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Significance of CD44 Expression in Gastrointestinal Stromal Tumors in Relation to Disease Progression and Survival

Kai-Hsi Hsu · Hung-Wen Tsai · Yan-Shen Shan · Pin-Wen Lin

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Abstract
Background CD44 is a transmembrane glycoprotein belonging to the cell-adhesion molecule family. It has been identified as being involved in tumor progression and metastasis, and its expression has been found to be of prognostic significance in several human malignancies. The aim of this study was to assess CD44 expression in gastrointestinal stromal tumors (GISTs), the most common mesenchymal tumor of the gastrointestinal tract.
Methods Between January 1995 and March 2006, 92 patients undergoing surgical resection for GIST in National Cheng Kung University Hospital were evaluated. To study the significance of CD44 expression, immunohistochemical staining of CD44 in tumor specimens was performed, and the clinicopathological information of patients was reviewed.
Results Fifty-nine of 81 patients (73%) showed positive CD44 expression. Loss of CD44 expression was associated with disease progression ($p = 0.019$). Kaplan-Meier analysis revealed better progression-free survival among patients with strong CD44 expression (++ and +++) ($p = 0.034$), absence of disease progression ($p < 0.001$), and lower risk, according to National Institutes of Health (NIH) Consensus Criteria for GIST risk stratification ($p = 0.003$). Multivariate analysis demonstrated that high-risk status was the only independent risk factor for disease progression and the only independent predictor for a poor progression-free survival ($p = 0.023$ and 0.045, respectively).
Conclusions It is demonstrated that high-risk status by NIH criteria is significantly associated with disease progression and poor progression-free survival in GIST.

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CD44 is an 80–200 kDa, type I transmembrane protein that is encoded by a single 20-exon gene located on chromosome 11p13. It was first identified in 1982 as a surface glycoprotein in lymphocytes and was later found to be expressed in many cell types of epithelial and mesenchymal tissues. Most normal cells ubiquitously express the smallest, so-called standard isoform (CD44s) transcribed from exon 1 to 5 and exon 16 to 20 [1–3]. Many variant isoforms (CD44v1–CD44v10) also exist by alternate messenger-RNA splicing of exon 6 to 15 in various combinations, encoding a product that is inserted into the ectodomain of the CD44 molecule. These variants are more restricted in their expression in different tissues [4, 5]. CD44 is a major cellular adhesion molecule for hyaluronic acid (HA), an extracellular matrix component, and it has been implicated in a variety of physiologic and pathophysiologic activities, including matrix adhesion, cell migration, cell differentiation, cell survival, signal transduction, presentation of growth factors or cytokines, tumor cell growth, proliferation, invasion, and metastasis [3, 6–8]. The role of CD44 in cancer progression and metastasis was first reported in a mouse animal model in 1991 [9]. Al-

though the role of CD44 variant isoforms (CD44v) in tumor biology remains relatively controversial, the expression of CD44 has been found to be associated with both favorable and unfavorable clinical outcomes in certain human tumors [10–16].

Gastrointestinal stromal tumors (GISTs) are the most common type of mesenchymal tumor of the gastrointestinal tract. They are derived from the interstitial cells of Cajal (ICC) in the myenteric plexus of the gut. Most GISTs have gain-of-function mutations of the proto-oncogene KIT, which encodes a 145-KD transmembrane tyrosine kinase KIT [17, 18]. Mutation of different exons of the KIT gene activates the tyrosine kinase activity of KIT, leading to ligand-independent kinase activity and cell resistance to apoptosis [19, 20]. KIT mutations were present in 90% of GISTs, and in GIST without KIT mutations, gain-of-function of the platelet-derived growth factor (PDGF) receptor α (PDGFRA) can be found in about one third of cases [21, 22]. The diagnosis of GIST depends on the positive KIT immunostaining, the mutation study for KIT or the PDGFRA gene. Complete surgical resection is the only curative treatment for primary resectable GIST. However, the recurrence rate after operation has been reported to be as high as 24%, and prognosis in patients with recurrent disease is dismal, with a median survival of 9–12 months [19, 23, 24]. Imatinib, a tyrosine kinase receptor inhibitor, that has been proved to be effective in the management of advanced GIST, is currently under investigation for its role as adjuvant therapy in the prevention of recurrence or metastasis in resectable GIST. It is therefore important to identify factors associated with higher risk for disease progression, which may serve as indicators for adjuvant imatinib therapy. Because CD44 has been found to be involved in a variety of tumor activities, especially in tumor progression and metastasis, one of the goals of the present study was to evaluate the expression of CD44 in resectable GIST and verify its possible clinical significance.

