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Original Article

Prognostic role of neuroendocrine cell differentiation in human gastric carcinoma

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Abstract: The objective of the current study was to investigate the significance and biologic characteristic of neuroendocrine cell differentiation (NED) in gastric carcinoma by comparing the prognosis and clinicopathologic characteristics between patients with or without NED. Retrospective analyses of neuroendocrine markers, neuron specific enolase (NSE), chromogranin A (CgA), and synaptophysin (Syn) were performed in 174 human gastric carcinoma patients. NED association was found in 21.3% gastric carcinoma patients, with or without NED, and was correlated with tumor location, cancer emboli, infiltrative depth, TNM stage and distant metastasis ($P < 0.05$ in each case). The 1-year and 3-year survival rate of the patients who suffered from gastric carcinoma with NED were significantly lower than those without NED. The overall survival time of patients with NED was shorter than those with gastric carcinoma without NED, with a significant difference between the two types ($P = 0.037$). Cumulatively, gastric carcinoma patients with NED had shorter postoperative survival time and poorer prognosis.

Keywords: Gastric carcinoma, neuroendocrine differentiation, prognosis, neuron specific enolase, synaptophysin, chromogranin A

Introduction

Neuroendocrine tumors can result in either pure endocrine tumors or mixed neoplasms harboring characteristics of both non-neuroendocrine features and neuroendocrine differentiation [1]. Gastric carcinoma with neuroendocrine differentiation (NED) is characterized by differentiated neuroendocrine cells scattered in the form of single cells or cell nests distributed between gastric carcinoma cells, accompanied by cancer tissue ingredients. The incidence of gastric carcinoma with NED is still a matter of debate with studies indicating ranges between 18.7% [2] to 53% [3].

Histological differentiation of gastric tumors are the clinical recommendation for predicting gastric carcinoma prognosis and it has been explicitly shown before that degree of differentiation positively correlated with gastric carcinoma aggressiveness [4-7]. However, there have been very few studies that evaluated the correlation of NED and disease progression in gastric carcinoma patients. Hence, the objective of the current study was to perform immunohistochemical staining-mediated detection of gastric carcinoma associated with NED, conduct comprehensive follow-up, and evaluate the prognostic significance of NED-associated gastric carcinoma.

Materials and methods

Study design, patient enrollment

The current study was approved by the Institutional Review Board of the People's Liberation Army General Hospital. We selected 174 patients who were diagnosed with gastric carcinoma and who received a gastrectomy at the Department of General Surgery, People's Liberation Army General Hospital between January 2006 and December 2008. The classic neuroendocrine tumor, neuroendocrine carcinoma and mixed gonadal neuroendocrine cancer were excluded from this study. Data was retrospectively collated using medical records and telephone interviews, follow-up time being up to 75 months (until September 2011). We analyzed patient's age, gender, tumor size, tumor location, cancer stage, pathological clas-

