RESEARCH ARTICLE

Editorial Process: Submission:04/16/2024 Acceptance:07/12/2024

The Overexpression of *NUSAP1* and *GTSE1* Could Predict An Unfavourable Prognosis and Shorter Disease Free Survival in ccRenal Cell Carcinoma

Maram El-Hussieny, Dalia M. Thabet*, Heba M. Tawfik, Mariana F. Gayyed, Nisreen D. Toni

Abstract

Background: Although it has been reported that *NUSAP1* and *GTSE1* are highly expressed in different types of tumors and associated with malignant progression and poor clinical prognosis, their significances with clinicopathological data and correlations with patients' survival in ccRCC are still poorly understood. Therefore, in our study we attempted to evaluate the link between *NUSAP1* and *GTSE1* in ccRCC and to correlate their immunoexpression with clinicopathological parameters and the patients' survival to identify their significance as potential therapeutic targets, indicators for tumor progression, and patients' prognosis. **Method:** *NUSAP1* and *GTSE1* were examined in 100 ccRCC patients by immunohistochemistry. The association between *NUSAP1* and *GTSE1* immunoreactivity and clinicopathological variables were evaluated. The disease free survival (DFS) was examined by the Kaplan-Meier method. The multivariate Cox regressions was estimated to detect the prognostic role of both proteins. **Results:** We detected high *NUSAP1* and *GTSE1* expression in 60% and 62% of the cases, respectively. A significant association was detected between *NUSAP1* and *GTSE1* immunoexpression and size (p=0.007 and p=0.026, respectively), Fuhrman grade (p=0.022 and p=0.004, respectively), tumor stage (p=0.003 and p=0.019, respectively), TILs (p=0.026 and p=0.04 respectively), capsular invasion (p=0.002 and p=0.009, respectively), Distant metastasis (p=0.007 and p=0.009, respectively), and DFS (p=0.007 and 0.009, respectively). Multivariate Cox regression showed that high *NUSAP1* and *GTSE1* expression levels were independently associated with an unfavourable poor prognosis of ccRCC cases. **Conclusion:** We demonstrated that *NUSAP1* and *GTSE1* overexpression was closely related to the poor prognostic clinicopathological features of ccRCC and predicted an unfavorable prognosis. Therefore, *NUSAP1* and *GTSE1* might act together as potential futuristic prognostic indicators and therapeutic targets for ccRCC patients. However, further analysis in molecular studies on larger scale are mandatory to highlight the interactive crosstalk regulatory mechanisms between both markers and their combined effect on ccRCC.

Keywords: ccRCC- *NUSAP1*- *GTSE1*- immunohistochemistry- prognosis

Asian Pac J Cancer Prev, **25 (7)**, 2551-2559

Introduction

Renal cell carcinoma (RCC) is one of the most lethal urological malignancies and is the 9th commonest cancer worldwide, accounting for 2-3% of all malignant tumors [1]. It constitutes > 90% of all renal malignancies [2]. The clear cell RCC (ccRCC) subtype is considered the most predominant variant of RCC [3]. Worldwide, it ranks as the 9th and as the 14th most frequent cancer among males and females respectively [4].

In Egypt, it was reported by National Cancer Institute that RCC represents about 0.8% of newly diagnosed cancers and represents about 6% of newly diagnosed malignancies in the genitourinary system [5]. The incidence of RCC is higher in-between older age group [6]. Renal cancer is a multifactorial malignant disease. Between 25% and 33% of patients present with metastasis at the time of ccRCC diagnosis [7]. A proportion of cases who underwent nephrectomy were presented with either recurrence or metastasis. The consequence of RCC differs widely, suggesting the need for appropriate, precise and accurate prognostic parameters. Up to date, the best prognostic system for overall survival (OS) is the TNM staging system [8]. However, the need to improve the management of patients. and detection of new reliable prognostic parameters is mandatory.

Nucleolar and spindle associated protein1 (*NUSAP1*) gene is located at cytogenetic bands 15q14 [9]. Its

*Department of Pathology, Faculty of Medicine, Minia University 61511, El-Minia, Egypt. *For Correspondence: daliathabit18@gmail.com*

localization depends on the cell cycle. When it appears in interphase G2 cells, it is localised in the nucleus and concentrates within the nucleoli [10]. After nuclear envelope breakdown during meiosis, soluble *NUSAP1* is released into the cytoplasm and localised on the microtubules in prometaphase, gradually concentrating at microtubule plus ends near the chromosomes until the anaphase [11].

NUSAP1 is overexpressed in numerous types of malignant tumors compared to the non-neoplastic tissue counterpart [12]. Down-regulation of *NUSAP1* expression was linked to inhibition of proliferation, which defines it as a proto-oncogene [13]. It also influences migration, invasion, as well as metastasis via affecting the cytoskeleton [14]. Its overexpression was noticed as a poor prognostic indicator [15].

G2 And S-Phase Expressed 1 (*GTSE1*) gene was mapped to chromosome 22q13.2-q13.3 [16]. *GTSE1* is predominantly located within the cytoplasm and cytoskeleton, including tubulin or microtubules [17]. It controls microtubule dynamics through suppressing microtubule depolymerase MCAK, which is fundamental for chromosome stability, alignment and spindle integrity during mitosis [18].

GTSE1 protein participated in cellular response to DNA-damaging agents by regulating p53 function and stability during the S and G2 phases of the cell cycle [19]. Also, it was observed that its ability to control apoptosis appeared to be restricted during the S and G2 phases of cell cycle [20]. Previous studies detected *GTSE1* expression in various malignancies and its expression was a poor prognostic indicator [21, 22]. The Aim of this study was evaluation of the link between *NUSAP1* and *GTSE1* in ccRCC and correlation their immunoexpression with clinicopathological parameters and the patients' survival to identify their significance as potential therapeutic targets, indicators for tumor progression, and pa¬tients' prognosis.

