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Clinical, pathological and prognostic implications of USP22, SIRT1 and E-cadherin expression in papillary thyroid cancer (PTC) and adjacent non-neoplastic tissue

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Abstract

Introduction: Ubiquitin-specific peptidase 22 (USP22) is described as a stem cell (CSC) marker which is involved in many biological processes, including cancer development, cellular growth and differentiation. Sirtuin 1 (SIRT1) controls a set of biologic processes that range from metabolic homeostasis to cancer. E-cadherin is a calcium-dependent intercellular adhesion molecule. Clinically, USP22, SIRT1 and E-cadherin have been studied to predict prognosis of a variety of cancers but the detailed roles of their expression in papillary thyroid cancer (PTC) and their relation to cancer invasion, metastases and recurrence are still not fully explained.

Aim of the study: To evaluate the expression of USP22, SIRT1 & E-cadherin in PTC tissues and adjacent non-neoplastic thyroid tissue and to correlate their expression with histopathology, clinical, pathological and prognostic parameters of PTC patients.

Methods: We have assessed USP22, SIRT1 & E-cadherin expression using immunohistochemistry in 40 cases with PTC in both malignant tissue and adjacent non-neoplastic tissue, analyzed the relationships between their levels of expression, clinic-pathological parameters, prognosis and survival of patients.

Results: High protein expression levels of both USP22, SIRT1 in addition to low E-cadherin expression in PTC were associated with larger tumors, extra-thyroidal extension, vascular invasion, lymphatic spread ($p < 0.001$), existence of distant metastases ($p = 0.005$ & 0.012 respectively), higher stage of the disease ($p = 0.012$ & 0.042 respectively) and worse five-years overall survival rates ($p < 0.001$).

Conclusion: Patients having advanced PTC with unfavorable prognosis had high levels of both USP22, SIRT1 in addition to low E-cadherin expression.

Keywords: Papillary-thyroid-carcinoma, USP22, SIRT1, E-cadherin, Immunohistochemistry, Prognosis

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Introduction

Thyroid cancer constitutes 90% of endocrine malignancies and 2.5% of all malignancies, with female predominance; includes well-differentiated thyroid carcinomas (DTC) (papillary and follicular) that represent the most frequent subtypes (85–90% of total thyroid cancers). Papillary thyroid cancer (PTC) is the most widely recognized well differentiated thyroid cancer (DTC). PTC has a good prognosis with a 5-year survival rate of 98.1% for all combined subtypes and stages (Faugeras et al. 2018). However, some cases of PTC show early recurrence, invasion, spread to multiple lymph node and distant metastasis (Wang et al. 2013). DTCs can be cured with surgery and radioiodine but certain subtypes of DTCs behave like poorly differentiated thyroid cancers (PDTCs) and are resistant to both radioiodine therapy and chemotherapy (Malaguarnera et al. 2018). Consequently, it is critical to identify those subtypes of PTC with higher risk for invasion and metastasis and to detect novel targeted therapy for them. Ubiquitin-specific peptidase 22 (USP22) is a discovered novel deubiquitinase (DUB) family member. USP22 is a stem cell (CSC) marker which is found to be involved in plethora of biological processes, as cancer development, cell cycle regulation and transcriptional activation (Melo-Cardenas et al. 2016). USP22 is moderately expressed in various normal tissues, like heart and skeletal muscle, while it is weakly expressed in liver and lung tissues (Melo-Cardenas et al. 2016; Wang et al. 2017). Sirtuin 1 (SIRT1) is a member of sirtuin family of nicotinamide adenine dinucleotide (NAD+)- dependent deacetylases which controls a set of biologic processes that range from metabolic homeostasis and aging to cancer (Armour et al. 2013; Shin et al. 2014). It was found that there was association between USP22 and SIRT1 in cancer progression as USP22 deubiquitinates and stabilizes the expression of SIRT1 in a variety of cancer types and expression of both markers might be primarily incriminated in chemotherapy resistance in cancer cells (Ao et al. 2014). E-cadherin is an intercellular calcium-dependent adhesion molecule which impairment of its expression may be consistent with cancer cell invasion and metastasis (Naito et al. 2001).

Aim of the study

To evaluate the expression of USP22, SIRT1 & E-cadherin in PTC tissues and adjacent non-neoplastic thyroid tissue and to correlate their expression with histopathology, clinical, pathological and prognostic parameters of PTC patients.

Patients and methods

This is a prospective cohort study where; one hundred patients which were diagnosed with thyroid gland swelling were admitted to general-surgery department, faculty of-medicine, Zagazig-university hospitals, total thyroidectomy and diagnostic frozen section was done to all patients intra-operatively and the forty cases, that were found to have PTC were subjected to block-neck-dissection and were sent to pathology-department faculty of medicine Zagazig university to complete the diagnosis and subsequent research. Routine H & E staining was done then immunohistochemistry was done to evaluate tissue protein expression of USP22, SIRT1, and E-cadherin in both malignant tissue and adjacent non-neoplastic tissue of the same patients. Patients were followed-up for 5 years, from October 2013 to October 2018, in both departments of clinical oncology& nuclear medicine and medical oncology, faculty of medicine, Zagazig University. Full patient pathological and clinical data were found in patients records. We used T-N-M staging-system modified by the AJCC *Cancer Staging; the seventh* edition for surgical staging of PTC (Edge et al. 2010) analyzed the relationships between expression of USP22, SIRT1, E-cadherin in both malignant tissue and adjacent non-neoplastic tissue, clinic-pathological parameters (tumor size, grade, stage and histopathological subtype), prognosis and survival of patients.

The histopathologic subtypes of PTCs in the study are PTC conventional and PTC follicular variant other variants are not evaluated in relation to the included biomarkers due to their rarity Table 1.

Table 1 The clinicopathological features of all the studied patients

	No.	%
Age Group		
< 40y	18	45.0%
≥40y	22	55.0%
Sex		
M	11	26.8%
F	29	74.3%
Histopathological subtype		
PTC conventional	36	45.0%
PTC follicular variant	4	5.0%
Adjacent non-neoplastic Thyroid tissue	40	50.0%
Group		
PTC	40	50.0%
Adjacent non-neoplastic Thyroid tissue	40	50.0%

Immunohistochemical staining

The technique of streptavidine-biotin was used (Hsu et al. 1981). Paraffin-embedded sections (4 mm) were deparaffinized in xylene and rehydrated in a graded series of ethanol solutions. The sections were subsequently submerged in EDTA (pH 8) and autoclave at 121uC for 5 min to retrieve the antigenicity. Endogenous peroxidase was antagonized and blocked with 3% H2O2 for 15 min. After washing with phosphate buffered saline (PBS), the sections were incubated overnight with primary goat monoclonal anti-USP22 antibody (ab71732; dilution 1:50), primary rabbit monoclonal anti- SIRT1 antibody (ab32441; dilution 1:150) (Abcam, Cambridge, MA, USA, and primary mouse monoclonal anti-E-Cadherin antibody (Beijing Zhong Shan Biotechnology Co.Ltd., Beijing, China; dilution 1:50), sections from breast carcinoma, colon cancer and normal colonic mucosa are used as positive control for USP22, Sirt1 and E-cadherin respectively while the negative controls were done by using the non-immune serum instead of primary antibodies. The results of staining were evaluated by two pathologists who were blinded to the clinical data.

