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Clinicopathological and Prognostic Values of Gankyrin, Snail1, and IDH1 Expression in Renal Cell Carcinoma Patients

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Abstract

Background: Gankyrin is an oncoprotein incriminated in cancer growth, invasion, and spread. Snail1 is associated with mesenchymal features acquisition that is related to invasion and metastasis of malignant cells. Isocitrate dehydrogenase 1(IDH1) has been found to be mutated in several cancers, which leads to altered cellular metabolism and tumorgenesis. The present study aimed to assess Gankyrin, Snail1, and IDH1 expression patterns and compare them to clinicopathological and prognostic parameters.

Method: In our prospective cohort study, the samples taken from 60 renal cell carcinoma (RCC) patients were processed, diagnosed, graded, staged, and subjected to immunohistochemistry for Gankyrin, Snail1, and IDH1. The patients were chosen, treated, and followed up from January 2015 to December 2019. Overall survival (OS) and progression-free survival (PFS) were assessed.

Results: High expression levels of Gankyrin were positively associated with high grade ($P = 0.003$), stage ($P = 0.033$), and size of the tumor ($P = 0.049$), in addition to lymph nodes metastasis ($P = 0.01$), distant metastases ($P = 0.007$), higher incidence of tumor progression, unfavorable 5-year PFS, and OS rates ($P < 0.001$). High expression levels of Snail1 were positively associated with high grade ($P = 0.004$) and stage ($P = 0.023$) of the tumors, on top of lymph nodes metastasis ($P = 0.003$), distant metastases ($P = 0.002$), higher incidence of tumor progression, poorer five-year PFS, and OS rates ($P < 0.001$). High expression levels of IDH1 were negatively associated with low grade ($P = 0.002$), stage, and size of the tumor and lymph nodes metastasis ($P < 0.001$), distant metastases ($P = 0.041$), lower incidence of tumor progression ($P = 0.013$), better five-year PFS, and OS rates ($P < 0.041$).

Conclusion: We indicated the associations between poor RCC pathological parameters, unfavorable patients' outcome, high Gankyrin, high Snail1, and reduced IDH1 expression.

Keywords: Carcinoma, Renal cell, Immunohistochmistry, Prognosis

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Introduction

Renal cell carcinoma (RCC) is the most prevalent adult malignant kidney tumor which is ranked as the 6th and 10th most common malignancy in males and females, respectively.[1] It is usually localized to the kidney and in most cases, it is surgically resectable, but recurrent. Metastatic RCC have limited treatment options and poorer prognosis.[2] RCC is a heterogeneous tumor with marked variability between patients, which is important in individualized management strategies.[3]

Before, RCC risk stratification and determining treatment strategies were known to depend on clinical-pathological parameters, such as grade, stage, size, the presence of necrosis, and predictive biomarkers of the clinical outcome of RCC patients, yet none of these markers are conclusive in accurate decision making for therapy, which paves the way to novel prognostic and predictive markers for RCC outcomes.[4] Gankyrin is an onco-protein encoded by PSMD10 gene that is incriminated in cancer growth, invasion, and spread.[5]

Epithelial-mesenchymal transition (EMT) is the process playing a pivotal role in cancer invasion and spread.[6] EMT has been found to be controlled by numerous factors; the most important one is believed to be Snail1 that is a zinc-finger transcription factor, which is found to be overexpressed in plenty of malignancies. Snail1 is incriminated in mesenchymal features acquisition, which is associated with invasion and metastasis of malignant cells.[7] Isocitrate