Materials and Methods

Tissue Specimens

One hundred cases of formalin-fixed, paraffin-embedded radical nephrectomy specimens of ccRCC were included in the current study. They were randomly selected from pathology laboratory of Minia University Hospital in the period between January 2012 and December 2017. The study was approved by the ethical committee of the faculty of Medicine, Minia university (Approval No. 476/10/2022).

Clinicopathological data were obtained from pathology reports. Nuclear grade was revised according to Fuhrman nuclear grading system and categorized into 4 grades [23]. Stage, size and LNM were determined according to AJCC TNM system [24]. TILs were categorized into absent, mild, moderate, and marked. Absent; no inflammatory cells at the tumor's invasive margin, mild; mild and patchy infiltrate, moderate; prominent band-like inflammatory reaction at the invasive margin, and marked; florid cup-like infiltrate at the invasive edge with frequent destruction of tumor cells [25].

Immunohistochemical procedure

Briefly, sections were cut 5 μm thick on positively charged slides, de-paraffinized, and rehydrated. Slides were immersed in 3% hydrogen peroxide for 30 min then rinsed in PBS solution. Citrate buffer (pH 6.0) was used for antigen retrieval using the microwave. Then, slides were left to cool at room temperature and washed in PBS solution. Primary anti-*NUSAP1* Polyclonal rabbit antibody (1:200, catalogue number 7764R, USA, Bioss Antibodies).and primary anti- *GTSE1* Polyclonal rabbit antibody (1:200, catalogue number: 2516R, USA, Bioss Antibodies) were added. Sections were then incubated in a humidity chamber overnight at 4°C. Afterwards, slides rinsed with PBS before treatment with secondary antibody for 30 min. After a wash in PBS, streptavidin-biotin complex reagent was applied for 30 min. The 3,3-diaminobenzidinetetra hydro-chloride (DAB) was added, then sections were washed in distilled water. Lastly, slides were stained with hematoxylin, dehydrated, cleared with xylene, and coverslipped.

Evaluation of immunostaining

Slides were examined by pathologists (D. Thabet, M. Gayyed and M. El-Hussieny), independently in a blind fashion to clinicopathological data using an Olympus light microscope, Japan.

Cytoplasmic *NUSAP1* staining was detected and considered as positive expression. Also, *GTSE1* cytoplasmic staining was considered positive. The intensity of staining was scored and stratified as follows: 0: no staining; 1+: weak staining; 2+: moderate staining; and 3+: strong staining. The percentage of positive cells was scored as follows: 0 <10%; 1 = 10–25%; 2 =26–50%; 3=51–75%; and 4 >75%. The final score was detected by multiplying the staining intensity by the percentage score. Concerning *NUSAP1*; a score of 1–4 was considered as low *NUSAP1* expression, and a score of 5–12 as high expression [26]. Regarding GETS1; a score ≥ 6 was detected as high expression, whereas < 6 as low expression [27].

Statistical analysis

Data were analyzed using SPSS version 20. Chi-square and Fisher's exact tests were used to compare categorical variables. Correlation between *NUSAP1* and GETS1 was evaluated using Spearman's correlation coefficient. The relationship between markers expression and their clinical outcomes was estimated through multivariate analysis. DFS curves were estimated using Kaplan-Meier curves, while the differences in the survival curves were compared using Log-Rank test. By using Cox regression model, the multivariate analysis was done. P value of < 0.05 was considered significant.

Results

This study included 100 ccRCC cases. The mean age ± standard deviation (SD) of the studied cases was 57.82 ± 9.45 and the median age was 57 years (ranged from 41-79 years). The age of the patients was classified into two main groups according to the median age. Other Clinicopathological characteristics were listed in Table 1.

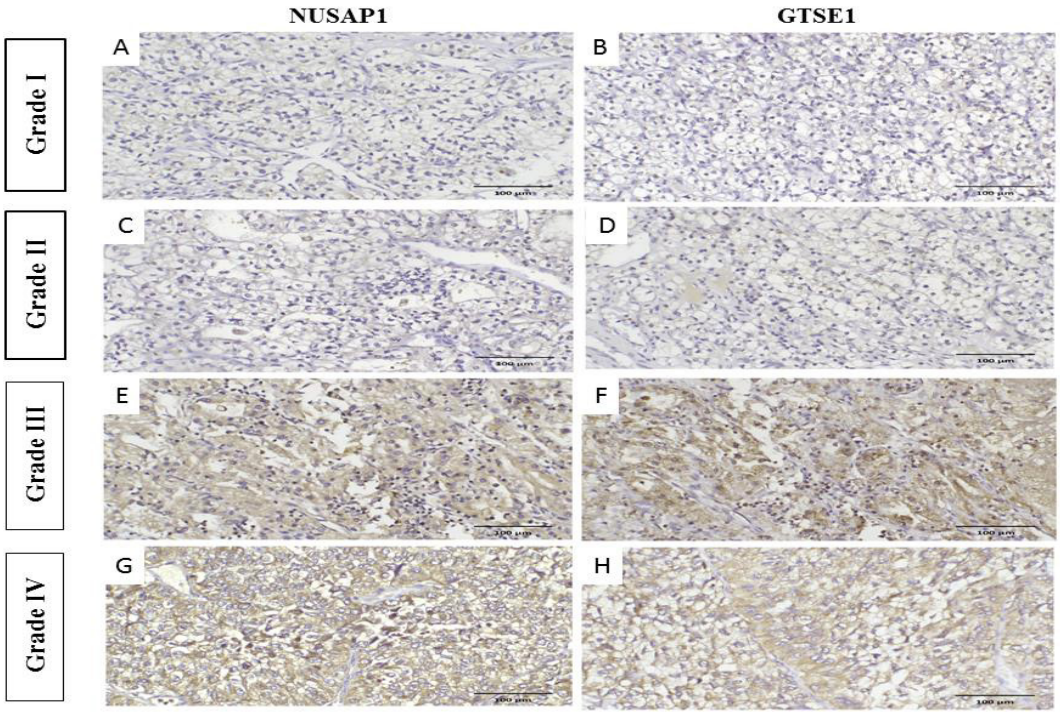

Figure 1. Immunohistochemical Expression of *NUSAP1* and *GTSE1* in ccRCC: Low expression in grade 1 (A&B) and grade 2 (C&D) of both *NUSAP1* and *GTSE1* respectively. High expression in grade 3 (E&F) and grade 4 (G&H) of both *NUSAP1* and *GTSE1* respectively (magnification X200) (scale bar= 100µ)

