Gastric Cancer (2003) 6: 203–209
DOI 10.1007/s10120-003-0249-0

Original article

Carcinogenesis of gastric endocrine cell carcinoma: analysis of histopathology and *p53* gene alteration

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Abstract

Background. **Endocrine cell carcinoma of the stomach is characterized by endocrine differentiation and aggressive biological behavior, and is frequently accompanied by an adenocarcinoma component. Because the carcinogenic pathway and genetic alterations remain unclear, we investigated the histogenesis of this tumor by histopathological and *p53* gene analysis.**

Methods. **The materials were 68 gastric endocrine cell carcinomas and 30 carcinoid tumors, which were resected from 93 Japanese patients for histopathological and immunohistochemical investigation. We also analyzed the concordance of *p53* mutational status between the associated adenocarcinoma and endocrine cell carcinoma components, using microdissection and direct sequencing techniques.**

Results. **An adenocarcinoma component was associated with 70.6% (48/68) of endocrine cell carcinomas, of which 42 (87.5%) were of well- to moderately differentiated type, while 36 of these 42 (85.7%) demonstrated histological continuity with the endocrine cell carcinoma components. Overexpression of p53 protein was observed in 58.8% (20/34) of cases. Common *p53* mutational status between the two components was revealed in 73.3% (11/15) of cases analyzed. In contrast, carcinoid tumors did not exhibit p53 protein overexpression (0/15) or gene mutation (0/5).**

Conclusion. **These data suggest that gastric endocrine cell carcinomas predominantly arise from endocrine precursor cell clones occurring in preceding adenocarcinoma components (particularly the differentiated type), transforming into endocrine cell carcinoma during rapid clonal expansion under the influence of *p53* gene alteration.**

Key words Endocrine cell carcinoma · Carcinoid tumor · Carcinogenesis · p53 · Stomach

Introduction

Histologically and biologically, endocrine cell tumors in the stomach are divided into two groups: (a) carcinoid tumor (CD), a low-grade malignancy, and (b) endocrine cell carcinoma (ECC), which is synonymous with small-cell carcinoma or poorly differentiated neuroendocrine carcinoma (PDNC), a high-grade malignancy [1,2]. CD is characterized by trabecular, ribbon-like, or solid structures composed of uniformly small cells containing abundant amounts of cytoplasm with many neuroendocrine granules, including rare mitotic figures that generally behave in a low-grade malignant manner [1–4]. Conversely, ECC is characterized by solid, large anastomosing trabecular structures composed of atypical cells larger than those of CD, with polymorphic nuclei, many mitotic figures, and aggressive biological behaviors, such as frequent vascular permeation and distant metastasis, even from an early stage [1,2,5].

Iwafuchi et al. [5] reported that ECC frequently contains an adenocarcinomatous component, and proposed that ECC may be generated from a coexisting adenocarcinoma component. Whether or not ECC originates from adenocarcinoma precursor cells remains to be proven. A *p53* mutation is well known to be the most common genetic alteration in ordinary gastric carcinomas [6–8]. However, no studies have reported on the genetic events contributing to the development of gastric ECC. In order to clarify the pathway of gastric ECC carcinogenesis, we analyzed the histopathological organization in a large number of cases, particularly in regard to the relationship between adenocarcinoma and ECC components. We also analyzed the concordance of the *p53* mutational pattern between the two tissue components, using microdissection and direct sequencing techniques, this being the first time such a study has been done.

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Received: March 10, 2003 / Accepted: July 3, 2003

Subjects, materials, and methods

In this study, each ECC and CD diagnosis was established on the basis of the typical histological findings as described in the WHO blue book [1,2]. These findings were confirmed by diffuse positivity for at least two well-known endocrine cell markers, including Grimelius' argyrophil stain, chromogranin A immunostain, and neuron-specific enolase immunostain.

