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Combined EZH2 and p-STAT3 protein expressions in renal cell carcinoma and their significances

Ola A. Harb, Ola A. Megahed, Rham Z. Ahmed, Ahmed A. E. Obaya

ABSTRACT

Aims: It is important to detect prognostic markers for effective management of renal cell carcinoma (RCC) patients. Enhancer of zeste-homolog-2 (EZH2) and signal-transducer and activator of transcription 3 (STAT3) are a members of transcription factors which had an essential role in carcinogenesis. Our aim was to assess EZH2 and p-STAT3 as tissue protein markers expressions in RCC patients, exploring their significance in disease progression and patient survival. Methods: In our prospective cohort study, we evaluated EZH2 and p-STAT3 tissue protein expressions using immunohistochemistry in 60 paraffin blocks of RCC, followed our patients for five years, analyzed correlations between the levels of markers expressions, clinicopthological parameters, disease progression and patients survival rates. Results: EZH2 high expression was positively correlated with higher grade (p < 0.001), advanced AJCC stage (p = 0.004), lymph node metastases (p = 0.002) and cancer progression (p < 0.001). The p-STAT3 high expression was significantly positively correlated with tumor grade, AJCC stage, lymph node (p < 0.001) and distant metastases (p = 0.004 and cancer progression (p < 0.001). Survival rates were shorter in patients with high EZH and p-STAT3 expressions than cases with low expressions. Expression of both EZH and p-STAT3 was positively correlated to each other (p < 0.001). Conclusion: EZH2 and p-STAT3 are markers of poor prognosis and can be used as therapeutic targets in RCC patients.

Keywords: Renal cell carcinoma (RCC), EZH2 and p-STAT3, Immunohistochemistry

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INTRODUCTION

Globally, renal cell carcinoma (RCC) is the fifth common malignancy and it formed about 2–3% of all cancers in adults [1, 2]. Patients that are having early stages RCC is increasing, but 30% of RCC are liable to develop metastatic and incurable disease [3]. Identification of novel predictive and prognostic biomarkers for RCC is a must, which could help to discover recent beneficial therapeutic agents for patients with variable clinicopathological parameters. TNM stage,

cancer size and Fuhrman grade are the usual factors that could predict patients' outcome [4]. But it was found that RCC patients that had the same stage can have different outcomes and that have not been understood yet. The studying of epigenetic-changes could explain cancer biological criteria and gives chances for discovering novel therapeutic agents for RCC [5]. Enhancer of zeste homolog 2 (EZH2) that have been mapped on chromosome 7q-35, is a polycomb group (PcG) genes family member which have allowed the gene expression patterns transmission to daughter cells with a stability. It is as a histone methyl-transferase that can silence many different tumor suppressor genes. The EZH2 have recently found to play essential roles in RCC oncogenesis [6]. But, the exact mechanism of its action is still uncertain.

Signal-transducer and activator of transcription (STAT) proteins are large family of transcription factors and STAT3 which is one member of such family have controlled many normal cellular processes as cell proliferation, survival, differentiation, and even inflammation [7], also it can be expressed in many malignant human tumors [8]. STAT3 activation stimulated cancer cells proliferation, invasion, carcinogenesis and also maintenance of tumor promoting inflammatory microenvironment [9]. Many cancer stimulatory STAT3 functions were discovered, e.g., drugs resistance [10], role in epigenetic regulations [11] and cancer stem cells [12]. All previously discovered data showed that STAT3 formed an important novel therapeutic target for patients with RCC [13]. But its exact prognostic role in such type of cancer is still controversial.

Our objective in this study was to assess the EZH2 and p-STAT3 as tissue protein markers expressions in RCC patients, exploring their significance in disease progression and patient survival.

MATERIALS AND METHODS

In our prospective cohort study, formalin-fixed, paraffin embedded tissue samples retrieved from 60 RCC patients were processed and diagnosed in Pathology Department, Faculty of Medicine, Zagazig University. We have followed patients for five years, between December 2011 and December 2016. Biopsies have been taken by radical nephrectomy and pelvic lymphadenectomy which have been done in Urology Department, Zagazig University-hospitals. We recorded the detailed clinico-pathological data for all cases. We used the TNM classification for pathologic RCC staging [14] and Fuhrman classification for histologic-grading [15, 16]. Our study followed local-ethics-committee-guidelines. We followed patients for five years from December 2011 and December 2016 in medical-oncology, clinical-oncology and nuclear medicine departments, faculty of medicine, Zagazig University. The follow-up deadline was December 2016.

Immunohistochemical staining

We used streptavidine-biotin technique of immunohistochemical staining [17], where we incubated tissue sections with primary mouse monoclonal anti-EZH2 (1:100; cell signaling technology, MA, Danvers, USA) and primary rabbit polyclonal antibody p-STAT3 (1:50; cell signaling technology, MA, Beverly), which detected only STAT3-phosphorylated-form. We have used sections from carcinomas of the breast and heart tissues as positive controls for EZH2 and p-STAT3 respectively. For negative controls we have removed the primary antibodies then replaced them with phosphate buffered saline. All slides were read by two pathologists.

Evaluation of EZH2- immunohistochemical-staining

We calculated the intensity of stain as zero that equals negative, one equals weak, two equals moderate and three equals strong stain intensity, the extent of immune-positive cells have been calculated as one (0–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%) [18].

Evaluation of immunohistochemical expression of p-STAT3

Nuclear p-STAT3 immuno-expression was scored as:

- no expression equals
- 0 weak-positive expression when, <10% of nuclei were positive;
- moderate-positive, 10–50% was positive;
- strong-positive, more than 90% were positive [19].

We have multiplied intensity and extent of both markers expression to reach scores from 0–12, the final cut-off stain-score was four, scores less than four have been considered low expressions and a scores equal to or more than four have been considered as high expression.

