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Original Article

Proliferation markers RacGAP1 and Ki-67 in gastrointestinal stromal tumors by immunohistochemistry with respect to clinicopathological features and different risk stratification systems

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Abstract: RacGAP1 is a protein associated with cell proliferation, cell growth regulation, cell transformation and metastasis. The present study was designed to evaluate RacGAP1 expression in gastrointestinal stromal tumors (GISTs) for the first time in the literature and to determine its association with some predictive clinicopathological features, Ki-67 proliferation index, and risk stratification systems of Armed Forces Institute of Pathology (AFIP) and modified National Institutes of Health (NIH). Paraffin-embedded tissues of 100 GISTs were investigated, retrospectively. High (≥10%) Ki-67 proliferation index, higher mitotic count, high cellularity, small intestinal location, and high-risk groups according to both AFIP and modified NIH criteria were found to be correlated with RacGAP1 positivity in the univariate analysis (all *P value*s <0.05). The association between RacGAP1 expression and higher cellularity was supported by the multivariate analysis (P=0.023). High (≥10%) Ki-67 proliferation index was correlated with higher nuclear pleomorphism, necrosis, ulceration, small intestinal location, greater tumor size, higher mitotic count, and high risk group according to AFIP and NIH criteria in the univariate analysis (all *P value*s <0.05). The correlation of Ki-67 proliferation index and mitotic count and high risk group according to AFIP criteria was confirmed by the multivariate analysis (all *P value*s <0.05). In conclusion, higher RacGAP1 expression and Ki-67 index might be considered as effective complementation of risk stratification systems and unfavorable clinicopathological features in predicting poor outcome of GISTs. However, the utility of RacGAP1 expression in GISTs should be further validated in larger cohorts of patients with long-term follow-up data.

Keywords: Clinicopathological features, gastrointestinal stromal tumor, immunohistochemistry, Ki-67, RacGAP1

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract that comprises 1-3% of all gastrointestinal malignant tumors [1, 2]. GISTs are characterized by a wide spectrum of tumors with variable malignant potential and unpredictable behavior, limiting the use of standard tumor-node-metastasis system in assessment of the prognosis [3-6]. Accordingly, several prognostic factors have been investigated in GISTs, while a risk stratification based assessment of the malignant potential is commonly used in the clinical setting [6, 7]. Most prevalent risk stratification systems include the National Institutes of Health (NIH) consensus criteria, the modified NIH consensus criteria, and the Armed Forces Institute of Pathology (AFIP) criteria [8-10].

Although high Ki-67 proliferation index, tumor necrosis, tumor rupture, and nuclear pleomorphism have been claimed to be the poor prognostic factors for GIST, there is still no reliable prognostic marker to predict the clinical behavior of GISTs in terms of malignant potential, progression and recurrence as well as resistance to therapy [9, 11-13].

RacGAP1 [Rac guanosine triphosphatase (GTPase)-activating protein 1] is a member of

GTPase activating proteins (GAPs) family localized in the mitotic spindle at metaphase and involved in inactivation of many Rho-mediated signals [14-16]. RacGAP1 has been considered to have crucial roles in cytokinesis during normal cell cycle as well as in cell growth regulation, differentiation, cell transformation, invasion, migration, tumorigenesis, progression, recurrence and metastasis [14-16]. Considerable interest exists in the potential prognostic role RacGAP1 overexpression in various tumors and its presence and poor prognostic role has recently been confirmed in breast cancer, hepatocellular carcinoma, gastric cancer, colorectal cancer, epithelial ovarian cancer, invasive cervical cancer, high grade meningioma and bronchopulmonary neuroendocrine neoplasms [14-18].

Increased mitotic potential and high Ki-67 proliferation index are the main characteristics of malignant tumors indicating progression and metastasis, while Rho GTPases have been related to prognostic markers, such as histological grade and proliferation index owing to their effect on Rac inactivation [5, 10, 14]. Hence, in a limited number of studies on the role of RacGAP1 expression in tumor proliferation, RacGAP1 upregulation was reported to be associated with high Ki-67 proliferation index for some tumors including epithelial ovarian carcinomas, invasive cervical cancer, high grade meningioma, and lung neuroendocrine tumors [14-16].

Besides, Ki-67 and RacGAP1 has been considered to be candidate proliferation-associated markers in breast cancer, being members of a cell cycle associated sub-network associated with a poor prognosis, while with regulatory differences according to their positions in network [19, 20].

However, no studies to date have investigated the presence and potential prognostic role of RacGAP1 in GIST. The present study was therefore designed to evaluate RacGAP1 expression in patients with GISTs for the first time in literature, and to determine its association with clinicopathologic prognostic factors, Ki-67 proliferation index and risk stratification.

Materials and methods

Study population

A total of 100 patients (mean ± SD age: 58.3±12.4 years, 53% were females) diagnosed with GIST between January 2008 and December 2014 at Gazi University School of Medicine, Ankara (n=96) and Bozok University School of Medicine, Yozgat (n=4) were included in this retrospective study. The GIST diagnosis were established based on the standard immunohistochemical diagnosis and differential diagnosis panel antibodies including CD117, CD34, SMA (smooth muscle actin), desmin, S-100 and Ki-67, and confirmed by positive immunohistochemical staining for CD117 and/or CD34 in accordance with histopathological features of GIST.

Presence of available records on clinicopathological characteristics and accessible pathology material were the inclusion criteria of the study. Patients with no accessible pathologic material (referral patients) were excluded from the study.

The study was conducted in full accordance with local Good Clinical Practice guideline and current legislations, while the permission was obtained from Bozok University Ethics Committee for the use of patient data for publication purposes.

Study parameters

Data on patient demographics (age, gender), clinicopathological features of GISTs [tumor size (cm), mitotic count in 50 high power fields (HPFs), cell types, cellularity (mild, moderate, high), nuclear pleomorphism (mild, moderate, high), necrosis, hemorrhage, ulceration, tumor location and growth pattern (expansive, infiltrative)] were retrieved from pathology reports and re-evaluated. GISTs were further evaluated for re-assessment of Ki-67 proliferation index scores, immunohistochemical analysis of RacGAP1 expression and risk stratification based on AFIP and modified NIH criteria.