Evaluation of immunohistochemical expression of USP22, SIRT1 and E-cadherin

We have considered positive cytoplasmic, nuclear and membranous expression as positive for USP22, SIRT1 and E-cadherin respectively. We have assessed the stain extent (percentage of tumor cells stained positive) and gave them scores as follows: 0, 0%; 1, 1 to 30%; 2, 31 to 60%; and 3, > 60%. We have assessed the stain intensity and gave them scores as follows: 0, negative stain; 1, weak stain; 2, moderate stain; and 3, strong stain. We have obtained the final staining score of combination of the extent (E) and intensity (I) of stain by multiplying values of both E × I called EI and the results varied from 0 to 9 for each spot. According to the final scores and for easy statistical analysis tumor tissues were divided into two types: low-level USP22 group (with a score ≤ 3) and high-level USP22 group (with a score > 3) (Zhao et al. 2016a; Cao et al. 2014; Ma et al. 2015).

Statistical analysis

The collected results were statistically analyzed using the program of Statistical Package for Social Science (SPSS) version 24.

Normal distribution of data was assessed using the Shapiro Walk test. Qualitative data were represented as frequencies and percentages. Chi square test (χ2) and Fisher exact were used to calculate differences between qualitative variables. Spearman's Rho Rank correlation test was sused for correlating variables. The (+) sign indicates a direct correlation & (−) sign indicates an inverse correlation. *P*-value ≤0.05 represents significant differences, $p < 0.001$ represents highly significant differences, while, $P > 0.05$ represents non-significant differences.

Survival analysis

Kaplan and Meier method was used to estimate overall (OS) and progression free survival (PFS) and log rank test was used to compare survival curves. Cox proportional hazards regression models was summarized with hazard ratios and 95% confidence intervals (CIs) for multivariate analysis.

Results

Patients criteria

Summary of patients' clinical data is presented in Tables 1 and 2.

Forty patients were included in the study; 11 (24.8%) males and 29 (74.2%) females with age ranged from (21–53) years; (Mean: 38.9 ± 10.4 years), 36 (90%) cases were diagnosed conventional PTC and 4 (10%) cases were follicular variant of PTC.

Expression of USP22, SIRT1 and E-cadherin were evaluated in sections from both malignant tissues and adjacent non-neoplastic tissues of all the studied cases. Patients were followed for 58 months with range (24–60) months.

Evaluation of USP22 expression in all the studied samples:

- High nuclear expression of USP22 was detected in 25 out of 40 (62.5%) cases of PTC and in 4 out of 40 cases of adjacent non-neoplastic thyroid tissue ($p < 0.001$). High expression of USP22 was found more in malignant thyroid tissue than in the adjacent non-neoplastic thyroid. The sensitivity and specificity of USP22 in detection of PTC was 62.5 and 90% respectively. Figure 1
- High expressions of USP22 in PTC was associated with larger size of the cancer ($p = 0.014$), multifocality ($p = 0.004$), capsular invasion ($p = 0.002$), extra-thyroidal extension ($p = 0.007$), vascular invasion ($p = 0.038$), L.N spread ($p = 0.001$), presence of distant metastases ($p = 0.032$) and advanced stage of the tumor ($p < 0.001$). Figure 1
- High expression of USP22 in PTC was associated with higher incidence of recurrence of the disease after successful therapy (p = 0.001), worse 5-year disease free survival (DFS) rate of patients ($p = 0.003$). Figure 4; Tables 4 and 5

Evaluation of SIRT1 expression in all the studied samples:

Table 2 USP22, SIRT1 and E-cadherin expression in all the studied samples (No 80)

	PTC $N = 40$	Adjacent non-neoplastic Thyroid tissue $N = 40$		Total $N = 80$		P
	No.	%	No.	%	No.	%	
USP 22							
Low	15	37.5%	36	90.0%	51	63.8%	< 0.001
High	25	62.5%	4	10.0%	29	36.3%	
SIRT1							
Low	21	52.5%	32	80.0%	53	66.3%	0.009
High	19	47.5%	8	20.0%	27	33.8%	
E-cadherin							
Low	25	62.5%	11	27.5%	36	45.0%	0.002
High	15	37.5%	29	72.5%	44	55.0%	

- High cytoplasmic expression of SIRT1 was detected in 19 out of 40 (44.4%) cases of PTC and in 8 out of 40 cases of adjacent non-neoplastic thyroid tissue ($p = 0.009$). High expression of SIRT1 was found more in malignant thyroid tissue than in the adjacent non-neoplastic thyroid. The sensitivity and specificity of SIRT1 in detection of PTC was 47.5 and 80% respectively.
- High expressions of SIRT1 in PTC was associated with larger size of the cancer ($p = 0.011$), multifocality ($p = 0.002$), capsular invasion ($p = 0.012$), extra-thyroidal extension (p = 0.01), vascular invasion ($p = 0.015$), L.N spread ($p < 0.001$), presence of distant metastases ($p = 0.042$) and advanced stage of the tumor (p < 0.001). Figure 2; Tables 2 and 3
- High expression of SIRT1 in PTC was associated with higher incidence of recurrence of the disease after successful therapy ($p = 0.006$), worse 5-year disease free survival (DFS) rate of patients (p = 0.01). Figure 4; Tables 4 and 5

Evaluation of E-cadherin expression in all the studied samples:

- High membranous expression of E-cadherin was detected in 15 out of 40 (37.5%) cases of PTC and in