The materials comprised 98 cases of endocrine cell tumor of the stomach, as follows: 68 ECC cases (15 early stage, 53 advanced) and 30 CD cases (all early stage), registered at the First Department of Pathology, Niigata University School of Medicine from 1980 through 2000. All tumors were surgically or endoscopically resected from 93 Japanese patients who had not had systemic adjuvant therapy; there were 68 patients with solitary ECC (54 men and 14 women; average age at surgery, 66.5 years) and 25 patients with CD (17 with sporadic CD and 8 with multifocal CD associated with type A gastritis; 11 men and 14 women; average age at endoscopic or surgical resection, 56.4 years). For the multifocal CD, we took 13 lesions from the 8 patients (2 CD lesions from each of 5 patients and 1 CD lesion from each of 3 patients). All tumors were fixed in 10% formalin solution immediately after resection, cut into 5-mm-thick sections along the largest diameter of each tumor, and embedded in paraffin.

One to three representative blocks were serially sectioned (2-μm-thick), and stained with hematoxylin-eosin (H&E) for general histopathological analysis and some blocks were reserved for examination of endocrine cell markers: Grimelius' argyrophil stain, chromogranin A immunostain, and neuron-specific enolase immunostain were used as the endocrine cell markers.

p53 immunohistochemistry

For the p53 immunohistochemistry (IHC) study, 15 of the 30 CD cases and 34 (8 early stage and 26 advanced) of the 68 ECC cases were randomly selected. A serial section, following the endocrine cell marker section, was immunostained for p53 protein (PAb1801, mouse monoclonal, 1:200; Oncogene Science, NY, USA), using the streptavidin-peroxidase complex method, as previously described [9]. Cells positive for p53 protein were defined as those with brownish nuclear staining, regardless of intensity. Expression of p53 protein was classified as follows: (−), negative; (+), scattered positive cells; (++), focal aggregates of positive cells; and (+++), positive cells distributed in most of the lesion. Staining patterns of (++) and (+++) were considered to represent overexpression of p53 protein, consistent with our previous reports [9,10].

Microdissection and DNA preparation

Of the 34 ECC and 15 CD cases on which p53 IHC was performed, 15 ECC (1 early stage, 14 advanced) and 5 CD cases were selected for *p53* gene analysis. One to four areas from the ECC components and one to three areas from the adenocarcinoma components, 60 areas in total, were sampled. Areas showing a histological shift from an adenocarcinoma component to an ECC component were excluded from the analysis. For CD, paraffin blocks were available for only 5 cases after repeated cutting, because of the rather small tumor size compared with ECC. One or two areas from each case were sampled, for 7 areas in total.

DNA extraction from paraffin sections was performed as follows: from each sample, five to ten serial unstained 10-μm-thick sections were dewaxed in xylene for 10 min, rehydrated in 90% ethanol for 5 min, and then briefly stained with hematoxylin. Lesions were dissected under direct observation with a microscope at 40× magnification, using sterilized, disposable 25-gauge needles (Terumo, Tokyo, Japan).

DNA was isolated using a DNA Isolator PS Kit (Wako, Osaka, Japan). Samples were precipitated with isopropyl alcohol and successively with 90% ethanol, dried, and then dissolved in 30 μl sterilized water.

Polymerase chain reaction (PCR)

DNA was amplified using nested PCR. The target sequences of the *p53* gene, exon 5 (codons 126–186), exon 6 (codons 187–224), exon 7 (codons 225–261), and exon 8 (codons 262–306) were each amplified independently with two sets of primers (Takara Shuzo, Kyoto, Japan) as previously described [10]. The first PCR product was used as the template for the second PCR, which was performed under the same conditions as the first. The product of the second PCR was electrophoresed for 20 min on 4% agarose gel (NuSieve 3:1 Agarose; FMC BioProducts, Rockland, ME, USA). An amplified band was cut out from the gel and subsequently purified using a Mermaid Kit (Bio101, La Jolla, CA, USA).

Direct DNA sequencing

All products of the second PCR were sequenced directly, using an Auto Load Solid Sequencing Kit (Pharmacia Biotech, Uppsala, Sweden) and an automated laser fluorescent sequencer (ALF DNA Sequencer II; Pharmacia) equipped with ALF Manager Version 2.5. Each PCR product was sequenced at least twice in both sense and antisense directions to confirm the reproducibility of the results.