STATISTICAL ANALYSIS

We expressed continuous and categorical variables by the mean±SD and median (range) and by number (percentage) respectively, checked continuous variables normality by Shapiro. Wilk test, used Pearson's chi-square test or Fisher's exact test for comparison between categorical variables percent, calculated progression free survival (PFS) as the time from diagnosis date to time at which any kind of progressions (local, or regional or distant metastasis) were detected, calculated overall survival (OS) as time from diagnosis date to date of death. Overall survival and progression free survival rates stratifications were done according to EZH2 and STAT3

expressions, estimated these rates by using the method of Kaplan Meier curve, and compared them by using two-sided log-rank test. We considered the *p*-value <0.05 as significant. We used SPSS 22.0 for windows (SPSS Inc., USA Chicago, IL) and MedCalc-windows (MedCalc-Software 13, Ostend, Belgium) in performing all statistics.

RESULTS

Patient characteristics

The clinical characteristics of our patients that were included in the study are summarized in Table 1.

We have included 60 patients with RCC which are reevaluated and diagnosed as 46 (76.7%) cases with clear cell renal cell carcinoma, 9 (15%) papillary renal cell carcinoma 5 (8.3%) chromophope renal cell carcinoma with age ranged from (40–77) years (Mean: 59.65±10.12 years). 48 (80%) patients were males and 12 (20%) were females.

Immunohistochemical results

EZH2 expression (Figure 1, Figure 2, Tables 2 and Table 3)

- High expression of EZH2 was detected in 30 out of 60 (50%) cases of RCC and was significantly positively correlated with increased age of the patient (p = 0.037), higher grade, T stage of the tumor (p < 0.001). Advanced AJCC stage (p = 0.004), presence of lymph nodes metastases (p = 0.002)
- There were no significant correlations between EZH2 expression with sex of the patient, histopathological subtypes or presence of distant metastases.

p-STAT3 expression (Figure 3, Figure 4, Table 2 and Table 3)

- High expression of p-STAT3 was detected in 31 out of 60 (51.7%) cases of RCC and was significantly positively correlated with higher grade, T stage of the tumor, advanced AJCC stage of the tumor, presence of lymph nodes (p<0.001) and distant metastases (p = 0.004).
- There were no significant correlations between p-STAT3 expression, age, sex of the patient or histopathological subtypes.

Progression and survival analysis in relation to EZH2 expression (Figure 5, Tables 4 and Table 5)

All cases with high EZH2 expression showed progression of the cancer while cases with low expression only three cases (10%) of them showed progression of the diseases (p<0.001).

- Progression free survival rate of all cases was 40 months and was 26 months only for cases with high EZH2 expression and 56 months for cases with low expressions (p<0.001).
- Five years overall survival rate of all cases was 44 months and was 30 months only for cases with high EZH2 expression and 57 months for cases with low expressions (p<0.001).

*Progression and survival analysis in relation to p-STAT3 expression (*Figure 5, Table 4 and Table 5)

- Twenty nine (93.5%) cases with high STAT3 expression showed cancer progression, while only four cases (13.8%) with low expression showed progression of the diseases (p<0.001).
- Progression free survival rate of all cases was 40 months and was 28 months only for cases with high p-STAT3 expression and 54 months for cases with low expressions (p<0.001).
- Five years overall survival rate of all cases was 44 months and was 31 months only for cases with high p-STAT3 expression and 57 months for cases with negative expressions (p<0.001).

We found a significant positive correlations between EZH2 and p-STAT3 expressions in RCC (p<0.001).

DISCUSSION

Pathologic stages have been considered the most important RCC prognostic factor. But, patients that had the same stage were still having variable outcomes. Cancer is a genetic disease which is initiated, promoted and controlled by alterations in many oncogenes and tumor suppressor genes. Epigenetic mutations which included histone-modifications and DNA-methylations were recently found to be significant as cancer promoting factors [20–22]. In genetic changes and mutations, the DNA sequence is altered by mutation which is difficult to be restored. On the contrary, epigenetic mutations could be restored by their inhibitors.

In the present study, we found that high expression of EZH2 have been significantly correlated with older age of the patient, higher tumor grade, advanced AJCC stage, presence of lymph node metastases, high incidence of disease progression and shortened overall survival and progression free survival rates.

Our results were similar to Xu et al., who found that EZH2 expression levels were increased with RCC progression, in high grade RCC, advanced TNM stage and presence of distant metastasis that suggested EZH2 as an important prognostic biomarker for RCC [23].

Our results were also similar to that of Liu et al., who found that EZH2 expression is a predictive of RCC patients' survival [24]. The prognostic values of TNM-stage in RCC are improved when combined with EZH2

Table 1: Clinicopathological features, EZH2 and p-STAT3 expressions in our patients

Characteristics	No.	%	Characteristics	No.	%
Age years			N		
Mean±SD	59.65	±10.12	No	34	56.7%
Median (Range)	64	(40–77)	N1	26	43.3%
≤ 55 years	24	40%	M		
> 55 years	36	60%	Mo	49	81.7%
Sex			M1	11	18.3%
Male	48	80%	AJCC Stage group		
Female	12	20%	Stage I	15	25%
Pathological type			Stage II	19	31.7%
Clear cell	46	76.7%	Stage III	12	20%
Papillary	9	15%	Stage IV	14	23.3%
Chromphobe	5	8.3%	EZH2		
Grade			low	30	50%
Grade I	15	25%	high	30	50%
Grade II	22	36.7%	STAT3		
Grade III	17	28.3%	Low	29	48.3%
Grade IV	6	10%	High	31	51.7%
Size			EZH2/p-STAT3		
<7 cm	17	28.3%	low/Low	25	41.7%
>7 cm	43	71.7	low/High	3	5%
T			high/Low	5	8.3%
T1	15	25%	High/High	27	45%
T2	21	35%	Follow-up months		
T3	17	28.3%	Mean±SD	37.48	±14.01
T4	7	11.7%	Median (range)	40.50	(12–58)