Patients and methods

From January 1995 to March 2006, 92 patients undergoing surgical resection for GIST in National Cheng Kung University Hospital were included in this retrospective study. Informed consent was obtained from all patients, and approval was obtained from the institute review board. The principle of surgery was to achieve R0 resection with complete tumor excision and grossly negative margins. The absence of tumor in the resection margin was documented microscopically in all patients postoperatively. Lymph node dissection was not performed unless enlarged nodes with suspected metastasis were present. Patients with initial metastasis or concurrent cancer other than GIST or multiple lesions were excluded. No patient received any form of neoadjuvant or adjuvant therapy. The diagnosis of GIST was confirmed by histopathologic examination, according to positive KIT immunohistochemical status, as well as microscopic morphology. Patients were regularly followed in the outpatient department for disease progression, which was defined as recurrence and/or metastasis. Abdominal ultrasonography or computed tomography (CT) was arranged every 3 months for the first year and annually thereafter. The first follow-up imaging study was arranged within 6 months after operation, depending on different patient conditions and complaints at outpatient visits. The time interval between each succeeding abdominal sonogram or CT scan was 3 months. We also categorized our cases according to National Institutes of Health (NIH) Consensus Criteria for GIST risk stratification: the very low-risk patients had tumors smaller than 5 cm with a mitotic count fewer than 5 mitoses per 50 high-power fields; intermediate-risk patients had tumors measuring less than 5 cm with 6 to 10 mitoses or tumors measuring 5 to 10 cm with fewer than 5 mitoses. High-risk patients are those with tumors larger than 5 cm and more than 5 mitoses or any lesions greater than 5 cm with more than 5 mitoses or any lesions greater than 10 cm or with more than 10 mitoses [25].

Formalin-fixed and paraffin-embedded blocks from the tumor specimen were cut into sections of 5-μm thickness in the pathology laboratory. The sections were deparaffinized and hydrated through graded concentrations of xylene and descending grades of ethanol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 10 min. The sections were then put in the citrate buffer (pH = 6.0), were boiled for 5 min twice, and were washed in phosphate saline buffer solution (PBS) (pH = 7.4). Sections were incubated for 1 h at room temperature with the primary monoclonal antibodies of CD44 (DF1485, Dako, Denmark) at 1:50 dilution. After repeated washing with PBS, the secondary anti-mouse antibodies and avidin-biotin complex were applied for 30 min each. Immunoreactivity was then visualized with chromogen AEC stain (3-amino-9-ethylcarbizole, Dako, Denmark), and the sections were counterstained with hematoxylin and mounted.

A tissue section of human tonsil was used as a positive control in each course of staining. In negative controls, the primary antibody was omitted. The expression of CD44 was graded as: negative (–); less than 10% positive cells (+); 10%–50% positive cells (++); > 50% positive cells (+++) (Fig. 1). Patients with CD44 expression graded as (–) and (+) were classified as being weak or negative for CD44 expression, or as having loss of CD44 expression. Those with CD44 of (++) or (+++) were classified as having strong CD44 expression, or as having positive CD44 expression.

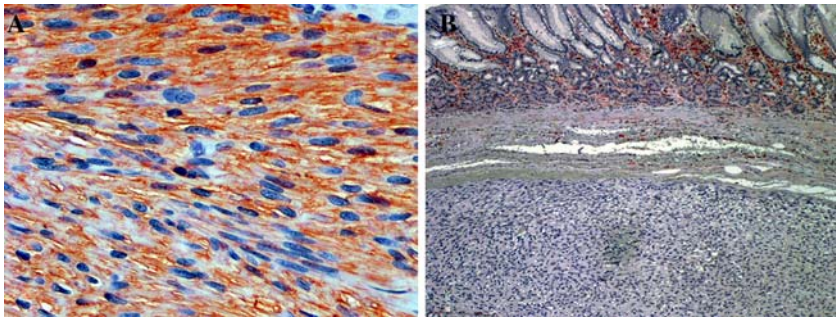

Fig. 1 Immunohistochemical staining for CD44 expression in gastrointestinal stromal tumor (GIST) showing +++ expression (**A**) and negative expression (**B**). Numerous CD44-positive lymphocytes seen among the intestinal glands in (**B**) can serve as internal positive control