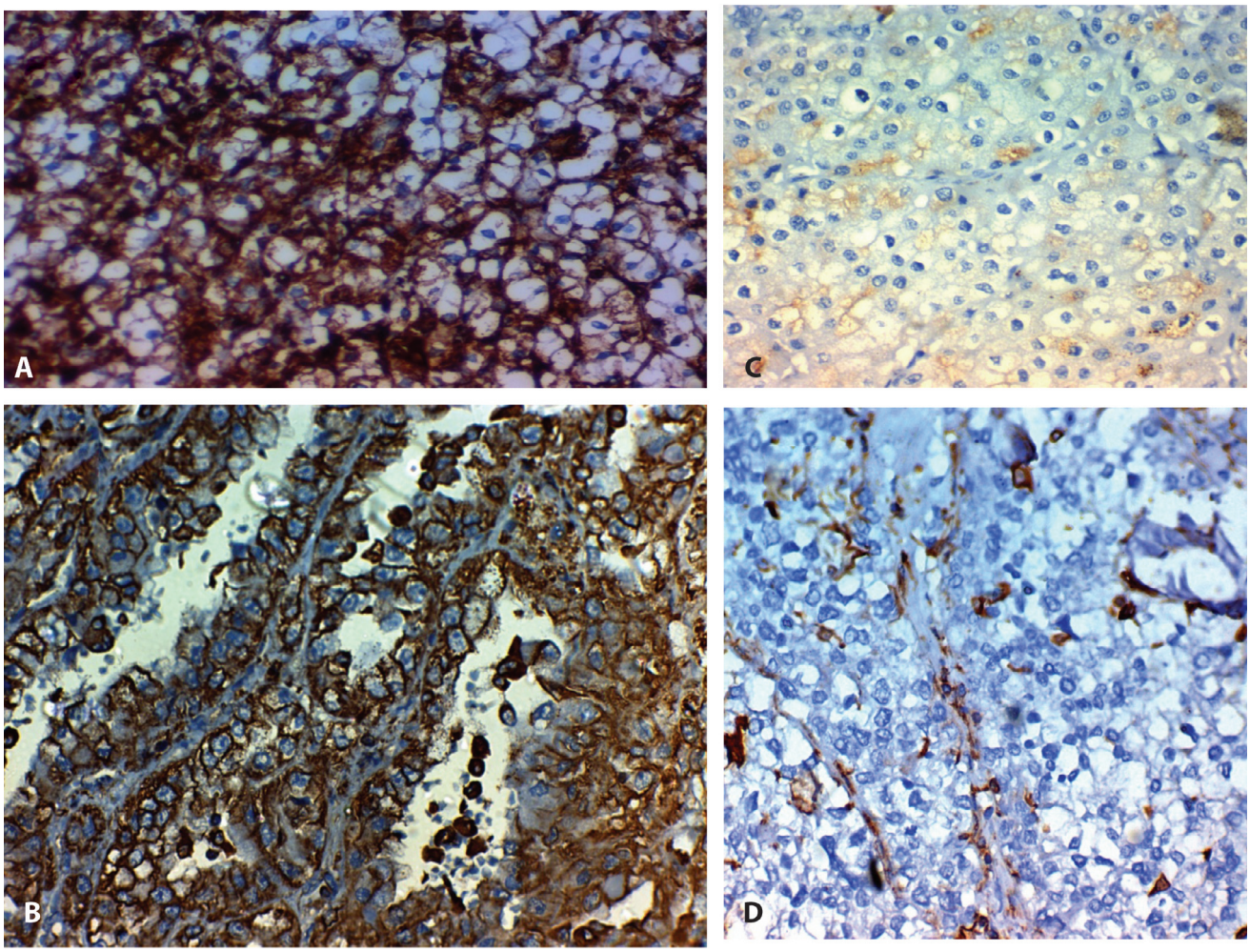

Figure 1. Immunohistochemical expression of Gankyrin in RCC: (A) Positive cytoplasmic expression in high grade and stage clear cell RCC 400×; (B) Positive cytoplasmic expression in high grade and stage papillary RCC 400×; (C) Low cytoplasmic expression in low grade and stage chromophope RCC 400×; (D) Negative cytoplasmic expression in low grade and stage clear cell RCC 400×.
RCC: Renal cell carcinoma

dehydrogenases (IDHs) are a family composed of three members: IDH1, 2, and 3. IDH1 is known to be responsible for conversion of isocitrate to alpha-ketoglutarate (aKG). IDH1 is mutated in many cancers, which leads to altered cellular metabolism and oncogenesis.[8]

The exact prognostic and predictive roles of Gankyrin, Snail1, and IDH1, as novel biomarkers