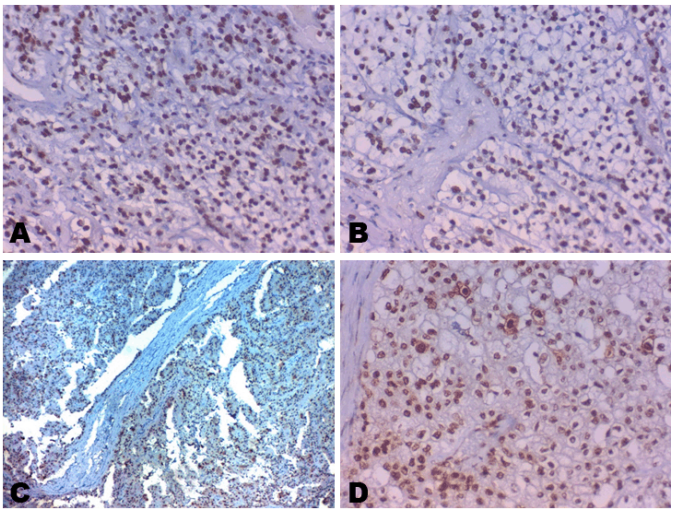

Figure 1: Immunohistochemical expression of EZH2 in renal cell carcinoma (RCC): (A) High expression in nucleus of clear cell RCC grade III x400 (B) High expression in nucleus of clear cell RCC grade II x400 (C) High expression in nucleus of papillary RCC grade II x200 (D) High expression in nucleus of chromophope RCC grade II x400.
Note: High EZH2 immunohistochemical expression in high grade and stage RCC.

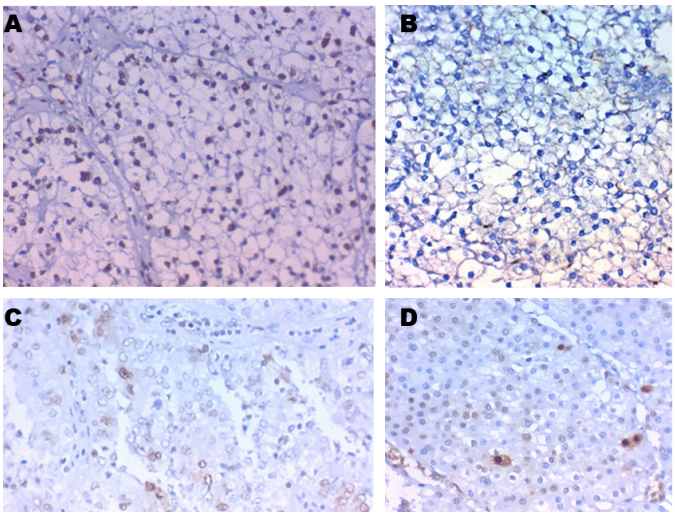

Figure 2: Immunohistochemical expression of EZH2 in renal cell carcinoma (RCC):(A) Low expression in nucleus of clear cell RCC grade II x400 (B) Low expression in nucleus of clear cell RCC grade I x400 (C) Low expression in nucleus of papillary RCC grade I x400. (D) Low expression in nucleus of chromophope RCC grade I x400.
Note: Low EZH2 immunohistochemical expression in Low grade and stage RCC

Table 2: Correlations between clinicopathological features, EZH2 and p-STAT3 expressions in our patients

Characteristics	All (N=60)		EZH2 low (N=30)		EZH2 High (N=30)		p-value	p-STAT3 Low (N=29)		p-STAT3 High (N=31)		p-value
	No.	%	No.	%	No.	%		No.	%	No.	%	
Age (years)												
Mean±SD	59.65	±10.12	57.57	±10.01	61.73	±9.97	0.037•	57.76	±10.18	61.42	±9.91	0.083•
Median (Range)	64	(40–77)	62.50	(40–77)	65.50	(40–75)		63	(40–77)	65	(40–75)	
≤ 55 years	24	40%	14	58.3%	10	41.7%	0.292‡	13	54.2%	11	45.8%	0.460‡
> 55 years	36	60%	16	44.4%	20	55.6%		16	44.4%	20	55.6%	
Sex												
Male	48	80%	24	50%	24	50%	1.000‡	24	50%	24	50%	0.605‡
Female	12	20%	6	50%	6	50%		5	41.7%	7	58.3%	
Pathological type												
Clear cell	46	76.7%	24	52.2%	22	47.8%	0.368‡	23	50%	23	50%	0.397‡
Papillary	9	15%	5	55.6%	4	44.4%		5	55.6%	4	44.4%	
Chromphobe	5	8.3%	1	20%	4	80%		1	20%	4	80%	
Grade												
Grade I	15	25%	14	93.3%	1	6.7%	<0.001§	12	80%	3	20%	<0.001§
Grade II	22	36.7%	11	50%	11	50%		11	50%	11	50%	
Grade III	17	28.3%	4	23.5%	13	76.5%		6	35.3%	11	64.7%	
Grade IV	6	10%	1	16.7%	5	83.3%		0	0%	6	100%	
Size												
<7 cm	17	28.3%	10	58.8%	7	41.2%	0.390‡	10	58.8%	7	41.2%	0.307‡
>7 cm	43	71.7	20	46.5%	23	53.5%		19	44.2%	24	55.8%	
T												
T1	15	25%	10	66.7%	5	33.3%	0.001§	10	66.7%	5	33.3%	<0.001§
T2	21	35%	14	66.7%	7	33.3%		15	71.4%	6	28.6%	
T3	17	28.3%	6	35.3%	11	64.7%		4	23.5%	13	76.5%	
T4	7	11.7%	0	0%	7	100%		0	0%	7	100%	
N												
No	34	56.7%	23	67.6%	11	32.4%	0.002‡	24	70.6%	10	29.4%	<0.001‡
N1	26	43.3%	7	26.9%	19	73.1%		5	19.2%	21	80.8%	
M												
Mo	49	81.7%	27	55.1%	22	44.9%	0.095‡	28	57.1%	21	42.9%	0.004‡
M1	11	18.3%	3	27.3%	8	72.7%		1	9.1%	10	90.9%	
AJCC Stage group												
Stage I	15	25%	10	66.7%	5	33.3%	0.004§	10	66.7%	5	33.3%	<0.001§
Stage II	19	31.7%	13	68.4%	6	31.6%		14	73.7%	5	26.3%	
Stage III	12	20%	4	33.3%	8	66.7%		4	33.3%	8	66.7%	
Stage IV	14	23.3%	3	21.4%	11	78.6%		1	7.1%	13	92.9%	
EZH2												
Negative	30	50%						26	86.7%	4	13.3%	<0.001‡
Positive	30	50%						3	10%	27	90%	
p-STAT3												
Low	29	48.3%	26	89.7%	3	10.3%	<0.001‡					
High	31	51.7%	4	12.9%	27	87.1%						

