunohistochemical expression in the cytoplasm of clear cell RCC Grade 3, ×400; (b) High immunohistochemical expression in the cytoplasm of papillary RCC Grade 3, ×400; (c) High immunohistochemical expression in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

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

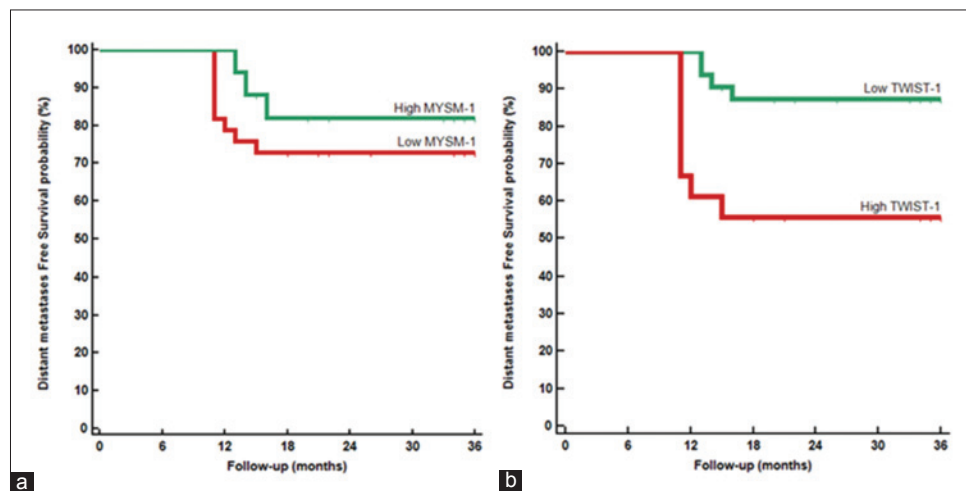


Figure 5: Kaplan-Meier plot of distant metastasis-free survival: (a) Stratified according to MYb-like, Swirn and Mpn domain-containing protein-1; (b) Stratified according to Twist-1

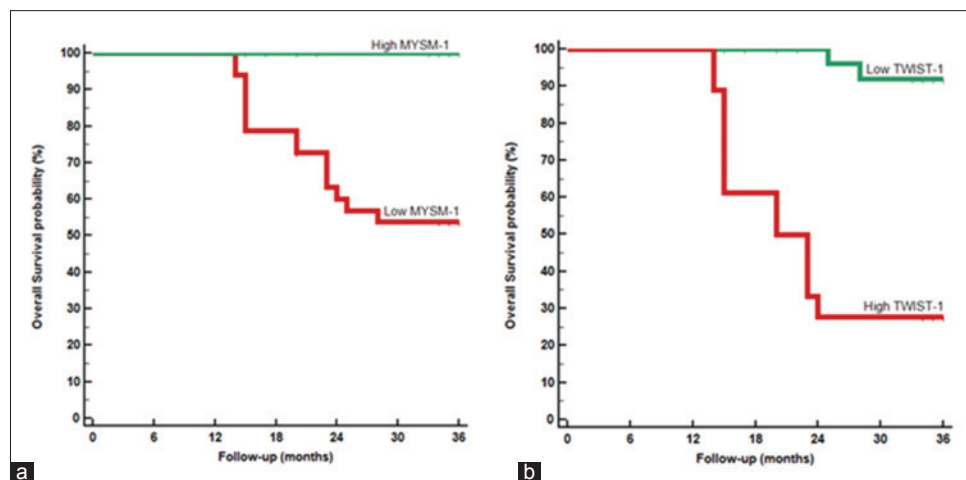


Figure 6: Kaplan-Meier plot of overall survival: (a) Stratified according to MYb-like, Swirn and Mpn domain-containing protein-1; (b) Stratified according to Twist-1

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.

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