Statistical methods

We calculated 95% confidence intervals using Casella's procedure with the StatXact version 4 program (Cytel Software, Cambridge, MA, USA).

Results

Histopathological findings

An adenocarcinoma component was associated with 48 of the 68 ECC cases (70.6%; 95% confidence interval [CI], 58.3%–81.0%). In detail, adenocarcinoma was observed in 73.3% (11/15; 95% CI, 44.9%–92.2%) of early-stage ECC, and in 69.8% (37/53; 95% CI, 55.7%–81.7%) of advanced ECC. The depth of adenocarcinoma invasion was limited to the submucosa in 85.4% of the 48 cases (41/48; 95% CI, 72.2%–93.9%). Forty-two of the 48 cases (87.5%; 95% CI, 74.8%–95.3%) demonstrated well- to moderately differentiated adenocarcinoma, of which 85.7% (36/42; 95% CI, 71.5%–94.6%) revealed histological continuity with the ECC component in the submucosal layer (Fig. 1). The remaining 6 cases demonstrated mixed-type adenocarcinoma, consisting of an undifferentiated part and a well- to moderately differentiated part; in all 6 cases, the latter part was located adjacent to the ECC component in the submucosal layer.

No CD cases demonstrated adenocarcinoma components in the same tumor (0/30; 95% CI, 0%–11.6%).

p53 IHC

p53 protein overexpression was observed in 58.8% (20/34; 95% CI, 40.7%–75.4%) of ECC cases. The p53 protein expression pattern coincided with both the adenocarcinoma component and the ECC component in 95.0% (19/20; 95% CI, 75.1%–99.9%) (Table 1) (Fig. 2).

None of the CD cases exhibited p53 overexpression (0/15; 95% CI, 0%–21.8%).

p53 *gene analysis*

p53 DNA analysis was performed in 15 ECC cases; an identical *p53* mutational pattern between the adenocarcinoma component and the ECC component was observed in 8 of these cases (cases 1–8). We identified three different mutational patterns in the ECC component in these 8 cases: 6 cases (cases 1–6) had a single mutational pattern, 1 (case 7) had double mutations (Fig. 3), and 1 (case 8) had triple mutations. The latter two cases demonstrated other mutation(s) in addition to the identical mutation. Of these 8 cases, case 6 revealed a deletion of codon 209, while the other 7 cases demonstrated point mutations (Table 2). In terms of the base-pair spectrum, 23 mutations in total were observed: 13 transitions (C to T, 4; G to A, 4; A to G, 3; T to C, 2), and 10 transversions (G to T, 5; C to A, 4; A to T, 1) (Table 2). Conversely, no mutations were detected in the adenocarcinoma component or the ECC component in 7 cases (cases 9–15): 3 cases (cases 9–11) demonstrated no evidence of p53 protein overexpression, and IHC revealed overexpression of both components in 4 cases (cases 12–15) (Table 2).

In contrast to the ECC cases, no *p53* mutations were found in CD cases (0/5; 95% CI, 0%–52.2%).

Table 1. p53 protein expression pattern in gastric endocrine cell carcinomas

Tumor	p53 protein expression: Ad part	p53 protein expression: ECC part	No. of cases (%)
ECC with adenocarcinoma component	3+	3+	10/20 (50.0)
	+	3+	1/20 (5.0)
	+	+	7/20 (35.0)
	−	−	2/20 (10.0)
ECC without adenocarcinoma component		3+	9/14 (64.3)
		+	2/14 (14.3)
		−	3/14 (21.4)

ECC, endocrine cell carcinoma; Ad, adenocarcinoma

Discussion

In the present study, 70.6% (48/68; 95% CI, 58.3%–81.0%) of ECC cases displayed adenocarcinoma components in the mucosa and/or submucosa, of which 87.5% (42/48; 95% CI, 74.8%–95.3%) were of well- to moderately differentiated type. Furthermore, 85.7% (95% CI, 71.5%–94.6%) of the differentiated adenocarcinoma components in the submucosal layer showed a shift to ECC components. In the mixed type adenocarcinoma, the differentiated part was located adjacent to the ECC component in the submucosa. These histopathological findings led us to presume that gastric ECC is derived from a preceding adenocarcinoma (particularly of differentiated type), and transforms into the ECC phenotype in the submucosa. As for the approximately 30% of ECC cases without an adenocarcinoma component, it is conceivable that, after or during histological transformation, the adenocarcinoma component may become necrotic and desquamate in association with ulcerative change, while the ECC component develops rapidly in the submucosa and deeper layers.

