

Genetic Alterations in Goblet Cell Carcinoids of the Vermiform Appendix and Comparison with Gastrointestinal Carcinoid Tumors

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Goblet cell carcinoid is a relatively rare neuroendocrine tumor of the vermiform appendix with poorly understood molecular pathogenesis. We studied the clinicopathologic features and genetic alterations, including allelic loss of chromosomes 11q, 16q, and 18q; sequencing of the *K-ras*, β -catenin, and *DPC4* (*SMAD4*) genes; and p53 overexpression and loss of *DPC4* by immunohistochemistry; in 16 goblet cell carcinoids. We compared the allelic loss in goblet cell carcinoids to those in 18 gastrointestinal carcinoid tumors. For goblet cell carcinoids, appendiceal perforation was the most common (70%, 7/10) clinical presentation. The mean tumor size was 2.0 ± 1.5 cm (range, 0.4 to 4.5 cm). The tumor invaded to appendiceal serosa in 50% (8/16) of patients, and two patients had metastasis in lymph nodes or adjoining viscera. With mean follow-up of 24 ± 14 months (median, 23 mo), 1 of 10 patients had died of disease, and 2 others had tumor recurrence. All four patients with metastases, recurrences, and/or death from disease had serosal involvement at presentation ($P = .02$). Loss of heterozygosity of chromosome 11q was present in 25% of goblet cell carcinoids, 14% of ileal carcinoid tumors, and 9% of nonileal carcinoid tumors; of chromosome 16q in 38%, 29%, and 0 ($P = .02$); and of chromosome 18q in 56%, 86%, and 9% ($P = .002$), respectively. No mutations of *K-ras*, β -catenin, or *DPC4* genes; p53 overexpression; or loss of staining for *DPC4* was present in any tumors. These findings suggest that allelic loss of chromosomes 11q, 16q, and 18q in goblet cell carcinoids and ileal carcinoids may have

an important role in the pathogenesis of these tumors.

KEY WORDS: Appendix, β -Catenin, *DPC4*, Goblet cell carcinoid, *K-ras*, Loss of heterozygosity of 18q, p53, 11q, 16q.

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Goblet cell carcinoid (International Classification of Diseases for Oncology, ICD-O 8243/3) is a relatively rare neuroendocrine tumor of the vermiform appendix, comprising approximately 6% of appendiceal carcinoids (1, 2). First described in 1974 (3), goblet cell carcinoid is currently considered an amphicrine tumor with histopathologic and ultrastructural features that overlap between typical carcinoid and adenocarcinoma (2). Although goblet cell carcinoid was previously considered a variant of carcinoid tumor, current evidence suggests that goblet cell carcinoid is a distinct tumor with a histogenesis different from that of the typical carcinoid, reportedly arising from the multipotential epithelial crypt stem cells (4, 5). In contrast, the typical carcinoid tumor of the appendix is thought to originate in the subepithelial neuroendocrine cells of neural crest derivation (6, 7). Furthermore, the clinical behavior of appendiceal goblet cell carcinoid is considered intermediate between that of typical carcinoid tumors and that of appendiceal adenocarcinomas, with an overall 5-year survival rate of 73–84% (8, 9). All these clinical and histopathologic data suggest that goblet cell carcinoid is a distinctive clinicopathologic entity.

Because of its rarity, little is known about the molecular pathogenesis of goblet cell carcinoid. In contrast, rapid advances have been made in characterization of molecular events underlying the tumorigenesis of related tumors, such as typical carcinoids and adenocarcinomas of the intestinal tract. Whereas mutations of *K-ras* proto-oncogene are frequent in adenocarcinomas of the colorectum (10, 11), appendix (12–14), and duodenum (15, 16),

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they are absent in typical carcinoid tumors of appendix and small bowel and in appendiceal goblet cell carcinoids (15, 17, 18). More controversial are the results reported for alterations in goblet cell carcinoids of the *p53* gene, a tumor suppressor gene frequently altered in colorectal neoplasia. One study reported the presence of *p53* gene point mutations without immunohistochemical detection of *p53* in both typical carcinoid tumors and goblet cell carcinoids of the appendix (18). In contrast, another study failed to demonstrate such alterations (17). Deregulation of β -catenin signaling is an important event in colorectal carcinoma (19–21). Overexpression of β -catenin leads to its translocation to cell nucleus together with Tcf/Lef-1 and formation of a bipartite transcriptional factor that activates transcription of many target genes, including *c-myc* and *cyclin D1* (20). Allelic loss of chromosomes 18q, 16q, and 11q is present in typical midgut carcinoids (22), and allelic loss of chromosome 18q is frequent in colorectal carcinomas (23). *DPC4* (*Smad4*) gene mutations are present in about 50% of pancreatic (24) and in about 20% of colorectal carcinomas (25). However, the role of β -catenin and *DPC4* in the pathogenesis of goblet cell carcinoid is unknown.

Because these genetic alterations have not been assessed in appendiceal goblet cell carcinoid, we studied cases for mutations of *K-ras*, β -catenin, and *DPC4* genes; *p53* overexpression, and loss of *DPC4* staining by using immunohistochemistry; and allelic loss of chromosomes 11q, 16q, and 18q as well as clinicopathological associations.