• Mann Whitney U test; ‡ chi-square test; § chi-square test for trend

Table 3: Correlations between clinicopathological features, expressions of both markers together in our patients

Characteristics	All		EZH2/p-STAT3								p-value
	(N=60)		low/Low (N=25)		low/High (N=3)		high/Low (N=5)		High/High (N=27)		
	No.	%	No.	%	No.	%	No.	%	No.	%	
Age (years)											
Mean ± SD	59.65	±10.12	57	±10.38	57	±9.53	62.80	±7.32	61.81	±10.18	0.155•
Median (Range)	64	40–77	62	40–77	58	47–66	65	50–68	66	40–75	
≤ 55 years	24	40%	12	50%	2	8.3%	1	4.2%	9	37.5%	0.409‡
> 55 years	36	60%	13	36.1%	1	2.8%	4	11.1%	18	50%	
Sex											
Male	48	80%	22	45.8%	1	2.1%	3	6.3%	22	45.8%	0.095‡
Female	12	20%	3	25%	2	16.7%	2	16.7%	5	41.7%	
Pathological type											
Clear cell	46	76.7%	19	41.3%	3	6.5%	5	10.9%	19	41.3%	0.554‡
Papillary	9	15%	5	55.6%	0	0%	0	0%	4	44.4%	
Chromphobe	5	8.3%	1	20%	0	0%	0	0%	4	80%	
Grade											
Grade I	15	25%	12	80%	1	6.7%	1	6.7%	1	6.7%	<0.001§
Grade II	22	36.7%	10	45.5%	1	4.5%	1	4.5%	10	45.5%	
Grade III	17	28.3%	3	17.6%	0	0%	3	17.6%	11	64.7%	
Grade IV	6	10%	0	0%	1	16.7%	0	0%	5	83.3%	
Size											
<7 cm	17	28.3%	9	52.9%	0	0%	2	11.8%	6	35.3%	0.433‡
>7 cm	43	71.7	16	37.2%	3	7%	3	7%	21	48.8%	
T											
T1	15	25%	9	60%	0	0%	2	13.3%	4	26.7%	0.001§
T2	21	35%	13	61.9%	0	0%	2	9.5%	6	28.6%	
T3	17	28.3%	3	17.6%	3	17.6%	1	5.9%	10	58.8%	
T4	7	11.7%	0	0%	0	0%	0	0%	7	100%	
N											
No	34	56.7%	21	61.8%	0	0%	4	11.8%	9	26.5%	<0.001‡
N1	26	43.3%	4	15.4%	3	11.5%	1	3.8%	18	69.2%	
M											
Mo	49	81.7%	24	49%	1	2%	5	10.2%	19	38.8%	0.009‡
M1	11	18.3%	1	9.1%	2	18.2%	0	0%	8	72.7%	
AJCC Stage group											
Stage I	15	25%	9	60%	0	0%	2	13.3%	4	26.7%	0.001§
Stage II	19	31.7%	12	63.2%	0	0%	2	10.5%	5	26.3%	
Stage III	12	20%	3	25%	1	8.3%	1	8.3%	7	58.3%	
Stage IV	14	23.3%	1	7.1%	2	14.3%	0	0%	11	78.6%	

• Mann Whitney U test; ‡ chi-square test; § chi-square test for trend

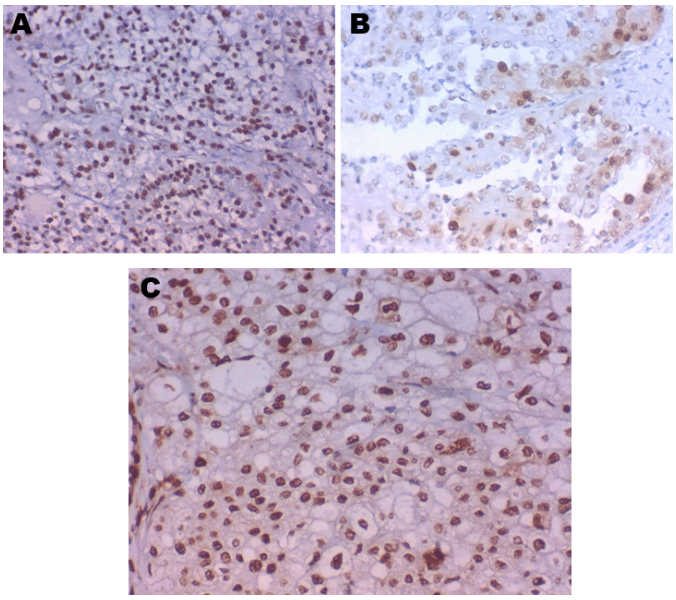

Figure 3: Immunohistochemical expression of p-STAT3 in renal cell carcinoma (RCC): (A) High expression in nucleus of clear cell RCC grade III x400 (B) High expression in nucleus of papillary RCC grade II x400 (C) High expression in nucleus of chromophope RCC grade II x400.
Note: High EZH2 immunohistochemical expression in high grade and stage RCC.