It is reported that *p53* gene mutation is the most common event implicated in gastric carcinogenesis [6,11], in addition to an early genetic event, particularly

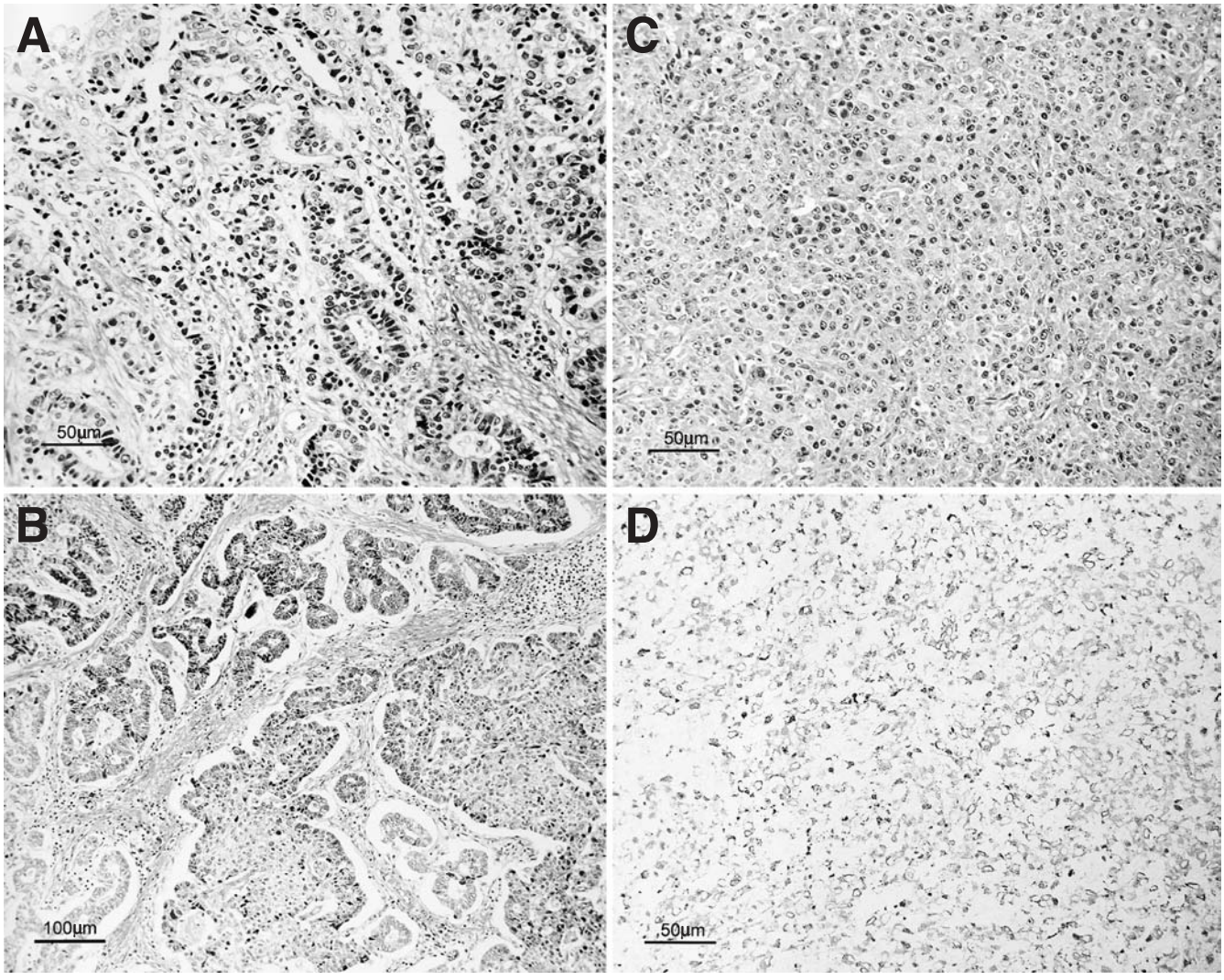

Fig. 1. **A** Adenocarcinoma component of well-differentiated type associated with gastric endocrine cell carcinoma (ECC). **B** Histological shift from adenocarcinoma component to ECC component. There is a zone of transition between the two components. **C** ECC component, demonstrating solid alveolar structure. **D** ECC, showing diffuse positivity for Grimelius' argyrophil stain. **A** and **C** H&E, ×200; **B** H&E, ×100; **D** ×200

in differentiated gastric adenocarcinomas [7]. In the present study, p53 protein overexpression was observed in 58.8% (95% CI, 40.7%–75.4%) of ECC cases by IHC, and the p53 protein expression pattern corresponded with both the adenocarcinoma component and the ECC component in 95.0% (95% CI, 75.1%–99.9%) of these cases. We obtained the two separate tissue components by microdissection and analyzed the concordance of the *p53* mutational pattern between them, using a direct sequencing method. Mammalian *p53* gene sequencing has revealed five highly conserved