CASE REPORT

Concurrent occurrence of gastric adenocarcinoma and duodenal neuroendocrine cell carcinoma: a composite tumour or collision tumours ?

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Abstract

***Background*—Neuroendocrine cell (NEC) carcinoma is occasionally accompanied by adenocarcinoma but the relationship between these two morphologically distinct tumours is unclear. Two hypotheses have arisen regarding the mechanism for the association of adenocarcinoma and NEC carcinoma. One is that both are derived from a common multipotential epithelial stem cell. The second hypothesis is that adenocarcinoma and NEC carcinoma arise from a multipotential epithelial stem cell and a primitive NEC, respectively.**

***Aims*—To elucidate the relationship between the two histologically distinct tumours, adenocarcinoma of the stomach and NEC carcinoma of the duodenum.**

***Patient/methods*—We present a case in which the tumour extended across the pyloric ring, the gastric portion of which revealed adenocarcinoma while the duodenal portion showed argyrophil NEC carcinoma. The two histologically distinct lesions of the tumour were examined by immunohistochemistry and genetic analysis of *p53*.**

***Results*—The gastric region was negative for chromogranin A staining but positive for carcinoembryonic antigen (CEA) staining. In contrast, the duodenal region was positive for chromogranin A but negative for CEA. All tumour regions showed a point mutation in *p53* gene at exon 7 (GGC (glycine)→GTC (valine) at codon 245). The distal portion of the duodenal tumour showed an additional point mutation in *p53* gene at exon 5 (GCC (alanine)→GTC (valine) at codon 129).**

***Conclusions*—The two histologically distinct tumours, adenocarcinoma of the stomach and NEC carcinoma of the duodenum, appear to be derived from a common epithelial cell.**

(*Gut* 2001;**48**:853–856)

Keywords: neuroendocrine cell carcinomas; adenocarcinoma; composite tumour; collision tumours

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Accepted for publication 17 October 2000

It is well known that neuroendocrine cell (NEC) carcinomas are occasionally accompanied by adenocarcinomas in the gastrointestinal tract.[1 2] Morphologically, such lesions are classified into two subgroups: composite-type tumours, in which both components appear to be mixed haphazardly,[2–4] and collision-type tumours, which are considered as double tumours with a "side by side" or "one upon another" pattern.[5 6] According to such morphological classification, two hypotheses have arisen regarding the mechanism for the association of adenocarcinoma and NEC carcinoma. One is that both are derived from a common multipotential epithelial stem cell, the NEC carcinoma component resulting from differentiation from the adenocarcinoma to the NEC phenotype during tumour progression.[7] The second hypothesis is that adenocarcinoma and NEC carcinoma arise from a multipotential epithelial stem cell and a primitive NEC, respectively, and that they exist next to each other coincidentally.[8] To test which hypothesis is correct, it is essential to use both immunohistochemical and genetic studies but few reports have approached the adenocarcinoma–NEC carcinoma spectrum in such a way.[9]

Here we report a patient with a tumour in the pyloric region, extending to the duodenum beyond the pyloric ring, which histologically showed adenocarcinoma in the pylorus and NEC carcinoma in the duodenum. To elucidate the relationship between the two histologically distinct lesions of this tumour, we performed immunohistochemical and genetic analysis of the two lesions.

Case report

A 63 year old man presented with nausea. Laboratory findings showed no abnormalities on urine, peripheral blood, or serum examination. Upper gastrointestinal endoscopy suggested a pedunculated tumour in the pyloric region, with ulcer formation extending to the duodenum beyond the pyloric ring. Biopsy from the gastric portion of the tumour revealed

Abbreviations used in this paper: CEA, carcinoembryonic antigen; NEC, neuroendocrine cell; PCR-SSCP, polymerase chain reaction-single stranded conformational polymorphism.

adenocarcinoma whereas that from the duodenal portion revealed NEC carcinoma.

Following a diagnosis of gastroduodenal carcinoma, distal gastrectomy with lymph node dissection was performed. Gross examination of the surgical specimen showed an adjacent spherical tumour across the pyloric ring (fig 1A). Although the infrapyloric lymph node, the lymph nodes in the posterior groups along the common hepatic artery, and those on the posterior surface of the pancreatic head were positive for metastasis, the cut end of the resected sample showed negative results. In view of the patient's wishes and quality of life, additional pancreaticoduodenectomy was not performed. The postoperative course was uneventful and the patient was discharged from hospital on day 45 day after surgery. The following studies were approved by the Dokkyo University Surgical Pathology Committee, and the patient gave informed consent.