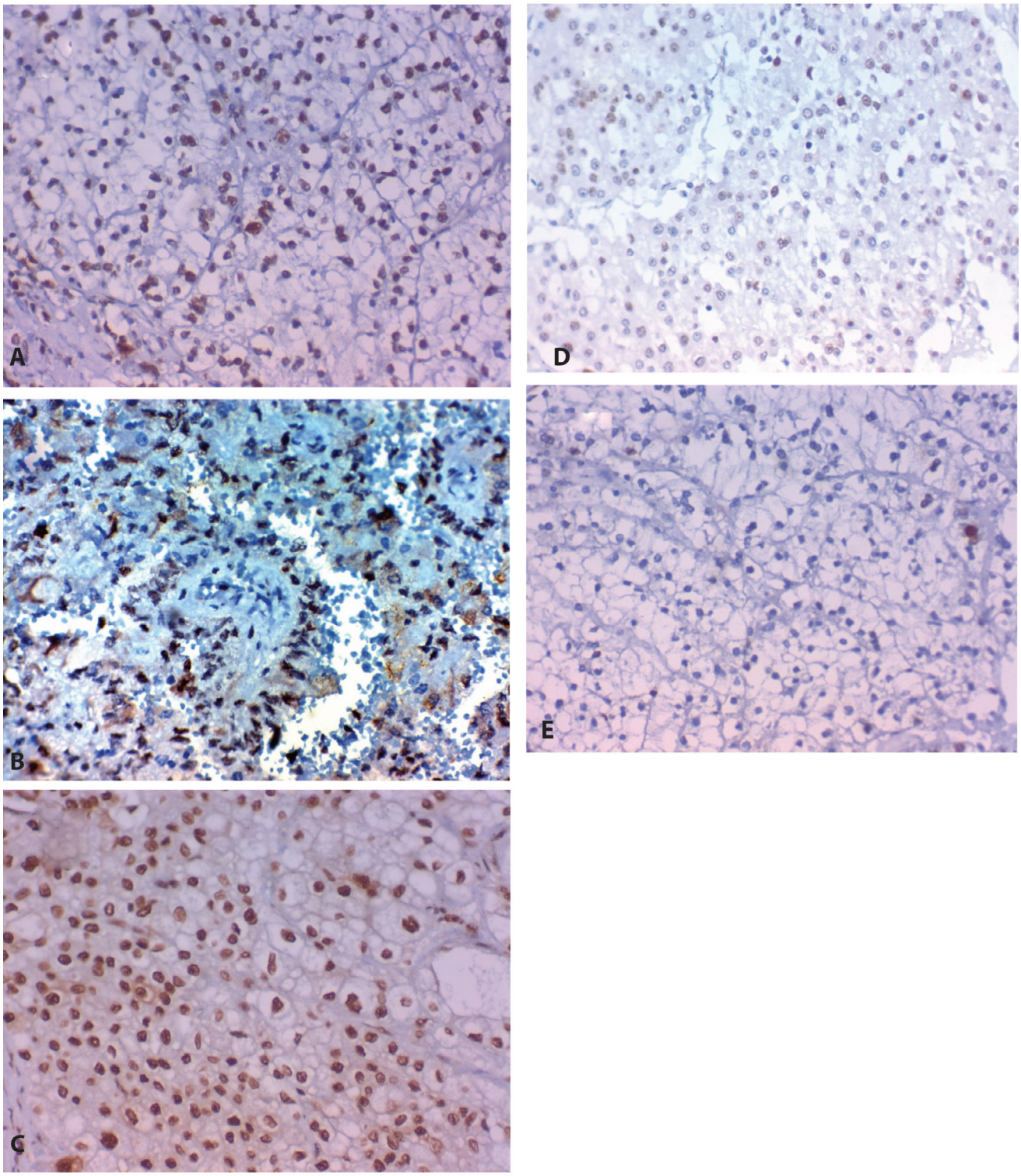

Figure 2. Expression of Snail1 in RCC: (A) Positive nuclear expression in high grade and stage clear cell RCC grade 400×; (B) Positive nuclear expression in high grade and stage papillary RCC 400×; (C) Low nuclear expression in low grade and stage chromophope RCC 400×; (D) Negative nuclear expression in low grade and stage clear cell RCC 400×.
RCC: Renal cell carcinoma

Table1. Association of clinic-pathological features with Gankyrin, Snail1, and IDH1 expression in the RCC patients

RCC		Gankyrin		*P*	Snail1		*P*	IDH1		*P*
		Negative	Positive		Negative	Positive		Negative	Positive	
		23	37		25	35		42	18	
Age	<55y	10 (43.4%)	13 (35.1%)	0.285	11 (44.0%)	12 (34.2%)	0.547	15 (35.7%)	8 (44.4%)	0.51
	>55y	13 (56.5%)	24 (64.8%)		14 (56.0%)	23 (56.7%)		27 (64.2%)	10 (55.5%)	
Sex										
	Male	16 (69.5%)	31 (83.7%)	0.119	18 (72.0%)	29 (82.8%)	0.255	32 (76.1%)	15 (83.3%)	0.406
	Female	7 (30.4%)	6 (16.2%)		7 (28.0%)	6 (17.1%)		10 (23.8%)	3 (16.6%)	
Grade										
	1	10 (43.4%)	4 (10.8%)	0.003	10 (40.0%)	4 (11.4%)	0.004	6 (14.2%)	8 (44.4%)	0.002
	2	10 (43.4%)	13 (35.1%)		12 (48.0%)	11 (31.4%)		13 (30.9%)	10 (55.5%)	
	3	3 (13.04%)	13 (35.1%)		2 (8.0%)	14 (40.00%)		16 (38.09%)	0 (0.0%)	
	4	0 (0.0%)	7 (18.9%)		1 (4.0%)	6 (17.1%)		7 (16.6%)	0 (0.0%)	
Size										
	<7cm	9 (39.1%)	8 (21.6%)	0.049	9 (36.0%)	8 (22.8%)	0.647	5 (11.9%)	12 (66.6%)	<0.001
	>7cm	14 (60.8%)	29 (78.3%)		16 (64.0%)	27 (77.1%)		37 (88.09%)	6 (33.3%)	
T										
	1	8 (34.7%)	8 (21.6%)	0.046	8 (32.0%)	8 (22.8%)	0.025	3 (7.1%)	13 (72.2%)	<0.001
	2	11 (47.8%)	8 (21.6%)		12 (48.0%)	7 (20.00%)		14 (33.3%)	5 (27.7%)	
	3	5 (21.7%)	10 (27.02%)		5 (20.0%)	10 (28.5%)		15 (35.7%)	0 (0.0%)	
	4	1 (4.3%)	9 (24.3%)		0 (0.0%)	10 (28.5%)		10 (23.8%)	0 (0.0%)	
N										
	0	17 (73.9%)	16 (43.2%)	0.01	19 (76.0%)	14 (40.00%)	0.003	15 (35.7%)	18 (100.0%)	<0.001
	1	6 (26.08%)	21 (56.7%)		6 (24.0%)	21 (60.00%)		27 (64.2%)	0 (0.0%)	
M										
	0	23 (100.0%)	24 (64.8%)	0.007	25 (100.0%)	22 (62.8%)	0.002	29 (69.04%)	18 (100.0%)	0.041
	1	0 (0.0%)	13 (35.1%)	0 (0.0%)	13 (37.1%)	13 (30.9%)	0 (0.0%)			
Stage										
	I	8 (34.7%)	5 (13.5%)	0.033	8 (32.0%)	5 (14.2%)	0.023	1 (2.3%)	12 (66.6%)	<0.001
	II	9 (39.1%)	12 (32.4%)		12 (48.0%)	9 (25.7%)		17 (40.4%)	4 (22.2%)	
	III	6 (26.08%)	9 (24.3%)		5 (20.0%)	10 (28.5%)		13 (30.9%)	2 (11.1%)	
	IV	0 (0.0%)	11 (29.7%)	0 (0.0%)	11 (31.4%)	11 (26.1%)	0 (0.0%)			