domains, four of which fall within exons 5 through 8. Because this is where most mutations occur [12], our study analysis focused here. We discovered an identical *p53* mutational pattern in the adenocarcinoma component and the ECC component in 8 of the 15 cases examined. Three other cases did not show any *p53* gene mutation or p53 protein overexpression in either of the two components; thus, these can be regarded as *p53* gene wild-type. In all 15 cases analyzed, concordance of *p53* mutational status between the two components reached 73.3% (11 of 15; 95% CI, 44.9%–92.2%). It is conceivable that, according to the *p53* mutational status, in most ECC tumors, the adenocarcinoma component and the ECC component share the same cell of origin. Furthermore, we found 2 interesting cases that exhibited double or triple mutations in the ECC component, which implies that other mutations occur, in addition to the precursor cell clone transformation from adenocarcinoma into the ECC phenotype.

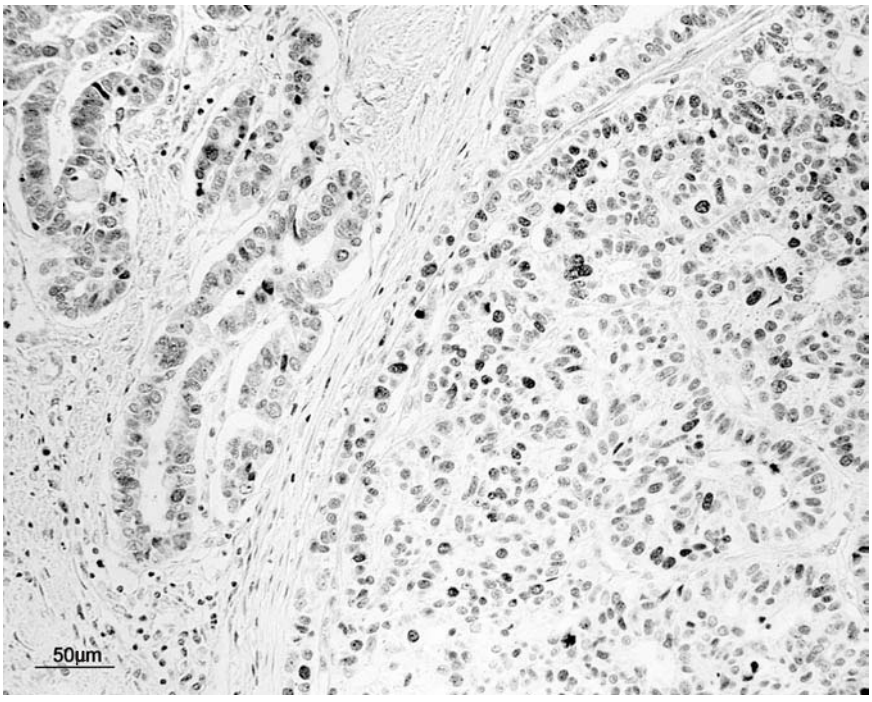

Fig. 2. p53 protein overexpression is observed in the adenocarcinoma component and in the transitional zone shifting to the ECC component. p53 immunohistochemistry, ×200