Methods

HISTOPATHOLOGICAL EXAMINATION

Tissue was fixed in 10% buffered formalin and embedded in paraffin. Sections 3 μm thick were cut and subjected to haematoxylin-eosin and Grimelius' staining. Immunohistochemical staining was performed with the avidin-biotin complex immunoperoxidase method using the following antibodies: monoclonal mouse antihuman chromogranin A (dilution 1:100) antibody (Dako Japan Co., Kyoto, Japan), monoclonal antihuman carcinoembryonic antigen (CEA; dilution 1:50) antibody (Dako Japan Co), and polyclonal antihuman p53 protein (dilution 1:2000) antibody (NCL-p53pCM1; Novocastra Lab., Newcastle upon tyne, UK).

GENETIC ANALYSIS OF *p53* BY POLYMERASE CHAIN REACTION-SINGLE STRANDED CONFORMATIONAL POLYMORPHISM AND DIRECT SEQUENCING

Mutations of the *p53* gene in the gastroduodenal carcinoma were investigated by polymerase chain reaction-single stranded conformational polymorphism (PCR-SSCP)[10] and direct sequencing. Tissues from six parts of the gastroduodenal carcinoma were examined (fig 1A). DNA was extracted from 10 mm paraffin sections of each tissue using a DNA isolator PS kit (Wako, Japan). One pair of oligonucleotide primers for each of four exons of *p53* were prepared: exon 5, 5'-TCTTCCTGCAGTACTC CCCT-3' (sense) and 5'-AGCTGCTCACCA TCGCTATC-3' (antisense); exon 6, 5'-TGAT TCCTCACTGATTGCT CT-3' (sense) and 5'-GAGACCCCAGTTGCAAACC-3' (antisense); exon 7, 5'-TTGTC TCCTAGGTTG GCTCT-3' (sense) and 5'-GCTCCTGAC CTGGAGTCTTC-3' (antisense); and exon 8, 5'-GCTTCTCTTTTCCTATCCTGA-3' (sense) and 5'-CGCTTCTTGTCCTGCT TGC-3' (antisense). The PCR reaction mixtures containing 0.1 μg of DNA sample and 25 pmol of each primer were subjected to amplification followed by 40 cycles of 94°C for 30 seconds, 60°C for one minute, and 72°C for one minute. Two microlitres of each amplified product were mixed with 6 μl of loading buffer (95% formamide, 10 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) and denatured by heating at 95°C for 10 minutes. A 5 μl aliquot of each sample was loaded onto a 18% polyacrylamide gel (60 W for five hours at 20°C) and stained using a Plus One DNA silver staining kit (Pharmacia Biotech, Sweden). PCR products were also analysed by direct sequencing according to the protocol of Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Chiba, Japan).

Results

The tumour across the pyloric ring was resected by surgery (fig 1A). Histopathological examination revealed that the tumour in the gastric region was an adenocarcinoma while that in the duodenal region was NEC carcinoma, showing a typical mass composed of infiltrating nests of small uniform tumour cells