Statistical analysis

Comparison between two groups was done by an independent sample *t*-test for continuous variables and the χ^2 test for categorical variables. Survival curves were estimated using the Kaplan-Meier method. Comparison of progression-free survival between groups was performed by the log-rank test. Significant variables in univariate analyses were applied to multivariate analysis based on the Cox proportional hazards regression model to determine significant prognostic factors for progression-free survival. Univariate predictors of disease progression were entered into a multivariate logistic regression. Each model included age and gender as covariates. A value of $p < 0.05$ was considered significant. All statistical analyses were performed using the SPSS computer statistical software (SPSS software; version 13.0; Chicago, IL).

Results

Patient characteristics and CD44 expression

Of the 92 patients enrolled in the study, 3 patients with concurrent cancer were excluded, and 8 others were lost to follow-up during the early postoperative period. Thus 81 eligible patients were included in this study, 44 women and 37 men, with a median age of 61 years. The tumor was located in the stomach in 55 patients (67.9%) and in the intestine in 26 patients (32.1%). After having been categorized into four risk groups according to the National Institutes of Health (NIH) Consensus criteria, the 81 patients were further divided two major groups: one with high risk and the other with lower risk, in which the very low-, low-, and intermediate-risk groups were included (Table 1). Three types of cell morphology were identified under microscopic examination: spindle cell type (74.1 %), epithelioid cell type (22.2 %), and mixed type (3.7 %). There was no significant association between cell morphology types and CD44 expression ($p = 0.232$) (Table 2). Fifty-nine of the 81 patients (73%) showed positive CD44 expression. Strong CD44 expression was noted in 54 patients (66.7%), and 27 patients (33.3%) presented with weak or negative CD44 expression (Fig. 1). Table 2 summarizes the clinicopathological data in these patients according to their CD44 expression status. There was no significant difference between gastric and intestinal GIST in terms of CD44 expression status ($p = 0.886$). Patients in the high-risk group showed a significant loss of CD44 expression ($p = 0.001$). Weak or no CD44 expression also correlated significantly with tumor progression ($p = 0.019$). The mean tumor size was 7.0 cm (range: 0.5–28 cm). Patients with negative or weak CD44 expression have larger tumors than patients with strong CD44 expression ($p = 0.032$) (Table 2).

Disease progression and survival

Disease progression after surgery was documented in 13 patients after a median follow-up of 47.4 months (range: 4.9–169.9 months). Loss of CD44 expression and classification as high-risk were significant predictors of disease progression after surgery ($p = 0.019$ and 0.002, respectively) (Table 3). Under multivariate analysis, high risk became the only independent risk factor for disease progression ($p = 0.023$) (Table 4). At 1 year, 3 years, and 5 years, the rate of progression-free survival for these 81 patients was 96.6%, 94%, and 83.8%, respectively, whereas the 1-year, 3-year, and 5-year survival for patients with disease progression were 91.9%, 67.4%, and 22.4%, respectively. The mean and median progression-free survival was 55.5 and 46.4 months, respectively. Kaplan-

Table 1 Patient categorization according to National Institutes of Health (NIH) Consensus Criteria for GIST risk stratification

Risk group			
Lower risk	Very low risk	4 (4.9%)	55 (67.9%)
	Low risk	30 (37%)	
	Intermediate risk	21 (26%)	
High risk	High risk	26 (32.1%)	26 (32.1%)
Total		81 (100%)	

GIST gastrointestinal stromal tumors

Table 2 Demographics and clinicopathological data in 81 patients with GIST with respect to CD44 expression