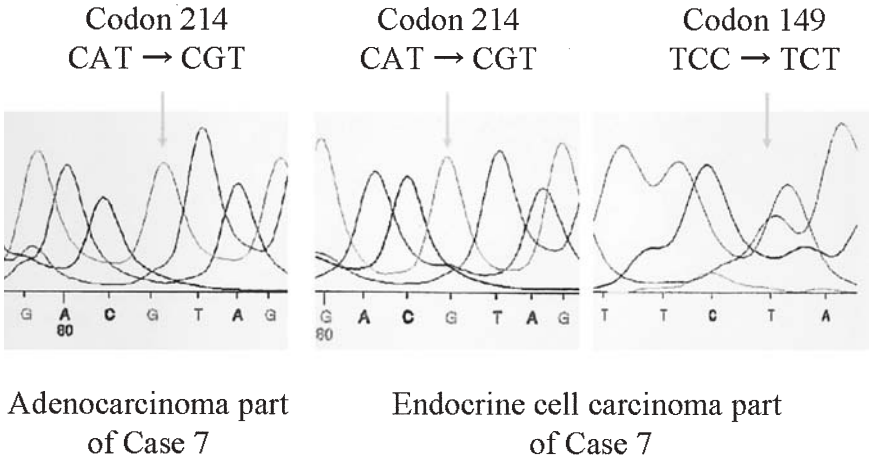

Fig. 3. DNA direct sequencing revealed an identical mutational pattern (codon 214: CAT to CGT) between an adenocarcinoma component and an ECC component. Another mutational pattern (codon 149: TCC to TCT) was also present in the ECC component

However, 4 of 15 cases displayed p53 protein overexpression despite an absence of *p53* gene mutation. There are two possible ways to account for this. One explanation is a false-positive IHC result; that is, posttranslational mechanisms stabilize the wild-type *p53* by complex formation and lead to inactivation with mdm-2 products [13] or viral protein [14]. Another theory is that a *p53* mutation may exist in exons other than 5 through 8. Further research needs to be conducted on such cases. In regard to the base-change spectrum, we found that 13 of the observed 23 point mutations (56.5%; 95% CI, 34.5%–76.8%) in ECC were of the transition type. This agrees with prior studies showing base transitions to be the most common point mutations, with a 50% to 91% frequency in gastric adenocarcinoma [7,8,11,15,16].

Our histological findings and *p53* gene analysis strongly support the hypothesis that most gastric ECCs are generated from precursor cell clones that occur in a preceding adenocarcinoma component (particularly of differentiated type) under the influence of *p53* gene alteration, and transform into ECC in the submucosa during rapid clonal expansion. Vortmeyer et al. [17] investigated loss of heterozygosity (LOH) of PDNC,

