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ARTICLE

Neuroendocrine Differentiation in Gastric Adenocarcinomas; Correlation with Tumor Stage and Expression of VEGF and p53

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Studies on neuroendocrine differentiation (NED) in conventional gastric adenocarcinomas and its significance on tumor behavior are limited. Our aim was to search for the expression of neuroendocrine differentiation in conventional gastric adenocarcinomas and correlate it with tumor type, stage and expression of VEGF and p53. Forty-two gastrectomy specimens with gastric adenocarcinoma were stained with chromogranin A to detect neuroendocrine differentiation and 45% of the cases were found to be NED (+). No significant correlation was found between NED and tumor type. However, NED was more frequent in advanced stage cases independently of tumor type. VEGF expression was also considerably more frequent in NED (+) tumors compared to NED (-) ones (84% vs. 56%). Moreover, we found a significant correlation between NED and the presence of lymph node metastases. P53 expression in NED (+) tumors was 68%. There was no significant correlation between VEGF and p53 in NED (+) cases. In conclusion, neuroendocrine differentiation is a frequent finding in conventional gastric adenocarcinomas, and although it does not seem to play a specific role in tumor progression, it seems that neuroendocrine cells are one of the factors contributing to angiogenesis by expressing VEGF, especially in advanced stage cases, affecting the incidence of lymph node metastases. Further studies with larger series should be performed to confirm this observation. (Pathology Oncology Research Vol 10, No 1, 47–51)

Keywords: Gastric adenocarcinoma, neuroendocrine, p53, VEGF

Introduction

The presence of neuroendocrine cells in normal gastric mucosa and tumors arising from these cells: carcinoids, neuroendocrine carcinomas or small cell undifferentiated carcinomas have been known for many decades. Another pattern in GI neoplasms is the presence of scattered tumor cells showing neuroendocrine differentiation in an otherwise typical adenocarcinoma. Although there are many studies on neuroendocrine tumors of gastric mucosa, studies on neuroendocrine differentiation (NED) in conventional gastric adenocarcinoma and its significance on tumor behavior is limited.

In conventional gastric adenocarcinomas, VEGF expression has been found to be an important prognostic factor, similarly to p53 expression which has also been shown to play a regulatory role in the control of angiogenesis through regulation of VEGF expression.[10,16] As neuroendocrine cells are known to express angiogenic factors like VEGF,[17] in this study we examined the presence of neuroendocrine differentiation in conventional gastric adenocarcinomas and correlate it with tumor type, tumor stage and expression of p53 and VEGF.

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Material and Methods

Tumor samples

42 gastrectomy specimens of 24 male and 18 female patients with gastric adenocarcinoma were included in our study. The patients' ages ranged between 42-80 years (average 61). Patients received neither chemotherapy nor radiation therapy before surgery. Each case was re-evaluated for histologic type (Lauren), depth of invasion and presence of lymph node metastasis. One representative tumor block from each case was selected for immunohistochemistry.

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Immunohistochemical techniques

Immunohistochemical staining was performed by the streptavidine-biotin method. Monoclonal antibodies for chromogranin A (Biogenex AM 126-5M), p53 (Biogenex AM 240-5M) and VEGF (Oncogene 6F25-100U6) were used. In brief, sections were deparaffinized and incubated with 0.3% hydrogen peroxide in methanol for 20 min to block endogenous peroxidase activity. For p53 and VEGF the sections were microwave treated for 15 min in citrate buffer (pH 6.0). After being washed with phosphate-buffered saline (PBS), sections were incubated with primary antibodies for 30 min in the case of chromogranin A, 2 h for p53 and 1 h for VEGF, at room temperature. Followed by two washes with PBS, sections were incubated with biotinylated secondary antibodies (anti-mouse; Zymed 85-9143) for 10 min at room temperature, followed by 2 washes and were treated with streptavidine-peroxidase reagent for 10 min at room temperature. Finally, specimens were incubated in PBS containing diaminobenzidine for 5 min.

Analysis of staining

The tumors were classified as having neuroendocrine differentiation (NED +) when at least some tumor cells stained positive for chromogranin A. The positivity was scored as 1+ when scattered amount (<10%), 2+ when moderate amount (10%-75%) of tumor cells were positive for chromogranin A, and 0 when there was no immunoreactivity observed (*Table 1*). Tumors with diffuse (more than 75%) neuroendocrine differentiation were not included in our study.