T: Tumor; N: Node; M: Metastases; IDH1: Isocitrate dehydrogenase 1; RCC: Renal cell carcinoma

for RCC, have not been yet understood.

Accordingly, the current work was conducted to assess Gankyrin , Snail1, and IDH1 expression patterns and degree in cells and tissue of RCC comparing these expression parameters to histopathological ones, such as grade, stage, clinical parameters, like patients age or sex, and prognostic parameters, like tumor progression and survival.

Methods

The current prospective cohort study comprised a total of 60 RCC patients who were operated in General Surgery and Urology Departments, Faculty of Medicine, Zagazig University hospitals. The Ethical Committee of Faculty of Medicine, Zagazig University approved the present work (Code No.: 2437). The surgically excised samples were sent to Pathology Department, Faculty of Medicine, Zagazig University, where they were processed, diagnosed, graded, staged, and subjected to immunohistochemistry (IHC) for Gankyrin, Snail1, and IDH1. The cases were staged according to tumor, node, and metastases (TNM) classification of the AJCC (the American Joint Committee on Cancer) 2010[9] and graded according to (European Association of Urology) EAU guidelines (2014) and ISUP (International Society of Urological Pathology, 2012) consensus.[10] The patients were selected, treated, and followed up from January 2015 to December 2019 in Clinical Oncology and Nuclear Medicine Department, Faculty of Medicine, Zagazig University. Ultimately, the survival (OS) and progression-free survival (PFS) were assessed. The subjects with localized disease underwent partial or radical nephrectomy according to its stage followed by active surveillance. The advanced and relapsed cases received Sunitinib as the first-line treatment (50 mg/day oral for 28 day every 6 weeks). Pazopinib was administered as the second-line treatment (800 mg/day oral either 1 hour before or two hours after meals). Palliative supportive care was done for those who failed on the second line treatment. Palliative radiotherapy and Zoledronic acid (Zomita) were

administered for the patients with bone metastasis. Written informed consent was obtained from all of our patients.

IHC

IHC was carried out using primary monoclonal antibodies, namely Anti-Gankyrin antibody (ab182576), Anti- Snail1 antibody (ab53519), and Anti-IDH1 antibody (ab215829) (dilution 1; 100, abcam, USA). We considered positive cytoplasmic expression as Gankyrin and IDH1 positive and positive nuclear expression as Snail1 positive.[11]

Assessment of Gankyrin, Snail1, and IDH1 expression in the stained tissues

The quantification of the expression of the included markers in the cells of RCC was performed utilizing a combined of multiplication of intensity and extent scores. The intensity scores were: 0 (no stain), 1 (weak stain), 2 (moderate stain), and 3 (strong stain); the percentage scores were: 1 (from zero to 25%), 2 (from 26 to 50%), 3 (from 51 to 75%), or 4 (more than or equal 75%). The cut-off value of the combined score equaled 6, above which was considered to be high, and below which was low expression.[12]

Statistical analysis

The collected data were computerized and statistically analyzed employing SPSS program (Statistical Package for Social Science) version 24. Chi square test (χ^2) and Fisher exact were used to determine the difference among the qualitative variables. Kaplan and Meier method were applied to estimate OS and PFS and log rank test compared with the survival curves (*P* value was considered to be significant at ≤ 0.05 levels and $P > 0.05$ indicated insignificant differences). OS is defined as the duration from the date of surgery to the date of death or last clinic visit and PFS is the duration from the date of surgery to the date when disease progression is identified via magnetic resonance imaging (MRI), computed tomography (CT), or the last clinic visit. Assessment of PFS and OS was done according to the markers. The univariate analysis was done using Cox proportional hazards analysis.