Immunoreactivity		CD44 expression			
		Strong	Weak or negative	Total	*p* Value
Number of patients		54 (66.7%)	27 (33.3%)	81 (100%)	
Gender	Male	24 (44.4%)	13 (48.1%)	37 (45.7%)	0.752
	Female	30 (55.6%)	14 (51.9%)	44 (54.3%)	
Age (years), mean ± SD (range)		60.5 ± 12.1 (38–81)	60.2 ± 14 (31–84)	60.4 ± 12.7 (31–84)	0.917
Symptoms and signs	Yes	44 (81.5%)	25 (92.6%)	69 (85.2%)	0.185
	Nil	10 (18.5%)	2 (7.4%)	12 (14.8%)	
Operative time (min), median (range)		105 (35–555)	147.5 (50–332)	115 (35–555)	0.056
Blood loss (ml), median (range)		100 (0–7500)	400 (0–2530)	100 (0–7500)	0.748
Operative methods	Gastrectomy	37 (68.5%)	18 (66.7%)	55 (67.9%)	0.402
	Small bowel resection	15 (27.8%)	6 (22.2%)	21 (26%)	
	Pancreatico-duodenectomy	2 (3.7%)	3 (11.1%)	5 (6.1%)	
Tumor location	Stomach	37 (68.5%)	18 (66.7%)	55 (67.9%)	0.866
	Intestine	17 (31.5%)	9 (33.3%)	26 (32.1%)	
Morphology	Spindle cell	43 (79.6%)	17 (63.0%)	60 (74.1%)	0.232
	Epithelioid	9 (16.7%)	9 (33.3%)	18 (22.2%)	
	Mixed	2 (3.7%)	1 (3.7%)	3 (3.7%)	
Tumor size (cm), mean ± SD (range)		6.2 ± 4.3 (0.5–28)	8.7 ± 5.1 (0.6–22)	7.0 ± 4.7 (0.5–28)	0.032
Disease progression	Yes	5 (9.3%)	8 (29.6%)	13 (16%)	0.019
	Nil	49 (90.7%)	19 (70.4%)	68 (84%)	
Risk group	High risk	11 (20.4%)	15 (55.6%)	26 (32.1%)	0.001
	Lower risk	43 (79.6%)	12 (44.4%)	55 (67.95)	

SD standard deviation

Meier analysis of progression-free survival in these 81 patients revealed that disease progression ($p < 0.001$) (Fig. 2), weak or negative CD44 expression ($p = 0.034$) (Fig. 3), and the high risk ($p = 0.003$) (Fig. 4) were significantly associated with poor progression-free survival. Multivariate analyses demonstrated that NIH high-risk categorization (odds ratio, 4.505, 95% confidence interval, 1.033 to 16.95; $p = 0.045$) was the only independent prognostic factor for progression-free survival (Table 5).

Discussion

Gastrointestinal stromal tumors are the most common mesenchymal tumor of the gastrointestinal tract, with an estimated incidence and prevalence of 14.5 and 129 cases per million, respectively [23]. The search for potential prognostic factors in GIST has been growing since the introduction of the term ‘‘GIST’ as a separate disease entity by Mazur and Clark [26] in 1983, one year after the identification of CD44 [2]. CD44 expression has been proposed to be both a favorable (in the majority) as well as an unfavorable prognostic factor in colorectal cancer, breast cancer, and many different human malignancies [16, 27–30]. Nevertheless, very few attempts have been made to discover an association between GIST and CD44. So far, our study is the largest series to explore the significance of CD44 expression in a particular patient cohort with resectable GIST. In the present study, 59 of the 81 patient (73%) with resectable GIST showed positive CD44 expression in tumor cells, and 92% of them were graded preoperatively as strong expression (++ and +++). This group of patients with strong CD44 expression was significantly related to the group with lower risk and was inversely associated with disease progression (Table 2). In the other group of negative or weak CD44 expression, or so-called loss of CD44 expression, survival was significantly worse (Fig. 2). This was in accordance with another study that addressed the issue of CD44 expression in human tumor, in which loss of CD44 expression, but not of CD44 variants, correlated with poor clinical outcome in 33 patients with gastric GIST [31]. However, the median follow-up of 7 months in this study, compared with that of 47.4 months in our series, was rather short for accurate interpretation of the results. Although loss of CD44 expression was significantly related to disease progression and disease-free survival in the univariate analysis ($p = 0.019$ and 0.034, respectively), it failed to demonstrate the same significance in the multivariate analysis ($p = 0.184$ and 0.137, respectively). If the clinical signifi-