Immunohistochemical expression of NUSAP1 and GTSE1 (Figures 1)

The cytoplasmic *NUSAP1* expression was considered positive. In the present study, 60% of the cases revealed high immunoreactivity. A significant association was detected between *NUSAP1* immunoexpression and size (p=0.007), Fuhrman grade (p=0.022), tumor stage (p=0.003), TILs (p=0.026), capsular invasion (p=0.002), Perinephric fat invasion (p=0.012), Distant metastasis (p=0.007), and DFS (p=0.007). Meanwhile no significant association was observed between *NUSAP1* immunostaining and age (p=0.465), gender (p=0.815), laterality (p=0.63), lymph node status (p=0.07), LVI

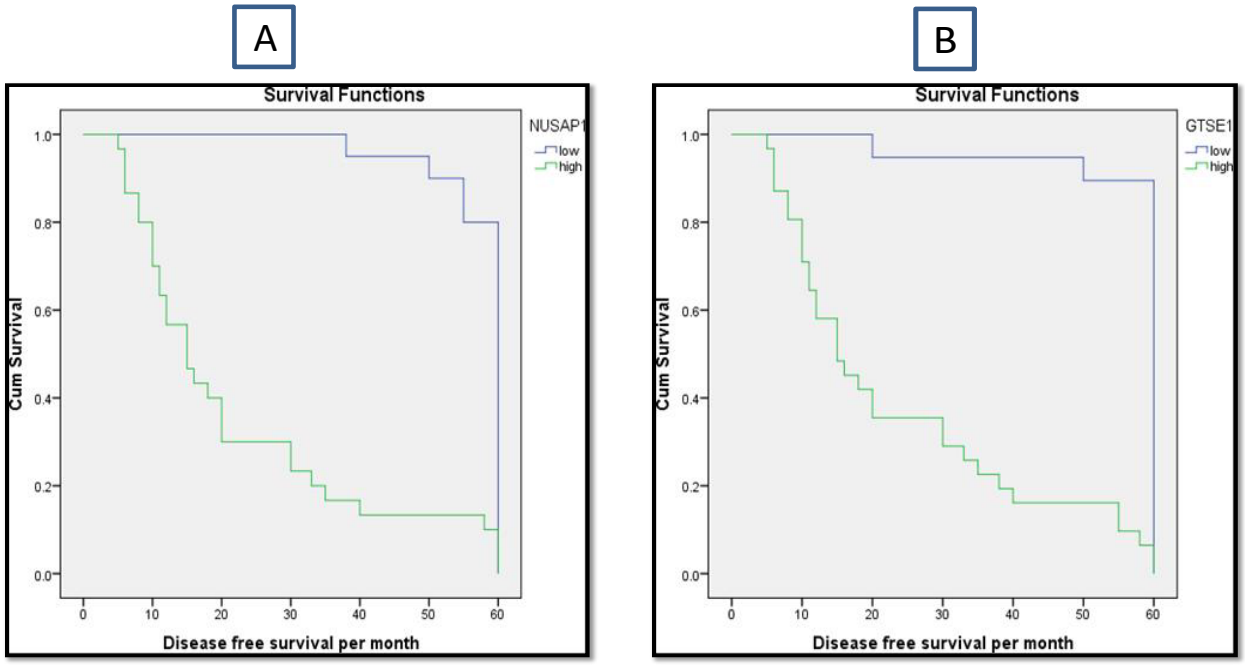

Figure 2. Kaplan- Meier Curve for DFS according to NUSAP1 (A) and *GTSE1* (B) Immunoexpression. Shorter DFS is associated with high expression.

Table 1. The Clinicopathological Data of ccRCC Patients (n=100)

Clinicopathological data		No.
Age(years)	≤ 57 years	66
	> 57 years	34
Gender	Male	58
	Female	42
Laterality	Right kidney	36
	Left kidney	64
Size (cm)	< 4 cm	22
	4.1-7 cm	46
	>7 cm	32
Fuhrman grade	1	18
	2	44
	3	22
	4	16
Tumor stage	I	22
	II	34
	III	26
	IV	18
Lymph node status	Nx	68
	N0	12
	N1	20
LVI	Positive	38
	Negative	62
Distant metastasis	Positive	18
	Negative	82
Capsular invasion	Positive	42
	Negative	58
Perinephric fat invasion	Positive	36
	Negative	64
Sinus fat invasion	Positive	16
	Negative	84
Renal vein invasion	Positive	16
	Negative	84
TILs	Negative	22
	Mild	22
	Moderate	32
	Marked	24
Coagulative tumor necrosis	Positive	40
	Negative	60
DFS	Positive	18
	Negative	82

(p=0.122), sinus fat invasion (p=0.345), tumor necrosis (p=0.556) or renal vein invasion (p=0.345).

Cytoplasmic expression of *GTSE1* was regarded as positive expression. In our study, 62% of the cases showed high immunostaining. A significant association was detected between *GTSE1* immunoexpression and size (p=0.026), Fuhrman grade (p=0.004), tumor stage (p=0.019), Lymph node status (p=0.021) TILs (p=0.04), capsular invasion (p=0.009), Distant metastasis (p=0.009), and DFS (p=0.009). Meanwhile no significant association was found between *GTSE1* immunostaining and age (p=0.74), gender (p=0.563), laterality (p=0.19), perinephric fat invasion (p=0.085), LVI (p=0.053), sinus fat invasion (p=0.975), tumor necrosis (p=0.812) or renal vein invasion (p=0.409) as shown in Table 2.