Clinicopathological features of GISTs was analyzed in the overall study population as well as with respect to RacGAP1 expression [negative (score 0) vs. positive (scores 1-3)], Ki-67 proliferation index (<10% vs. ≥10%) and risk stratification (low risk vs. high risk) groups. Risk stratification was also evaluated with respect to RacGAP1 expression and Ki-67 proliferation index. Multivariate logistic regression analysis was performed to determine risk factors predicting higher risk status, higher Ki-67 proliferation index and higher RacGAP1 expression.

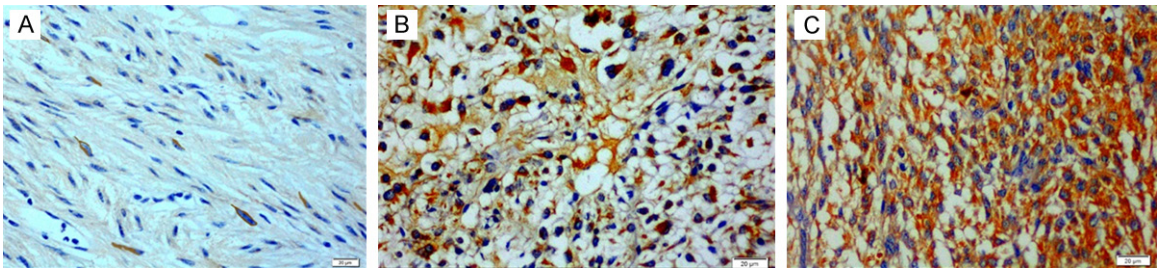

Figure 1. Photomicrographs of RacGAP1 expression in representative GIST cases. A: Score 1 RacGAP1 positivity in the tumor cells of a case with low risk category; B: Score 2 RacGAP1 positivity in the tumor cells of a case with low risk category; C: Score 3 RacGAP1 positivity in the tumor cells of a case with high risk category [Streptavidin-biotin-peroxidase method, original magnification 400×, each].

Immunohistochemistry

Paraffin blocks were cut into 4-μm sections, deparaffinized and dehydrated according to standard protocols. Then, immunohistochemistry was performed using the streptavidin-biotin-peroxidase method for RacGAP1 [1:500, rabbit polyclonal antibody; Abcam, Cambridge, United States of America] in automated stainer [Leica Bond-Max, Leica Biosystems, United Kingdom]. Testis tissue was used as a positive control of RacGAP1. For negative control, the primary antibody was omitted. Both cytoplasmic and nuclear staining was considered as positive for RacGAP1. Five random HPFs were examined to count immune-reactive cells under light microscope. Immunostaining of RacGAP1 was scored in accordance with the following criteria: score 0 = no staining, score 1 <10% staining, score 2 = 10% to 60% staining, score 3 = 61% to 100% staining.

Staining for Ki-67 was examined with 4× and 10× object lenses to identify the area of most intense staining (hot spot), while scoring was performed by counting at least 500 tumor cells in HPFs with a 40× object lens (BX53F, Olympus, Tokyo, Japan). All brown-stained nuclei, regardless of staining intensity, were counted as positive. Ki-67 proliferation index was evaluated in two groups (<10% and ≥10%) based on percentage of staining cells.

Risk stratification

Risk stratification was based on two different systems including AFIP criteria and modified NIH criteria [9, 10].

According to AFIP criteria that incorporate tumor site as well as tumor size and mitotic count, tumor size was categorized into four groups: <2 cm, >2 to ≤5 cm, >5 to ≤10 cm, and >10 cm, while mitotic count is classified into two groups: ≤5 or >5 mitoses per 50 HPFs [10].

In accordance with the modified NIH criteria, prognosis of GISTs was categorized into four subgroups (very low, low, intermediate or high risk) based on mitotic count and tumor size, similar to original NIH consensus criteria, and also on two additional prognostic factors incorporated into the modified model including primary tumor site and tumor rupture [8, 9]. Accordingly, cases with mitotic count >10/50 HPFs and tumor size >10 cm, or mitotic count >5/50 HPFs and tumor size >5 cm were classified as the high risk group for gastric tumors, whereas for non-gastric GISTs, tumor size >5 cm (regardless of the mitotic count) and tumor size >2 cm (if mitotic activity of >5/50 HPFs) were also classified into the high risk group. All cases with the presence of a tumor rupture were also classified as the high risk group.

For analysis purposes, high risk group was considered as “high-risk group” per se, while the remaining groups were combined under category of “low-risk groups” for both modified NIH and AFIP risk stratification models.

Statistical analysis

Statistical analysis was made using MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; 2013). Chi-square (χ^2) and Fisher-Exact tests were used for the comparison of categorical data, while numerical data were analyzed using Student-t test and Mann-Whitney U test for variables with normal distribution and for non-normally dis-

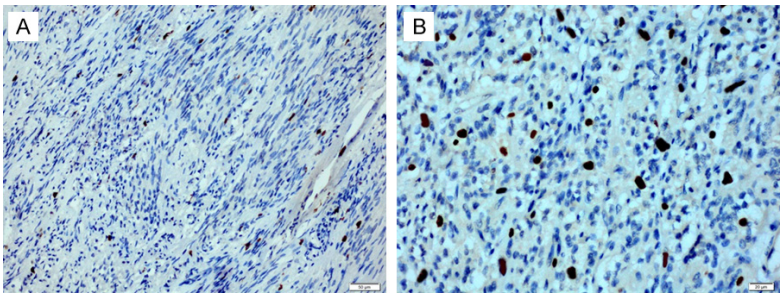

Figure 2. Photomicrographs of Ki-67 expression in demonstrative GIST cases. A: Low (<10%) Ki-67 proliferation index in the tumor cells of a case with low risk category; B: High (≥10%) Ki-67 proliferation index in the tumor cells of a case with high risk category [Streptavidin-biotin-peroxidase method, original magnification 200×, 400×, respectively].

tributed variables, respectively. Logistic regression analysis was performed with consideration of risk stratification, Ki-67 proliferation index and RacGAP1 expression (categorical) as the dependent variables while "Enter" was selected as the method and "simple first" as the categorical variable coding scheme. Predictors with possible influence on dependent variable were added as covariates. Data were expressed as "mean ± standard deviation (SD)", minimum-maximum, percent (%) and 95% confidence interval (CI) where appropriate. P<0.05 was considered statistically significant.