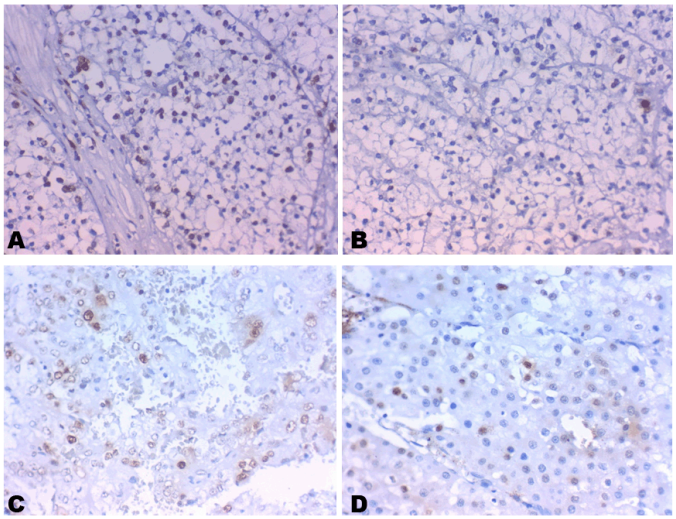

Figure 4: Immunohistochemical expression of p-STAT3 in renal cell carcinoma (RCC): (A) Low expression in nucleus of clear cell RCC grade II x400, (B) Low expression in nucleus of clear cell RCC grade I x400, (C) Low expression in nucleus of papillary RCC grade I x400, and (D) Low expression in nucleus of chromophobe RCC grade I x400.
Note: Low p-STAT3 immunohistochemical expression in Low grade and stage RCC.

Table 4: Correlation between EZH2 and p-STAT3 expressions and outcome of our patients

Characteristics	All (N=60)		EZH2 Low (N=30)		EZH2 High (N=30)		p-value	p-STAT3 Low (N=29)		p-STAT3 High (N=31)		p-value
	No.	(%)	No.	%	No.	%		No.	%	No.	%	
Progression												
Absent	27	45%	27	90%	0	0%	<0.001‡	25	86.2%	2	6.5%	<0.001‡
Present	33	55%	3	10%	30	100%		4	13.8%	29	93.5%	
Mortality												
Alive	36	60%	29	96.7%	7	23.3%	<0.001‡	28	96.6%	8	25.8%	<0.001‡
Died	24	40%	1	3.3%	23	76.7%		1	3.4%	23	74.2%	
Progression free survival												
Mean (month) (95% CI)	40 months (36–45)		56 months (53–58)		26 months (21–30)		<0.001†	54 months (51–58)		28 months (23–32)		<0.001†
12 month PFS	90%		100%		80%			100%		80.7%		
24 month PFS	75%		100%		50%			100%		51.6%		
36 month PFS	59.7%		93.1%		26.7%			89.3%		32.3%		
48 month PFS	40.9%		88.7%		0%			84.9%		6.5%		
Overall survival												
Mean (month) (95% CI)	44 months (40–49)		57 months (56–59)		30 months (25–35)		<0.001†	57 months (56–59)		31 months (26–36)		<0.001†
12 month OS	96.7%		100%		93.3%			100%		93.3%		
24 month OS	76.7%		100%		53.3%			100%		54.8%		
36 month OS	67.7%		100%		35.6%			100%		38.1%		
48 month OS	54.9%		94.4%		11.1%			94.7%		19.6%		

‡ Chi-square test; † Log rank test; 95%CI: 95% Confidence Interval;

Abbreviation: OS: Overall survival, PFS: Progression free survival

Table 5: Correlation between expressions of both markers together and outcome of our patients

Characteristics	All (N=60)		EZH2/p-STAT3 low/Low (N=25)		low/High (N=3)		Positive/Low (N=5)		High/High (N=27)		p-value
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	
Progression											
Absent	27	(45%)	24	(96%)	2	(66.7%)	1	(20%)	0	(0%)	<0.001§
Present	33	(55%)	1	(4%)	1	(33.3%)	4	(80%)	27	(100%)	
Mortality											
Alive	36	(60%)	25	(100%)	3	(100%)	3	(60%)	5	(18.5%)	<0.001§
Died	24	(40%)	0	(0%)	0	(0%)	2	(40%)	22	(81.5%)	
PFS											
Mean (month) (95%CI)	40 months (36–45)		57 months (55–59)		45 months (35–55)		38 months (32–44)		25 months (20–30)		<0.001†
12 month PFS	90%		100%		100%		100%		77.8%		
24 month PFS	75%		100%		100%		100%		44.4%		
36 month PFS	59.7%		96%		66.7%		60%		25.9%		
48 month PFS	40.9%		96%		66.7%		0%		0%		
OS											
Mean (month) (95% CI)	44 months (40–49)		58 months		51 months		45 months (43–47)		29 months (23–34)		<0.001†
12 month OS	96.7%		100%		100%		100%		92.6%		
24 month OS	76.7%		100%		100%		100%		48.2%		
36 month OS	67.7%		100%		100%		100%		29.2%		
48 month OS	54.9%		100%		100%		----		10.4%		

§ Chi-square test for trend; † Log rank test; 95%CI: 95% Confidence Interval

Abbreviation: OS: Overall survival, PFS: Progression free survival

expression levels. EZH2 expressions can be clinically applicable procedures for discrimination of patients with variable outcomes. It might lead to more individualized RCC patients' managements and improved patients suitable for systemic therapies. Wagener et al. proved that EZH2 is a novel poor prognostic bio-marker in RCC-patients [25] and it had been applied according to the REMARK criteria [26]. These included large samples size, long prospective follow-up periods of patients and predictive value description of marker.

Results similar to our study were found by other researchers that EZH2 expression have been correlated with poor clincopathological parameters and poor outcomes of patients with malignancies of different organs [27, 28].

Hinz and colleagues found different results from us and showed that EZH2 high levels of expression related to less aggressive criteria and associated with RCC patients' favorable prognosis when assessed by real-time PCR [29]. These conflicting results may be related to different detection methods of EZH2 expression levels.

Xu et al. explained how EZH2 over-expression allowed RCC progression by that it increased angiogenesis that lead to increased RCC in size, invasion and spread [23].

EZH2 gene silencing could suppress cancer cell growth and have induced cell cycle arrest and also apoptosis, but EZH2 overexpression could promote cancer cell growth and decreased apoptosis. That were in agreement with the previous results in which EZH2 knocking down would inhibit cancer cell proliferation and promote apoptosis [30, 31].