Correlation between the immunoreactivity of NUSAP1 and GTSE1

A statistically significant positive correlation was determined between *NUSAP1* and *GTSE1* (p <0.001, r= 0.701) (Table 3).

Survival analysis and prognostic significance

DFS of 100 cases of ccRCC patients was investigated. The variables analyzed included markers expression and some clinicopathological data included in our study. The time of DFS ranged from 5 months to 60 months with a mean ±SD of 36.4 ±0.4 months and a median survival time was 45 months. Patients with high *NUSAP1* and *GTSE1* immunoexpression had significantly shorter DFS than those patients with low expression (p= 0.007 and p=0.009, respectively) (Figure 2).

The multivariate analysis revealed that nuclear grade and tumor stage were both independent prognostic factors (p=0.01, p=0.027, respectively). Moreover, *NUSAP1* and *GTSE1* immunoexpression had an independent impact on the prognosis of the studied cases (p= 0.021 and p= 0.015, respectively), while the other variables were not statistically significant (Table 4).

Discussion

RCC is a heterogeneous disease and despite the recent advances in renal cancer treatment, the long-term survival of patients with advanced stage remains poor [28]. Due to the complexity of the disease, more studies are needed to give us the opportunity to understand the underlying molecular and genetic pathways of carcinogenesis and provide targeted therapies [29]. Also, tumor stage and grade are the most widely accepted prognostic factors combined approach including molecular pathways seems to improve the predictive accuracy [30].

NUSAP1 is a microtubule-associated protein which has microtubule-bundling and stabilizing activity [31]. *NUSAP1* also has roles in invasion and metastases [32]. In addition, it shares in functional interactions with pro-oncogenic pathways [33]. Although, the overexpression of *NUSAP1* has been found in various cancers, there is limited research concentrating on the expression and clinical significance of *NUSAP1* in RCC. In the current study, high NUSAP1 expression was detected in 60% of ccRCC. We found a significant association between NUSAP expression and the tumor size (p = 0.007). Regarding tumor grade, high *NUSAP1* expression was found in 50%, 72.7% and 100% of grade II, III and IV cases, respectively, displaying a significant association between high expression and Fuhrman grade. Also, 27.3% of stage I, 47.1% of stage II, 76.9% of stage III and 100% of stage IV cases showed high expression. These findings could be attributed to its anti-apoptotic role through the

Table 2. Association between *NUSAP1* and *GTSE1* Immunoexpression and Clinicopathological Data for the Patients with ccRCC (n=100)

		NUSAP1 expression			*GTSE1* expression		
Clinicopathological data	No.	Low	High	P value	Low	High	P value
Age (years)							
≤ 57	66	24 (36.4)	42 (63.6)	0.465	22 (33.3)	44 (66.7)	0.74
> 57	34	16 (47.1)	18 (52.9)		16 (47.1)	18 (52.9)	
Gender							
Male	58	24 (41.4)	34 (58.6)	0.815	26 (44.8)	32 (55.2)	0.563
Female	42	16 (38.1)	26 (61.9)		12 (28.6)	30 (71.4)	
Laterality							
Right kidney	36	16 (44.4)	20 (55.6)	0.63	18 (50)	18 (50)	0.19
Left kidney	64	24 (37.5)	40 (62.5)		20 (31.3)	44 (68.7)	
Size (cm)							
< 4 cm	22	16 (72.7)	6 (27.3)		16 (72.7)	6 (27.3)	
4.1 - 7 cm	46	20 (43.5)	26 (56.5)	0.007*	16 (34.8)	30 (65.2)	0.026*
> 7 cm	32	4 (12.5)	28 (87.5)		6 (18.8)	26 (81.2)	
Fuhrman grade							
I	18	12 (66.7)	6 (33.3)		14 (77.8)	4 (22.2)	
II	44	22 (50)	22 (50)	0.022*	20 (45.5)	24 (54.5)	0.004*
III	22	6 (22.3)	16 (72.7)		4 (18.2)	18 (81.8)	
IV	16	0 (0)	16 (100)		0 (0)	16 (100)	
Tumor stage							
I	22	16 (72.7)	6 (27.3)		16 (72.7)	6 (27.3)	
II	34	18 (52.9)	16 (47.1)	0.003*	14 (41.1)	20 (58.9)	0.019*
III	26	6 (23.1)	20 (76.9)		8 (30.8)	18 (69.2)	
IV	18	0 (0)	18 (100)		0 (0)	18 (100)	
Lymph node status							
Nx	68	34 (50)	34 (50)		32 (47.1)	36 (52.9)	
N0	12	4 (33.3)	8 (66.7)	0.07	6 (50)	6 (50)	0.021*
N1	20	2 (10)	18 (90)		0 (0)	20 (100)	
TILs							
Absent	22	14 (63.6)	8 (36.4)		12 (54.5)	10 (45.5)	
Mild	22	14 (63.6)	8 (36.4)	0.026*	16 (72.7)	6 (27.3)	
Moderate	32	6 (18.8)	26 (81.2)		6 (18.8)	26 (81.2)	0.04*
Marked	24	6 (25)	18 (75)		4 (16.7)	20 (83.3)	
LVI							
Positive	38	10 (26.3)	28 (73.3)		8 (21.1)	30 (78.9)	
Negative	62	30 (48.4)	32 (51.6)	0.122	30 (48.4)	32 (51.6)	0.053
Distant metastasis							
Present	18	0 (0)	18 (100)	0.007*	0 (0)	18 (100)	0.009*
Absent	82	40 (48.8)	42 (51.2)		38 (46.3)	44 (53.7)	
Capsular invasion							
Positive	42	6 (14.3)	36 (85.7)	0.002*	8 (19.1)	34 (80.9)	0.009*
Negative	58	34 (58.6)	24 (41.4)		30 (51.7)	28 (48.3)	
Perinephric fat							
Positive	36	6 (16.7)	30 (83.3)	0.012*	8 (22.2)	28 (77.8)	0.085
Negative	64	34 (53.1)	30 (46.9)		30 (46.9)	34 (63.1)	
Sinus fat invasion							
Positive	16	4 (25)	12 (75)	0.345	6 (37.5)	10 (62.5)	0.975
Negative	84	36 (42.9)	48 (57.1)		32 (38.1)	52 (61.9)	