Table 2. p53 analysis of gastric endocrine cell carcinomas

	Adenocarcinoma component					ECC component			
Case	*n*	Hist	Dep	IHC	Codon (sequence$^{\text{amino acid}}$)	*n*	Dep	IHC	Codon (sequence$^{\text{amino acid}}$)
1	3	W	m	3+	273 ($CGT^{Arg} \rightarrow TGT^{Cys}$)	2	sm	3+	273 ($CGT^{Arg} \rightarrow TGT^{Cys}$)
2	3	M	m	3+	244 ($GGC^{Gly} \rightarrow AGC^{Ser}$)	1	sm	3+	244 ($GGC^{Gly} \rightarrow AGC^{Ser}$)
						1	mp	3+	244 ($GGC^{Gly} \rightarrow AGC^{Ser}$)
						1	ss	3+	244 ($GGC^{Gly} \rightarrow AGC^{Ser}$)
3	1	M	m	3+	159 ($GCC^{Ala} \rightarrow TCC^{Ser}$)	1	mp	3+	159 ($GCC^{Ala} \rightarrow TCC^{Ser}$)
	1	M	m	3+	167 ($CAG^{Gln} \rightarrow CTG^{Leu}$)				
4	1	W	m	3+	ND	2	sm	3+	142 ($CCT^{Pro} \rightarrow CAT^{His}$)
	1	W	m	3+	142 ($CCT^{Pro} \rightarrow CAT^{His}$)				
5	1	W	m	3+	238 ($TGT^{Cys} \rightarrow TTT^{Phe}$)	1	sm	3+	238 ($TGT^{Cys} \rightarrow TTT^{Phe}$)
	1	W	m	3+	ND	1	mp	3+	238 ($TGT^{Cys} \rightarrow TTT^{Phe}$)
	2	P	sm	3+	ND				
6	1	M	m	+	209 ($AGA^{Arg} \rightarrow$ Del of AG)	1	sm	+	209 ($AGA^{Arg} \rightarrow$ Del of AG)
	1	M	m	+	ND	1	ss	+	209 ($AGA^{Arg} \rightarrow$ Del of AG)
	2	M	ss	+	209 ($AGA^{Arg} \rightarrow$ Del of AG)				
7	2	M	m	3+	214 ($CAT^{His} \rightarrow CGT^{Arg}$)	2	sm	3+	214 ($CAT^{His} \rightarrow CGT^{Arg}$) 149 ($TCC^{Ser} \rightarrow TCT^{Ser}$)
						1	mp	3+	214 ($CAT^{His} \rightarrow CGT^{Arg}$) 149 ($TCC^{Ser} \rightarrow TCT^{Ser}$)
8	1	M	m	3+	275 ($TGT^{Cys} \rightarrow TGC^{Cys}$)	1	mp	3+	275 ($TGT^{Cys} \rightarrow TGC^{Cys}$) 162 ($ATC^{Ile} \rightarrow ATA^{Ile}$) 177 ($CCC^{Pro} \rightarrow CCA^{Pro}$)
9	1	W	m	+	ND	1	mp	+	ND
10	2	M	m	+	ND	2	mp	+	ND
11	1	P	m	+	ND	1	sm	+	ND
12	1	M	m	3+	ND	1	sm	3+	ND
						1	mp	3+	ND
13	2	W	m	3+	ND	2	sm	3+	ND
14	2	M	m	3+	ND	3	sm	3+	ND
15	1	M	m	3+	ND	1	mp	3+	ND
						1	ss	3+	ND

ECC, endocrine cell carcinoma; *n*, number of samples; Hist, histological type; W, well-differentiated type; M, moderately differentiated type; P, poorly differentiated type; Dep, invasion depth; m, mucosa; sm, submucosa; mp, muscularis propria; ss, subserosa; IHC, immunohistochemistry; ND, mutation in exons 5–8 not detected

which is synonymous with ECC, in the large intestine. They found a concordance of LOH pattern between the PDNC component and the associated adenocarcinoma component; LOH of the *APC*, *DCC*, or *p53* genes involved the same allele in both tissue components in four of five informative colorectal PDNCs. They postulated that, for a small number of examined cases, the colorectal PDNC and associated adenocarcinoma were derived from the same cell origin. This is consistent with our gastric ECC findings.

However, it is reported that many gastric CD cases are formed by aggregations of endocrine cell micronests, predominantly composed of enterochromaffin-like cells, caused by the hypertrophic effect of hypergastrinemia, which is induced by extensive atrophic fundic gland mucosal change [18]. In the present study we could not find a CD case associated with the adenocarcinoma component, which presents little evidence for adenocarcinoma precursor cell-derived CD. In addition, we did not find evidence of a CD case exhibiting p53 protein overexpression or *p53* gene mutation, suggesting that CD development is independent of *p53* gene alteration. Our results reveal that CD and ECC are two distinct neoplasms, not only with respect to histological features or biological behavior but also in terms of tumorigenic pathway.

In conclusion, it is highly possible that gastric ECC arises predominantly from endocrine precursor cell clones occurring in preceding adenocarcinoma (particularly differentiated type) components, and transforms into ECC during rapid clonal expansion under the influence of *p53* gene alteration.