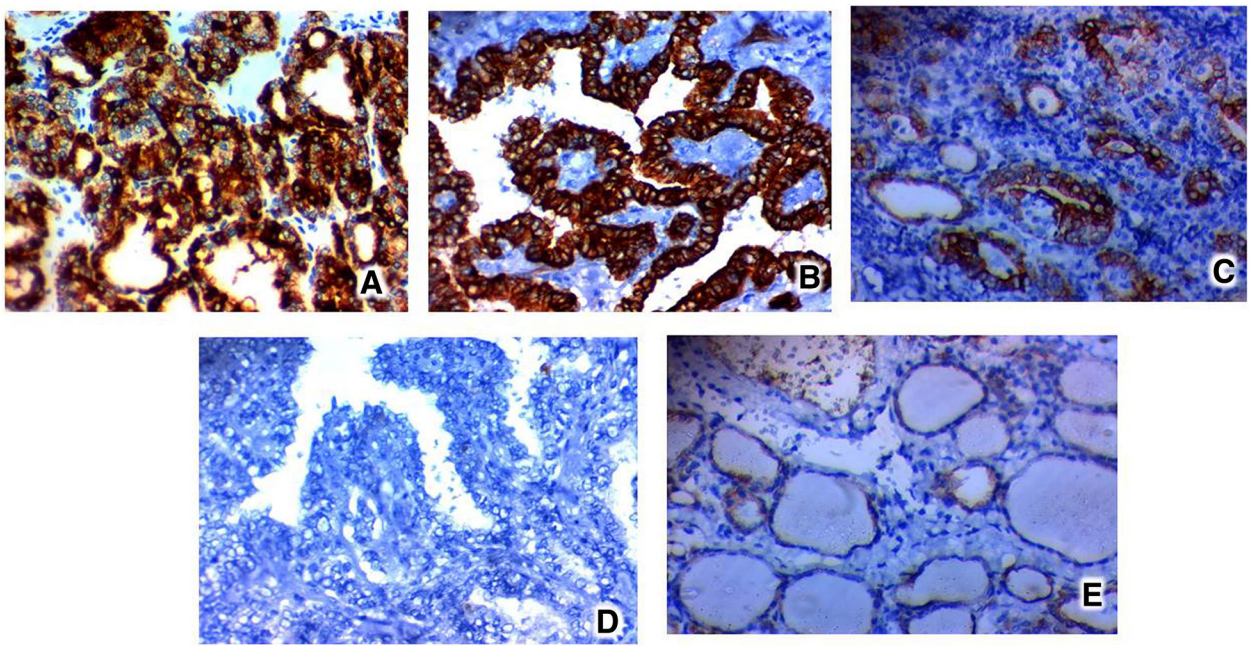

Fig. 1 Immunohistochemical expression of USP22 in papillary thyroid carcinoma (PTC) **a** High expression in the cytoplasm of poorly differentiated PTC stage IV × 400, **b** High expression in the cytoplasm of moderately differentiated PTC stage III × 400, **c** low expression in the cytoplasm of well differentiated follicular variant of PTC stage II× 400, **d** negative expression in the cytoplasm of well differentiated variant of PTC stage I× 400 **e** negative expression in adjacent non-neoplastic thyroid follicles × 400

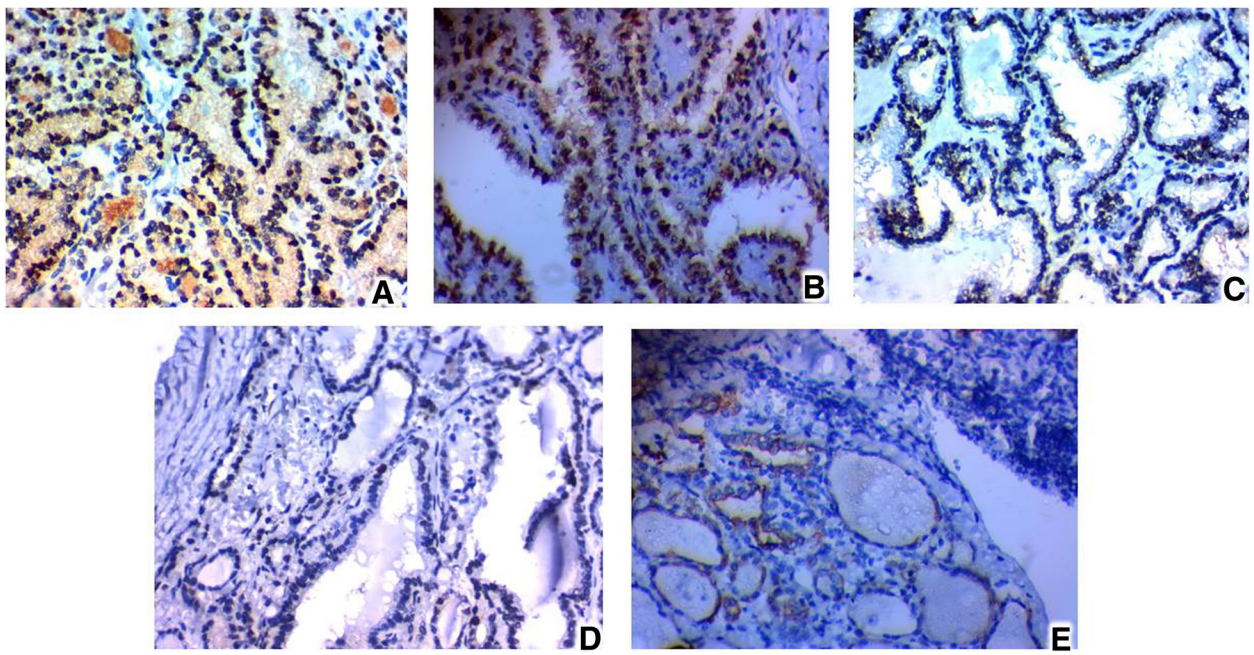

Fig. 2 Immunohistochemical expression of SIRT1 in in papillary thyroid carcinoma (PTC) **a** High expression in the nucleus of poorly differentiated PTC stage IV× 400, **b** High expression in the nucleus of moderately differentiated PTC stage III× 400, **c** low expression in the nucleus of well differentiated PTC stage IIx400, **d** low expression in the nucleus of well differentiated PTC stage Ix400 **e** negative expression in adjacent non-neoplastic thyroid follicles x400

29 out of 40 cases of adjacent non-neoplastic thyroid tissue (p = 0.002). High expression of E-cadherin was found more in the adjacent non-neoplastic thyroid than in malignant thyroid tissue. The sensitivity and specificity of E-cadherin in detection of PTC was 37.5 and 27.5% respectively.

- Low expression of E-cadherin in PTC was associated with larger size of the cancer ($p = 0.014$), multifocality ($p = 0.026$), capsular invasion ($p = 0.047$), extra-thyroidal extension ($p = 0.045$), vascular invasion ($p = 0.007$), L.N spread ($p = 0.008$), presence of distant metastases (p = 0.014), stage of the tumor ($p = 0.044$). Figure 3; Tables 2 and 3
- High expression of E-cadherin in PTC was associated with a lower incidence of recurrence of the disease after successful therapy (p = 0.02), favorable 5-year disease free survival (DFS) rate of patients ($p = 0.03$). Figure 4; Tables 4 and 5
- Univariate Cox regression analyses of different prognostic factors revealed that USP22, SIRT1 and E-cadherin are important predictive factors for disease-free survival (DFS) in PTC patients. Table 6
- Significant positive correlations was noted between USP22 and SIRT1 expression (r = + 0.530), an inverse correlation between USP22 and E-cadherin (r = – 0.253) and an inverse correlation between SIRT1 and E-cadherin expression (r = – 0.737) in all the studied samples ($p < 0.001$).
- No significant correlations were found between markers expressions and age, sex of our patients, histopathological type of the PTC, site of distant metastases, extent of performed surgery, dose of radioactive iodine used for the patients, response to therapy or 5 year overall survival rate of patients. Fig. 5; Tables 2, 3, 4 and 5

Discussion

Some PTC patients have died as a result of scanty discriminate diagnostic biomarkers and defective management plan. Understanding the novel process of PTC carcinogenesis and recognizing new targets which could prohibit PTC progression are the main focus of research in the refinement of PTC treatment (Zhao et al. 2016b). A thorough studying of the molecular mechanisms implicated in PTC initiation and progression is crucial to enhance present strategies and point out new molecular-targeted therapies.