Results

Clinicopathological features with respect to RacGAP1 expression and Ki-67 proliferation index

Overall, positive RacGAP1 expression (**Figure 1**) and high (≥10%) Ki-67 proliferation index (**Figure 2**) were noted in 69% and 19% of patients, respectively. Mean ± SD tumor size was 6.0±4.24 cm (5-10 cm in 41.0%) and mean ± SD mitotic count was 7.4±15.3 (≥5 mitosis in 33.3%) in 50 HPFs. Most of tumors were spindle cell type tumors (73.0%) with high-degree cellularity (44.0%), mild-to-moderate nuclear pleomorphism (73.0%), expansive growth pattern (92.0%) and gastric location (71.0%) with no signs of necrosis (70.0%), hemorrhage (97.0%) or ulceration (85.0%) (**Table 1**).

Significantly larger tumor size (9.16±5.76 cm vs. 5.26±3.46 cm, P<0.001), higher mitotic count (29.1±25.7 vs. 2.3±2.4, P<0.001) and higher likelihood of RacGAP1 positivity (89.5% vs. 64.2%, P=0.021), high degree nuclear pleomorphism (47.4% vs. 8.6%, P=0.001), necrosis (52.6% vs. 24.7%, P=0.025), ulceration (31.6% vs. 11.1%, P=0.036) and small intestinal location (36.8% vs. 8.6%, P=0.016) were noted in patients with high (≥10%) Ki-67 proliferation index compared to those with low (<10%) Ki-67 proliferation index (**Table 1**). No significant difference was noted between <10% and ≥10% Ki-67 proliferation index groups in terms of patient demographics, tumor cell type, degree of cellularity, presence of hemorrhage and growth pattern (**Table 1**).

Significantly higher mitotic count (9.3±16.9 vs. 3.8±11.5, P=0.002), higher likelihood of high degree cellularity (50.7% vs. 29.0%, P=0.018), high (≥10%) Ki-67 proliferation index (24.6% vs. 6.5%, P=0.017) and small intestinal location (18.8% vs. 3.2%, P=0.047) were noted in patients with positive RacGAP1 expression compared to those with negative RacGAP1 expression (**Table 1**). No significant difference was noted between RacGAP1-positive and RacGAP1-negative patients in terms of patient demographics, tumor size, tumor cell type, and degree of nuclear pleomorphism, presence of ulceration, hemorrhage or necrosis and growth pattern (**Table 1**).

Risk stratification with respect to RacGAP1 expression and Ki-67 proliferation index

High risk and low risk (very low-risk, low-risk and intermediate-risk) groups composed 22.0% and 76.0% of study population, respectively according to AFIP criteria; while 41.0% and 59.0% of patients were categorized into high risk and low risk groups, respectively according to modified NIH criteria (**Table 2**).

According to AFIP risk stratification, 73.7% (14 out of 19) of patients with high (≥10%) Ki-67 proliferation index and 30.3% (20 out of 66) of patients with score 2-3 positive RacGAP1 expression were classified in the high risk group (**Table 2**).

Table 1. Clinicopathologic features of GISTs in the overall study population and with respect to RacGAP1 expression and Ki-67 proliferation index