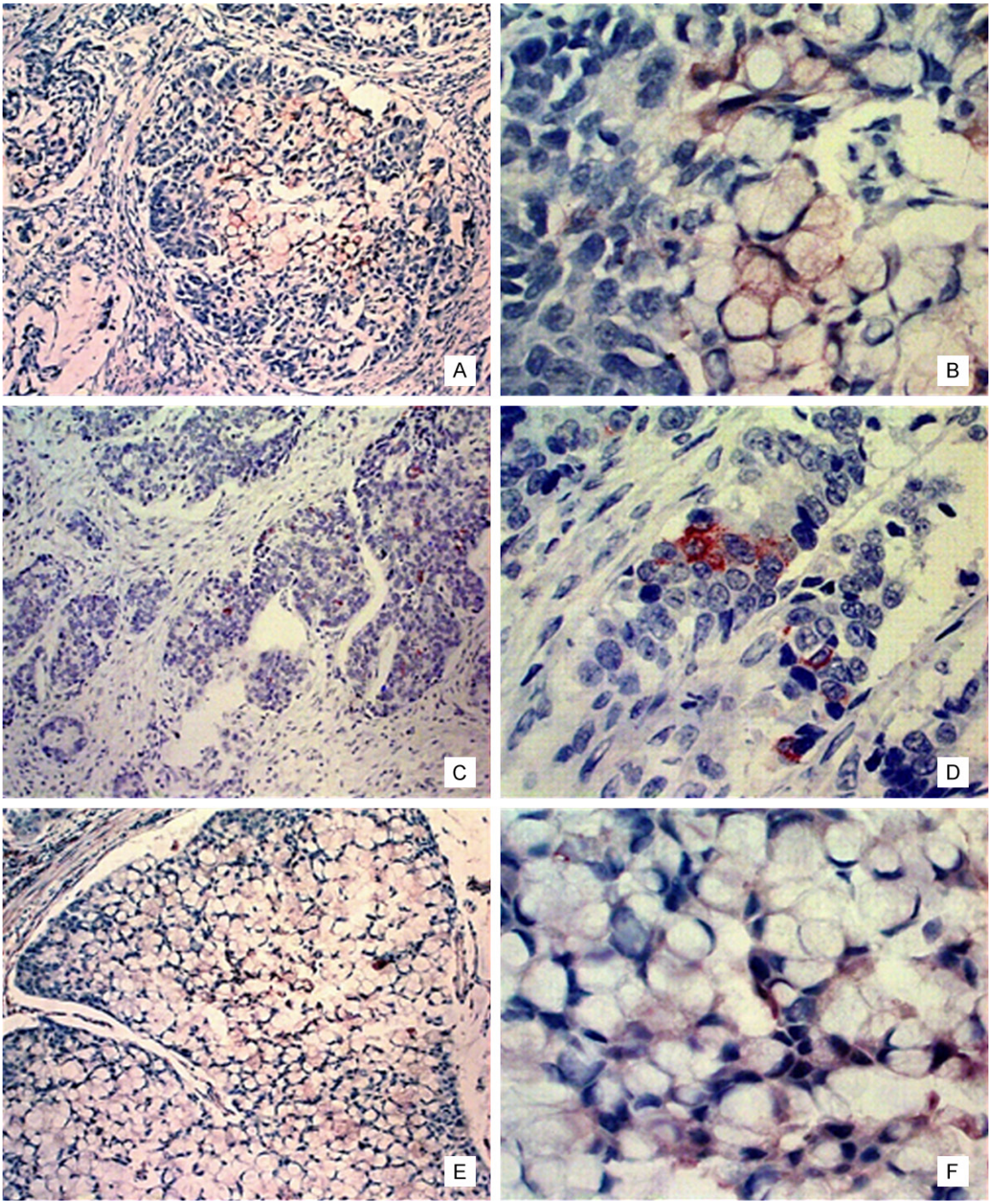

Figure 1. Immunohistochemical analysis of gastric carcinoma with neuroendocrine differentiation marker expression levels, including synaptophysin (Syn) (A, B), chromogranin A (CgA) (C, D), neuron-specific enolase (NSE) (E, F) at 100 and 400× magnifications, respectively.

sification, histological differentiation, and overall 1, and 3-year survival rates. The follow-up analyses included postoperative treatment and the program of choice, time to recurrence, time of death.

Inclusion and exclusion criteria

The patient inclusion criteria were as follows: (1) gastric carcinoma confirmed by histology; (2) patients underwent radical resection; (3)

Table 1. Correlation between the gastric carcinoma patients with or without NED and clinicopathologic features (n, %)