EZH2 plays an essential role in histone-methylations that could be reversed by its inhibitors. So, EZH2 represented a powerful novel bio-marker for prognosis prediction in RCC and would be included in well proved prognostic parameters to allow better expectation of patients' diagnosis, management and follow-up. EZH2 expression might have therapeutic role as it contributed to RCC growth and its silencing would have anti-proliferative and therapeutic effects, so EZH2 inhibitors could repress cancer growth [32].

STAT3 is an oncogene which played an important role in cancer progression and is activated in cancers of many organs. Special tyrosine residues phosphorylation

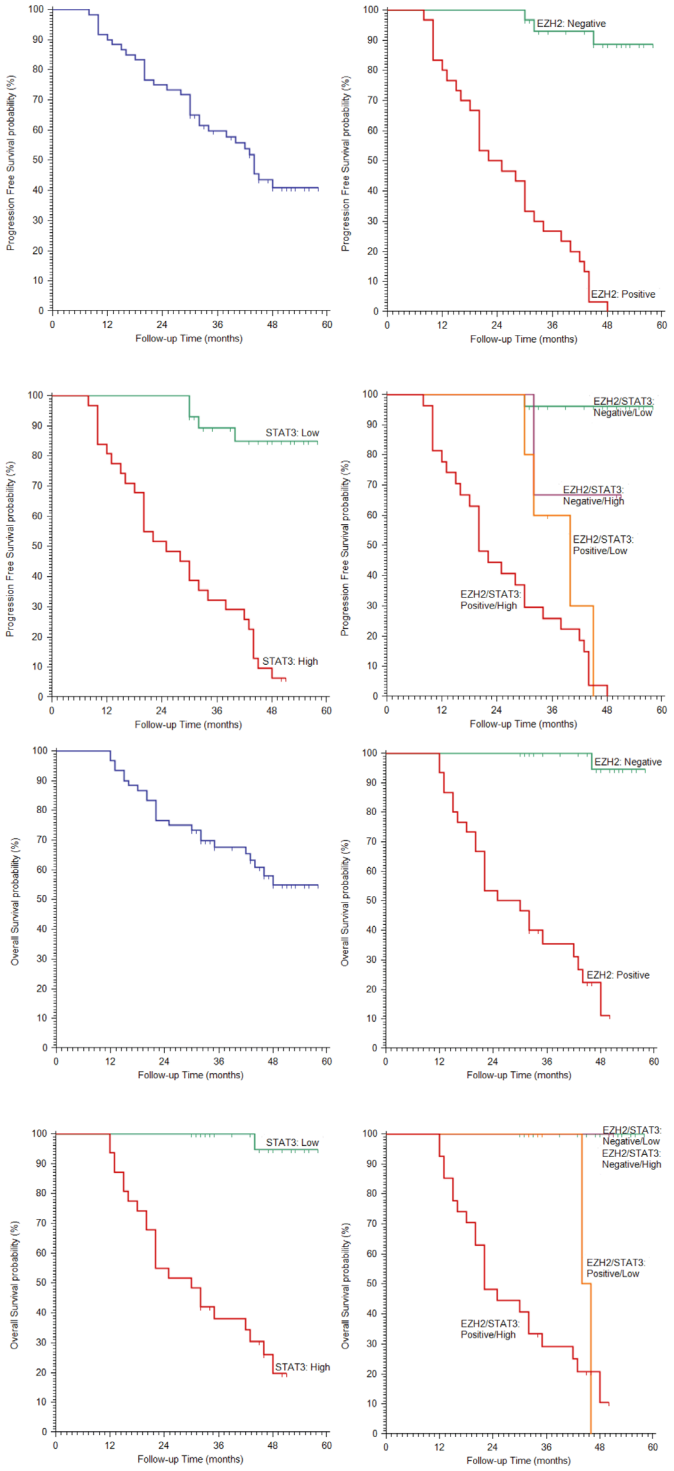

Figure 5: Kaplan–Meier plot of survival: Left panel; Progression Free Survival, Right panel; Overall Survival, (A, E) For all patients, (B, F) Stratified by EZH2, (C, G) Stratified by p-STAT3, and (D, H) Stratified by EZH2/p-STAT3.

of STAT3 is an essential step for its activation. When activated, p-STAT3 can induce many genes expression that are involved in cancer cell survival and proliferation. There are many studies investigated pstat3 expression in cancer, but the published results are conflicting especially in relation to patient prognosis, even in the same cancer type [33]. In that study we evaluated the prognostic significance of p-STAT3 expression in RCC patients, found that high expression of p-STAT3 was significantly positively correlated with, high grade, advanced AJCC stage, presence of L.N and distant metastases, cancer progression, shorter Progression free survival and overall survival rates.

These findings indicated that p-STAT3 high expression predicted a poorer clinical prognosis than in cases with low p-STAT3 expression. Similar results found by Charles Guo et al., who proved that in RCC A high level of p-STAT3 was associated with a poor prognosis [19], and Xu YH and Lu S, , have demonstrated that high p-STAT3 expression is a predictor of non-small-cell lung cancer patients poor prognosis [34], also Kun Ji, et al., findings indicated that the increased p-STAT3 expression predicted poor prognosis, high cancer grade, presence of lymph node and distant metastasis in patients having gastric cancer [33]. So, p-STAT3 has been considered a useful biomarker for expecting RCC patient prognosis. Also Li et al., found similar results esophageal carcinoma that high p-STAT3 expression have found to be correlated with advanced cancer stage, poor prognosis and shorten OS rate of patients [35].

