# Studies on the Relationship between Neuroendocrine Cellular Differentiation in Gastric Cancers and Post-operative Survival Time

Song He[1]
Qiao Yan[2]
Xudong Chen[1]
Jianbing Zhang[1]
Xiaoyun Lu[1]
Hongjia Pan[2]
Li Chen[2]

[1] Department of Pathology, Nantong Cancer Hospital, Nantong, Jiangsu 226361 China.
[2] Department of Pathology, Nantong University, Nantong, Jiangsu, China.

Correspondence to: Song He
E-mail: hesong515@sina.com

**OBJECTIVE**    To examine the ultrastructure of gastric cancer cells by the electron microscope, in order to assess the relationship between neuroendocrine differentiation and post-operative survival time.
**METHODS**    NSE, Syn and CgA immunohistochemical labeling was conducted in 168 cases with a common-type of gastric cancer. Electron microscopy was performed in 80 cases with positive immunohistochemical labeling. These cases were followed-up for over 5 years and the post-operative survival data analyzed.
**RESULTS**    Neuroendocrine granules were found by electron microscopy in 39 cases. The rate of neuroendocrine differentiation found was 23% (39/168), using routine diagnostic criteria and electron microscopy (REM). The post-operative survival time of gastric cancer patients with neuroendocrine differentiation was significantly shorter ($P$=0.0032) compared to those without neuroendocrine differentiation.
**CONCLUSION**    It is of significant clinical importance to determine if the neuroendocrine cells are differentiated in gastric cancers. The gastric cancer patients with neuroendocrine differentiation have a shorter post-operative survival time and a poorer prognosis. Electron microscopy is a reliable method of providing a diagnosis.

**KEYWORDS: gastric cancer, neuroendocrine, electron microscopy, post-operative survival time.**

## INTRODUCTION

Gastric carcinoma (GC) has a high incidence and is one of the most common malignant tumors of the digestive tract. This carcinoma causes the most deaths from malignant tumors in many areas of China. Though the accuracy of diagnosis and efficacy of treatment has continuously increased, it still has been difficult to improve the post-operative survival rate of GC patients. Over the past few years, continuous and in-depth research on GC neuroendocrine (NE) differentiation has been conducted, and precise evaluation of the prognosis of GC cases would be helpful to individualize treatment of patients. In our study, electron microscopic examinations were used to assess the neuroendocrine differentiation of the common-type of GC. Follow-up data were gathered to extensively investigate the relationship between the level of GC neuroendocrine differentiation and post-operative survival time.

## MATERIALS AND METHODS

### Clinical data

A total of 168 common-type GC cases were selected. These pa-

tients had undergone radical surgery and received follow-up in the Nantong Cancer Hospital, Jiangsu during the period from 1990 to 1995. The follow-up period was 60 months, with a deadline of May 2001. Following review of the 168 cases using the pathological sections, the patients were classified into a TNM clinical stage based on the WHO 2000 classification. The cases were then randomly divided into 5 groups according to their survival time as follows: Group A, ≤12 months; Group B, 13~24 months;  Group C, 25~36 months; Group D, 37~59 months and Group E, ≥60 months.

## Electron microscopy and judgment of the results

Labeling of NSE, Syn and CgA was conducted for the 168 common-type GC tumors, using an immunohistochemical indirect method. The immunohistochemical neuroendocrine examination was considered to be positive if there was expression in any one of the three markers. All 80 cases with positive neuroendocrine expression were selected, and an electron microscopic examination of the paraffin mounted sections of these positive cases was conducted using the following methods: an immunohistochemical positive area was chosen by microscopic examination, and localization was conducted on the related paraffin block. A no. 16 needle was used to punch out a tissue sample, which was deparaffinized three times in xylene (1.5 h each time) and hydrated in an alcohol gradient. The samples were fixed with osmic acid, after a 2.5% glutaral fixation and a washout with phosphate buffer. After dehydration and resin embedment, the tissues were cut into the semi-thin sections followed by toluidine blue staining, after which the region of interest was selected under the light microscope, and cut into ultra-thin sections. Following double staining with lead-uranium, the sections were photographed under a H-600EX-type transmission electron microscope. The NE differentiation was assured based on the identification of NE granules in several ultra-thin sections under the electron microscope.