Test of significance: Chi-Square test. * P - value < 0.05 is considered statistically significant TILs, tumor infiltrating lymphocytes; LVI, lymphovascular invasion; DFS, disease free survival

Table 2. Continued

Clinicopathological data	No.	*NUSAP1* expression Low	*NUSAP1* expression High	P value	*GTSE1* expression Low	*GTSE1* expression High	P value
Renal vein invasion							
Positive	16	4 (25)	14 (75)	0.345	4 (25)	12 (75)	0.409
Negative	84	36 (42.9)	48 (57.1)		34 (40.5)	50 (59.5)	
Tumor necrosis							
Present	40	14 (35)	26 (65)	0.556	14 (35)	26 (65)	0.812
Absent	60	26 (43.3)	34 (56.7)		24 (40)	36 (60)	
DFS							
Present	18	0 (0)	18 (100)	0.007*	0 (0)	18 (100)	0.009*
Absent	82	40 (48.8)	42 (51.2)		38 (46.3)	44 (53.7)	

Test of significance: Chi-Square test. * P - value < 0.05 is considered statistically significant TILs, tumor infiltrating lymphocytes; LVI, lymphovascular invasion; DFS, disease free survival

Table 3. Correlation between Immunoreactivity of *NUSAP1* and *GTSE1* (n=100)

		GTSE1 expression Low (%)	High (%)	Total	P value
NUSAP1 expression	Low (%)	34 (85)	6 (15)	40	
	High (%)	4 (6.7)	56 (93.3)	60	<0.001*
Total		38 (38)	62 (62)	100	

Test of significance: Spearman correlation test; * P - value < 0.05 is considered statistically significant

Table 4. Multivariate Survival Analysis in ccRCC

	Sig.	Exp(B)	95.0% CI for Exp(B) Lower	Upper
Age	0.13	0.621	0.335	1.151
Tumor size	0.379	0.625	0.22	1.778
LN	0.255	1.344	0.808	2.237
Grade	0.01*	0.573	0.376	0.873
Stage	0.027*	2.846	1.125	7.201
NUSAP1	0.021*	2.86	1.176	6.959
GTSE1	0.015*	3.178	1.254	8.052

Test of significance: Cox regression test * P - value < 0.05 is considered statistically significant; CI, confidence interval.

inhibition of P53, leading to cell survival and uncontrolled proliferation [34]. Moreover, its proliferative role through PI3K/AKT pathway could explain the impact of *NUSAP1* on tumor differentiation and progression [35]. *NUSAP1* overexpression has a significant effect on tumorogenesis through microtubule bundling and cell cycle arrest at G2/M check point which led to progression of mitotic process of proliferating process [36].

Our findings are in accordance with those observed in other tumors. Zhu et al. that detected a statistically significant association between expression and tumor grade in glioma [37]. Also, Hou et al., reported a significant association with tumor size in urothelial carcinoma [38], and Guo et al., reported that tumors with advanced stage and marked TILs showed high *NUSAP1* expression in gastric carcinoma [36, 39]. In the current study, 100% of the cases with positive distant metastasis showed high *NUSAP1* expression. *NUSAP1* was supposed to regulate numerous EMT-related markers, such as E-cadherin, N-cadherin and vimentin [40]. In this context, a significant association was found in patients with distant metastasis in lung adenocarcinoma [41]. This might be linked to the role of *NUSAP1* in cell invasion through Wnt/β-catenin signalling pathways [34]. *NUSAP1* also influences cell migration by affecting the cytoskeleton. Up to our knowledge, there is no previous study demonstrated the relationship between *NUSAP1* and perinephric fat invasion and capsular invasion.

NUSAP1 is related with shorter DFS and the multivariate regression analysis confirmed it as an independent poor prognostic factor in ccRCC. To the best of our knowledge, it is the 1st study that is concerned with the prognostic effect of *NUSAP1* on survival and its relation to different clinicopathologic parameters via multivariate analysis in ccRCC.

GTSE1 has an essential role in cell cycle regulation and microtubules-based activity. Furthermore, there is strong evidence that the aberrant *GTSE1* expression could have an essential effect on the invasion and cancers progression [42]. However, it has limited utility as a prognostic marker in ccRCC.

Namely, upregulation of *GTSE1* shows a statistically significant association with the aggressive features of ccRCC, such as Fuhrman grade, tumor size, stage, capsular invasion and metastasis. This could be attributed to the anti-apoptotic abilities of *GTSE1* through its role as a negative regulator of p53 expression leading to uncontrolled proliferation [43]. Furthermore, *GTSE1* has a function in tumor progression through cell cycle arrest at G0/G1 phase and activation of G2/M phase, which subsequently led to enhancement of mitotic process in proliferating cells [44]. Also, *GTSE1* is recognized to advance the microtubule elongation by gathering at plus end; this increases the invasive potential in carcinomas [45]. These results are in line with Lin et al., who reported a link between *GTSE1* and tumor grade and stage in breast cancer [21].

Furthermore, *GTSE1* is suggested to induce cellular migration and proliferation through upregulation of Wnt/β-catenin signaling pathway [46]. To our knowledge,

there is no previous study that demonstrated the relevance between *GTSE1* and capsular invasion in ccRCC.