Table 3 Univariate analysis for disease progression in 81 patients with GIST

		Disease progression		*p* Value
		Yes (n = 13)	Nil (n = 68)	
Gender	Male	7	30	0.519
	Female	6	38	
CD44 expression	Strong	5	49	0.019
	Weak or negative	8	19	
Age (years), mean ± SD (range)		62.9 ± 11.6 (44–79)	60.0 ± 12.9 (31–84)	0.419
Symptoms and signs	Yes	6	38	0.360
	Nil	7	30	
Operative time (min), median (range)		114.5 (85–555)	115 (35–332)	0.329
Blood loss (ml), median (range)		125 (0–7500)	100 (0–6000)	0.466
Tumor size (cm), mean ± SD (range)		9.5 ± 6.5 (3–28)	6.6 ± 4.2 (0.5–22)	0.131
Operative methods	Gastrectomy	8	47	0.865
	Small bowel resection	4	17	
	Pancreatico- duodenectomy	1	4	
Tumor location	Stomach	8	47	0.592
	Intestine	5	21	
Risk group	High risk	9	17	0.002
	Lower risk	4	51	

Table 4 Multivariate analysis of risk factors for disease progression after surgery in 81 patients with GIST

Parameters	Disease progression		
	OR	95% CI	*p* Value
Age	1.021	0.971–1.073	0.427
Gender: male/female	1.198	0.325–4.405	0.787
CD44 expression: weak or negative/strong	2.501	0.647–9.657	0.184
Risk group: high risk/lower risk	5.000	1.255–20.00	0.023

OR odds ratio; *CI* confidence interval

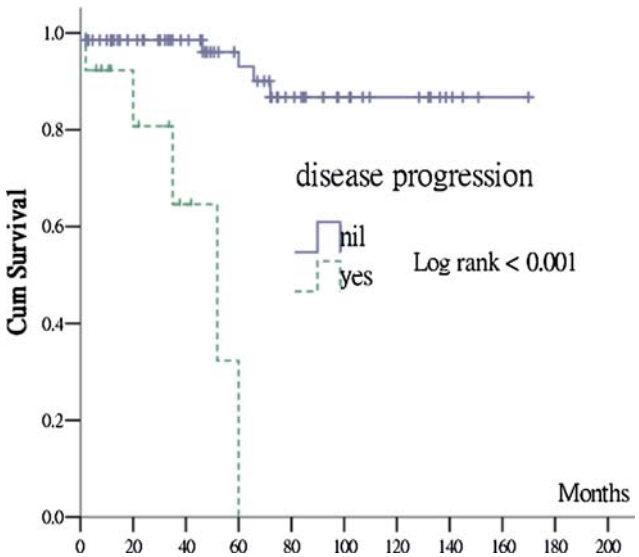

Fig. 2 Kaplan-Meier progression-free survival analysis for disease progression

cance and prognostic value of CD44 expression are to be more accurately verified, more patients may be necessary for further analysis in a prospective study.

The exact mechanism whereby CD44 contributes to disease progression and poor outcome in GIST is unclear. One explanation is that as a major adhesion molecule, CD44 can help retain the integrity of cell–cell and cell–matrix adhesion, anchoring the abnormal proliferating cells in place, preventing detachment of the basement membrane, and blocking subsequent infiltration or invasion of the surrounding tissues and metastasis [32]. Recently it was noted that the proteolytic cleavage of CD44 ectodomain contributes to the regulation of cell attachment and migration and may be involved in the malignancy of human tumors [33]. This finding raises the possibility that decreased or even absent CD44 expression in cell membrane may also be due to high percentage of tumor cells with CD44 ectodomain cleavage as a result of ongoing invasion or metastatic activities in these cells. The result is a poor prognosis for these patients. This effect can be observed in our study, where we observed an increased rate of tumor progression after surgery in patients with weak or negative CD44 expression. The fact that loss of CD44 expression was associated with a significantly increased tumor size also led us to suspect that CD44 might also play a role in tumor cell proliferation in GIST. These hypotheses, along with the underlying upstream and downstream events affecting the CD44 pathways in GIST, await further elucidation and investigation.