Results

Table 1 depicts the patients' data and association with the expression patterns of the included markers.

Gankyrin immune expression in the samples

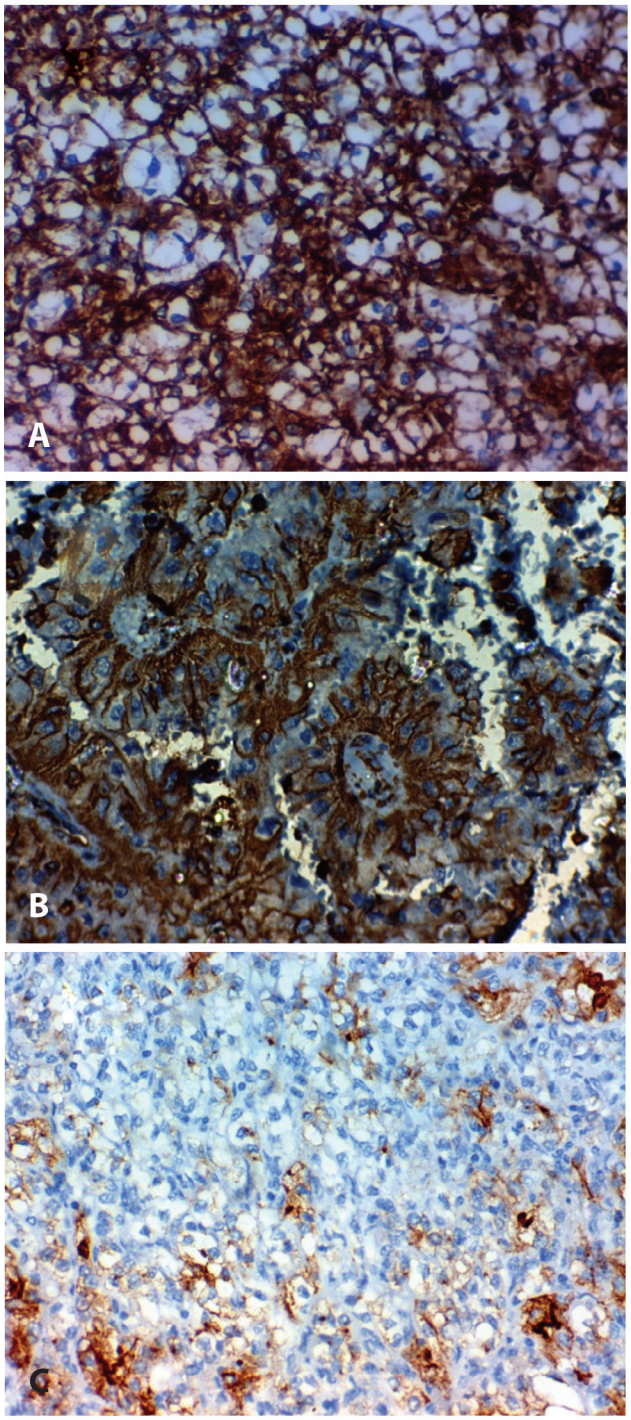

Figure 3. Immunohistochemical expression of IDH1 in RCC: (A) High cytoplasmic expression in low grade and stage clear cell RCC 400×; (B) High cytoplasmic expression in low grade and stage papillary RCC 400×; (C) Low cytoplasmic expression in high grade and stage clear cell.
RCC: Renal cell carcinoma; IDH1: Isocitrate dehydrogenase 1

Table 2. The outcome of the RCC patients concerning Gankyrin, Snail1, and IDH1 expression

RCC		Total	Gankyrin		*P*	Snail1		*P*	IDH1		*P*
		N=60	Negative	Positive		Negative	Positive		Negative	Positive	
			23	37		25	35		42	18	
Progression											
	Absent	28 (46.6%)	19 (82.6%)	9 (24.3%)	<0.001	20 (80.0%)	8 (22.8%)	<0.001	16 (38.09%)	12 (66.6%)	0.013
	Present	32 (53.3%)	4 (17.3%)	28 (75.6%)		5 (20.0%)	27 (77.1%)		26 (61.9%)	6 (33.3%)	
Survival status											
	Alive	38 (63.3%)	23 (100.0%)	15 (40.5%)	<0.001	25 (100.0%)	13 (37.1%)	<0.001	21 (50.0%)	17 (94.4%)	0.041
	Died	22 (36.6%)	0 (0.0%)	22 (59.4%)		0 (0.0%)	22 (62.8%)		21 (50.0%)	1 (5.5%)	

IDH1: Isocitrate dehydrogenase 1; RCC; Renal cell carcinoma

retrieved from the RCC patients and its association with clinicopathological parameters (Figure 1).

