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Original Article

Expression of CD133 as a cancer stem cell marker in invasive gastric carcinoma

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Summary

Introduction. Gastric cancer is considered to be the fourth most common malignancy worldwide and the second cause of cancer deaths. Regarding the cancer stem cells (CSCs) theory, they are a small group of tumor cells with unrestricted self-renewal and differentiation abilities that help tumor formation. There is an interest in the utility of CD133 as a promising marker to detect the tumor stem cell population for a variety of solid malignancies including gastric cancer. Tumors that express stem cell markers such as CD133 are found to be more aggressive tumors with poor prognosis and high liability for recurrence. This study aimed to evaluate the immunohistochemical expression of CD133 in invasive gastric carcinoma and study the relation between CD133 immunohistochemical expression and different clinicopathological parameters.

Material and methods. 77 cases of gastric carcinoma were collected from the surgical pathology unit at the Gastroenterology Center, Mansoura University, Egypt. CD133 expression in tumor tissue was evaluated by immunohistochemistry.

Results. CD133 expression positively correlated with tumor metastasis and recurrence. Multivariate analysis revealed CD133 positivity to be an independent prognostic factor for tumor recurrence (P = 0.03).

Conclusion. CD133 is a good marker that can predict tumor recurrence and metastasis in gastric carcinoma. Even though, studies regarding CSCs are still in their initial stages especially those related to CD133 in gastric cancer.

Key words

CD133 • Stem cells • Gastric cancer • Immunohistochemistry

Introduction

Gastric cancer is one of the most aggressive human malignancies. It is considered by the WHO as the fourth most common cancer worldwide and the second leading cause of cancer deaths [1]. According to The Egyptian National Cancer Institute, it is the fourteenth most common cancer representing 1.8% of cases in both sexes [2]. Tumor recurrence and metastasis are the main causes of death in gastric cancer patients, regardless of surgical intervention and post-operative adjuvant therapy [3]. The overall 5-year survival rate is less than 20% as most cases are usually diagnosed late and are incompatible for curative surgery. Therefore, there is a critical need to find more effective methods to eliminate cancer cells [4].

CSCs correspond to a subpopulation of cells within the tumor defined by self-renewal, asymmetrical division, and differentiation properties, giving rise to the more or less differentiated cells composing the tumor mass [5]. CSCs were first known in the early half of the 2000s in solid cancer. A treatment targeting CSCs has become a challenge for radical cure of cancer. Therefore, it is important to identify and isolate CSCs using specific markers known as CSCs markers [6].

Different markers have been found to be expressed on the surface of CSCs. CD133 is one of these markers that has retained much attention and importance [7]. CD133 receptors on gastrointestinal cancer cells was firstly recognized by Smith et al. [8]. It was found that an antibody-drug conjugate against this receptor cause delayed growth response in gastric cancer cells [9].

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Materials and methods

Tissue specimens

This retrospective study was performed using gastrectomy specimens from 77 cases of gastric carcinomas that were collected during the period from January 2012 until September 2015 from the archive of the pathology department. None of the patients had received preoperative adjuvant therapy, chemotherapy or radiotherapy. The protocol was approved by the Ethical Committee of Mansoura University, and performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained.

Histopathology

The paraffin blocks were re-cut in 5-µm-thick sections and stained with hematoxylin and eosin and were independently reviewed by two pathologists for confirmation of diagnosis.

Immunohistochemistry

For immunohistochemical staining for CD133, 5-µm-thick tissue sections were cut and mounted on coated slides. The sections were deparaffinized in xylene then rehydrated in a series of decreasing concentration of ethanol. After that, heat-induced antigen retrieval was done using a pressure cooker and EDTA buffer (PH 9) and immersed in peroxidase-blocking solution (3% hydrogen peroxide) for 15-20 minutes to inhibit endogenous peroxidase activity, the slides were washed in phosphate buffer saline (PBS). The slides were – then – incubated with the Anti-CD133, mouse monoclonal antibody (1:40, clone AC133, Miltenyi Biotech, Bergisch Gladbach, Germany) that was used as primary antibody for CD133 detection for 1 hour at room temperature, followed by incubation with the secondary antibody (poly horseradish peroxidase conjugate for mouse/rabbit) for 15 minutes at room temperature. Diaminobenzedene (DAB) was the chromogen used to visualize the stain. The slides were then counterstained with hematoxylin, dehydrated, coversliped, and finally mounted with DPX [10]. Both normal gastric mucosa and normal kidney tissue were used as replace internal bypositive [11 12]. Negative control was done by substituting the primary antibody with phosphate buffer saline [13].