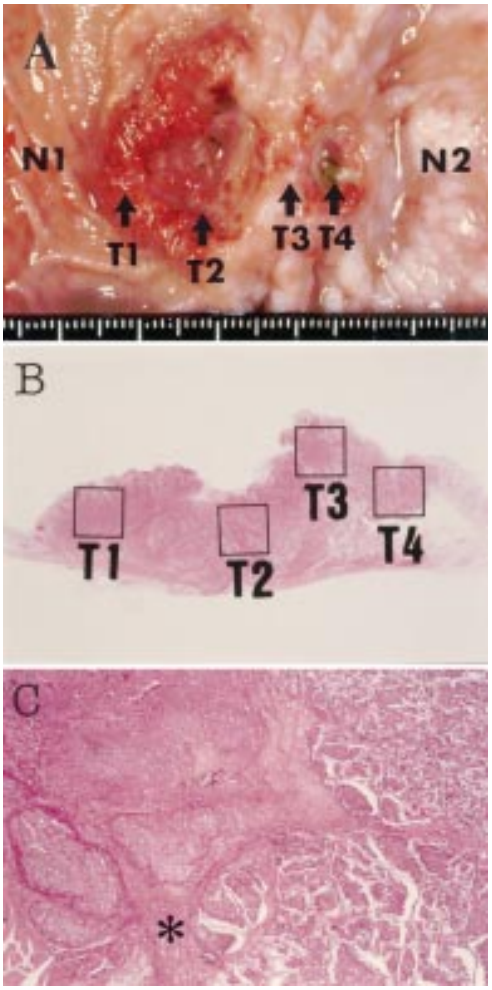

Figure 1 (A) Macroscopic view of the resected specimen showing a spherical tumour located at the pre-pylorus and duodenum. Tissues from six parts in and around the gastroduodenal carcinoma were microdissected to analyse p53 gene mutations: normal duodenum (N1), anal side of duodenal tumour (T1), centre of duodenal tumour (T2), tumour at the pyloric ring (T3), tumour of the stomach (T4), and normal stomach (N2). (B) Distinct areas (T1–T4) which were analysed for p53 gene mutation are indicated by the solid lines. (C) The well circumscribed tumour cell nests in the duodenal region revealed typical medullar growth of neuroendocrine carcinoma (left side). Poorly differentiated adenocarcinoma in the stomach showed small acinar pattern (right side). The pyloric sphincter (asterisk) clearly divides the two histologically distinct lesions.

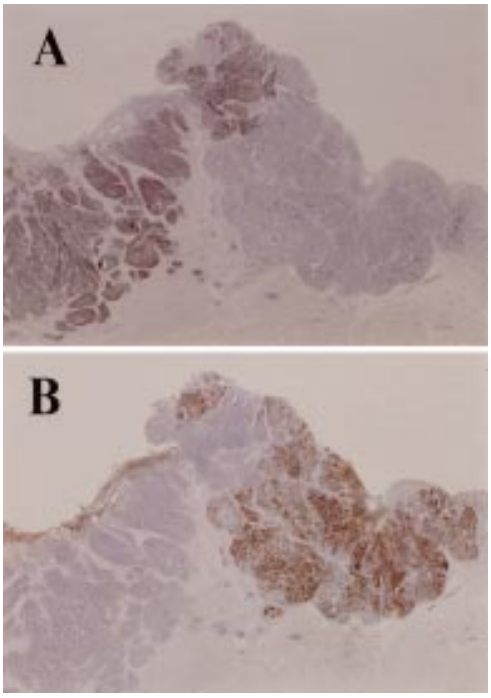

Figure 2 Immunohistochemical staining of the gastroduodenal tumour. (A) The duodenal lesion (neuroendocrine cell carcinoma) was positive for chromogranin A but the gastric lesion (adenocarcinoma) gave a negative result. (B) In contrast, the gastric lesion was positive for carcinoembryonic antigen but the duodenal lesion was negative.

(fig 1B, C). These neuroendocrine tumour cells were not seen in the gastric antrum. The two regions of the tumour were distinctly divided by the pyloric sphincter (fig 1B, C). The gastric region was negative for chromogranin A staining but positive for CEA staining. In contrast, the duodenal region was positive for chromogranin A but negative for CEA (fig 2).

We examined nuclear accumulation of p53 protein in tumour cells by immunohistochemical staining. We detected nuclear accumulation of the p53 protein in most tumour cells in the adenocarcinoma component (fig 3A). In contrast, only a few cells in the NEC carcinoma region were stained by the anti-p53 antibody (fig 3B). Although the entire area of the NEC carcinoma is not shown in fig 3B, there was no difference in p53 staining pattern in the different portions of the NEC carcinoma (T1, T2, and T3).

Genetic alterations of *p53* (exons 5, 6, 7, and 8) were further investigated in these two tumours using PCR-SSCP. All tumour regions (T1–T4) showed the same abnormal band in the *p53* gene at exon 7. Only the distal part of the duodenal tumour (T1) showed an abnormal band in the *p53* gene at exon 5 in addition to that seen in exon 7 (data not shown). We repeatedly examined mutation of exon 5 of *p53* in the T2 and T3 regions of NEC carcinoma by PCR-SSCP but failed to detect the mobility shift of the band from exon 5 of *p53* in these regions. We also examined the mutation of the *p53* gene in the normal parts of the samples (N1 and N2) by PCR-SSCP. No mobility shift was observed in the samples prepared from the