In our study, we detected that *GTSE1* expression was linked with shorter DFS and the multivariate regression analysis proved it as a poor independent prognostic factor. An earlier study clarified the association of *GTSE1* expression in ccRCC with prognosis. They reported that high *GTSE1* expression is associated with lower DFS, and this association was statistically significant [27]. In our study, Fuhrman grade and stage were adverse prognostic clinical factors, and this is in agreement with previous studies done on gastric carcinoma [36, 47].

According to our results, there is a statistically significant positive correlation between *NUSAP1* and *GTSE1* expression. To the best of our knowledge, it is the first time to study the correlation between the expressions of them. *NUSAP1* and *GTSE1* have significant impact on cancer progression through their effect on EMT [34, 48]. High levels of *NUSAP1* or *GTSE1* are accompanied by reduced levels of epithelial indicators such as desmoplakin and E-cadherin, and elevated levels of mesenchymal markers as N-cadherin and vimentin [26, 27]. Additionally, both markers were reported to function in the PI3K/AKT signaling pathway [49, 50]. It was indicated that *NUSAP1* or *GTSE1* knockdown decreased the phosphorylation of PI3K and AKT. Therefore, both markers knockdown suppressed cancer cell proliferation, migration, and invasion by inhibiting the PI3K/Akt signaling [51]. Both *NUSAP1* and *GTSE1* may function through Wnt/β-catenin signaling pathways. It was found that *NUSAP1* and *GTSE1* overexpression remarkably elevated the levels of active β-catenin expression in the cytoplasm, enhanced the transcription activity of β-catenin and remarkably decreased β-catenin expression in the nucleus [46].

In conclusion, we demonstrated that *NUSAP1* and *GTSE1* overexpression was closely related to the poor prognostic clinicopathological features of ccRCC and predicted an unfavorable prognosis. Therefore, *NUSAP1* and *GTSE1* might act together as potential futuristic prognostic indicators and therapeutic targets for RCC patients. However, further analysis in molecular studies on larger scale are mandatory to highlight the interactive crosstalk regulatory mechanisms between both markers and their combined effect on ccRCR.

Author Contribution Statement

The laboratory work was done by DMT and ME. DMT, NDT, MFT and HMT analysed and interpretated the data. All authors shared in the manuscript writing and approval of the final version.

Acknowledgements

Approval

It is not a part of an approved student thesis.

Ethical Declaration

The study was approved by the ethical committee of the faculty of Medicine, Minia university (Approval No. 476/10/2022).

Data Availability Statement

Data is available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Attalla K, Weng S, Voss MH, Hakimi AA. Epidemiology, risk assessment, and biomarkers for patients with advanced renal cell carcinoma. Urol Clin North Am. 2020;47(3):293-303. https://doi.org/10.1016/j.ucl.2020.04.002.
2. Piana A, Andras I, Diana P, Verri P, Gallioli A, Campi R, et al. Small renal masses in kidney transplantation: Overview of clinical impact and management in donors and recipients. Asian J Urol. 2022;9(3):208-14. https://doi.org/10.1016/j.ajur.2022.06.001.
3. Abu-Ghanem Y, Powles T, Capitanio U, Beisland C, Järvinen P, Stewart GD, et al. The impact of histological subtype on the incidence, timing, and patterns of recurrence in patients with renal cell carcinoma after surgery—results from recur consortium. Eur Urol Oncol. 2021;4(3):473-82. https://doi.org/10.1016/j.euo.2020.09.005.
4. Usher-Smith J, Simmons RK, Rossi SH, Stewart GD. Current evidence on screening for renal cancer. Nat Rev Urol. 2020;17(11):637-42. https://doi.org/10.1038/s41585-020-0363-3.
5. Abdu Allah AM, El-Hefnway SM, Alhanafy AM, Zahran AM, Kasem HE. Leptin receptor gene (a/g) polymorphism rs1137101 and renal cell carcinoma. Mol Cell Biochem. 2018;448(1-2):137-44. https://doi.org/10.1007/s11010-018-3320-1.
6. Abd El-Fadeal NM, Ellawindy A, Jeraiby MA, Qusti SY, Alshammari EM, Alzahrani AK, et al. Hsp70 expression signature in renal cell carcinoma: A clinical and bioinformatic analysis approach. Genes. 2023;14(2):355. https://doi.org/10.3390/genes14020355.
7. Dudani S, De Velasco G, Wells JC, Gan CL, Donskov F, Porta C, et al. Evaluation of clear cell, papillary, and chromophobe renal cell carcinoma metastasis sites and association with survival. JAMA Netw Open. 2021;4(1):e2021869-e. https://doi.org/10.1001/jamanetworkopen.2020.21869.
8. Saarenheimo J, Eigeliene N, Andersen H, Tiirola M, Jekunen A. The value of liquid biopsies for guiding therapy decisions in non-small cell lung cancer. Front Oncol. 2019;9:129. https://doi.org/10.3389/fonc.2019.00129.
9. Kotian S, Banerjee T, Lockhart A, Huang K, Catalyurek UV, Parvin JD. Nusap1 influences the DNA damage response by controlling brca1 protein levels. Cancer Biol Ther. 2014;15(5):533-43. https://doi.org/10.4161/cbt.28019.
10. Hensley PJ, Kyprianou N. Nuclear spindles pave the way to metastasis. Oncotarget. 2018;9(16):12544. https://doi.org/10.18632/oncotarget.23728.
11. Vanden Bosch A, Raemaekers T, Denayer S, Torrekens S, Smets N, Moermans K, et al. Nusap is essential for chromatin-induced spindle formation during early embryogenesis. J Cell Sci. 2010;123(19):3244-55. https://doi.org/10.1242/jcs.063875.
12. Yadav DK, Sharma A, Dube P, Shaikh S, Vaghasia H, Rawal RM. Identification of crucial hub genes and potential molecular mechanisms in breast cancer by integrated bioinformatics analysis and experimental validation. Comput Biol Med. 2022;149:106036. https://doi.org/10.1016/j.compbiomed.2022.106036.