The scope of cancer epigenetics is currently the concern of significant progress in cancer research. Histone modifications play a role in tumorigenesis as well as cancer diagnosis, treatment and patients' follow-up. Ubiquitination which is one of the histone modifications in the tail region of histones is involved in transcription and DNA damage repair. Lately, a novel deubiquitinating enzyme; USP22, a presumed cancer stem cell marker, has been recognized to have ubiquitin hydrolase activity

Table 3 Correlations between clinicopathological features, USP22, SIRT1 and E-cadherin expression in PTC patients (N = 40)

	USP 22				p	SIRT1				p	E-cadherin				p
	Low		High			Low		High			Low		High		
	N = 15		N = 25			N = 21		N = 19			N = 25		N = 15		
Age.Group															
< 40y	6	40.0%	14	56.0%	0.327	7	33.3%	13	68.4%	0.027	15	60.0%	5	33.3%	0.102
= > 40y	9	60.0%	11	44.0%		14	66.7%	6	31.6%		10	40.0%	10	66.7%	
Sex															
M	5	33.3%	6	24.0%	0.522	6	28.6%	5	26.3%	0.873	7	28.0%	4	26.7%	0.927
F	10	66.7%	19	76.0%		15	71.4%	14	73.7%		18	72.0%	11	73.3%	
Histo-pathological subtype															
PTC conv	14	93.3%	22	88.0%	0.586	20	95.2%	16	84.2%	0.246	22	88.0%	14	93.3%	0.586
PTC FV	1	6.7%	3	12.0%		1	4.8%	3	15.8%		3	12.0%	1	6.7%	
Stage															
I	6	40.0%	1	4.0%	< 0.001	7	33.3%	0	0.0%	< 0.001	3	12.0%	4	26.7%	0.044
II	7	46.7%	4	16.0%		9	42.9%	2	10.5%		4	16.0%	7	46.7%	
III	1	6.7%	13	52.0%		2	9.5%	12	63.2%		12	48.0%	2	13.3%	
IV	1	6.7%	7	28.0%		3	14.3%	5	26.3%		6	24.0%	2	13.3%	
Tumor size															
<4 cm	13	86.7%	12	48.0%	0.014	17	81.0%	8	42.1%	0.011	12	48.0%	13	86.7%	0.014
= > 4 cm	2	13.3%	13	52.0%		4	19.0%	11	57.9%		13	52.0%	2	13.3%	
Surgery (thyroidectomy)															
Total	6	40.0%	14	56.0%	0.203	10	47.6%	10	52.6%	0.121	14	56.0%	6	40.0%	0.094
Subtotal	5	33.3%	2	8.0%		6	28.6%	1	5.3%		2	8.0%	5	33.3%	
Total + BND	4	26.7%	8	32.0%		4	19.0%	8	42.1%		9	36.0%	3	20.0%	
Lobectomy	0	0.0%	1	4.0%		1	4.8%	0	0.0%		0	0.0%	1	6.7%	
Multifocality	2	13.3%	15	60.0%	0.004	4	19.0%	13	68.4%	0.002	14	56.0%	3	20.0%	0.026
LN involvement	4	26.7%	20	80.0%	0.001	6	28.6%	18	94.7%	< 0.001	19	76.0%	5	33.3%	0.008
Vascular invasion	1	6.7%	9	36.0%	0.038	3	14.3%	7	36.8%	0.015	8	32.0%	2	13.3%	0.007
Capsular invasion	1	6.7%	14	56.0%	0.002	4	19.0%	11	57.9%	0.011	12	48.0%	3	20.0%	0.047
Extrathyroid extension	1	6.7%	12	48.0%	0.007	3	14.3%	10	52.6%	0.01	11	44.0%	2	13.3%	0.045
Distant metastasis	1	6.7%	7	28.0%	0.032	3	14.3%	5	26.3%	0.042	6	24.0%	2	13.3%	0.014
Lung Mets	1	6.7%	3	12.0%	0.559	2	9.5%	2	10.5%	0.871	3	12.0%	1	6.7%	0.559
Bone Mets	1	6.7%	1	4.0%	0.731	1	4.8%	1	5.3%	0.911	2	8.0%	0	0.0%	0.251
Brain Mets	0	0.0%	1	4.0%	0.423	0	0.0%	1	5.3%	0.274	1	4.0%	0	0.0%	0.423
Mediastinal Mets	1	6.7%	0	0.0%	0.2	0	0.0%	1	5.3%	0.274	1	4.0%	0	0.0%	0.423

(Wang et al. 2013). Expanding evidence has demonstrated that the USP family members are expressed in tumors, donating that, they are possible therapeutic targets in cancer therapy (Ma et al. 2015). In spite of the fact that USP22 was reported as a marker of "death-from-cancer" and was unusually elevated in many tumors (Ma et al. 2015), the molecular mechanisms implying the increased expression of USP22 in the course of PTC progression and poor prognosis are yet elusive. The epithelial-to-mesenchymal transition (EMT) is a mechanism in which epithelial cells are deprived of cell-adhesive properties, characterized by suppression of E-cadherin expression, raised mesenchymal gene expression and cellular mobility. It is essential for distinguishable biological mechanisms: organ fibrosis, embryogenesis and cancer metastases (Kalluri and Weinberg 2009). USP22 was reported to have a role in epithelial-mesenchymal transition (EMT) (Zhao et al. 2016a). Implying the presence of a potential relation between SIRT1 and EMT; the role of SIRT1 in cancer progression has been shown in various studies to be cell type dependent and complex (Simic et al. 2013).

Table 4 Correlations between USP22, SIRT1 and E-cadherin expression and outcome of PTC patients (N = 40)

	USP 22				p	SIRT1				p	E-cadherin				p
	Low		High			Low		High			Low		High		
	N = 15		N = 25			N = 21		N = 19			N = 25		N = 15		
Response															
PD	0	0.0%	0	0.0%	0.378	0	0.0%	0	0.0%	0.564	0	0.0%	0	0.0%	0.692
SD	0	0.0%	1	4.0%		0	0.0%	1	5.3%		1	4.0%	0	0.0%	
PR	0	0.0%	2	8.0%		1	4.8%	1	5.3%		1	4.0%	1	6.7%	
CR	15	100.0%	22	88.0%		20	95.2%	17	89.5%		23	92.0%	14	93.3%	
Overall Response															
NoCR	0	0.0%	3	12.0%	0.163	1	4.8%	2	10.5%	0.489	2	8.0%	1	6.7%	0.877
CR	15	100.0%	22	88.0%		20	95.2%	17	89.5%		23	92.0%	14	93.3%	
Recurrence															
No	13	86.7%	7	28.0%	0.001	15	71.4%	5	26.3%	0.006	9	36.0%	11	73.3%	0.02
Yes	2	13.3%	15	60.0%		5	23.8%	12	63.2%		14	56.0%	3	20.0%	
Outcome															
Censored	13	86.7%	20	80.0%	0.591	19	90.5%	14	73.7%	0.163	19	76.0%	14	93.3%	0.162
Died	2	13.3%	5	20.0%		2	9.5%	5	26.3%		6	24.0%	1	6.7%	

Clinically increased expression of USP22 & SIRT-1 in addition to E-cadherin down regulation has been studied to predict progression, recurrence, metastasis, and poor survival of a variety of cancers after diagnosis (He et al. 2015; Li et al. 2017) but the detailed prognostic roles of their expression in PTC and their relation to cancer invasion, metastases and recurrence after consecutive treatment are still not fully explained.