Assessment of CD133 immunostaining

CD133 show membranous and cytoplasmic pattern of expression [14]. We consider the pattern of expression as the more dominant type was seen in the specimen [6]. CD133 results were scored to as the sum of CD133 intensity of staining reaction in tumor cells plus CD 133 percent of expression in tumor cells. CD133 intensity was graded from zero to three at which (0 for negative expression, 1 for mild intensity, 2 for moderate intensity and 3 for strong intensity) and CD133 percent of expression also was graded from zero to three at which (0 for negative percent, 1 for > 10% of tumor cells, 2 for 10% to < 50% of tumor cells and 3 for ≥ 50% of tumor cells) [15]. The receiver operating characteristic (ROC) curve was used to set the cut-off point (Fig. 1) [16 17]. ROC curves facilitate the detection of the threshold value that corresponds to maximum sensitivity with minimal loss of specificity and above which a test result should be considered positive for some outcome [16].

Statistical analysis

Statistical analysis was done by using IBM SPSS, version 20.0. The relation between CD133 expression and the clinicopathological parameters were assed as a univariate analysis. Multivariate analysis was done using binary logistic regression test to detect the predictors of CD133 expression.

Results

Examination of the clinicopathological data

Seventy-seven cases of gastric carcinomas were involved in this study. The mean age of the studied cases was 54.44 years, and the youngest patient at time of diagnosis was 24 year old, while the oldest was 81 years. The cases showed male predominance as it included 45 males (58.4%) and 32 females (41.6%). The tumor size of the excised biopsies ranged from 1 cm to 18 cm in its maximum diameter with mean tumor size was (6.05 ± 3.3 cm). Grade II adenocarcinoma was the most common histology. The intestinal type was also the predominant according to Laurén classification. Fungating tumor growth pattern was the commonest. The tumor depth of invasion in most of the cases was T3 (75.3%), lymph node metastasis were positive in 57 cases (74.0%), while 10 cases only (13.0%) out of total number of the cases showed distant metastasis. Regarding TNM staging most of the cases were present in stage III (39%). Nineteen cases were positive for lymphovascular invasion (24.7%) and 17 cases were positive for perineural invasion (22.1%), 11 cases (14.3%) showed tumor recurrence (Tab. I).

Immunohistochemistry

ROC curve analysis was used to determine the clinically relevant threshold for CD133 positivity, the significant cut off was < 3.5, 32 cases showed CD133 score (0-3) that consider negative and about 45 cases showed CD133 score (4-6) that consider positive. Re-