13. Lin M, Ye M, Zhou J, Wang ZP, Zhu X. Recent advances on the molecular mechanism of cervical carcinogenesis based on systems biology technologies. Comput Struct Biotechnol J. 2019;17:241-50. https://doi.org/10.1016/j.csbj.2019.02.001.
14. Bell R, Barraclough R, Vasieva O. Gene expression meta-analysis of potential metastatic breast cancer markers. Curr Mol Med. 2017;17(3):200-10. https://doi.org/10.2174/1566524017666170807144946.
15. Deng Y, Ma H, Hao J, Xie Q, Zhao R. Mcm2 and nusap1 are potential biomarkers for the diagnosis and prognosis of pancreatic cancer. BioMed Res Int. 2020;2020:8604340. https://doi.org/10.1155/2020/8604340.
16. Monte M, Collavin L, Lazarevic D, Utrera R, Dragani T, Schneider C. Cloning, chromosome mapping and functional characterization of a human homologue of murine gtse-1 (b99) gene. Gene. 2000;254(1-2):229-36. https://doi.org/10.1016/s0378-1119(00)00260-2.
17. Aseervatham J. Cytoskeletal remodeling in cancer. Biology. 2020;9(11):385. https://doi.org/10.3390/biology9110385.
18. Xie C, Xiang W, Shen H, Shen J. Gtse1 is possibly involved in the DNA damage repair and cisplatin resistance in osteosarcoma. J Orthop Surg Res. 2021;16:1-11. https://doi.org/10.1186/s13018-021-02859-8.
19. Mohammadzadeh A, Mirza-Aghazadeh-Attari M, Hallaj S, Saei AA, Alivand MR, Valizadeh A, et al. Crosstalk between p53 and DNA damage response in ageing. DNA Repair. 2019;80:8-15. https://doi.org/10.1016/j.dnarep.2019.05.004.
20. Ruan X, Zheng J, Liu X, Liu Y, Liu L, Ma J, et al. Lncrna linc00665 stabilized by taf15 impeded the malignant biological behaviors of glioma cells via stau1-mediated mrna degradation. Mol Ther Nucleic Acids. 2020;20:823-40. https://doi.org/10.1016/j.omtn.2020.05.003.
21. Lin F, Xie YJ, Zhang XK, Huang TJ, Xu HF, Mei Y, et al. Gtse1 is involved in breast cancer progression in p53 mutation-dependent manner. J Exp Clin Cancer Res. 2019;38:1-16. https://doi.org/10.1186/s13046-019-1157-4. .
22. Xiong J, Zhang J, Li H. Identification of g2 and s phase-expressed-1 as a potential biomarker in patients with prostate cancer. Cancer Manag Res. 2020:9259-69. https://doi.org/10.2147/CMAR.S272795.
23. Delahunt B, Eble JN, Egevad L, Samaratunga H. Grading of renal cell carcinoma. Histopathology. 2019;74(1):4-17. https://doi.org/10.1111/his.13735.
24. Delahunt B, Eble JN, Samaratunga H, Thunders M, Yaxley JW, Egevad L. Staging of renal cell carcinoma: Current progress and potential advances. Pathology. 2021;53(1):120-8. https://doi.org/10.1016/j.pathol.2020.08.007.
25. Mohammed ZM, Going JJ, Edwards J, McMillan DC. The role of the tumour inflammatory cell infiltrate in predicting recurrence and survival in patients with primary operable breast cancer. Cancer Treat Rev. 2012;38(8):943-55. https://doi.org/10.1016/j.ctrv.2012.04.011.
26. Gao S, Yin H, Tong H, Zhan K, Yang G, Hossain MA, et al. Nucleolar and spindle associated protein 1 (nusap1) promotes bladder cancer progression through the tgf-β signaling pathway. Onco Targets Ther. 2020:813-25. https://doi.org/10.2147/OTT.S237127.
27. Chen W, Wang H, Lu Y, Huang Y, Xuan Y, Li X, et al. Gtse1 promotes tumor growth and metastasis by attenuating of klf4 expression in clear cell renal cell carcinoma. Lab Invest 2022;102(9):1011-22. https://doi.org/10.1038/s41374-022-00797-5.
28. Wolf MM, Kimryn Rathmell W, Beckermann KE. Modeling clear cell renal cell carcinoma and therapeutic implications. Oncogene. 2020;39(17):3413-26. https://doi.org/10.1038/s41388-020-1234-3.
29. Yang Y, Li S, Wang Y, Zhao Y, Li Q. Protein tyrosine kinase inhibitor resistance in malignant tumors: Molecular mechanisms and future perspective. Signal transduct target. 2022;7(1):329. https://doi.org/10.1038/s41392-022-01168-8.
30. Andersson-Evelönn E, Vidman L, Källberg D, Landfors M, Liu X, Ljungberg B, et al. Combining epigenetic and clinicopathological variables improves specificity in prognostic prediction in clear cell renal cell carcinoma. J Transl Med. 2020;18(1):1-11. https://doi.org/10.1186/s12967-020-02608-1.
31. Zhou Q, Lee KJ, Kurasawa Y, Hu H, An T, Li Z. Faithful chromosome segregation in trypanosoma brucei requires a cohort of divergent spindle-associated proteins with distinct functions. Nucleic Acids Res. 2018;46(16):8216-31. https://doi.org/10.1093/nar/gky557.
32. Gordon CA, Gong X, Ganesh D, Brooks JD. Nusap1 promotes invasion and metastasis of prostate cancer. Oncotarget. 2017;8(18):29935. https://doi.org/10.18632/oncotarget.15604.
33. Ciraku L, Esquea EM, Reginato MJ. O-glcnacylation regulation of cellular signaling in cancer. Cell Signal. 2022;90:110201. https://doi.org/10.1016/j.cellsig.2021.110201.
34. Sun L, Shi C, Liu S, Zhang E, Yan L, Ji C, et al. Overexpression of nusap1 is predictive of an unfavourable prognosis and promotes proliferation and invasion of triple-negative breast cancer cells via the wnt/β-catenin/emt signalling axis. Gene. 2020;747:144657. https://doi.org/10.1016/j.gene.2020.144657.
35. Zhang J, Fan J. Prazosin inhibits the proliferation, migration and invasion, but promotes the apoptosis of u251 and u87 cells via the pi3k/akt/mtor signaling pathway. Exp Ther Med. 2020;20(2):1145-52. https://doi.org/10.3892/etm.2020.8772.
36. Ge Y, Li Q, Lin L, Jiang M, Shi L, Wang B, et al. Downregulation of nusap1 suppresses cell proliferation, migration, and invasion via inhibiting mtorc1 signalling pathway in gastric cancer. Cell Biochem Funct. 2020;38(1):28-37. https://doi.org/10.1002/cbf.3444.
37. Zhu T, Xie P, Gao YF, Huang MS, Li X, Zhang W, et al. Nucleolar and spindle-associated protein 1 is a tumor grade correlated prognosis marker for glioma patients. CNS Neurosci Ther. 2018;24(3):178-86. https://doi.org/1111/cns.12803.
38. Hou J, Lu Z, Liu X, Luo B, Qu G, Xu Y, et al. Increased nusap1 expression is associated with lymph node metastasis and survival prognosis in bladder urothelial carcinoma. Sci Rep. 2022;12(1):7003. https://doi.org/10.1038/s41598-022-11137-4.
39. Guo H, Zou J, Zhou L, Zhong M, He Y, Huang S, et al. Nusap1 promotes gastric cancer tumorigenesis and progression by stabilizing the yap1 protein. Front Oncol. 2021;10:591698. https://doi.org/10.3389/fonc.2020.591698.
40. Ling B, Wei P, Xiao J, Cen B, Wei H, Feng X, et al. Nucleolar and spindle-associated protein 1 promotes non-small cell lung cancer progression and serves as an effector of myocyte enhancer factor 2d. Oncol Rep. 2021;45(3):1044-58. https://doi.org/10.3892/or.2020.7918.
41. Wang YS, Yin JZ, Zhou C, Shi XQ, Zhao XW, Li B, et al. Nusap1 is a Prognostic Biomarker and Correlated to Immune Infiltration in lung Adenocarcinoma. 2023.
42. Zheng Y, Shi Y, Yu S, Han Y, Kang K, Xu H, et al. Gtse1, cdc20, pcna, and mcm6 synergistically affect regulations in cell cycle and indicate poor prognosis in liver cancer. Anal Cell Pathol. 2019;2019:1038069. https://doi.org/10.1155/2019/1038069.