Immunoreactivity for p53 was assessed as positive when tumor cells showed a complete nuclear staining pattern (*Figure 1*). Immunostaining for VEGF was considered positive when unequivocal staining of membrane or cytoplasm was seen in tumor cells (*Figure 2*). Evaluation of the density of staining for p53 and VEGF was made semi-quantitatively as follows: +++: > 75%; ++: 25-75%; +: < 25% of the tumor cells show positive staining; -: very scattered or no staining.

Statistics

Chi-square and Fisher exact tests were used.

Results

NED was detected in 19 of 42 specimens (45%); 7 of them had scattered and 12 moderate amount of chromogranin A positive tumor cells. Ten (53%) of these 19 cases were diffuse type and 9 (47%) were intestinal type tumors (*Figures 3, 4*). This ratio in NED (-) tumors was 48% and 52%, respectively. Statistically there was no significant correlation between the histologic type and neuroendocrine differentiation of the tumors.

When correlated with stage, 63% of NED (+) tumors vs. 39% of NED (-) ones were found to be of advanced stage (stages 3+4), while 11% of NED (+) vs. 39% of NED (-) tumors were of stage 1 (*Table 2*). Although the percentage of tumors with neuroendocrine differentiation was consid-

Table 1. **Characteristics of the NED(+) tumors**

Cases	*Stage*	*Tumor type*	*LN met*	*Chromogranin A*	*p53*	*VEGF*
1	2	D	+	+	–	+
2	1B	A	–	++	++	+++
3	2	D	+	+	++	+++
4	4	A	+	+	+++	++
5	3A	D	+	+	+	+
6	3A	A	+	+	–	+
7	3A	A	+	+	–	+
8	3A	A	+	+	++	+
9	3A	D	+	++	+++	–
10	2	D	+	+	+++	+
11	3B	A	+	++	–	+
12	4	D	+	+	+	+
13	2	D	+	+	–	++
14	4	D	+	+	–	++
15	3A	A	+	+	+++	++
16	3A	A	+	++	+	++
17	1B	A	+	+	+	++
18	2	D	–	++	+++	–
19	3A	D	+	++	+++	–

erably higher in advanced stage tumors, this difference was not statistically significant.

Lymph node metastasis was found in the case of 89% of NED (+) tumors, and in 50% of them NED was retained in the metastatic lymph nodes (*Figure 5*). On the other hand, lymph node metastasis was found in 56% of NED (-) tumors. This correlation was statistically significant ($p< 0.01$) (*Table 3*).