## Statistical analysis

The cases with identified NE granule, were classified into a NE differentiation (NED) group, and those without apparent NE granules, and/or with negative immunohisto- chemical NE markers were classified into a NE undifferentiated (NEU) group. The data related to the number of months of survival for the 168 cases and microscopic results were analyzed using Stata 7.0 medical software. The log-rank test of the survival time was carried out, and a Kaplan-Meier survival curve was produced, with α=0.05.

# RESULTS

## Follow-up

The follow-up results of the 168 GC cases were as follows: there were 99 cases with a survival of over 5 years, and 69 cases of less than 5 years (21 cases survived ≤ 12 months, 23, 13-24 months, 15 25-36 months, and 10 37-59 months). Microscopic observation of the 80 cases with positive immunohistochemical samples was conducted, and NE granules were found in 39 of these cases. Based on the electron microscopic results, the rate of detection of the differentiated NE GC was 23% (39/168 cases). For the number of differentiated cases in the 5 survival groups see Table 1.

**Table 1. Relationship between NED of the GC and survival time of the patients.**

| Group | NED | NEU |
|-------|-----|-----|
| A | 7 | 14 |
| B | 10 | 13 |
| C | 5 | 10 |
| D | 2 | 8 |
| E | 15 | 84 |
| Total | 39 | 129 |

## Ultrastructure form

Electron microscopy showed that the number of the NE cells varied in quantity, and the cells and cell nuclei varied in size, with an indiscriminate polarity. Most of the chromatin was in the form of euchromatin, and large nucleoli were present. The endocrine granules lost their polarity in the cytoplasm, with a granule diameter ranging from 80 to 500 nm, and a disparate number of granules (Figs.1~3). These endocrine granules were divided into three types: in type-I, the secretory granules (SGs) were pantomorphic and small, with a high electron density. The average diameter ranged from 100 to 200 nm, similar to the EC cell. The type-II SGs were round, and their average diameter ranged from 100 to 250 nm, with an integrated limiting membrane, and usually with a dense core and areola zone. Some were vesiculiform, with an irregular low electron-density core in the center. A rich rough endoplasmic reticulum (RER) and polyribosomes could be seen in the cytoplasm, just like the ECL cells. The type-III SGs were the major type, having a diameter ranging from 150 to 500 nm. Some were vacuolar, with a floccose core of a low electron density. Some had a moderate electron density. Granules of different types could sometimes be found in the same tumor.
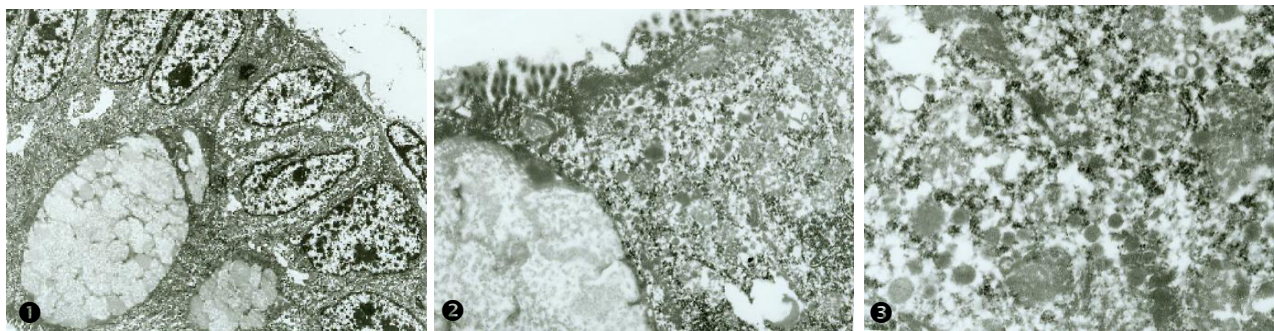
**Fig.1.** Large and irregular nucleoli of adenocarcinoma cells found by electron microscopy, with overt mucosal secretion ×2,500.
**Fig.2.** Non-homogeneous distribution of the NE granules in the cytoplasm, with a loss of polarity ×10,000.
**Fig.3.** SGs were round, with an inequality of size and an integrated limiting membrane. The dense core and empty-ring can be seen ×20,000.
**Fig.4.** Comparison of the post-operative Kaplan-Meier curves between the GC patients with and without NED.
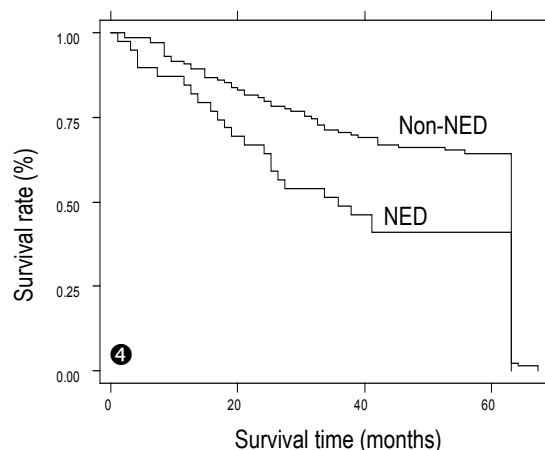
## Relationship between NE differentiation of the GC and post-operative survival time