High expression levels of Gankyrin in the tissues of the RCC were found to be positively associated with high Fuhrman grade ($P = 0.003$), advanced stage ($P = 0.033$), large size of the tumor ($P = 0.049$), the presence of lymph nodes metastasis ($P = 0.01$), and distant metastases ($P = 0.007$).

Association with prognostic and outcome parameters

The patients with a high expression of Gankyrin showed a higher incidence of tumor progression and poorer five-year PFS and OS rates ($P < 0.001$) than those with a low expression of Gankyrin (Table 2 –Figure 4A and 5A).

Snail1 immune expression in the samples retrieved from the RCC patients (Figure 2)

Association with clinicopathological parameters

High expression levels of Snail1 in the tissues of the RCC were positively associated with high Fuhrman grade ($P = 0.004$), advanced stage ($P = 0.023$), the presence of lymph nodes metastasis ($P = 0.003$), and distant metastases ($P = 0.002$).

Association with prognostic and outcome parameters

The subjects with a high expression of Snail1 indicated a higher incidence of tumor progression and poorer five-year PFS and OS rates ($P < 0.001$) than those with a low expression of Snail1 (Table 2 –Figure 4B and 5B).

IDH1 immune expression in the samples retrieved from the RCC patients (Figure 3)

Association with clinicopathological parameters

High expression levels of IDH1 in the tissues of RCC were negatively associated with low Fuhrman grade ($P = 0.002$), advanced stage, larger size of the tumor, the presence of lymph nodes metastasis ($P < 0.001$), and distant metastases ($P = 0.041$).

Association with prognostic and outcome parameters

The patients with high expression of IDH1 showed a lower incidence of tumor progression ($P = 0.013$) and better five-year PFS and OS rates ($P < 0.041$) than those with a low expression of IDH1 (Table 2 –Figure 4C and 5C).

Univariate analysis

High expression of Gankyrin and Snail1 and low IDH1 expression were unfavorable independent predictors for OS and PFS of the RCC patients (OS, $P < 0.001$ for Gankyrin and Snail1 and $P = 0.019$ for IDH1; PFS, $P < 0.001$ for Gankyrin and Snail1 and $P = 0.003$ for IDH1). In addition, TNM stage and grade were considered as independent predictors for both OS and PFS (Table 3).

Discussion

This study demonstrated that Gankyrin expression was correlated with progression and poor patients' prognosis. Additionally, it considered a poor prognostic risk factor for RCC progression and unfavorable survival. Similar results were reported by Wang et al.[4] in RCC patients and Huang et al.[12] in gastric cancer patients. Wang et al.[4] and Huang et al.[12] respectively showed that high Gankyrin expression levels led to promotion of RCC and gastric carcinogenesis, since it is incriminated in tumor high rate of growth, vascular invasion, and metastasis of cancer cells in addition to the association with poor survival.

Wang and Cheng[11] reported similar results.

They considered Gankyrin as a potential predictive and prognostic marker for several tumors. Wang et al.[4] added Gankyrin to established clinical prognostic parameters, as nuclear grade and TNM stage, which resulted in the improvement of prognostic accuracy in expecting survival rates of RCC patients.

Thus, we suggested that Gankyrin might be considered as a predictor of RCC patients' outcome, particularly if combined with current prognostic parameters.

Previous researchers have studied novel predictors of RCC outcome, which might allow the establishment of selection of better treatment strategies for RCC patients and improving their prognosis.[13, 14] However, the results were not conclusive.

In the present study, we assessed the expression of three novel markers, namely Gankyrin, Snail1, and IDH1. Gankyrin has been previously found to be of a significant oncogenic role in proliferation of tumor cells; its expression has been found to increase in plethora of tumors as hepatocellular carcinoma (HCC). This is because Gankyrin is able to increase the activity of hypoxia-inducible factor-1(HIF-1), promote the production of VEGF, and stimulate tumor angiogenesis.[15]

Moreover, high expression of Gankyrin has been found to result in the inhibition of p53 and pRb, which are established as tumor suppressor genes.[16] Gankyrin has several oncogenic roles due to activation of several pathways incriminated in RCC carcinogenesis, for instance, Wnt/β-Catenin, STAT3/Akt, NF-κB, and RhoA/ROCK, which explain why the increased expression of Gankyrin leads to aggressive phenotype RCC.[4]

Based on our results, inhibition of Gankyrin, as a novel management strategy for RCC, could be considered as a therapeutic strategy.