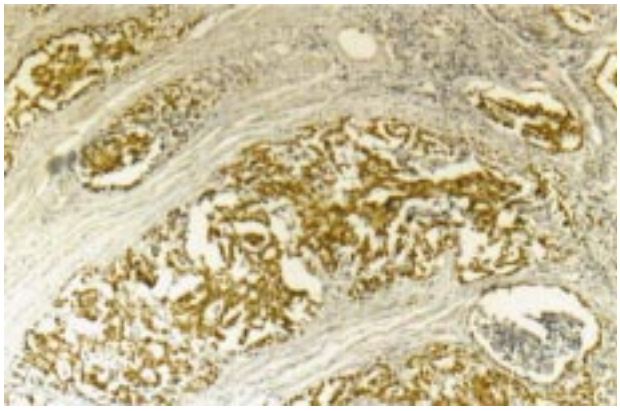

Figure 1. *p53 immunoreactivity (DAB X100)*

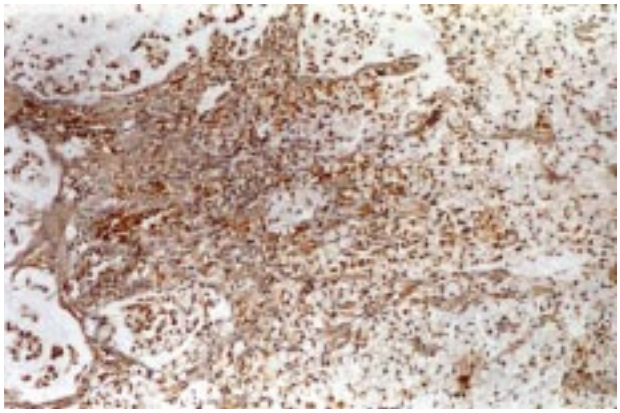

Figure 2. *VEGF immunoreactivity (DAB X100)*

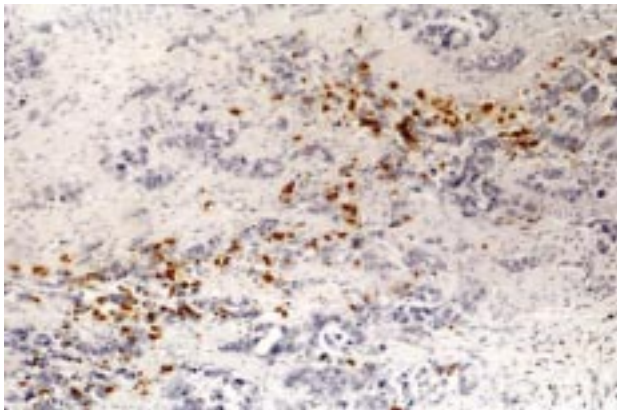

Figure 3. *Chromogranin A immunoreactivity in intestinal type gastric adenocarcinoma (X100)*

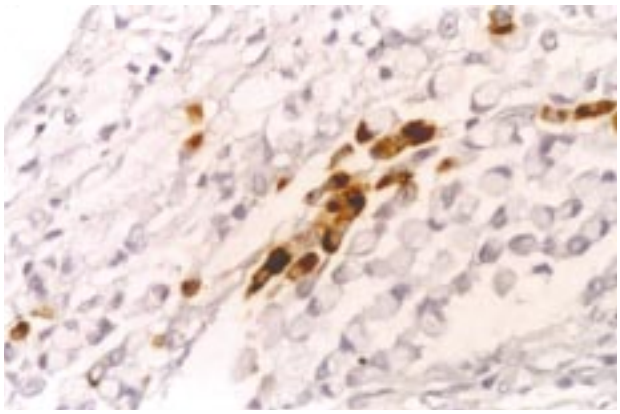

Figure 4. *Chromogranin A immunoreactivity in diffuse type gastric adenocarcinoma (X200)*

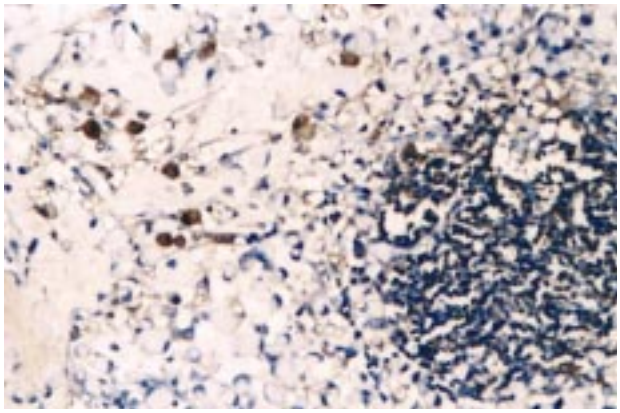

Figure 5. *Chromogranin A immunoreactivity in lymph node metastasis of diffuse type gastric adenocarcinoma (X100)*

We had follow-up records available in the case of 11 (26%) patients. Of these 11 patients, 5 (45%) died of disease within 2 years. The tumors showed neuroendocrine differentiation in 3 (60%) of them.

Overall immunoreactivity for p53 in NED (+) and NED (-) tumors were 68% and 43%, respectively. Although NED (+) tumors showed more frequent expression of p53, there was no statistically significant correlation between the presence of neuroendocrine differentiation and p53 expression (*Table 4*). When only p53 (+) tumors were analyzed, there was also no significant correlation found between different staining densities and NED.