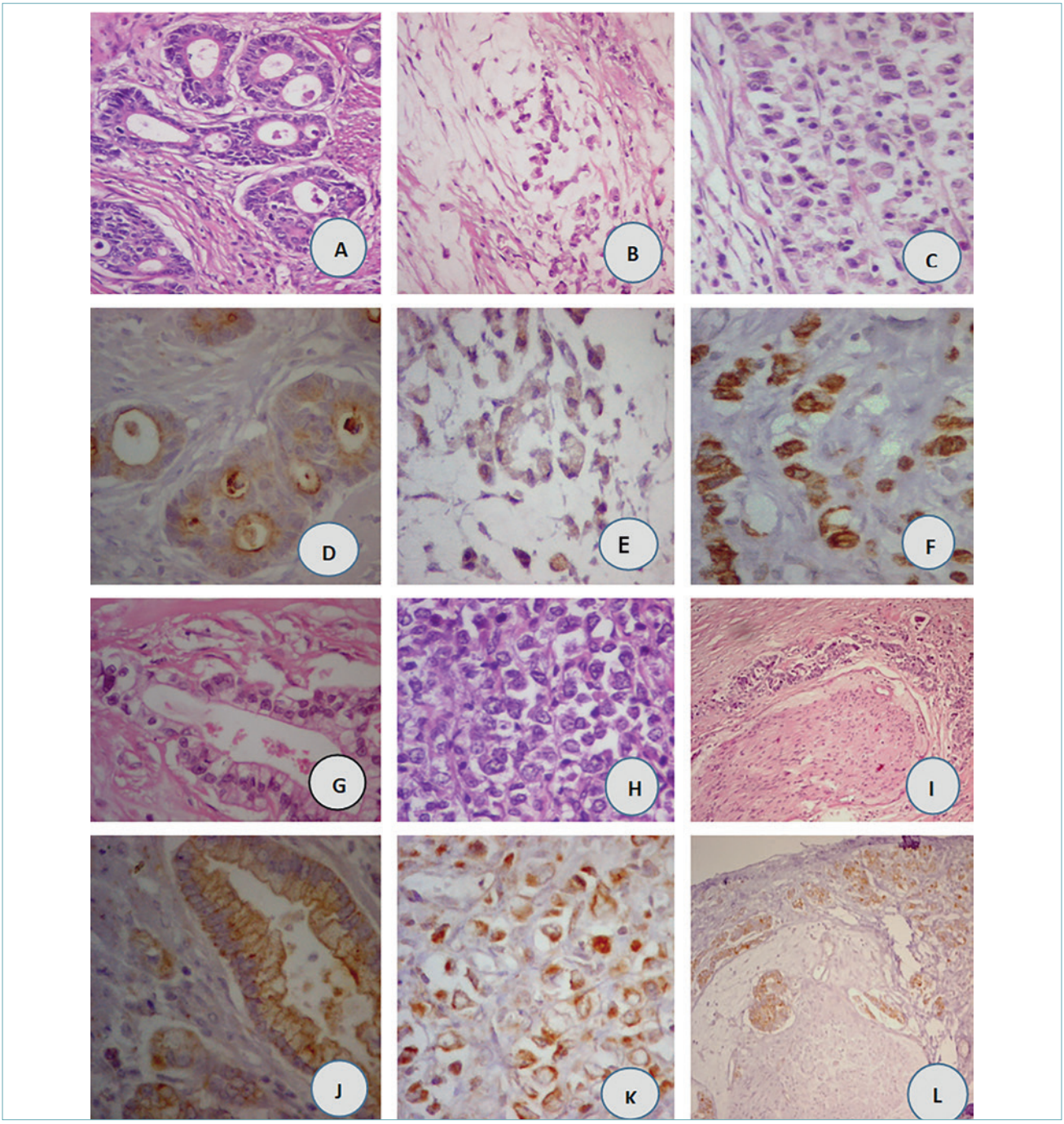

Fig. 1. A) Grade II adenocarcinoma (H&E x 200 original magnification). **B)** Mucoid adenocarcinoma (H&E x 200 original magnification). **C)** Signet ring carcinoma (H&E x 400 original magnification). **D)** CD133, membranous reaction (luminal) with moderate intensity in 10 to > 50% of tumor cells, score 4 (x 400 original magnification). **E)** CD133, cytoplasmic reaction with mild intensity in 10 to > 50% of tumor cells, score 3 (x 200 original magnification). **F)** CD133, cytoplasmic reaction with strong intensity in > 50% of tumor cells, score 6 (x 400 original magnification). **G)** Grade I adenocarcinoma of the stomach (H&E x 400 original magnification). **H)** Undifferentiated adenocarcinoma of the stomach (H&E x 400 original magnification). **I)** Perineural invasion by tumor cells (H&E x 100 original magnification). **J)** CD133, membranous reaction (luminal) with strong intensity in > 50% of tumor cells, score 6 (x 400 original magnification). **K)** CD133, cytoplasmic reaction with strong intensity in > 50% of tumor cells, score 6 (x 400 original magnification). **L)** CD133, positive in tumor cells (x 100 original magnification).

Tab. I. Clinicopathological features of the studied cases.