Panobinostat (LBH589) has been considered a novel anticancer agent that acts through the downregulation of the pathway of Gankyrin/STAT3/Akt.[17] Additionally, the newly-detected cjoc42 binds to Gankyrin and inhibits its activity in order to prevent p53 protein reduction, which leads to restoration of p53-dependent sensitivity to DNA damage and

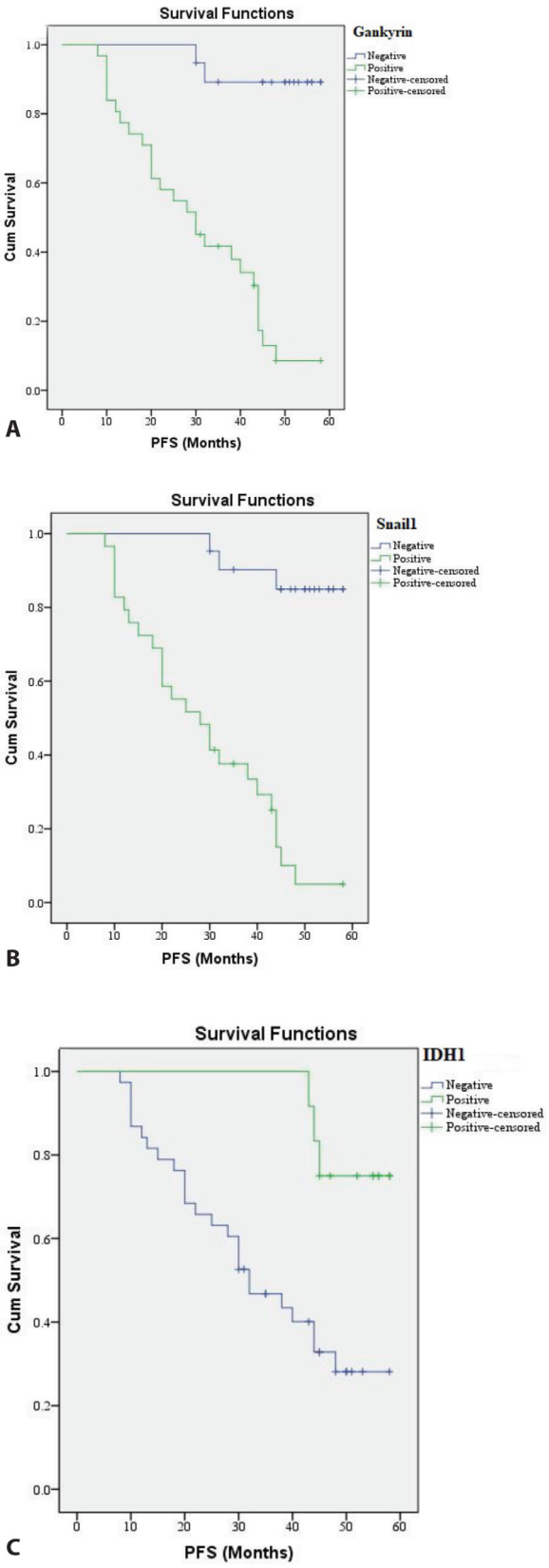

Figure 4. This figure shows: A) 5-year PFS regarding Gankyrin expression; B: 5-year PFS regarding Snail1 expression; C: 5-year PFS regarding IDH1 expression.

PFS: Progression-free survival, Cum: Cumulative; IDH1: Isocitrate dehydrogenase 1

apoptosis.[18] Meanwhile, all the mechanisms of tumorgenecity of Gankyrin are not fully understood. In the current study, we tried to shed light on the association between Gankyrin and the EMT transcription factor Snail1.

Overexpression of Snail1 was observed to be positively associated with Ganykrin expression, high grade, and progression of RCC.[8,7,19-23] We obtained similar results regarding the positive associations between Snail1 expression and RCC stage and grade and progression. There are several established mechanisms regarding the role of Snail1 in RCC progression through activation of EMT and disturbances in the axis of IDH/Snail1, as it has been previously shown that the molecular signature of IDH1low/Snail high is a poor prognostic biomarker in breast cancer,[24] yet the roles of IDH1 was not sufficiently described in RCC. IDH1 is an NADP-dependent enzyme that controls oxidation-dependent cell damage. It is considered as a tumor suppressor factor and its inactivation has an essential role in oncogenesis.[25] IDH1 has been studied in numerous types of cancer, yet its roles in RCC still remains unclear.

In the current study, we found a significant inverse association between IDH1 expression, Gankyrin expression, Snail1 expression, and poor outcome of RCC patients.

Furthermore, we showed that levels of IDH1 expression are a risk factor for the survival of RCC patients. Laba et al.[8] obtained similar results, in addition to stating that the expression of IDH1 in RCC increased the prognostic accuracy of the established risk factors, including stage, grade, and tumor size.