Characteristics	With NED (n = 37)	Without NED (n = 137)	*P* value
Gender			
Male	32 (86.5%)	114 (83.2%)	0.630
Female	5 (13.5%)	23 (16.8%)	
Age (years)			
≤ 40	1 (2.7%)	11 (8%)	0.270
41-65	20 (54.1%)	83 (60.6%)	
> 65	16 (43.2%)	43 (31.4%)	
Type of gastrectomy			
D1	18 (48.6%)	90 (65.7%)	0.058
D2	19 (51.4%)	47 (34.3%)	
Total gastrectomy			
Yes	1 (2.7%)	18 (13.1%)	0.131
No	36 (97.3%)	119 (86.9%)	
Tumor size (cm)			
< 4	10 (27%)	34 (24.8%)	0.961
4-7	20 (54.1%)	77 (56.2%)	
≥ 8	7 (18.9%)	26 (20%)	
Tumor location			
Cardiac	21 (56.8%)	63 (46.0%)	0.0007
Corpus	11 (29.7%)	15 (11.0%)	
Corpus distal	5 (13.5%)	59 (43%)	
Histological differentiation			
Well differentiated	2 (5.4%)	5 (3.7%)	0.562
Moderately differentiated	5 (13.5%)	28 (20.4%)	
Poorly differentiated	30 (81.1%)	104 (75.9%)	
Lauren's classification			
Intestinal type	20 (54.1%)	76 (55.5%)	0.988
Diffuse type	12 (32.4%)	43 (31.4%)	
Mixed type	5 (13.5%)	18 (13.1%)	
Perineural invasion			
Yes	5 (13.5%)	10 (7.3%)	0.256
No	32 (86.5%)	127 (92.7%)	
Surgical margin			
R0	29 (78.4%)	107 (78.1%)	0.971
R1	8 (21.6%)	30 (21.9%)	
Cancer emboli			
Yes	15 (40.5%)	19 (13.9%)	0.000
No	22 (59.5%)	118 (86.1%)	
Depth of invasion, T stage			
T1	0 (0%)	11 (8%)	0.000
T2	2 (5.4%)	34 (24.8%)	
T3	21 (56.8%)	86 (62.8%)	
T4	14 (37.8%)	6 (4.4%)	
Lymph node metastasis			

patient's tissues were adequate to be detected; (4) patients had not received any treatments prior to surgery; and, (5) all data of patient medical history, record of surgery, pathological report and follow-up were available. The exclusion criteria were as follows: (1) histological diagnosis revealed gastric sarcoma, interstitialoma, lymphoma, etc; (2) patient received either neoadjuvant chemotherapy or radiotherapy before surgery; (3) patients underwent palliative surgery; (4) surgical resection margin was R2; (5) surgical tissue samples were not enough to be tested; (6) patients died from other reasons for unexpected outcomes; (7) patient had suffered from other malignancies before gastric carcinoma; and, (8) patients were lost to follow-up.

Diagnosis and confirmation of neuroendocrine cell differentiation

All tumor sections were fixed with 10% formalin and embedded immediately in paraffin post-resection. The staining procedure followed for immunohistochemistry was as follows: Tissue sections were de-waxed and washed with serial washes of alcohol and water before being immersed in 3% hydrogen peroxide for 10 minutes (for getting rid of endogenous peroxidase) and subsequently washed for three times with distilled water. The specimens were then subjected to pressure repair, cooled at room temperature for 20 minutes, washed with distilled water for three times before being washed with phosphate buffered saline (PBS) for 15 minutes. The slides were blocked using goat serum at 37°C for 20 minutes in a humidor. Subsequently the slides were incubated with anti-chromogranin A (CgA), anti-synaptophysin (Syn), or anti-neuron-specific enolase (NSE) at 4°C overnight. Following three washes with PBS at room temperature, color was developed using 3, 3'-diaminobenzidine (DAB). The slides were counterstained with hematoxylin. NED of gastric carcinoma was diagnosed when (a) hematoxylin staining revealed neuroendo-

LN = 0	10 (27%)	48 (35%)	0.482
LN = 1-6	12 (32.4%)	47 (34.3%)	
LN ≥ 7	15 (40.6%)	42 (30.7%)	
Distant metastasis			
Yes	8 (21.6%)	4 (2.9%)	0.000
No	29 (78.4%)	133 (97.1%)	
TNMs stage			
I	1 (2.7%)	28 (20.4%)	0.024
II	10 (27%)	26 (19%)	
III	18 (48.7%)	69 (50.4%)	
IV	8 (21.6%)	14 (10.2%)	
Postoperative chemotherapy			
Yes	17 (45.9%)	84 (61.3%)	0.093
No	20 (54.1%)	53 (38.7%)	