Characteristics	Overall (n=100)	RacGAP1 expression						Ki-67 proliferation index		
		Negative	Positive				*P value* negative vs. total positive	<10% (n=81)	≥10% (n=19)	*P value*
		Score 0 (n=31)	Score 1 (n=3)	Score 2 (n=16)	Score 3 (n=50)	Total (n=69)				
Gender, n (%)										
Female	53 (53.0)	19 (61.3)	2 (66.7)	9 (56.3)	23 (46.0)	34 (49.3)	0.287[1]	44 (54.3)	9 (47.4)	0.618[1]
Male	47 (47.0)	12 (38.7)	1 (33.3)	7 (43.8)	27 (54.0)	35 (50.7)		37 (45.7)	10 (52.6)	
Age (year)										
Mean ± SD	58.3±12.4	60.9±13.5	53.7±3.2	53.7±12.8	58.4±11.6	57.3±12.0	0.249[2]	58.1±1.8	59.3±10.9	0.700[2]
Median (min-max)	58.5 (21-84)	60 (21-84)	55 (50-56)	52.5 (32-80)	59 (27-77)	58 (27-80)		59 (21-84)	58 (40-82)	
RacGAP1, n (%)										
Score 0	31 (31.0)	-	-	-	-	-		29 (35.8)	2 (10.5)	0.021[1]
Score 1	3 (3.0)	-	-	-	-	-	-	3 (3.7)	0 (0)	
Score 2	16 (16.0)	-	-	-	-	-		9 (11.1)	7 (36.8)	
Score 3	50 (50.0)	-	-	-	-	-		40 (49.4)	10 (52.6)	
Mean ± SD	1.8±1.3	-	-	-	-	-		1.7±1.4	2.3±0.9	0.231[3]
Median (min-max)	2.5 (0-3)	-	-	-	-	-		2 (0-3)	3 (0-3)	
Ki-67, n (%)										
<10%	81 (81.0)	29 (93.5)	3 (100.0)	9 (56.3)	40 (80.0)	52 (75.4)	0.017[1]	-	-	-
≥10%	19 (19.0)	2 (6.5)	0 (0.0)	7 (43.8)	10 (20.0)	17 (24.6)		-	-	
Tumor size (cm)										
Mean ± SD	6.0±4.24	5.13±3.40	3.83±2.36	5.82±3.51	6.74±4.9	6.51±4.59	0.140[3]	5.26±3.46	9.16±5.76	<0.001[3]
Median (min-max)	5.1 (0.4-25)	5.0 (0.5-14)	3.0 (2.0-6.5)	5.35 (0.4-15)	5.5 (0.4-25)	5.5 (0.4-25)		4.8 (0.4-16.5)	6.5 (2.0-25)	
n (%)										
<2 cm	10 (10.0)	4 (12.9)	0 (0.0)	1 (6.3)	5 (10.0)	6 (8.7)	0.314[1]	10 (12.3)	0 (0.0)	0.020[1]
2-5 cm	34 (34.0)	11 (35.5)	2 (66.7)	5 (31.3)	16 (32.0)	23 (33.3)		31 (38.3)	3 (15.8)	
5-10 cm	41 (41.0)	14 (45.2)	1 (33.3)	9 (56.3)	17 (34.0)	27 (39.1)		31 (38.3)	10 (52.6)	
>10 cm	15 (15.0)	2 (6.5)	0 (0.0.0)	1 (6.3)	12 (24.0)	13 (18.8)		9 (11.1)	6 (31.6)	
Mitotic count (in 50 HPFs)										
Mean ± SD	7.4±15.3	3.8±11.5	1.7±2.1	10.4±16.3	8.9±17.3	9.3±16.9	0.002[3]			
Median (min-max)	3 (0-80)	1 (0-65)	1 (0-4)	5.5 (0-65)	3 (0-80)	3 (0-80)		2.3±2.4	29.1±25.7	<0.001[3]
n (%)										
<5	67 (67.0)	27 (87.1)	3 (100.0)	7 (43.8)	30 (60.0)	40 (57.9)	0.001[1]	65 (80.2)	2 (10.5)	<0.001[1]
≥5	33 (33.0)	4 (12.9)	0 (0.0)	9 (56.3)	20 (40.0)	29 (42.1)		16 (19.8)	17 (89.5)	
Cell type, n (%)										
Spindle	73 (73.0)	26 (83.9)	3 (100.0)	10 (62.5)	34 (68.0)	47 (68.1)	0.071[1]	61 (75.3)	12 (63.2)	0.347[1]
Mixed	21 (21.0)	3 (9.7)	0 (0.0)	4 (25.0)	14 (28.0)	18 (26.1)		16 (19.8)	5 (26.3)	
Epithelioid	6 (6.0)	2 (6.5)	0 (0.0)	2 (12.5)	2 (12.5)	4 (5.8)		4 (4.9)	2 (10.5)	

Cellularity, n (%)										
Mild	33 (33.0)	14 (45.2)	3 (100.0)	4 (25)	12 (24.0)	19 (27.5)	0.018[1]	30 (37)	3 (15.8)	0.065[1]
Moderate	23 (23.0)	8 (25.8)	0 (0.0)	2 (12.5)	13 (26.0)	15 (21.7)		20 (24.7)	3 (15.8)	
High	44 (44.0)	9 (29.0)	0 (0.0)	10 (62.5)	25 (50.0)	35 (50.7)		31 (38.3)	13 (68.4)	
Nuclear pleomorphism (atypia), n (%)										
Mild	62 (62.0)	19 (61.3)	3 (100.0)	8 (50)	32 (64.0)	43 (62.3)	0.314[1]	55 (67.9)	7 (36.8)	0.001[1]
Moderate	11 (11.0)	2 (6.5)	0 (0.0)	2 (12.5)	7 (14.0)	9 (13.0)		9 (11.1)	2 (10.5)	
High	16 (16.0)	4 (12.9)	0 (0.0)	4 (25.0)	8 (16.0)	12 (17.4)		7 (8.6)	9 (47.4)	
Absent	11 (11.0)	6 (19.4)	0 (0.0)	2 (12.5)	3 (6.0)	5 (7.2)		10 (12.3)	1 (5.3)	
Necrosis, n (%)										
Present	30 (30.0)	8 (74.2)	0 (0.0)	5 (31.3)	17 (34.0)	22 (31.9)	0.363[1]	20 (24.7)	10 (52.6)	0.025[1]
Absent	70 (70.0)	23 (25.8)	3 (100.0)	11 (68.8)	33 (66.0)	47 (68.1)		61 (75.3)	9 (47.4)	
Hemorrhage, n (%)										
Present	3 (3.0)	0 (0)	0 (0.0)	1 (6.3)	2 (4.0)	3 (4.3)	0.549[1]	3 (3.7)	0 (0.0)	1.00[1]
Absent	97 (97.0)	31 (100.0)	3 (100.0)	15 (93.8)	48 (96.0)	66 (95,7)		78 (96.3)	19 (100)	
Ulceration, n (%)										
Present	15 (15.0)	5 (16.1)	0 (0.0)	3 (18.8)	7 (14.0)	10 (14.5)	1.000[1]	9 (11.1)	6 (31.6)	0.036[1]
Absent	85 (85.0)	26 (83.9)	3 (100.0)	13 (81.3)	43 (86.0)	59 (85.5)		72 (88.8)	13 (68.4)	
Tumor location, n (%)										
Colon	4 (4.0)	1 (3.2)	0 (0)	0 (0)	3 (6.0)	3 (4.3)	0.047[1]	3 (3.7)	1 (5.3)	0.016[1]
Stomach	71 (71.0)	28 (90.3)	2 (66.7)	10 (62.5)	31 (62.0)	43 (62.3)		62 (76.5)	9 (47.4)	
Small intestine	14 (14.0)	1 (3.2)	0 (0)	2 (12.5)	11 (22)	13 (18.8)		7 (8.6)	7 (36.8)	
Duodenum	7 (7.0)	1 (3.2)	1 (33.3)	2 (12.5)	3 (6.0)	6 (8.7)		5 (6.2)	2 (10.5)	
Esophagus	4 (4.0)	0 (0)	0 (0)	2 (12.5)	2 (4.0)	4 (5.8)		4 (4.9)	0 (0.0)	
Growth pattern, n (%)										
Expansive	92 (92.0)	29 (93.5)	3 (100.0)	12 (75.0)	48 (96.0)	63 (91.3)	0.713[1]	76 (93.8)	16 (84.2)	0.174[1]
Infiltrative	8 (8.0)	2 (6.5)	0 (0.0)	4 (25.0)	2 (4.0)	6 (8.7)		5 (6.2)	3 (15.8)	

HPFs: High power fields. [1]Fisher Exact test, [2]Student t test, [3]Mann-Whitney U test.