In this study, we demonstrated that USP22 expression is found to be higher in PTC tissues than those in surrounding non-neoplastic tissues, indicating up-regulation in malignancy. Additionally we found that, high USP22 expression is associated with larger tumor size, presence of extra-capsular invasion, multi-focality, lymph node and distant metastasis. This strengthens the hypothesis that USP22 might act as an oncogene in

Table 5 Correlations between USP22, SIRT1 and E-cadherin expression and PTC patients' survival analysis

		Survival Time (Months)				Survival rate (%)	P
		(95% CI)	Means ±SE		Median ± SE (95% CI)		
5-year Overall Survival							
USP 22	Low	56.9 ± 2.4	(52.3–61.56)	NR		86.7	0.571
	High	56.1 ± 1.7	(52.68–59.49)	NR		79.2	
SIRT1	Low	57.8 ± 1.7	(54.45–61.17)	NR		90.5	0.149
	High	54.8 ± 2.2	(50.4–59.16)	NR		72.2	
E-cadherin	Low	54.6 ± 2.2	(50.34–58.83)	NR		75	0.142
	High	59.3 ± 0.6	(58.07–60.6)	NR		93.3	
Overall		56.4 ± 1.4	(53.66–59.16)	NR		82.1	
5-year Disease-Free Survival							
USP 22	Low	55.9 ± 2.9	(50.16–61.57)	NR		86.7	0.003
	High	51 ± 1.8	(47.4–54.51)	50 ± 1.7	(46.72–53.28)	31.8	
SIRT 1	Low	55.3 ± 2.3	(50.85–59.75)	NR		75	0.01
	High	50.2 ± 2.2	(45.85–54.5)	52 ± 5.4	(41.51–62.49)	29.4	
E-cadherin	Low	50.5 ± 2.3	(45.95–55.01)	53 ± 3	(47.13–58.87)	39.1	0.03
	High	57 ± 1.6	(53.81–60.19)	NR		78.6	
Overall		52.9 ± 1.6	(49.72–56.17)	NR		54.1	

NR Not reached

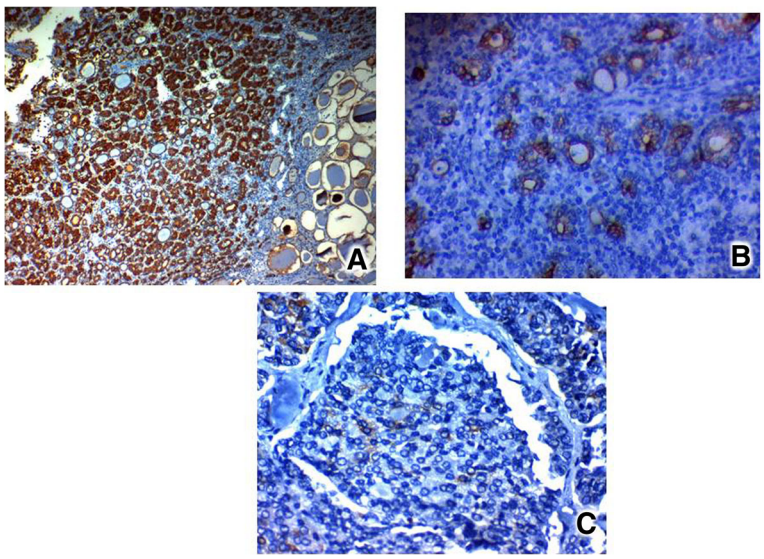

Fig. 3 Immunohistochemical expression of E-cadherin in papillary thyroid carcinoma (PTC) **a** High membranous expression in non-neoplastic thyroid follicles × 100, **b** High membranous expression in non-neoplastic thyroid follicles x400, **c** low membranous expression in poorly differentiated PTC stage IVx400

PTC. In Kaplan–Meier survival analysis of our patients we have proved that the OS rate of patients with high USP22 expression was shorter than that of patients with low USP22 expression. Univariate analyses showed that high USP22 expression in PTC tissues was a significant predictor of OS rate. Moreover, multivariate analysis demonstrated that USP22 expression is an independent risk factor in the prognosis of PTC patients.

These results suggested that the detection of increased USP22 expression might help to identify PTC patients with a poor prognosis and that USP22 could be a novel prognostic marker for PTC patients that were in concordance with Wang et al., (Wang et al. 2013).

USP22 levels were associated with higher incidence of lymph node metastasis, suggesting that it may serve as a promoter in PTC.

In our study we have confirmed the predictive and prognostic power of USP22 in addition to its diagnostic potential in PTC as its expression is increased in malignant tissue more than the adjacent non-neoplastic tissue. Therefore, USP22 may be a future diagnostic, prognostic marker and also a therapeutic target to improve the prognosis of such malignancy.

Our results were similar to results of Zhao et al., (Zhao et al. 2016a), in anaplastic thyroid carcinoma (ATC) that was carried out to determine the role of USP22 in ATC progression. They have found that USP22 was repeatedly overexpressed in ATC tissues, associated with clinicopathological characteristics, and was considered an independent prognostic indicator in ATC patients. Following USP22 silencing, the proliferation and invasion of ATC cells were significantly decreased, whereas ATC cell apoptosis was markedly elevated.

Expanding evidence demonstrated that USP22 provides a crucial function in a variety of pathological mechanisms and can be utilized as an encouraging diagnostic and/or prognostic marker of cancers that was similar to our results.

Zhao et al., (Zhao et al. 2016a), also evaluated the predictive strength to further affirm the potential clinical value of USP22. Kaplan–Meier analysis showed that the five-year overall survival rate in patients with increased USP22 protein expression was lower than that found in patients with lower USP22 protein levels. Accordingly, USP22 may be considered a future diagnostic and/or prognostic marker of ATC patients. There are several mechanisms by which USP22 permits tumor progression. In this study, we demonstrated that USP22 expression was higher in PTC tissues than those in adjacent non-cancerous tissues, indicating its clinical significance. USP22 levels were positively associated with clinicopathological characteristics, as size of the tumor, extrathyroidal extension, vascular invasion, lymphatic spread, existence of distant metastases and stage of the disease, suggesting that USP22 may serve as an oncogenic function in promoting the progression of PTC.