The post-operative survival time of the GC cases with NED was significantly shorter compared to non-NED cases ($\chi^2 = 8.68$, $P=0.0032<0.01$). Fig. 4 shows that the difference in survival time between the two groups occurred at about the 25th month after operation. This difference became more obvious 40 months after operation.

## DISCUSSION

Gastric NE tumors, which are a type of tumor that originates from the gastric NE cells, can secrete a variety of hormones. Diagnosis of these tumors mainly depends on the histological form and immunohistochemical NE labeling. However differentiated gastric adenocarcinomas are difficult to identify by a light microscope as less than 50% of the differentiated NE cells occur in the tumor proper of a gastric adenocarcinoma. These cells exist dispersedly as individual cells or cell nests, which is characteristic of these cancers[1]. Both ultrastructural observation and immunohistochemical staining are of significant importance in identifying NED. Electron microscopy is the most reliable way to distinguish the NE granules, and this method can be used as a basis for the final diagnosis of NED.

Those with a NE expression in the common-type GC can be identified by its neuroendocrine differentiation of GC, which is classified as an adenocarcinoma[2]. Based on the previous literature, the expression of NE immunohistochemical markers in GC are inconsistent at present. For instance, researchers from overseas[3] conducted Syn and CgA labeling of 70 gastric adenocarcinoma cases, showing that 20%~30% displayed neuroendocrine differentiation. The findings of Li and Zheng[4] showed that the percentage was 11%~43%. In our study of 168 cases of gastric adenocarcinoma using immunohistochemical NE labeling and electron microscopy, we found the following degrees of expression: NSE, 30.4%, Syn, 28%; CgA, 21.4%, and in 23% of these cases NE granules were detected.