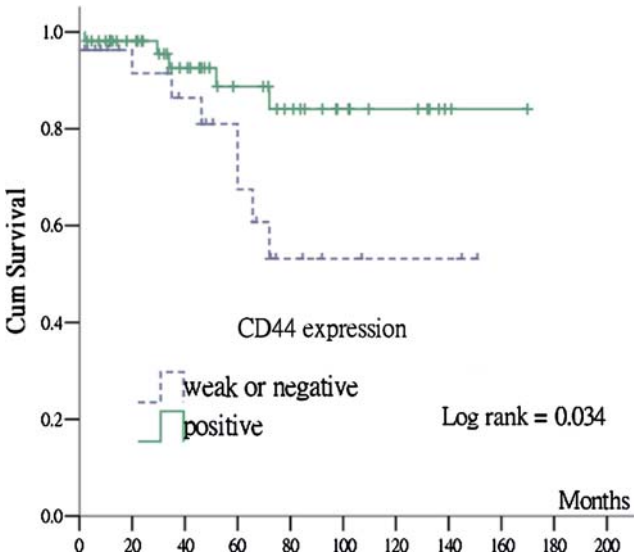

Fig. 3 Kaplan-Meier progression-free survival analysis for CD44 expression

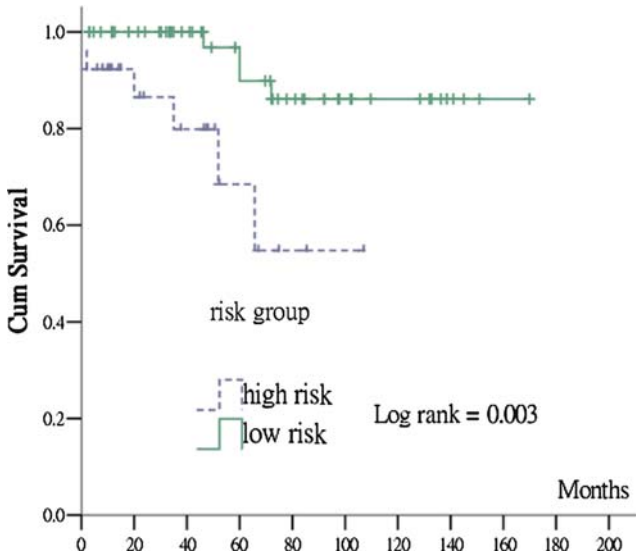

Fig. 4 Kaplan-Meier progression-free survival analysis for two risk groups

Many studies have been carried out to evaluate possible clinical, pathological, and molecular markers responsible for aggressive disease behavior and tumor progression. This is especially true after the emergence of effective imatinib treatment in advanced GIST and the promising adjuvant therapy trials ongoing in patients with high-risk GIST [19, 20, 34]. Risk stratification of patients with GIST after surgery is therefore essential for establishing inclusion criteria for possible adjuvant therapy. Many specific clinicopathological and molecular parameters have been examined for their prognostic significance, such as tumor location, tumor necrosis, cellularity, nuclear pleomorphism, cellular atypia, epitheloid cell type, Ki-67 staining, MIB-1 Ag labeling index, Bcl-2 expression, DNA ploidy, Cox-2 expression, p53 expression, vascular endothelial growth factor expression, telomerase activity, KIT mutation, or chromosomes alterations [35–42]. More large-scale studies are necessary for verification of the reproducibility and statistical consistency of these variables. At present, the NIH Consensus Criteria that incorporated the two reliable parameters, tumor size and mitotic count, has been generally agreed as a standard for GIST risk assessment [25]. In the present study, we have further confirmed its prognostic value in resectable GIST; the patients in our series who were classified as being at high risk according to these criteria, in fact did carry a significantly higher risk of disease progression and a worse progression-free survival. The impact of loss of CD44 expression (odds ratio, 2.996; 95% confidence interval, 0.705–12.74; $p = 0.137$) on progression-free survival was not as significant as that of NIH high-risk status (odds ratio, 4.505; 95% confidence interval, 1.033–16.95; $p = 0.045$).

Table 5 Multivariate analysis of prognostic factors for progression-free survival in 81 patients with GIST

Parameters	Progression-free survival		
	OR	95% CI	*p* Value
Age	1.020	0.962–1.080	0.513
Gender: male/female	1.172	0.302–4.255	0.853
CD44 expression: weak or negative/strong	2.996	0.705–12.74	0.137
Risk group: high risk/lower risk	4.505	1.033–16.95	0.045

In conclusion, the results from our study show that, although loss of CD44 expression in GIST correlates with disease progression after surgery and is associated with high-risk status as well as poor prognosis, current evidence from multivariate analysis do not support its role as a single prognostic factor for clinical outcomes. Prospective studies with more patients could be conducted for further evaluation of the prognostic value of CD44 expression. In the present study, classification as high risk according to NIH criteria was the only independent predictive factor for both disease progression and poor progression-free survival in patients with resectable GIST.

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