Table 2. Risk stratification with respect to RacGAP1 expression and Ki-67 proliferation index

Risk stratification models	Total (n=100)	RacGAP1 expression				Ki-67 proliferation index	
		Score 0 (n=31)	Score 1 (n=3)	Score 2 (n=16)	Score 3 (n=50)	<10% (n=81)	≥10% (n=19)
AFIP-risk stratification, n (%)							
Low risk groups	76 (76.0)	28 (90.3)	3 (100.0)	9 (56.3)	36 (72.0)	72 (88.9)	4 (21.0)
Very low-risk	14 (14.0)	6 (19.4)	1 (33.3)	1 (6.2)	6 (12.0)	13 (16.0)	1 (5.3)
Low-risk	44 (44.0)	17 (54.8)	2 (66.7)	5 (31.3)	20 (40.0)	44 (54.3)	0 (0)
Intermediate-risk	18 (18.0)	5 (16.1)	0 (0.0)	3 (18.8)	10 (20.0)	15 (18.5)	3 (15.8)
High-risk group (*High-risk per se)*	22 (22.0)	2 (6.4)	0 (0.0)	6 (37.5)	14 (28.0)	8 (9.9)	14 (73.7)
Insufficient Data	2 (2.0)	1 (3.2)	0 (0.0)	1 (6.2)	0 (0.0)	1 (1.2)	1 (5.3)
Modified NIH-risk stratification, n (%)							
Low risk groups	59 (59.0)	24 (77.4)	3 (100.0)	7 (43.7)	25 (50.0)	57 (70.4)	2 (10.5)
Very low-risk	10 (10.0)	4 (12.9)	0 (0.0)	1 (6.3)	5 (10.0)	10 (12.3)	0 (0.0)
Low-risk	28 (28.0)	10 (32.3)	2 (66.7)	4 (25.0)	12 (24.0)	27 (33.3)	1 (5.3)
Intermediate-risk	21 (21.0)	7 (22.6)	1 (33.3)	2 (12.5)	8 (16.0)	20 (24.7)	1 (5.3)
High-risk group (*High-risk per se)*	41 (41.0)	7 (22.6)	0 (0.0.0)	9 (56.3)	25 (50.0)	24 (29.6)	17 (89.5)

AFIP: Armed Force Institute of Pathology, NIH: National Institutes of Health.

According to modified NIH risk stratification, 89.5% (17 out of 19) of patients with high (≥10%) Ki-67 proliferation index and 51.5% (34 out of 66) of patients with score 2-3 positive RacGAP1 expression were categorized into the high risk group (**Table 2**).

Clinicopathological features with respect to risk stratification

According to AFIP-based risk stratification, high-risk group had significantly higher mean ± SD RacGAP1 scores (2.4±0.9 vs. 1.7±1.4, P= 0.043), tumor size (10.44±5.32 cm vs. 4.66± 2.85 cm, P<0.001), mitotic count (20.6±22.2 vs. 3.6±10.3, P<0.001) along with higher rates of ≥10% Ki-67 proliferation index (63.6% vs. 5.3%, P<0.001), high-degree cellularity (72.7% vs. 35.5%, P=0.009), high-degree nuclear pleomorphism (36.4% vs. 9.2%, P=0.027), necrosis (50.0% vs. 22.4%, P=0.016), ulceration (31.8% vs. 9.2%, P=0.014) and small intestinal location (36.4% vs. 8.2%, P=0.009) (**Table 3**).

According to modified NIH risk stratification criteria, high-risk group had significantly higher mean ± SD RacGAP1 scores (2.3±1.1 vs. 1.5±1.4, P=0.017), tumor size (9.08±4.51 cm vs. 3.87±2.33 cm, P<0.001), mitotic count (15.5±21.5 vs. 1.7±1.9, P<0.001) along with higher rates of ≥10% Ki-67 proliferation index (41.5% vs. 3.4%, P<0.001), high-degree cellularity (63.4% vs. 30.5%, P=0.006), high-degree nuclear pleomorphism (34.1% vs. 3.4%, P= 0.001), necrosis (46.3% vs. 18.6%, P=0.003) and small intestinal location (31.7% vs. 1.7%, P=0.001) (**Table 3**).

Multivariate logistic regression analysis for factors predicting high risk status, higher Ki-67 proliferation index and RacGAP1 overexpression

Presence of ulceration (OR, 51.7; 95% CI, 2.08 to 1287.12; P=0.016), larger tumor size (OR, 1.07; 95% CI, 1.02 to 1.13; P=0.005) and higher Ki-67 proliferation index (OR, 59.3; 95% CI, 1.61 to 2184.01, P=0.026) were associated with increased likelihood of being categorized into high-risk group according to AFIP criteria (**Table 4**).

Larger tumor size (OR, 1.75; 95% CI, 1.05 to 2.93, P=0.033) and higher mitotic count (OR, 2347.85; 95% CI, 1.33 to 4150482.35, P= 0.042) were associated with increased likelihood of being categorized into high-risk group according to modified NIH criteria (**Table 4**).