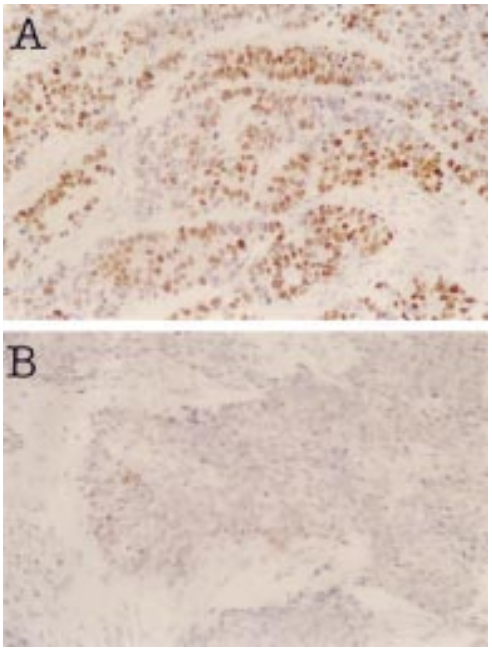

Figure 3 Immunohistochemical staining for p53 protein. (A) Strong nuclear accumulation of the p53 protein in most of the tumour cells in the adenocarcinoma component was observed. (B) Only a few cells in the neuroendocrine cell (NEC) carcinoma region were positive for p53 protein. There was no difference in p53 staining pattern in the different portion (T1, T2, and T3) of the NEC carcinoma (data not shown).

Table 1 Sequencing analyses of p53 gene mutations

Tumour region	*Exon 5*	*Exon 7*
T1	GCC→GTC at codon 129	GGC→GTC at codon 245
T2	Wild-type	GGC→GTC at codon 245
T3	Wild-type	GGC→GTC at codon 245
T4	Wild-type	GGC→GTC at codon 245

T1, anal side of the duodenal tumour; T2, centre of the duodenal tumour; T3, pyloric ring; T4, tumour of the stomach.

normal parts (N1 and N2). To confirm mutations of *p53* in tumour cells, PCR products were analysed using DNA sequencing (table 1). All tumour regions (T1–T4) showed a point mutation in exon 7 (GGC (glycine)→GTC (valine) at codon 245). The distal portion of the duodenal tumour (T1) showed an additional point mutation in exon 5 (GCC (alanine)→GTC (valine) at codon 129).

Discussion

NEC carcinoma is reported to occur throughout the gastrointestinal tract.[1] In general, NEC carcinoma is characterised by its affinity for silver, the presence of endocrine secretary granules, and positive staining for pan-neuroendocrine cell markers such as chromogranin A.[11 12] The duodenal part of the tumour in our present case showed characteristic silver staining and positive immunoreactivity to chromogranin A, confirming the diagnosis of NEC carcinoma. In this case, the NEC carcinoma of the duodenum was accompanied by adenocarcinoma in the pyloric region of the stomach, both of which were clearly distinguished not only by haematoxylin and eosin staining but also by immunohistochemistry, suggesting that the present tumour may be classified as a collision-type tumour.[5 6]

From a histological viewpoint, it is considered that the collision-type tumour consists of adenocarcinoma and NEC carcinoma that have arisen independently from a multipotential epithelial stem cell or a primitive NEC, respectively.[8] In contrast, the composite-type tumour is believed to consist of adenocarcinoma and NEC carcinoma that have arisen from a common multipotential epithelial stem cell in the epithelium with biphenotypic differentiation.[7] To clarify the oncogenic association between adenocarcinoma and NEC carcinoma more precisely, we performed genetic analysis of the *p53* gene. A common point mutation at exon 7 of the *p53* gene was present throughout the entire tumour, strongly suggesting that both adenocarcinoma in the stomach and NEC carcinoma in the duodenum had originated from a common epithelial stem cell. In this type of tumour it may be difficult to determine whether adenocarcinoma or NEC carcinoma is the primary lesion. In our present case, in addition to a common *p53* gene mutation at exon 7, we found an additional mutation at exon 5 of *p53* in the distal part of the duodenal NEC carcinoma. As this mutation must have occurred after the common mutation at exon 7 of *p53* seen in the entire tumour, the NEC carcinoma of the duodenum may have developed from a phenotypic change of adenocarcinoma cells to endocrine cells during tumour progression. The fact that even typical gastric adenocarcinomas often contain cells with a neuroendocrine phenotype may lend support to this possibility.[1]

By immunohistochemical staining of p53 protein, we observed strong nuclear staining in most tumour cells of the adenocarcinoma and faint nuclear staining in a few tumour cells of NEC carcinoma. However, we detected the same mutation of the *p53* gene at exon 5 in the adenocarcinoma region (T4) and in the NEC carcinoma regions (T1, T2, and T3), and an additional mutation of *p53* gene at exon 7 only in a T1 sample, distal portion of NEC carcinoma. The possible reasons for these observations are: (a) although p53 proteins in T1, T2, and T3 portions contain the same amino acid substitutions, expression of p53 associated proteins such as MDM2 and p14ARF may be different in cells with different histopathological features, and the stability of the p53 protein may be changed in these cells; furthermore, some cells in the T1 portion may receive the second mutation of the *p53* gene because this portion is a proliferation edge of the NEC carcinoma but these second mutations of the *p53* gene did not alter the stability of the p53 protein or the phenotype of the cells; (b) although we could not detect additional mutations in T2 and T3 portions in the NEC carcinoma, the same additional mutation of the *p53* gene occurred in the entire part of the NEC carcinoma and these second mutations may alter the phenotype of the cells and the stability of the p53 protein.