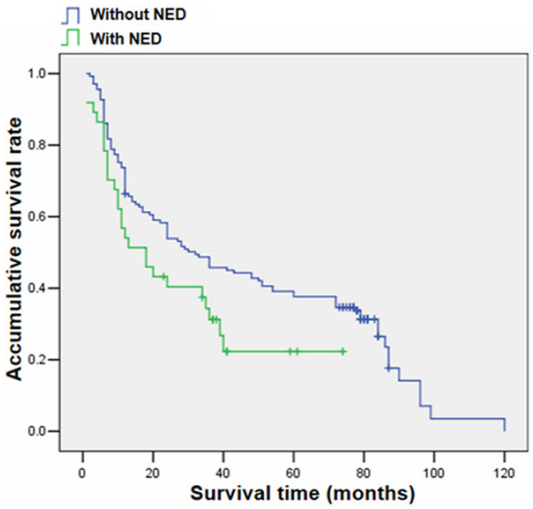

Figure 2. Kaplan-Meier survival analysis between the patients who have gastric carcinoma with or without neuroendocrine cell differentiation (NED).

crine cells in cancer tissue dispersed in the form of single cells or cell nests, and (b) the tissues were positive for one of the three markers, CgA, Syn and NSE.

Statistical methods

The data obtained was tested using the χ^2 test. The survival time was defined as the day of gastric surgery to the last follow-up or time of death. Survival analysis was performed using the Kaplan-Meier method. Log-rank rank test was conducted to analyze significant difference of survival time. Cox proportional hazards model was used to assess the correlation of various clinicopathological factors and survival of patients with gastric carcinoma, with or without NED. Statistical analysis application SPSS17.0 software was used for all statistical analyses. A P value of < 0.05 was considered statistically significant.

Results

Histological findings

Out of the 174 cases of gastric carcinoma enrolled in the current study, 37 cases (21.3%) expressed at least one of the three tested NED markers. CgA positive expression was observed in 16 cases, Syn positive expression in 30 cases, and NSE positive expression was observed in 12 cases (**Figure 1**).

Clinicopathological findings

The clinicopathological findings are summarized in **Table 1**. In gastric carcinoma patients with NED, the male to female ratio was 32:5. The age distribution was 38 to 81 years old, with more than 50% of the cases in the age range of 41-65 years. Both groups (with and without NED) had similar clinical symptoms, inclusive of abdominal discomfort, abdominal pain, and bloating. All patients underwent surgical resection, out of which 101 patients (58%) had received postoperative chemotherapy.

The location of the tumor was more common in parts cardia in both groups; however there were more tumors located in gastric body than antrum in the NED group as opposed to the non-NED group. Between the two groups of patients, there was no statistically significant difference in terms of gender, age, type of gastrectomy, total gastrectomy, tumor size, degree of tumor differentiation, Lauren's classification, perineural invasion, surgical margin, regional lymph node metastasis, and post-operative chemotherapy treatment. However, tumor location (P = 0.0007), cancer emboli (P = 0.000), depth of invasion (P = 0.000), distant metastasis (P = 0.000) and TNM stage (P = 0.024) were significantly associated with neuroendocrine cell differentiation (**Table 1**).

Survival analysis

As shown in **Figure 2**, in postoperative gastric carcinoma patients with NED, the survival rate was significantly lower than that associated

Table 2. Survival analysis of prognostic factors in gastric carcinoma by parametric regression model

Prognostic variables	RR	95% CI	*P* value
Group (with or without NED)	0.521	0.416-0.653	< 0.001
Gender	1.169	0.857-1.595	0.324
Age	0.952	0.822-1.103	0.513
Tumor location	0.979	0.889-1.078	0.664
Histological differentiation	1.11	0.957-1.287	0.169
TNM stage	1.009	0.907-1.122	0.869
Postoperative chemotherapy	1.117	0.931-1.338	0.234

with non-NED patents. NED was correlated to poorer prognosis, with 1 and 3-year survival rates significantly lower than the non-NED group of gastric carcinoma ($P = 0.037$). Postoperative survival time of gastric carcinoma patients with NED was significantly lower than that associated without NED.

Multivariate analysis affecting the prognosis of gastric carcinoma

The parametric regression model was used to assess and analyze the clinicopathological factors associated with the prognosis of gastric carcinoma patients, with or without NED. The results showed that neither of the patient's gender, age, tumor location, histological differentiation, TNM stage, or postoperative chemotherapy were independent factors for prognosis of gastric carcinoma ($P > 0.05$). However, NED was an independent factor affecting the prognosis of gastric carcinoma (**Table 2**).