Different results from us were found by Woo et al., that patients with p-STAT3 expression had better survival rates than those with low expression [36], and such discrepancy in the study of Woo et al, may be due to different cases number, fewer positive expression rates of p-STAT3 in cancer cases, or different clones of the antibody used in IHC [33]. Aberrant STAT3 activation had been found in a wide range of cancers [37]. p-Stat-3 is a downstream target of Janus-Kinase 2 (JAK2) [38]. JAK-STAT pathways are important oncogenic signaling cas-cades which included Janus kinase (JAK) non-receptor tyrosine kinase family and STAT transcription factors family [39], that were activated by tyrosine and serine residues phosphorylation by up-stream kinases [40]. Our results are in line with that activation of p-STAT3 is a promising RCC prognostic maker and is considered an essential novel therapeutic target for discovering novel anticancer treatments. In RCC, inhibition of STAT3 activity by protein tyrosine kinase inhibitors demonstrates a suppressive effect on cancer cells [41]. So, further studies are needed to evaluate to which degree STAT3 represented a recent prognostic biomarker and provided targeted and effective approaches in RCC management.

Many studies had found that STAT3 and EZH2 are markedly related to cancer growth, invasion, and spread [42]. It was found that IL-6/STAT3 signaling pathway played an essential role in epigenetic changes regulation during carcinogenesis, by controlling epigenetic enzymes expression like EZH2 [43].

Our findings demonstrate that there is a significant direct relation between EZH2 and STAT3 expressions

in RCC and over-expression of both markershave been found to be related to worsened clinicopathological parameters and poor patient prognosis, suggesting that a combination of STAT3 and EZH2 expression could predict disease outcome.

That was similar to Pan et al., which found similar results in gastric carcinoma, and that inhibition of STAT3 have down-regulated EZH2 at tissue protein expression levels, also this study demonstrated that STAT3 regulated EZH2 protein expression by binding to its promoter, that was in line with the results of Qiu et al., in colorectal carcinoma cells [44]. Also, STAT3 inhibition stimulated apoptosis by EZH2 suppression, through caspase-3/9 activation. Our results proved the results of previous studies which found that EZH2 and STAT3 over-expression had apoptosis antagonizing effects by activation of the Akt/Bad/Bcl-xL apoptotic pathway [43].

CONCLUSION

High levels of EZH2 and p-STAT3 expression have been found to be correlated with poor prognosis in renal cell carcinoma patients, so that the panel of both markers served as molecular prognostic biomarkers and therapeutic targets for such type of cancer. Further studies are required to explore the functional roles of these molecules as new therapeutic targets.

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Author Contributions

Ola A. Harb – Substantial contributions to conception and design, Acquisition of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Ola A. Megahed – Substantial contributions to conception and design, Acquisition of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Rham Z. Ahmed – Substantial contributions to conception and design, Acquisition of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Ahmed A.E. Obaya – Substantial contributions to conception and design, Acquisition of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

REFERENCES

1. DeSantis CE, Lin CC, Mariotto AB, et al. Cancer treatment and survivorship statistics, 2014. CA Cancer J Clin 2014 Jul–Aug;64(4):252–71.
2. An H, Xu L, Zhu Y, et al. High CXC chemokine receptor 4 expression is an adverse prognostic factor in patients with clear-cell renal cell carcinoma. Br J Cancer 2014 Apr 29;110(9):2261–8.
3. Stewart GD, O'Mahony FC, Powles T, Riddick AC, Harrison DJ, Faratian D. What can molecular pathology contribute to the management of renal cell carcinoma? Nat Rev Urol 2011 May;8(5):255–65.
4. Ficarra V, Galfano A, Mancini M, Martignoni G, Artibani W. TNM staging system for renal-cell carcinoma: Current status and future perspectives. Lancet Oncol 2007 Jun;8(6):554–8.
5. Jonasch E, Futreal PA, Davis IJ, et al. State of the science: An update on renal cell carcinoma. Mol Cancer Res 2012 Jul;10(7):859–80.
6. Niu X, Zhang T, Liao L, et al. The von Hippel-Lindau tumor suppressor protein regulates gene expression and tumor growth through histone demethylase JARID1C. Oncogene 2012 Feb 9;31(6):776–86.
7. Hirano T, Ishihara K, Hibi M. Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors. Oncogene 2000 May 15;19(21):2548–56.
8. Crescenzo R, Abate F, Lasorsa E, et al. Convergent mutations and kinase fusions lead to oncogenic STAT3 activation in anaplastic large cell lymphoma. Cancer Cell 2015 Apr 13;27(4):516–32.
9. Yu H, Lee H, Herrmann A, Buettner R, Jove R. Revisiting STAT3 signalling in cancer: New and unexpected biological functions. Nat Rev Cancer 2014 Nov;14(11):736–46.
10. Lee HJ, Zhuang G, Cao Y, Du P, Kim HJ, Settleman J. Drug resistance via feedback activation of Stat3 in oncogene-addicted cancer cells. Cancer Cell 2014 Aug 11;26(2):207–21.
11. Ernst M, Thiem S, Nguyen PM, Eissmann M, Putoczki TL. Epithelial gp130/Stat3 functions: An intestinal signaling node in health and disease. SSemin Immunol 2014 Feb;26(1):29–37.
12. Kim E, Kim M, Woo DH, et al. Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. Cancer Cell 2013 Jun 10;23(6):839–52.
13. Yan S, Li Z, Thiele CJ. Inhibition of STAT3 with orally active JAK inhibitor, AZD1480, decreases tumor