Age	N	%
≤ 55	37	48.1
< 55	40	51.9
Sex		
Male	45	58.4
Female	32	41.6
Tumor size		
Median ± SD	6.05 ± 3.3	
Mean (min-max)	5.0 (1.0-18.0)	
Tumor depth of invasion		
T1	3	3.9
T2	14	18.2
T3	58	75.3
T4	2	2.6
Regional LN metastasis		
Negative (N0)	20	26.0
Positive (N1, N2, N3)	57	74.0
Distant metastasis		
M0	67	87.0
M1	10	13.0
P TNM stage		
I	10	13
II	27	35.1
III	30	39
IV	10	13.0
Lymphovascular invasion		
Positive	19	24.7
Negative	58	75.3
Perineural invasion		
Positive	17	22.1
Negative	60	77.9
Tumor recurrence		
Positive	11	22.2
Negative	66	77.8
Histological type and grade		
Grade 1 adenocarcinoma	5	11.1
Grade 2 adenocarcinoma	34	44.1
Grade 3 adenocarcinoma	15	19.4
Mucoid adenocarcinoma	6	7.79
Signet ring carcinoma	11	14.28
Undifferentiated carcinoma	6	7.79
Lauren classification		
Intestinal	60	77.9
Diffuse	17	22.1
Ming classification		
Fungating	35	45.5
Infiltrating	14	18.2
Malignant ulcer	28	36.4

garding CD133 pattern of expression ten cases were negative. Thirty five cases showed membranous pattern and thirty two cases showed cytoplasmic pattern (Tab. II). Examples of the staining patterns are shown in Figure 1.

Tab. II. Immunohistochemical characteristics.

CD 133 score	N	%
0-3	32	41.6
4-6	45	58.4
CD133 pattern		
Negative	10	13
Membranous	35	45.5
cytoplasmic	32	41.6

Table III describes the relation between CD133 score and some of the clinico-pathological features. There was a significant relationship between CD133 score and distant metastasis as nine out of ten cases with distant metastasis showed high CD133 score (4-6) with *p* value = 0.03. Also there was a significance between CD133 score and tumor recurrence as ten out of elven cases with tumor recurrence showed high CD133 score with P value (0.02). Multivariate analysis using binary logistic regression test demonstrated that cases with tumor metastasis have 7.75 time incidence of expression of CD133 (P = 0.058), while cases with tumor recurrence have 8.86 time incidence of CD133 expression (p = 0.04), CD133 is an independent prognostic factor for tumor recurrence (Tab. IV).

Discussion

CD133 was considered as one of the most prominent CSC markers that was found to be over expressed in hepatocellular carcinoma, gastric cancers, colorectal cancer, pancreatic cancer and other types of cancer. In the current study, CD133 positivity was found in 58.4% of cases. It was found that presence of CD133 positive staining in minor populations of cells may be indicative of cancer stem-like cells which lead to tumor recurrence and metastasis. Our study aimed to study the relation between CD133 immunohistochemical expression and different clinicopathological parameters.

This study showed a significant relation between CD133 score and tumor distant metastasis and recurrence and this agreed with the study by Zhao et al. that reviewed 336 gastric carcinoma cases, 46 cases showing distant metastasis, 38 cases of which showed CD133 overexpression [10]. It also agreed with the results of a meta-analysis based study including 26 studies with a total of 4729 cases, a relation between CD133 overexpression and tumor metastasis was found [18]. Also, significant relation was found between CD133 score and tumor recurrence as 10 of 11 cases with tumor recurrence had score (4-6) with P value = 0.02, it was also found that CD133 is an inde-

Tab. III. The relation between CD133 score and the clinicopathological parameters.