Discussion

Gastric carcinoma is one of the common malignant tumors of the digestive tract, with global increases in incidence and mortality rates. Although the diagnosis and treatment methods are well established, and are being continuously improved, it has been almost impossible to improve the survival rate of gastric carcinoma patients. In the present study we found statistically significant difference among the two groups of gastric carcinoma patients, with or without gastric NED, in the tumor location, cancer emboli, depth of invasion, distant metastasis and TNM stage.

The stomach is the most important human neuroendocrine organ, and plays an important role in the regulation of normal physiological function and is closely related to the occurrence of the disease. The 4th edition of WHO digestive tumor classification in 2010 revised nomenclature and classification of neuroendocrine tumors [8]; gastrointestinal and pancreatic neuroendocrine tumors were classified as neuroendocrine tumors (neuroendocrine tumor, NET), neuroendocrine carcinoma (NEC), and mixed adenoneuroendocrine carcinoma (MANEC) [8]. In addition, adenocarcinoma with scattered neuroendocrine cells confirmed by immunohistochemistry cannot be included in the neuroendocrine tumors and named as adenocarcinoma with NED, which would still be classified as adenocarcinoma.

Gastric carcinoma with NED is difficult to determine with light microscope, the diagnosis is based on immunohistochemistry results of neuroendocrine markers. We used immunohistochemical method to detect chromogranin A (chromogranin A), synaptophysin (Syn) and neuron-specific enolase (NSE) to determine NED. According to the literature, CgA is the most sensitive and specific marker to neuroendocrine (NE) cells [9]. However, there is no uniform standard as to the specific NE markers tested. Thus our logic was that using a multitude of markers would improve diagnosis and decrease false-positives. The results of this study found that, through combined detection of CgA, Syn and NSE, the NED positive expression rate was 21.3%. Currently, H&E staining based morphological screening is routinely used to identify NE tumors, rather than using immunohistochemical staining; in turn, perhaps significantly reducing the detection rate of gastric carcinoma patients with NED.

The clinical benefit in recommending treatment or prognosis seems to be controversial. Song et al. [10] showed that gastric carcinoma patients with NED had shorter postoperative survival time and overall poorer prognosis. However, Eren et al. [11] showed that in gastric adenocarcinoma, NED did not seem to affect the prognosis. The later also suggested that NED might actually up-regulate the expression of vascular endothelial growth factor (VEGF) and affect the incidence of lymph node metastasis to promote neoangiogenesis. In the present study, we found that whether gastric carcinoma is accomplished with NED is associated with tumor location, cancer emboli, depth of invasion, distant metastasis, and TNM stage. Cox

proportional hazards model assessment found that patient's age, tumor size, regional lymph node metastasis and distant metastasis are independent factors for prognosis of gastric carcinoma ($P < 0.05$), while with or without NED is not. Therefore, we believe that NED may aid in tumor cell invasion and plays a certain role in the metastasis process of gastric carcinoma.

Treatment of gastric carcinoma with NED is mainly based on surgery, and the choice of surgical resection depends on the pathological type, tumor size, tumor location, depth of invasion, lymph node involvement and distant metastasis of primary tumor. Post-operative chemotherapy is often adopted according to the pathological results and the NCCN (National Comprehensive Cancer Network) guidelines. However, there is very little study on the chemotherapy of gastric carcinoma patients accomplished with NED. The correlation between its sensitivity to chemotherapy and NED is currently not yet clear and still needs to be further studied.

In summary, we believe that it is an important method to determine the common gastric carcinoma using morphological screening combined with immunohistochemistry for detection of NE markers. One potential limitation of our current study is that we did not evaluate whether neuroendocrine differentiation was also preserved in locoregional lymph node metastasis, which needs to be further pursued. NED in gastric carcinoma may be an important adverse prognostic factor. Assessment whether gastric carcinoma is accompanied by NED would be of great value for predicting prognosis and in clinical choice of adjuvant therapy. However, the basis of the molecular pathology of this type of gastric carcinoma and postoperative treatment program need further study.

Disclosure of conflict of interest

None.

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