Higher mitotic count (OR, 1.73; 95% CI, 1.23 to 2.43, P=0.002) and higher degree of cellularity (OR, 2.21; 95% CI, 0.66 to 7.38, P=0.023) were found to predict high Ki-67 proliferation index

Table 3. Clinicopathologic features of GISTs and with respect to risk stratification according to AFIP and modified NIH criteria

Characteristics	Risk stratification					
	AFIP			Modified NIH		
	Low risk groups	High risk group	*P value*	Low risk groups	High risk group	*P value*
Gender, n (%)						
Female	42 (55.3)	11 (50.0)	0.809[1]	35 (59.3)	18 (43.9)	0.156[1]
Male	34 (44.7)	11 (50.0)		20 (40.7)	23 (56.1)	
Age (year)						
Mean ± SD	58.4±13.3	57.2±9.3	0.617[2]	59.1±12.5	57.1±12.3	0.434[2]
Median (min-max)	59 (21-84)	58 (40-74)		59 (21-84)	58 (27-82)	
RacGAP1						
Mean ± SD	1.7±1.4	2.4±0.9	0.043[4]	1.5±1.4	2.3±1.1	0.017[4]
Median (min-max)	2 (0-3)	3 (0-3)		2 (0-3)	3 (0-3)	
Ki-67						
<10%	72 (94.7)	8 (36.4)	<0.001[3]	57 (96.6)	24 (58.5)	<0.001[3]
≥10%	4 (5.3)	14 (63.6)		2 (3.4)	17 (41.5)	
Tumor size (cm)						
Mean ± SD	4.66±2.85	10.44±5.32	<0.001[4]	3.87±2.33	9.08±4.51	<0.001[4]
Median (min-max)	4.25 (0.4-14)	10.75 (2.0-25)		3.3 (0.4-10.0)	7.8 (2.0-25)	
Mitotic count (in 50 HPFs)						
Mean ± SD	3.6±10.3	20.6±22.2	<0.001[4]	1.7±1.9	15.5±21.5	<0.001[4]
Median (min-max)	2 (0-65)	10 (3-80)		1 (0-9)	6 (0-80)	
Cell type, n (%)						
Spindle	57 (75)	15 (68.2)	0.694[3]	45 (76.3)	28 (68.3)	0.552[3]
Mixed	15 (19.7)	5 (22.7)		10 (16.9)	11 (26.8)	
Epithelioid	4 (5.3)	2 (9.1)		4 (6.8)	2 (4.9)	
Cellularity, n (%)						
Mild	30 (39.5)	3 (13.6)	0.009[3]	24 (40.7)	9 (22)	0.006[3]
Moderate	19 (25)	3 (13.6)		17 (28.8)	6 (14.6)	
High	27 (35.5)	16 (72.7)		18 (30.5)	26 (63.4)	
Nuclear pleomorphism, n (%)						
Mild	51 (97.1)	11 (50.0)	0.027[3]	42 (71.2)	20 (48.8)	0.001[3]
Moderate	9 (11.8)	2 (9.1)		8 (13.6)	3 (7.3)	
High	7 (9.2)	8 (36.4)		2 (3.4)	14 (34.1)	
Absent	9 (11.8)	1 (4.5)		7 (11.9)	4 (9.8)	
Necrosis, n (%)						
Present	17 (22.4)	11 (50.0)	0.016[1]	11 (18.6)	19 (46.3)	0.003[1]
Absent	59 (77.6)	11 (50.0)		48 (81.4)	22 (53.7)	
Hemorrhage, n (%)						
Present	3 (3.9)	0 (0)	1.00[3]	3 (5.1)	0 (0)	0.267[3]
Absent	73 (96.1)	22 (100)		56 (94.9)	41 (100)	
Ulceration, n (%)						
Present	7 (9.2)	7 (31.8)	0.014[3]	7 (11.9)	8 (19.5)	0.394[3]
Absent	69 (90.8)	15 (68.2)		52 (88.1)	33 (80.5)	
Tumor location, n (%)						
Colon	3 (4.1)	1 (4.5)	0.009[3]	1 (1.7)	3 (7.3)	0.001[3]
Duodenum	4 (5.5)	1 (4.5)		4 (6.8)	3 (7.3)	
Esophagus	2 (2.7)	2 (9.1)		1 (1.7)	3 (7.3)	
Small intestine	6 (8.2)	8 (36.4)		1 (1.7)	13 (31.7)	
Stomach	58 (79.4)	10 (45.4)		52 (88.1)	19 (46.3)	
Growth pattern, n (%)						
Expansive	72 (94.7)	19 (86.4)	0.186[3]	55 (93.2)	37 (90.2)	0.713[3]
Infiltrative	4 (5.3)	3 (13.6)		4 (6.8)	4 (9.8)	

HPFs: High power fields, AFIP: Armed Force Institute of Pathology, NIH: National Institutes of Health. [1]Chi Square Test, [2]Student t test, [3]Fisher Exact test, [4]Mann-Whitney U test.

Table 4. Multivariate logistic regression analysis for clinicopathologic features significantly associated with risk stratification and Ki-67 proliferation index and RacGAP1 expression in the univariate analysis