The mechanism of neuroendocrine differentiation of carcinoma cells is still unknown. It has been reported that *menin* is a gene responsible for NEC tumorigenesis[13] but it is not always involved in the development of NEC carcinoma.[14 15] In this study, although we did not analyse alteration of *menin* in the tumour, it is possible that the second mutation at exon 5 of *p53* gene may have been associated with neuroendocrine differentiation in the adenocarcinoma. The NEC component of the present tumour was clearly distinguishable from adenocarcinoma at the pyloric ring—not only an anatomical junction but also an environmental border between these two luminal organs. Indeed, many biological factors, such as acidity, capacity for food storage, and mobility differ between the two organs.

In summary, our molecular pathological approach clearly demonstrated that the adenocarcinoma of the stomach and the NEC carcinoma of the duodenum were derived from the same epithelial stem cells, and that an additional mutation in the *p53* gene may be involved in the neuroendocrine differentiation in the adenocarcinoma.

1 Chejfec G, Falkmer S, Askensten U, *et al.* Neuroendocrine tumors of the gastrointestinal tract. *Pathol Res Pract* 1988;**183**:143–54.
2 Roger LW, Murphy RC. Gastric carcinoids and carcinomas: Morphologic correlates of survival. *Am J Surg Pathol* 1979; **3**:195–202.
3 Ulich TR, Kollin M, Lewin KJ. Composite gastric carcinoma. *Arch Pathol Lab Med* 1988;**112**:91–3.
4 Levendoglu H, Cox CA, Nadimpalli V. Composite (adenocarcinoid) tumors of the gastrointestinal tract. *Dig Dis Sci* 1990;**35**:519–25.
5 Yamashina M, Flinner RA. Concurrent occurrence of adenocarcinoma and carcinoid tumour in the stomach: a composite tumour or collision tumours? *Am J Clin Pathol* 1985;**83**:233–6.
6 Corsi A, Bosman C. Adenocarcinoma and atypical carcinoid: morphological study of a gastric collision-type tumour in the carcinoma-carcinoid spectrum. *Ital J Gastroenterol* 1995;**27**:303–8.
7 Lattes R, Grossi C. Carcinoid tumors of the stomach. *Cancer* 1956;**9**:698–711.
8 De Lellis RA, Dayal Y. Neuroendocrine system. In: Sternberg SS, ed. *Histology for pathologists*. New York: Raven Press, 1992:359.
9 Vortmeyer AO, Lubensky IA, Merino MJ, *et al.* Concordance of genetic alterations in poorly differentiated colorectal neuroendocrine carcinomas and associated adenocarcinomas. *J Natl Cancer Inst* 1997;**89**:1448–51.
10 Soong R, Iacopetta BJ. A rapid and nonisotopic method for the screening and sequencing of *p53* gene mutations in formalin-fixed, paraffin-embedded tumors. *Mod Pathol* 1997;**10**:252–8.
11 Ferrari L, Seregni E, Martinetti A, *et al.* Chromogranin A measurement in neuroendocrine tumors. *Int J Biol Markers* 1998;**13**:3–9.
12 Baudin E, Gigliotti A, Ducreux M, *et al.* Neuron-specific enolase and chromogranin A as markers of neuroendocrine tumours. *Br J Cancer* 1998;**78**:1102–7.
13 Chandrasekharappa SC, Guru SC, Manickam P, *et al.* Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science* 1997;**276**:404–7.
14 Debelenko LV, Emmert-Buck MR, Zhuang Z, *et al.* The multiple endocrine neoplasia type I gene locus is involved in the pathogenesis of type II gastric carcinoids. *Gastroenterology* 1997;**113**:773–81.
15 Toliat MR, Berger W, Ropers HH, *et al.* Mutations in the MEN I gene in sporadic neuroendocrine tumours of gastroenteropancreatic system. *Lancet* 1997;**350**:1223.