At present the biologic tumor features are used as a basis for determining the post-operative survival of the patients with a NE tumor[5]. Extensive research is being conducted on NE cells of GC by studying the relationship between NE cells and GC malignancy to improve prognosis of GC cases. Eren et al.[6] studied 42 cases of gastric adenocarcinoma, showing that NE cells were one of the characteristics of a malignant tumor. They showed a correlation between VEGF and p53 expression and the clinical progression and lymph node metastasis of the tumors. Nevertheless the data form previous literature on post-operative survival time of GC patients with the NED, were not

quite in agreement. Findings from Liu et al.[7] indicated that the post-operative survival time of the patients with gastric adenocarcinoma and positive NED was shorter compared to the patients with negative NED. In 2003, Naritomi et al.[8] reached a conclusion similar to that of Liu et al.[3] which had been reported previously[9,10]. It was suggested that there was no correlation between GCs with or without NED and the patient prognosis. Therefore it remains to be determined if NED of GC can be a practical guide for clinical treatment and for formation of a prognosis. However, it's worth noting that immunohistochemical methods had been employed in studies of NED in GC by almost all researchers overseas and in China. A number of NE markers, such as NSE, Syn and CgA etc. were examined but the results were not always in agreement, and the criteria for an immunohistochemical positive-reaction varied, resulting in uncertainty in the reliability and the value of NED for GC diagnosis.

It is well-known that ultrastructural electron microscopic analysis can provide reliable evidence for diagnosing a NE tumor and the NED of a tumor, but no reports have been published in China or abroad concerning the relationship of NED with survival time. In our study of GC cases using electron microscopy, we compared survival time of cases with and without NED, showing that survival time of the GC cases with NED was significantly shorter ($P$=0.0032<0.01) compared to those without NED. Because of the poor prognosis in NED cases, active therapy for these cases should be adopted.

In general, pathologic confirmation of NED in the common-type of GC is of important clinical significance because of the need for aggressive post-operative symptomatic treatment. The ultrastructural findings, specific for NE granules, provide reliable evidence for a diagnosis, so it is highly recommended, as far as possible to conduct an ultrastructural examination of the tissues in GC cases in the hospital and clinic that have the proper equipment.

## REFERENCES

1   Kamiya N, Akakura K, Suzuki H, et al. Pretreatment serum level of neuron specific enolase (NSE) as a prognostic factor in metastatic prostate cancer patients treated with endocrine therapy. Eur Urol 2003; 44: 309–314.
2   Tang CL. Development of gastric carcinoma with neuroendocrine differentiation. J Intl Pathol Clin Med 2007; 27: 130–132 (Chinese).
3   Fujii A, Kamiakito T, Takayashiki N, et al. Neuroendocrine tissue-specific transcription factor, BETA2/NeuroD, in gastric carcinomas: a comparison with chromogranin A and synaptophysin expressions. Pathol Res Pract 2003; 199: 513–519.
4   Li HZ, Zheng LR. Immunohistochemical observation of the common-type gastric carcinoma with neuroendocrine differentiation. Chin J Clin Gastroenterol. 2005; 17:206–208 (Chinese).
5   Kaltsas GA, Besser GM, Grossman AB. The diagnosis and medical management of advanced neuroendocrine tumors. Endocrine Reviews 2004; 25: 458–511.
6   Eren F, Celikel C, Gulluoglu B. Neuroendocrine differentiation in gastric adenocarcinomas; correlation with tumor stage and expression of VEGF and p53. Pathol Oncol Res 2004; 10: 47–51.
7   Liu JR, Wei SL, Gao GD, et al. Clinical pathologic significance of neuroendocrine cell in gastric carcinoma. Chin J Cancer Prev Treat 1996; 23: 347–349.
8   Naritomi K, Futami K, Arima S, et al. Malignant potential regarding mucin phenotypes and endocrine cell differentiation in gastric adenocarcinoma. Anticancer Res 2003; 23: 4411–4422.
9   Zhu B, Zhang CN, Chen YQ. Immunohistochemical research on p53 expression in neuroendocrine differentiation of the gastric cancer. J Clin Surg 1997; 35: 298 (Chinese).
10  Li J, Zhang XH, Meng K, et al. Clinical pathologic studies on neuroendocrine carcinoma of the stomach and gastric carcinoma with neuroendocrine differentiation. J Med Postgraduate 2003; 16: 327–328 (Chinese).