	AFIP criteria (low risk/high risk)			Modified NIH criteria (low risk/high risk)			Ki-67 proliferation index (<10%/≥10%)			RacGAP1 expression (negative/positive)		
	Univariate	Multivariate analysis		Univariate	Multivariate analysis		Univariate	Multivariate analysis		Univariate	Multivariate analysis	
	P value	*P value*	OR (95% CI LB-UB)	*P value*	*P value*	OR (95% CI LB-UB)	*P value*	*P value*	OR (95% CI LB-UB)	*P value*	*P value*	OR (95% CI LB-UB)
Gender	0.809[1]	-	-	0.156[1]	-	-	0.618[3]	-	-	0.290[3]	-	-
Age	0.617[2]	-	-	0.434[2]	-	-	0.700[2]	-	-	0.249[2]	-	-
Nuclear pleomorphism	0.027[3]	0.626	-	0.001[3]	0.590	-	0.001[3]	0.817	-	0.314[3]	-	-
Hemorrhage	1.00[3]	-	-	0.267[3]	-	-	1.00[3]	-	-	0.549[3]	-	-
Necrosis	0.016[1]	0.363	-	0.003[1]	0.459	-	0.025[3]	0.599	-	0.363[3]	-	-
Ulceration	0.014[3]	0.016	51.7 (2.08-1287.12)	0.394[3]	-	-	0.036[3]	0.705	-	1.00[3]	-	-
Tumor location	0.009[3]	0.504	-	0.001[3]	0.385	-	0.016[3]	0.978	-	0.047[3]	0.309	-
Cellularity	0.009[3]	0.911	-	0.006[3]	0.296	-	0.065[3]	-	-	0.018[3]	0.023	2.21 (0.66-7.38)
Growth pattern	0.184[3]	-	-	0.713[3]	-	-	0.174[3]	-	-	0.713[3]	-	-
Cell type	0.694[3]	-	-	0.552[3]	-	-	0.347[3]	-	-	0.071[3]	-	-
Tumor size	<0.001[4]	0.005	1.07 (1.02-1.13)	<0.001[4]	0.033	1.75 (1.05-2.93)	<0.001[4]	0.240	-	0.140[4]	-	-
Mitotic count	<0.001[4]	0.900	-	<0.001[4]	0.042	2347.85 (1.33-4150482.35)	<0.001[4]	0.002	1.73 (1.23-2.43)	<0.001[4]	0.536	-
RacGAP1 expression	0.043[4]	0.521	-	0.017[4]	0.250	-	0.231[4]	-	-	-	-	-
Ki 67 proliferation index	<0.001[3]	0.026	59.3 (1.61-2184.01)	<0.001[4]	0.065	-	-	-	-	0.017[3]	0.177	-

CI: confidence interval, LB: lower bound, UB: upper bound. [1]Chi Square Test, [2]Student t test, [3]Fisher Exact test, [4]Mann-Whitney U test. Dependent Variables: AFIP (0: low 1: high; Hosmer and Lemeshow test P=0.295; Model P<0.05), Modified NIH (0: low 1: high; Hosmer and Lemeshow test p=1.00; Model P<0.05), Ki-67 (0:<10% 1:>10%; Hosmer and Lemeshow test P=0.209; Model P<0.05), RacGAP1 (0: negative 1: positive; Hosmer and Lemeshow test P=0.296; Model P<0.05).

(≥10%) and positive RacGAP1 expression, respectively (**Table 4**).

Discussion

Representing the first study in the literature addressing the RacGAP1 expression in GISTs, our findings confirmed the expression of the RacGAP1 protein in 69.0% of overall cases and in 89.4% of cases with high Ki-67 proliferation index. High Ki-67 proliferation index was evident in 19.0% of overall cases and in 24.6% of RacGAP1 positive cases. High-degree cellularity and high mitotic count were the only factors that found to predict presence of RacGAP1 expression and high Ki-67 proliferation index, respectively in multivariate analysis.

In a past study conducted with 57 GIST cases, 54% of patients were reported to be in the high risk group based on tumor location, tumor size and mitotic count with original NIH criteria and AFIP criteria [8, 10, 21]. In another study with 249 GIST cases, high risk was reported in 47%, moderate risk in 23%, low risk in 16% and very low risk in 3% of patients based on modified NIH criteria [9, 22].

In our study, risk stratification via modified NIH risk criteria revealed two-fold higher prevalence of high risk category (41.0% vs. 22.0%) as compared with AFIP criteria. This seems in agreement with the re-classification of some non-gastric GISTs formerly grouped as the intermediate risk as the high risk group in modified NIH risk stratification as well as inclusion of additional prognostic factors such as primary tumor site, non-radical resection and tumor rupture [6, 23]. Besides, when compared to AFIP system, NIH system has been considered to over-grade gastric tumors and down-grade a subset of non-gastric tumors [23].

Correlation between RacGAP1 upregulation and high Ki-67 proliferation index was reported in the past studies for various tumor types including epithelial ovarian carcinomas, invasive cervical cancer, high grade meningioma, and lung neuroendocrine tumors [14-16]. Although RacGAP1 positivity was significantly associated with tumor location, high mitotic count, high-degree cellularity and high Ki-67 proliferation index in univariate analysis in our study population, multivariate analysis findings revealed that higher degree of cellularity (OR, 2.21; 95% CI, 0.66 to 7.38, P=0.023) was the single determinant of RacGAP1 overexpression.

Hence, while RacGAP1 expression was associated with poor prognostic factors such as high mitotic count, high-degree cellularity and high (≥10%) Ki-67 proliferation index and more likely to be present in case of high risk than low-risk status, multivariate analysis findings seem to indicate lack of its prognostic significance apart from prediction of high degree cellularity in GISTs.

Notably, high-degree cellularity was not amongst the determinants of high-risk category in AFIP and modified NIH risk stratification in our study. Hence, while RacGAP1 scores were significantly higher in high risk than in low risk groups, only one-third of patients and half of patients with score 2-3 positive RacGAP1 expression were categorized into high-risk group according to AFIP and modified NIH criteria, respectively. This seems to indicate that while high risk group patients are more likely to have RacGAP1 overexpression, not every patient with RacGAP1 overexpression is expected to be classified in high risk category in risk stratification. Also, approximately 10% of our patients with score 2-3 positive RacGAP1 expression were categorized into very-low risk category. Given the association of RacGAP1 expression with poor prognostic factors in the univariate analysis, this seems to emphasize the uncertainty in consideration of a specific tumor as definitely benign in risk stratification systems which use the term “very low risk” instead of “benign” [23]. Moreover, high RacGAP1 expression in breast cancer was reported to be associated with grade I and II but not grade III tumors, indicating the likelihood of the potential role of Rac function in the early stages of tumor progression [24].