Zhao et al., (Zhao et al. 2016b), found that USP22 has been shown to enhance the proliferation of thyroid cancer cell by promoting cell cycle progression, which was confirmed by the observed G1 phase arrest and accompanying reduction in the S and G2/M phases when USP22 was depleted. So, they demonstrated that USP22

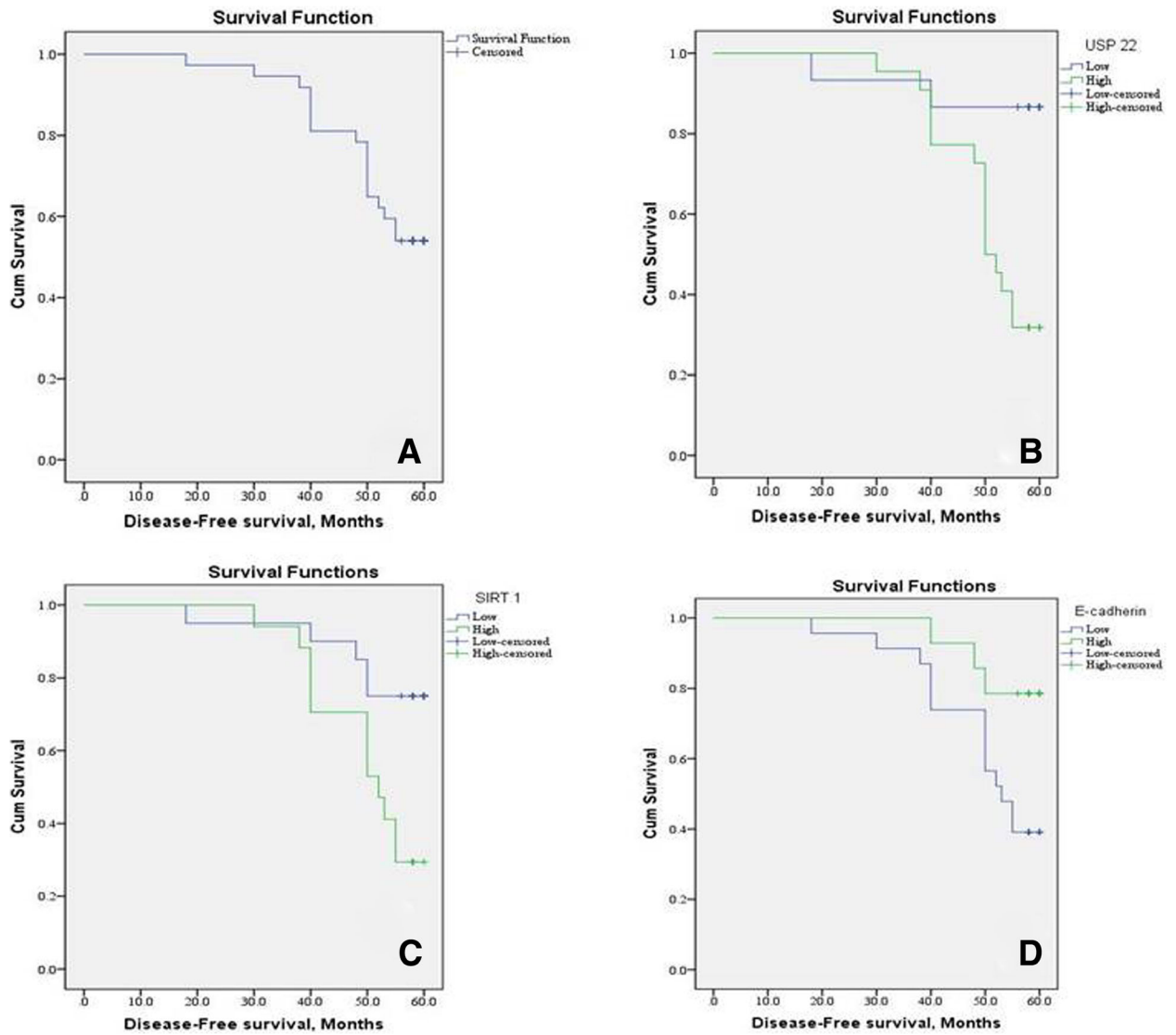

Fig. 4 Kaplan Meir survival curves of Disease free survival (DFS) of the studied papillary thyroid carcinoma (PTC) patients **a** DFS rate of all the studied PTC cases **b** DFS rate stratified according to USP22 expression **c** DFS rate stratified according to SIRT1 expression **d** PFS rate stratified according to E-cadherin expression

silencing inhibited the proliferation of PTC cells by adjusting the Rb/E2F pathway.

Consistent with our report, Wang et al., (Wang et al. 2013), results revealed that the expression levels of USP22 mRNA and protein in PTC tissues were both higher than those in non-cancerous tissues. These observations support the hypothesis that USP22 may function as an oncogene in PTC and also propose that USP22 actively participated in the tumorigenesis of PTC. Moreover, USP22 promotes cell cycle progression by regulating the PI3K/Akt/cyclin D2/Rb pathway in PTC cells (Zhao et al. 2016b).

Additionally, Zhao et al., (Zhao et al. 2016b), results stated that USP22 knockdown induce PTC cell apoptosis, as evidenced by results of caspase-3 activity and TUNEL assays. Further studies were needed to determine whether the well characterized deubiquitinating enzyme activity of USP22 plays a role in the PTC apoptotic machinery.

Similarly, recent studies demonstrated the ability of USP22 in anticipating metastatic spread and high possibility of treatment failure in human malignancies (Wang et al. 2017; Ma et al. 2015; Li et al. 2017). Furthermore, Wang et al., (Wang et al. 2017), proved that USP22 could induce cisplatin resistance in lung cancer progression.

Our results are explained by results of previous studies presented by Ma et al., (Ma et al. 2015), who

Table 6 Univariate and multivariate Cox regression analyses of different prognostic factors for disease-free survival (DFS) in PTC patients

	Univariate			Multivariate (stepwise)		
	HR	95.0% CI	*P*	HR	95.0% CI	*P*
Age < 40 vs >40Y	1.67	0.63–4.39	0.302			
Sex M vs F	1.05	0.37–2.99	0.924			
Histopathologicalsubtype	1.14	0.26–5.01	0.857			
USP22, low vs. high	6.54	1.49–28.79	0.013	5.25	1.15–23.93	0.032
SIRT1, low vs. high	3.48	1.22–9.92	0.02			
Ecadherin, low vs. high	0.29	0.08–1	0.05			
LN involvement	3.31	1.22–9.01	0.019			
Vascular invasion	3.69	1.06–12.9	0.041			
Capsular invasion	4.03	1.52–10.67	0.005			
Extra-thyroid extension	2.23	0.86–5.81	0.1			
LN involvement	2.63	1.01–6.86	0.049			
Stage	2.92	1.51–5.63	0.001			
Distant metastasis	4.66	1.56–13.9	0.006	3.06	1.01–9.29	0.048
Surgery	1.14	0.65–2	0.641			
Tumor size	3.21	1.17–8.78	0.023			

HR Hazard ratio, *CI* Confidence interval

demonstrated that USP22- mediated protein stabilization of BMI1 promotes gastric CSC stemness maintenance and GC progression, thereby providing the logical basis for USP22 targeting as a possible therapeutic approach against GC.

Moreover, Li et al., (Li et al. 2017), presented data supporting the ability of USP22 to promote colorectal (CRC) metastasis via EMT induction. The pivotal findings of the study are as follows: (i) USP22 promotes CRC cell migration and invasion by inducing EMT, (ii) USP22 increases AP4 transcription to induce EMT and enhance CRC cell metastasis to the lungs and (iii) USP22 overexpression is related to CRC progression, liver metastasis and poor outcomes of patients.