43. Silvestro S, Diomede F, Chiricosta L, Zingale VD, Marconi GD, Pizzicannella J, et al. The role of hypoxia in improving the therapeutic potential of mesenchymal stromal cells. A comparative study from healthy lung and congenital pulmonary airway malformations in infants. Front Bioeng Biotechnol. 2022;10:868486. https://doi.org/10.3389/fbioe.2022.868486.
44. Liu B, Pan CF, Ma T, Wang J, Yao GL, Wei K, et al. Long non-coding rna ak001796 contributes to cisplatin resistance of non-small cell lung cancer. Mol Med Rep. 2017;16(4):4107-12. https://doi.org/10.3892/mmr.2017.7081.
45. Singh D, Schmidt N, Müller F, Bange T, Bird AW. Destabilization of long astral microtubules via cdk1-dependent removal of gtse1 from their plus ends facilitates prometaphase spindle orientation. Curr Biol. 2021;31(4):766-81. e8. https://doi.org/10.1016/j.cub.2020.11.040.
46. Guo W, Zhu J, Zhu Y, Wang K. G2 and s phase-expressed-1 acts as a putative tumor promoter in cervical cancer by enhancing wnt/β-catenin signaling via modulation of gsk-3β. Environ Toxicol. 2021;36(8):1628-39. https://doi.org/10.1002/tox.23158.
47. Jiang Y, Chen F, Ren X, Yang Y, Luo J, Yuan J, et al. Rna-binding protein col14a1, tns1, nusap1 and ywhae are valid biomarkers to predict peritoneal metastasis in gastric cancer. Front Oncol. 2022;12:830688. https://doi.org/10.3389/fonc.2022.830688.
48. Wu X, Wang H, Lian Y, Chen L, Gu L, Wang J, et al. Gtse1 promotes cell migration and invasion by regulating emt in hepatocellular carcinoma and is associated with poor prognosis. Sci Rep. 2017;7(1):5129. https://doi.org/10.1038/s41598-017-05311-2.
49. Guo L, Zhang S, Zhang B, Chen W, Li X, Zhang W, et al. Silencing gtse-1 expression inhibits proliferation and invasion of hepatocellular carcinoma cells. Cell Biol Toxicol. 2016;32(4):263-74. https://doi.org/10.1007/s10565-016-9327-z.
50. Xu T, Ma M, Chi Z, Si L, Sheng X, Cui C, et al. High g2 and s-phase expressed 1 expression promotes acral melanoma progression and correlates with poor clinical prognosis. Cancer Sci. 2018;109(6):1787-98. https://doi.org/10.1111/cas.13607.
51. Zhang F, Meng J, Jiang H, Feng X, Wei D, Meng W. Gtse1 facilitates the malignant phenotype of lung cancer cells via activating akt/mtor signaling. Anal Cell Pathol. 2021;2021:5589532. https://doi.org/10.1155/2021/5589532.