Accordingly, while RacGAP1 expression was not associated with any of clinicopathological prognostic factors other than high degree cellularity in multivariate analysis, given that GISTs show an unpredictable biological behavior and clinical course [5], prognostic role of RacGAP1 expression in GISTs should be further addressed in larger cohorts.

Past studies reported diagnostic and prognostic value of high Ki-67 proliferation index in

GISTs [5, 21, 25]. Ki-67 proliferation was shown to predict risk of metastasis and the malignant potential of GISTs, while Ki-67 index over 10% was associated with poor prognosis [25-30].

In a past study among patients with GISTs, 37.8% of patients in the overall study population and 60% of the cases belonging to the high risk group were reported to have Ki-67 proliferation index >10%, according to original NIH criteria [21].

Although larger tumor size, higher mitotic count and higher likelihood of RacGAP1 positivity, high degree nuclear pleomorphism, necrosis, ulceration and small intestinal location were noted in patients with high (≥10%) than with low (<10%) Ki-67 proliferation index in our study, multivariate analysis revealed that high mitotic count (OR, 1.73; 95% CI, 1.23 to 2.43, P=0.002) was the only factor predicting increased risk of having high Ki-67 proliferation index. Accordingly, 73.7% and 89.5% of our patients with high (≥10%) Ki-67 proliferation index were categorized into the high risk group via AFIP and modified NIH criteria, respectively. This seems consistent with our multivariate analysis findings which revealed higher Ki-67 proliferation index (OR, 59.3; 95% CI, 1.61 to 2184.01, P=0.026) and higher mitotic count (OR, 2347.85; 95% CI, 1.33 to 4150482.35, P=0.042) to be the determinants of high-risk category in AFIP and modified NIH risk stratification, respectively. It should also be noted that none of the patients with high Ki-67 proliferation index (≥10%) were classified in the very low risk category via modified NIH criteria and only one patient was considered in the very-low risk category according to AFIP criteria in our study. Hence, albeit identified in lower percentage of GIST cases in our overall cohort when compared to rate of RacGAP1 expression, high Ki-67 index seems to be superior to RacGAP1 expression in terms of specificity for high risk status.

Ki-67 has been suggested to be a non-location-specific prognostic factor in a past study, based on similarity of gastric and small-intestinal GISTs in terms of Ki-67 values [5]. Authors also considered lack of location specificity as an advantage of Ki-67 index to mitotic index which shows strong correlation with anatomic site of tumor [5]. In contrast, albeit not confirmed in multivariate analysis, higher prevalence of high Ki-67 index was associated with small intestinal location in our univariate analysis, indicating the likelihood of a location specific action.

Amongst the factors shown to be associated with higher likelihood of being categorized into high-risk group in univariate analysis in both models (higher RacGAP1 scores, mitotic count, ≥10% Ki-67 proliferation index, high-degree cellularity, high-degree nuclear polymorphism, necrosis and tumor location), only larger tumor size (OR, 1.07; 95% CI, 1.02 to 1.13; P=0.005) and higher Ki-67 proliferation index (OR, 59.3; 95% CI, 1.61 to 2184.01, P=0.026) for AFIP criteria and larger tumor size and higher mitotic count for modified NIH criteria were found to predict increased risk of high-risk status.

This seems consistent with consideration of the tumor size and mitotic count as the most important and reliable prognostic factors in GISTs, while importance of primary tumor localization has also been emphasized [5, 8, 10, 31, 32]. Indeed, univariate analysis in the present study revealed tumor location and mitotic count to be the two prognostic factors that showed significant association with all of the parameters studied including high risk status, high Ki-67 proliferation index and positive RacGAP1 expression. However, tumor location was not found to be a significant determinant for any of these parameters in the multivariate analysis, while mitotic count was a significant determinant only for high Ki-67 proliferation index and modified NIH based high risk status. This seems consistent with expected correlation between Ki-67 and mitotic activity index, as suggested to be a prognostic factor for malignant behavior in gastric GISTs [25, 33-36].

Stomach (40-60%) is considered as the most common site of GISTs, as followed by small bowel (30-40%), large bowel (5-15%), and esophagus (2%) [1]. Although primary tumor location was stomach in majority of cases in our study, small intestinal location was more likely in patients with high Ki-67 proliferation index, positive RacGAP1 expression and high risk status. This seems notable given the higher prevalence of clinically malignant behavior and poorer prognosis reported in intestinal GISTs than in gastric GISTs despite similar diameter and mitotic activity [1, 9, 10, 21, 37, 38].

In conclusion, to our knowledge, this is the first study on the RacGAP1 expression in GISTs as well as its association with clinicopathological prognostic factors, Ki-67 proliferation index and risk stratification. RacGAP1 positivity was significantly associated with tumor location, high mitotic count, high-degree cellularity and high Ki-67 proliferation index in univariate analysis. In addition, multivariate analysis supported the relation of higher degree of cellularity and RacGAP1 overexpression in patients with GISTs. While RacGAP1 scores were significantly higher in high risk than in low risk groups, only one-third and half of patients with RacGAP1 overexpression were classified into the high-risk group based on AFIP and modified NIH criteria, respectively and approximately 10% were categorized into very-low risk group. However, great majority of patients with high Ki-67 proliferation index were categorized in the high risk group along with multivariate analysis confirming the higher Ki-67 proliferation index and higher mitotic count as the determinants of high-risk category in AFIP and modified NIH risk stratification, respectively. Accordingly, albeit identified in a lower percentage of overall cases in our cohort when compared to RacGAP1 expression, high Ki-67 index seems to be superior to RacGAP1 expression in terms of specificity for high risk status in GISTs. Hence, given the great heterogeneity and unpredictability of behavior of GISTs, the utility of RacGAP1 expression as a potential predictive marker in GISTs should be further explored and validated in larger cohorts of risk stratified patients with long-term follow-up data.

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Disclosure of conflict of interest

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