The oncogenic roles of gene mutations of IDH1 and its loss, which leads to RCC carcinogenesis, have been reported in several types of cancer in previous reports,[26-28] which is in line with our findings. IDH1 forms an important pathway for regeneration of NADPH in normal cells that could be able to regulate glutathione (GSH) and thioredoxin levels in cells.[29] This protects cells from reactive oxygen species (ROS) associated with oxidative cell DNA damages.[30-31]

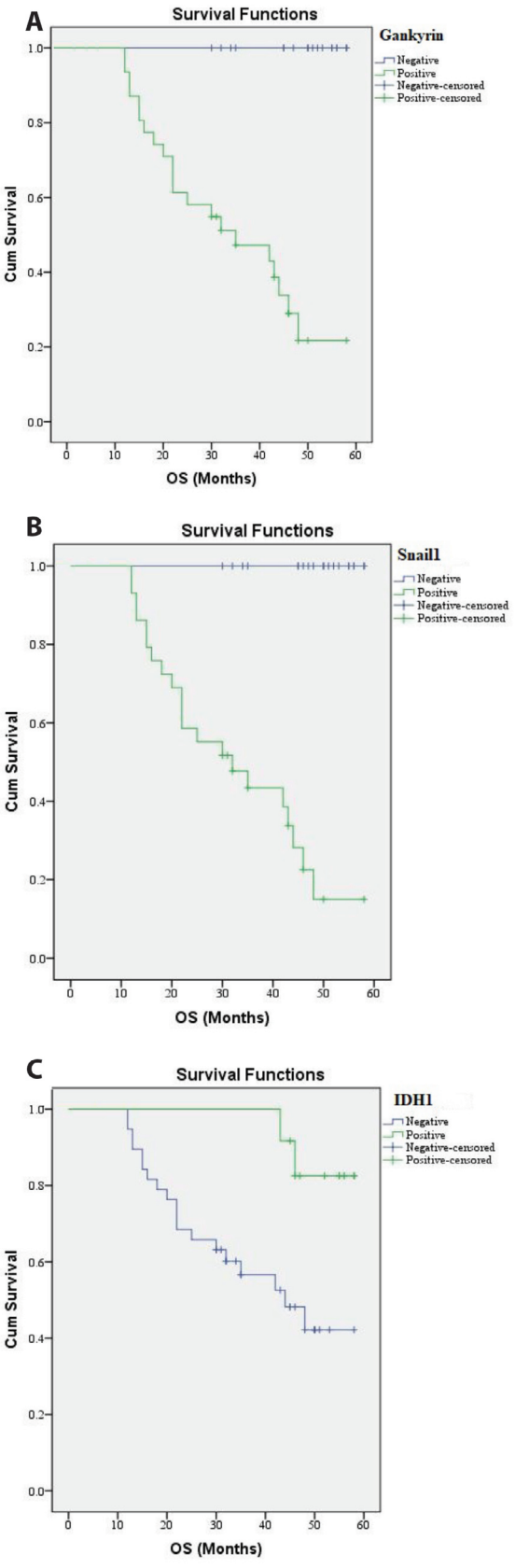

Figure 5. This figure shows A) 5-year OS regarding Gankyrin expression; B) 5-year OS regarding Snail1 expression; C) 5-year OS regarding IDH1 expression.
OS: Overall survival; Cum: Cumulative; IDH1: Isocitrate dehydrogenase 1

Table 3. Univariate analysis of overall and progression-free survival associated with the studied parameters

Variables	5-year Overall survival rate (%)	*P*-value	5-year Progression-Free survival rate (%)	*P*-value
Age group				
<55y	56.4%	0.748	57.0%	0.174
>55y	48.1%		29.3%	
Sex				
Male	51.2%	0.744	36.3%	0.368
Female	60%		60.0%	
Size				
<7 Cm	59.3%	0.249	53.8%	0.093
>7 Cm	55.4%		36.3%	
Grade				
1	80%	0.002	83.3%	0.003
2	63.5%		49.9%	
3	29.6%		46.7%	
4	20%		0.0%	
Stage				
Stage I	71.1%	0.004	63.6%	< 0.001
Stage II	65.5%		59.8%	
Stage III	45.5%		24.2%	
Stage IV	37.5%		0.0%	
Gankyrin				
Negative	100%	< 0.001	84.9%	< 0.001
Positive	15%		5%	
Snail1				
Negative	100%	< 0.001	89.4%	< 0.001
Positive	21.7%		8.7%	
IDH				
Negative	42.2%	0.019	75.0%	0.003
Positive	82.5%		28.1%	

IDH1: Isocitrate dehydrogenase 1

Limitations

The limited number of patients, short period of follow-up, and the use of only immunohistochemistry for markers evaluation are considered as the limitations in our study.

Hence, we could recommend that a prospective cohort study be conducted with a large number of cases and utilizing other methods of evaluation of the markers as gene studies.

Conclusion

In the current research, we exhibited the associations between poor RCC pathological parameters, unfavorable patients' outcome, high Gankyrin, high Snail1, and reduced IDH1 expression, which suggested the correlation between the development of EMT, disturbed apoptosis, oxidation, RCC progression, and dismal outcome. Thus, such markers could be useful for detecting the degree of aggressiveness of RCC and could facilitate the detection of targeted therapy.

Conflict of Interest

None declared.

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