Acknowledgments This study was supported by the Tsukada Grant for Niigata University Medical Research, and by a Grant-in-Aid (no.10770073) for Encouragement of Young Scientists from the Ministry of Education, Science, Sports, and Culture, Japan. We are

also grateful to Dr. Kohei Akazawa, Department of Medical Informatics of Niigata University Medical Hospital, for his helpful advice on statistical procedures.

References

1. Watanabe H, Jass JR, Sobin LH. Histological typing of oesophageal and gastric tumors. 2nd Ed. Berlin Heidelberg New York Tokyo: Springer; 1990: p 24–7.
2. Solcia E, Kloppel G, Sobin LH. Histological typing of endocrine tumors. 2nd Ed. Berlin Heidelberg New York Tokyo: Springer; 2000: p 61–4.
3. Nishikura K, Watanabe H, Iwafuchi M. PCNA index and nuclear morphometry for diagnosing higher malignancies of endocrine cell tumors in the large intestine. Acta Med Biol 1997;45:143–51.
4. Nishikura K, Watanabe H, Iwafuchi M, Ajioka Y, Hashidate H, Tanabe T, et al. Histological overview of the classification of gastric carcinoid (in Japanese with English summary). Stomach and Intestine 2000;35:1349–54.
5. Iwafuchi M, Watanabe H, Ishihara N, Noda Y, Ajioka Y. Histopathology of carcinoid tumor and endocrine cell carcinoma in gastrointestinal tract (in Japanese). Clin Gastroenterol 1990;5: 1669–81.
6. Tahara E. Molecular mechanism of stomach carcinogenesis. J Cancer Res Clin Oncol 1993;119:265–72.
7. Uchino S, Noguchi M, Ochiai A, Saito T, Kobayashi M, Hirohashi S. p53 mutation in gastric cancer: a genetic model for carcinogenesis is common to gastric and colorectal cancer. Int J Cancer 1993;54:859–64.
8. Renault B, Broek M, Fodde R, Wijnen J, Pellegata NS, Amadori D, et al. Base transitions are the most frequent genetic changes at p53 in gastric cancer. Cancer Res 1993;53:2614–7.
9. Ohashi Y, Watanabe H, Ajioka Y, Hatakeyama K. p53 immunostaining distinguishes malignant from benign lesions of the gallbladder. Pathol Int 1995;45:58–65.
10. Nakagawa S, Watanabe H, Ajioka Y, Nishikura K, Hitomi J, Hatakeyama K. Archival analysis of p53 protein overexpression and genetic mutation in esophageal squamous cell carcinoma. Acta Med Biol 1996;44:63–9.
11. Imazeki F, Omata M, Nose H, Ohto M, Isono K. *p53* gene mutations in gastric and esophageal cancers. Gastroenterology 1992; 103:892–6.
12. Soussei T, Fromentel CC, May P. Structural aspects of the p53 protein in relation to gene evolution. Oncogene 1990;5:945–52.
13. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. Cell 1992;69:1237–45.
14. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papilloma virus type 16 and 18 promotes degradation of p53. Cell 1990;63:1129–36.
15. Yokozaki H, Kuniyasu H, Kitadai Y, Nishimura K, Todo H, Ayhan A, et al. *p53* point mutations in primary human gastric carcinomas. J Cancer Res Clin Oncol 1992;119:67–70.
16. Poremba C, Yandell DW, Huang Q, Little JB, Mellin W, Schmid KW, et al. Frequency and spectrum of *p53* mutations in gastric cancer — a molecular genetic and immunohistochemical study. Virchows Arch 1995;426:447–55.
17. Vortmeyer AO, Lubensky IA, Merino MJ, Wang C, Pham T, Furth EE, et al. Concordance of genetic alterations in poorly differentiated colorectal neuroendocrine carcinomas and associated adenocarcinomas. J Natl Cancer Inst 1997;89:1448–53.
18. Itsuno M, Watanabe H, Iwafuchi M, Ito S, Yanaihara N, Sato K, et al. Multiple carcinoids and endocrine cell micronests in type A gastritis — their morphology, histogenesis, and natural history. Cancer 1989;63:881–90.