Overall immunoreactivity for VEGF was 84% in NED (+) tumors whereas it was 56% in NED (-) ones. Although this finding was not statistically significant, it was very close to significance ($p= 0.09$) (*Table 4*). In tumors expressing VEGF no significant correlation was found between different staining densities. In cases with neu-

Table 2. **Correlation of presence of NED and tumor stage (%)**

STAGE	*NED(+)*	*NED(-)*
1	2(11)	9(39)
2	5(26)	5(22)
3	9(47)	8(35)
4	3(16)	1(4)

Table 3. **Correlation of lymph node metastasis and presence of NED (%)**

	LN Met (+)	*LN Met (-)*
NED (+)	17(89)	2(11)
NED (-)	13(56)	10(44)

Table 4. **Correlation of overall reactivity to P53, VEGF and NED (%)**

	P53(+)	*P53(-)*	*VEGF(+)*	*VEGF(-)*
NED(+)	13(68)	6(32)	16(84)	3(16)
NED(-)	10(43)	13(56)	13(56)	10(44)

roendocrine differentiation, 60% of VEGF (+) tumors were found to be of advanced stage.

No correlation could be found between VEGF and p53 expressions in cases with neuroendocrine differentiation.

Discussion

In this study, for the immunohistochemical detection of neuroendocrine differentiation we used chromogranin A which has been shown to be a valuable marker to detect neuroendocrine cells.[12,13]

We found neuroendocrine differentiation in tumor cells of nearly half (45%) of the conventional gastric adenocarcinomas. This is consistent with other studies indicating that NED is a frequent finding in conventional gastric adenocarcinomas.[2,12,15] Waldum et al. suggested a correlation between gastric carcinoma of diffuse type and neuroendocrine differentiation,[18] but the frequent presence of NED did not show any preference among histologic types in our study; distribution of NED in diffuse type carcinomas were very similar to that of intestinal type carcinomas (52% vs. 47%).

When correlated with the tumor stage, we noticed that NED was more frequent in advanced stage disease independently of tumor type, but the number of cases in some stage groups were too small to determine appropriate statistical significance. However, this finding was also observed in the study of Ooi et al.[13] in gastric carcinomas and Allen et al.[1] in prostatic adenocarcinomas. Similarly to these authors, we think that it is probably due to the haphazard expression of multipotential stem cells, which is more common in advanced stages.

There are studies of neuroendocrine differentiation in different types of tumors like prostate,[3] colon,[7,14] breast,[11] lung,[6] esophagus,[4] etc. which showed correlation of NED with either prognosis or prognostic factors. However, as far as we know, our study is the first in English literature which correlated the presence of neuroendocrine differentiation and expression of VEGF and p53 in gastric adenocarcinomas. It is well known that p53 and VEGF are related to tumor progression,[8,19] VEGF being a major angiogenetic factor.[9] In gastric adenocarcinomas Maeda et al. suggested that p53 and VEGF might be useful indicators of prognosis, and they demonstrated that p53 expression closely correlated with VEGF expression.[16] In our study we found that a high proportion of tumors (84%) with NED showed VEGF expression, whereas this expression was lower (56%) in NED (-) tumors. This difference was not statistically significant but close to significance. It is known that many neuroendocrine cells such as ECL cells normally produce factors like VEGF that induce angiogenesis.[17,5] We can suggest that, besides other factors, neuroendocrine cells also contribute to VEGF expression in gastric adenocarcinomas. In fact, VEGF expression was more frequent in advanced stage cases in which neuroendocrine differentiation was also found to be more frequent.

We found that a high proportion of tumors with neuroendocrine differentiation showed lymph node metastasis and in half of them chromogranin A positive tumor cells were retained in the metastatic lymph nodes, which is an evidence that chromogranin A positive cells are organic components of the tumor. The significant correlation with lymph node metastasis and the presence of NED can be related to frequent expression of VEGF in NED (+) tumors. We could not find any significant correlation between p53 and VEGF expression in tumors with neuroendocrine differentiation. Similarly, no correlation could be found between the presence of neuroendocrine differentiation and p53 expression.

We had follow-up data available for only 11 patients. Five (45%) of them had died of disease within 2 years, and NED was found in tumors of 3 of these 5 patients. Unfortunately the number of patients with follow-up data was too small to determine any statistical correlation between survival and the presence of NED in gastric carcinomas.

As a result, our findings show that NED is a common finding in conventional gastric adenocarcinomas independently of histologic type. Although the presence of neuroendocrine differentiation does not seem to play a significant role in tumor progression, we can suggest that neuroendocrine cells may contribute to angiogenesis by expressing VEGF, especially in advanced stage cases,

affecting the incidence of lymph node metastases. However, further studies with larger series should be performed to confirm this observation.

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