growth in neuroblastoma and pediatric sarcomas in vitro and in vivo. Oncotarget 2013 Mar;4(3):433–45.
14. Novara G, Ficarra V, Antonelli A, et al. Validation of the 2009 TNM version in a large multi-institutional cohort of patients treated for renal cell carcinoma: Are further improvements needed? Eur Urol 2010;58(4):588–95.
15. Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. Am J Surg Pathol 1982 Oct;6(7):655–63.
16. Ljungberg B, Cowan NC, Hanbury DC, et al. EAU guidelines on renal cell carcinoma: The 2010 update. Eur Urol 2010 Sep;58(3):398–406.
17. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 1981 Apr;29(4):577–80.
18. Weichert W, Röske A, Gekeler V, et al. Association of patterns of class I histone deacetylase expression with patient prognosis in gastric cancer: A retrospective analysis. Lancet Oncol 2008 Feb;9(2):139–48.
19. Guo C, Yang G, Khun K, et al. Activation of Stat3 in renal tumors. Am J Transl Res 2009 Feb 28;1(3):283–90.
20. Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. Nat Rev Genet 2006 Jan;7(1):21–33.
21. Sparmann A, van Lohuizen M. Polycomb silencers control cell fate, development and cancer. Nat Rev Cancer 2006 Nov;6(11):846–56.
22. Moss TJ, Wallrath LL. Connections between epigenetic gene silencing and human disease. Mutat Res 2007 May 1;618(1–2):163–74.
23. Xu ZQ, Zhang L, Gao BS, et al. EZH2 promotes tumor progression by increasing VEGF expression in clear cell renal cell carcinoma. Clin Transl Oncol 2015 Jan;17(1):41–9.
24. Liu L, Xu Z, Zhong L, et al. Prognostic value of EZH2 expression and activity in renal cell carcinoma: A prospective study. PLoS One 2013 Nov 27;8(11):e81484.
25. Wagener N, Macher-Goeppinger S, Pritsch M, et al. Enhancer of zeste homolog 2 (EZH2) expression is an independent prognostic factor in renal cell carcinoma. BMC Cancer 2010 Oct 4;10:524.
26. McShane LM, Altman DG, Sauerbrei W, et al. Reporting recommendations for tumor MARKer prognostic studies (REMARK). Breast Cancer Res Treat 2006 Nov;100(2):229–35.
27. Xu K, Wu ZJ, Groner AC, et al. EZH2 oncogenic activity in castration-resistant prostate cancer cells is polycomb-independent. Science 2012 Dec 14;338(6113):1465–9.
28. Jene-Sanz A, Váraljai R, Vilkova AV, et al. Expression of polycomb targets predicts breast cancer prognosis. Mol Cell Biol 2013 Oct;33(19):3951–61.
29. Hinz S, Weikert S, Magheli A, et al. Expression profile of the polycomb group protein enhancer of Zeste homologue 2 and its prognostic relevance in renal cell carcinoma. J Urol 2009 Dec;182(6):2920–5.
30. Hubaux R, Thu KL, Coe BP, MacAulay C, Lam S, Lam WL. EZH2 promotes E2F-driven SCLC tumorigenesis through modulation of apoptosis and cell-cycle regulation. J Thorac Oncol 2013 Aug;8(8):1102–6.
31. Nakagawa S, Okabe H, Sakamoto Y, et al. Enhancer of zeste homolog 2 (EZH2) promotes progression of cholangiocarcinoma cells by regulating cell cycle and apoptosis. Ann Surg Oncol 2013 Dec;20 Suppl 3:S667–75.
32. Wagener N, Holland D, Bulkescher J, et al. The enhancer of zeste homolog 2 gene contributes to cell proliferation and apoptosis resistance in renal cell carcinoma cells. Int J Cancer 2008 Oct 1;123(7):1545–50.
33. Ji K, Zhang L, Zhang M, Chu Q, Li X, Wang W. Prognostic value and clinicopathological significance of p-Stat3 among gastric carcinoma patients: A systematic review and meta-analysis. Medicine (Baltimore) 2016 Feb;95(5):e2641.
34. Xu YH, Lu S. A meta-analysis of STAT3 and phospho-STAT3 expression and survival of patients with non-small-cell lung cancer. Eur J Surg Oncol 2014 Mar;40(3):311–7.
35. Li H, Xiao W, Ma J, et al. Dual high expression of STAT3 and cyclinD1 is associated with poor prognosis after curative resection of esophageal squamous cell carcinoma. Int J Clin Exp Pathol 2014 Oct 15;7(11):7989–98.
36. Woo S, Lee BL, Yoon J, et al. Constitutive activation of signal transducers and activators of transcription 3 correlates with better prognosis, cell proliferation and hypoxia-inducible factor-1a in human gastric cancer. Pathobiology 2011;78(6):295–301.
37. Proietti CJ, Rosemblit C, Beguelin W, et al. Activation of Stat3 by heregulin/ErbB-2 through the co-option of progesterone receptor signaling drives breast cancer growth. Mol Cell Biol 2009 Mar;29(5):1249–65.
38. Imada K, Leonard WJ. The Jak-STAT pathway. Mol Immunol 2000 Jan–Feb;37(1-2):1–11.
39. Darnell JE Jr. STATs and gene regulation. Science 1997 Sep 12;277(5332):1630–5.
40. Ihle JN. STATs and MAPKs: Obligate or opportunistic partners in signaling. Bioessays 1996 Feb;18(2):95–8.
41. Horiguchi A, Oya M, Marumo K, Murai M. STAT3, but not ERKs, mediates the IL-6-induced proliferation of renal cancer cells, ACHN and 769P. Kidney Int 2002 Mar;61(3):926–38.
42. Pan YM, Wang CG, Zhu M, et al. STAT3 signaling drives EZH2 transcriptional activation and mediates poor prognosis in gastric cancer. Mol Cancer 2016 Dec 9;15(1):79.
43. Ahn KS, Sethi G, Sung B, Goel A, Ralhan R, Aggarwal BB. Guggulsterone, a farnesoid X receptor antagonist, inhibits constitutive and inducible STAT3 activation through induction of a protein tyrosine phosphatase SHP-1. Cancer Res 2008 Jun 1;68(11):4406–15.
44. Qiu MZ, Wang ZQ, Zhang DS, et al. Comparison of 6th and 7th AJCC TNM staging classification for carcinoma of the stomach in China. Ann Surg Oncol 2011 Jul;18(7):1869–76.

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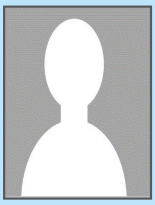

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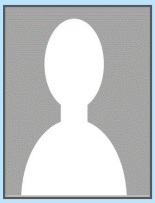

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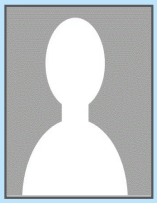

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