Zhao et al., (Zhao et al. 2016b), proved that miR-101 suppressed PTC tumorigenesis by targeting USP22. USP22 has been shown to promote the proliferation of lung cancer cells and human bladder cancer cell EJ by facilitating cell cycle progression, which was supported by the observed G1 phase arrest and concomitant reduction in the S and G2/M phase when USP22 was depleted (Zhao et al. 2016b). Previous study also showed that USP22 promotes cell cycle progression by positively regulating the PI3K/Akt pathway. Cyclin D2 expression and Akt activation were significantly suppressed upon USP22 silencing in ATC cells. These findings together with former reports indicating that PI3K/Akt signaling up regulates cyclin D2 via repression of the transcriptional factor FOXO, suggest that USP22 promotes cell cycle progression possibly via PI3K/Akt/cyclin D2 pathway in ATC cells (Zhao et al. 2016b).

By far most of cancer deaths are owing to metastasis, which an exceptionally confounded process including various molecules and signaling pathways. EMT is intimately associated to cancer cell invasion and metastasis. USP22 expression further promotes EMT by up-regulating ZEB1 and Snail in cancer cells though a process that involves focal adhesion kinase (FAK) signaling (Ning et al. 2014).

In our study we have found an inverse relation between USP22 expression in PTC and E-cadherin expression that was similar to results of Wang et al., (Wang et al. 2013), and Zhao et al.; (Zhao et al. 2016b) in PTC and Zhao et al., (Zhao et al. 2016a) in ATC and Hu et al., (Hu et al. 2015), in lung cancer.

EMT is a well-known central mechanism accounting for numerous cancers invasion and metastasis, which endows the epithelial cells with mesenchymal-like properties, e.g. raised cell motility, and reduced intercellular adhesion (Thiery 2002; Nieto 2002). Down-regulation of E-cadherin is characteristic for cells undergoing EMT (Cano et al. 2000). Zhao et al., (Zhao et al. 2016b), suggest that USP22 is considered a significant regulator of PTC metastasis by promoting EMT and down regulating E-cadherin expression. Moreover, Zhao et al., (Zhao et al. 2016a), results in ATC showed that USP22 depletion raises E-cadherin expression in ATC cells, which is similar to our findings.

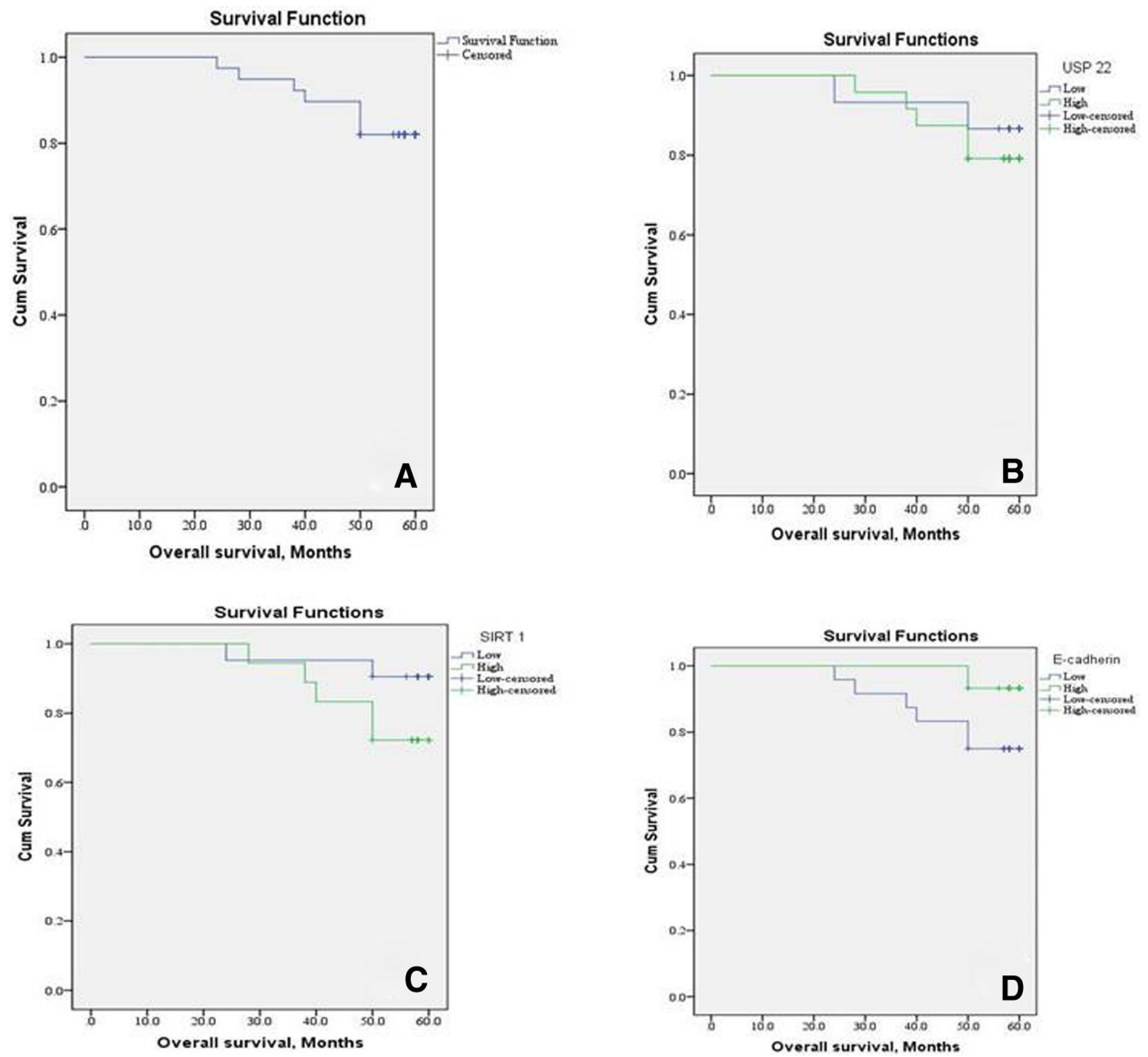

Fig. 5 Kaplan Meir survival curves of overall survival rate (OS) of the of the studied papillary thyroid carcinoma (PTC) patients **a** OS rate of all the studied PTC cases **b** OS rate stratified according to USP22 expression **c** OS rate stratified according to SIRT1 expression (**d**) OS rate stratified according to E-cadherin expression

Hu et al., 2015 have proved that USP22 enhances tumor progression and initiates epithelial-mesenchymal transition and down regulating E-cadherin expression in lung adenocarcinoma that was similar to our results in PTC.

The seven members (SIRT1-SIRT7) of the sirtuin family have emerged as a crucial regulator of multiple physiologic or pathologic events that include life-span extension, age-related disorders and cancer (Raynes et al. 2013). Studies have proposed that Sirt1 can act as a tumor suppressor or a promoter depending on its targets in particular signaling pathways or in specific malignancy and, therefore, its role remains uncertain (Kweon et al. 2014; Wu et al. 2016).

In the present study, we have noted that SIRT1 is overexpressed in PTC cells more than adjacent non neoplastic thyroid follicular cells and its expression is related to poor clinical parameters. Our results were similar to results of Kweon et al., (Kweon et al. 2014), in PTC and Zu et al., (Zu et al. 2016), in colorectal cancers. Consistent with our finding, Wu et al., (Wu et al. 2016), found that levels of SIRT1 mRNA and protein expression were higher in PTC than in the adjacent normal thyroid tissues. These findings

indicated that SIRT1 signaling pathway was involved in the development of PTCs.