Clinicopathological parameters	CD 133 0-3 (n = 32)		CD133 4-6 (n = 45)		test of significance
Age	**N**	**%**	**n**	**%**	
≤ 55	14	43.8	23	51.1	χ^2 = 0.41 p = 0.52
< 55	18	56.2	22	48.9	
Sex					
Male	22	68.8	23	51.1	χ^2 = 2.39 p = 0.12
Female	10	31.2	22	48.9	
Tumor size					
Median (Min-Max)	5.0(1.0-18.0)		6.0(1.0-18.0)		Z = 0.037 P = 0.97
Tumor depth of invasion					
T1	2	6.2	1	2.2	MC P = 0.39
T2	4	12.5	10	22.2	
T3	26	81.2	32	17.1	
T4	0	0.0	2	4.4	
Regional LN metastasis					
Positive NO	8	25.0	12	26.7	χ^2 = 0.03 P = 0.87
Negative N1,N2,N3	24	75.0	33	73.3	
Histological type and grade					
Grade 1 adenocarcinoma	0	0.0	5	11.1	MC P = 0.15
Grade 2 adenocarcinoma	14	43.8	20	44.4	
Grade 3 adenocarcinoma	6	18.8	9	20.0	
Mucoid adenocarcinoma	3	9.4	3	6.7	
Signet ring carcinoma	4	12.5	7	15.6	
Undifferentiated carcinoma	5	15.6	1	2.2	
Lauren classification					
Intestinal	23	71.9	37	82.2	χ^2 = 1.16 p = 0.28
Diffuse	9	28.1	8	17.8	
Ming classification					
Fungating	15	46.9	20	44.4	χ^2 = 0.09 P = 0.95
Infiltrating	6	18.8	8	17.8	
Ulcerative	11	34.4	17	37.8	
Distant metastasis					
M0	31	96.9	36	80.0	χ^2 = 4.7 P = 0.03*
M1	1	3.1	9	20.0	
PTNM					
I-II	15	46.9	22	48.9	χ^2 P = 0.86
III-IV	17	53.1	23	51.1	
Lymphovascular invasion	8	25.0	11	24.4	χ^2 = 0.003 p = 0.96
Perineural invasion	9	28.1	8	17.8	χ^2 = 1.16 p = 0.28
Tumor recurrence					
Negative	31	96.9	35	77.8	χ^2 = 5.57 p = 0.02*
Positive	1	3.1	10	22.2	

χ2 = Chi-square test * p value significant if < 0.05. MC = Monte Carlo z = Mann Whitney U test.

Tab. IV. Binary logistic regression to detect predictors of CD133 positive cases.

Predictors	Odds ratio	95% CI	P
Tumor recurrence (positive)	8.86	(1.07-73.18)	0.04*
Tumor metastasis (positive)	7.75	(0.93-64.6)	0.058

P: probability * p value significant < 0.05 CI: Confidence interval.

pendent prognostic factor for tumor recurrence.

This positive relationship between high CD133 expression and tumor aggressiveness suggests that CD133-positive cells have more cancer stem-like

cells. These cancer stem-like cells with their self-renewal and differentiation abilities could be responsible for tumor recurrence and metastasis even after traditional treatment [19]. It indicates that CD133 may be a promising prognostic markers in gastric adenocarcinoma [10].

This study found no significant relation between CD133 score and the sex and age. This finding agreed with that of Ishigami et al.; Zhao et al.; Poddar et al. [20 10 21] and in contrary to that of Nosrati et al. that reported positive correlation between CD133 and age over 65 years with $P < 0.001$ that is statistically highly significant [11]. Moreover, there was insignificant relation between CD133 expression and tumor size, histological type and grade and TNM staging. This finding disagreed with some studies including Ishigami et al., Zhao et al., Nosrati et al. and Poddar et al. that found significant relation between CD133 expression and tumor size, histological type, grade and TNM staging [20 10 11 21]. This discrepancy in significance may be related to different patient characteristics, genetics, race, environmental factors, and the application of different scoring methods for CD133 expression. A non-significant relation between lymphovascular invasion and CD133 expression was found in the current study that agreed with that of Nosrati et al. [11], but in contrast to that of Ishigami et al.; Yu et al. and Poddar et al. that reported significant relation between CD133 expression and lymphovascular invasion with p value = (0.027), (0.000) and (0.01) respectively that was considered significant [4 20 21].

In conclusion, CD133 is a good molecular biomarker that can predict tumor recurrence and metastasis in gastric carcinoma. Regardless, studies regarding CSCs are still in their initial stages especially those related to CD133 in gastric cancer. Further studies are required to confirm the role of CD133 in tumor recurrence and metastasis and consequently promote finding more appropriate treatment modalities involving targeted gene therapy.

Conflict of interest statement

None declared.

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