Moreover, studies indicated that SIRT1 is overexpressed in various human malignancies such as breast cancer (Elangovan et al. 2011) and prostate cancer (Huffman et al. 2007).

Distinctive results were recorded by Simic et al., (Simic et al. 2013), reporting that reduction of SIRT1 promoted metastasis of breast epithelial cells in an orthotopic model of breast cancer, EMT and cell motility in these cells in vitro.

Wu et al., (Wu et al. 2016), proved that aberrant expression of HIC/SIRT1 may be helpful in assessing the risk of developing PTC and may be considered a novel therapeutic target. Kweon et al., (Kweon et al. 2014), demonstrated that induction of SIRT1 expression differed among thyroid cancer cell lines which are associated with survival under genotoxic stress conditions.

Zu et al., (Zu et al. 2016), stated that SIRT1 participates in many vital biological processes such as apoptosis, senescence and metabolism. Zu et al., (Zu et al. 2016), meta-analysis demonstrated the prognostic role of SIRT1 for CRC clinical outcome with conflicting results regarding SIRT role in CRC. The presence of better prognosis or poor outcome of high SIRT1 expression in CRC made it necessary to perform a meta-analysis of OS.

The role of SIRT1 expression in cancer is still controversial (Zu et al. 2016). Previous studies have reported that high SIRT1 was reported to contribute to the tumorigenesis in breast cancer (Elangovan et al. 2011). Additionally SIRT1 is repeatedly up regulated in lymph node spread and TNM upstaging of CRC patients (Huffman et al. 2007), which was consistent with our results in PTC.

Other studies proved that SIRT1 inhibition resulted in tumor suppressor genes activation (Pruitt et al. 2006). On the other hand, it has been reported that activation of SIRT1 reduced tumorigenesis of various cancers like hepatocellular carcinoma (Wang et al. 2008). These studies indicated that SIRT1 played different roles in various cancers.

SIRT has several roles in cancer promotion. Simic et al., (Simic et al. 2013), results showed that SIRT1 led to EMT induction and E-cadherin degradation from the cell surface, thereby b-catenin release from the cadherin junctions to the nucleus, a characteristic of mesenchymal cells, which were in line with our results regarding the association between higher SIRT-1 levels, lower E cadherin levels and higher incidence of local invasion and distant metastases of PTC. The role of SIRT1 in cancer may depend on the tumor type. SIRT1 has been shown to protect against bowel cancers (Firestein et al. 2008). However, SIRT1 was associated with malignancy in prostate cancer and chronic myelogenous leukemia (Byles et al. 2012; Li et al. 2012). In cancer prostate, SIRT1 caused enhancement of cell migration and metastasis by combination with EMT transcription factor ZEB1 to suppress E-cadherin transcription after PC3 cancer cell tail injections (Byles et al. 2012).

SIRT1 suppress the TGF-b signaling pathway which is considered vital in EMT. Differentiation and activation of myofibroblasts was promoted by TGF-b, with subsequent scar and fibrous tissue formation, facilitating cancer progression and metastasis (Wendt et al. 2009). Furthermore, EMT stimulation by TGB-b has been associated with the selection and expansion of cancer breast stem cells (Mani et al. 2008; Morel et al. 2008; Ben-Porath et al. n.d.). Since disruption of E-cadherin by MMPs can mediate EMT (Zheng et al. 2009), metastasis (Onder et al. 2008). SIRT1 also leads to repression of a second pathway involved in EMT signaling (Chaturvedi and Hass 2011). SIRT1 is a multi-faceted enzyme which plays many roles in secretory organs cancers, e.g. thyroid, ovary and pancreas (Frazzi 2018).

Opposing results from Zu et al., (Zu et al. 2016), meta-analysis showed that SIRT1 expression was not associated with clinicopathological features except for depth of invasion, TNM stage and lymph node spread, while SIRT1 overexpression predicted a poor OS in CRC. Hence, SIRT1 may represent a novel biomarker for the poor prognosis of PTC.

Our results demonstrated SIRT 1 is overexpressed in PTC than in non-neoplastic thyroid tissue pointing to the possibility of presence of a diagnostic role of in thyroid malignancies in addition to its prognostic role, in concordance to results of Kweon et al., (Kweon et al. 2014), who investigated Sirt1 expression as a molecular marker to differentiate thyroid cancers from normal thyroid cells.

In our study, we have correlated the expression of USP22 with SIRT-1 and Ecadherin detecting a positive correlation between both USP22 with SIRT1. Their concurrent expression in PTC was not previously assessed. Other studies in lung cancer have studied the relation between both markers and chemoresistance. Wang et al., (Wang et al. 2017), reported that overexpression of USP22 may be involved in the process of chemoresistance through a variety of mechanisms. On the other hand, Liang et al., (Liang et al. 2014), demonstrated that USP22 was linked to the gemcitabine resistance in pancreatic cancer; moreover they suggested that cisplatin can suppress USP22 expression through p38/MAPK pathway in HeLa cells. USP22 controls androgen receptor (AR) accumulation and signaling, and is a predictor of androgen deprivation therapy in prostate adenocarcinoma (Schrecengost et al. 2014). SIRT1 expression was shown to be a strong predictor for poor prognosis in

NSCLC patients who underwent platinum-based chemotherapy; hence, interfering with SIRT1 expression significantly enhanced cisplatin chemosensitivity (Zhang et al. 2013). USP22 is reported to deubiquitinate and stabilize SIRT1, sharing in regulation processes by SIRT1 acetylation as cell proliferation, apoptosis and DNA damage repair. Wang et al., (Wang et al. 2017), study suggested that both USP22 and SIRT1 can induce cisplatin resistance.

Lin et al., (Lin et al. 2012), study demonstrated that USP22 is a deubiquitinase of SIRT1 that led to subsequent suppression of p53 transcriptional activity and p53-mediated cell apoptosis in response to DNA damage.

In conclusion, our results showed that both USP22 and SIRT1 is upregulated in PTC more than adjacent non-neoplastic thyroid tissue and their high expression correlated with tumor size, extracapsular invasion, multifocality, lymph node metastasis, distant metastasis, and TNM stage, as well as poor prognosis of PTC patients. We also demonstrated that USP22, SIRT1 and E-cadherin are independent prognostic factors in PTC patients. The results of or study and previous studies confirmed that USP22 and SIRT1 depletion reduced the growth and invasion of PTC cells by regulating the expression and activation of many pro-tumorigenesis molecules. These results suggest that USP22 and SIRT1 promote tumor development and metastasis, and highlight USP22 and SIRT1 as novel prognostic markers.

Limitations of the work and recommendations

The limitations of our study are; we have not included the other subtypes of PTC due to their rarity and the small number of included cases so we recommended performing an extended study on a large number of patients using different evaluation methods to prove our results.

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Consent for publication
Were taken from all the contributing authors.

Competing interests
The authors declare that they have no competing interests

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