MATERIALS AND METHODS

Case Material

Sixteen appendiceal goblet cell carcinoids (ICD-O 8243/3) were identified from the surgical pathology files of the University of Texas M.D. Anderson Cancer Center from patients undergoing resection between 1991 and 2002 and available formalin-fixed, paraffin-embedded tissue blocks (1). The patient records and histopathological findings were reviewed. The diagnosis of goblet cell carcinoid was confirmed in all cases using the World Health Organization classification of appendiceal tumors (1). Mixed adenocarcinoma-carcinoid tumors were excluded from the study. Goblet cell carcinoids were compared with 18 gastrointestinal carcinoid tumors, including 15 tumors from a previously reported study (26) and 3 appendiceal carcinoid tumors. The study was approved by the Surveillance Committee (institutional review board) of the University of Texas M.D. Anderson Cancer Center.

DNA Preparation

Genomic DNA was extracted from tumor and control nonneoplastic tissues after microdissection from 5- μ m sections of formalin-fixed, paraffin-embedded tissue as described previously (27).

LOH of Chromosomes 11q, 16q, and 18q

LOH of chromosomes 11q, 16q, and 18q was determined by use of dinucleotide microsatellite markers with PCR amplification using fluorescent dye-labeled primers (Invitrogen, Carlsbad, CA). Chromosome 11q was assessed by microsatellite markers D11S4191, D11S937, D11S4127, D11S912, and D11S968; chromosome 16q by D16S3136, D16S514, D16S3066, D16S505, and D16S520; and chromosome 18q by D18S474, D18S69, D18S64, D18S1147, D18S55, D18S61, D18S58, and D18S70, respectively. The 5' oligonucleotide was end-labeled with 6-FAM fluorescent dye. PCR was performed in 15- μ L reaction volumes containing 20 ng of DNA, 9 μ L of ABI Prism True Allele PCR Premix (Applied Biosystems, Foster City, CA), and 5 pmol of each primer. The cycling conditions were denaturation at 95° C for 12 minutes, 10 cycles (94° C for 15 s, 55° C for 15 s, 72° C for 30 s), 32 cycles (89° C for 15 s, 55° C for 15 s, 72° C for 30 s), and extension at 72° C for 10 minutes. A 0.25- μ L aliquot of each fluorescent-labeled PCR product was combined with 12 μ L of formamide and 0.5 μ L of GENESCAN 400HD [ROX] size standard (Applied Biosystems). The samples were then subjected to capillary electrophoresis on an ABI 3700 DNA Analyzer by using GENESCAN Analysis software (Applied Biosystems). Loss of a marker was considered to be present when the assay showed absence or decrease in intensity by >50% of one of two alleles from a tumor sample as compared with the paired control nonneoplastic tissue. LOH of chromosomal arms was defined by allelic loss of one or more polymorphic (informative) microsatellite markers present on that chromosomal arm.

DNA Sequencing of Exon 1 of *K-Ras*, Exon 3 of β -Catenin, and Exons 8–11 of *DPC4* Genes

Exon 1 of the *K-ras* proto-oncogene, exon 3 of the β -catenin gene, and exons 8–11 of the *DPC4* gene were amplified by PCR and sequenced using the primers and PCR conditions as described previously (28–30). DNA sequencing was performed on the PCR product and amplification primers for each exon with a commercial DNA sequencing kit according to the manufacturer's instructions (Applied Biosystems).

Immunohistochemistry for *p53* and *DPC4*

Expression of *p53* gene was assessed by using mouse monoclonal antibody D07 (1:250 dilution)

TABLE 1. Clinical and Histopathologic Characteristics of 16 Patients with Goblet Cell Carcinoid of the Appendix

Number	Sex/Age (years)	Clinical Presentation	Surgical Procedure	Tumor Size	Depth of Invasion	Lymph Node Metastases, Fraction	Recurrences (number, site)	Disease Free Survival	Status—Survival
1	F/50	NA ^a	Right ileocectomy	NA	Serosa and adjacent ileum ^b	Present, NA	NA	NA	DOD ^a —24 mo
2	M/31	NA	Appendectomy followed by right ileocectomy ^c	NA	Serosa	Absent, 0/8	3, small bowel, sigmoid colon, and abdominal wall	20 mo	AWD ^{a,d} —34 mo
3	M/41	Perforation	Appendectomy followed by right ileocectomy ^c	4.5 cm	Serosa	Absent, 0/13	1, omentum	32 mo	ANED ^a —47 mo
4	M/62	NA	Right ileocectomy	4.0 cm	Serosa	Present, 3 of 4	0	4 mo	ANED—4 mo
5	M/56	Perforation	Appendectomy	3.5 cm	Serosa	NA	NA	NA	Lost to F/U ^a
6	M/74	Perforation	Appendectomy	NA	Serosa	NA	NA	NA	Lost to F/U
7	F/56	NA	Appendectomy	2.0 cm	Serosa	NA	NA	NA	Lost to F/U
8	F/60	NA	Right ileocectomy	NA	Serosa	Absent, 0/2	0	6 mo	ANED—6 mo
9	F/49	Perforation	Appendectomy	2.5 cm	Serosa	NA	NA	NA	Lost to F/U
10	F/24	NA	Appendectomy	1.1 cm	Mesoappendix	NA	0	20 mo	ANED—20 mo
11	M/40	Perforation	Appendectomy and regional lymph node dissection	1.8 cm	Subserosa	Absent, 0/2	NA	NA	Lost to F/U
12	M/38	Perforation	Appendectomy	0.5 cm	Subserosa	NA	0	22 mo	ANED—22 mo
13	F/27	Abdominal pain	Appendectomy	0.6 cm	Subserosa	NA	0	12 mo	ANED—12 mo
14	F/59	Incidental ^e	Appendectomy and TAHBSO ^a for undifferentiated ovarian carcinoma	NA	Subserosa	NA	NA	NA	Lost to F/U
15	M/67	Incidental ^e	Appendectomy	NA	Muscularis	NA	0	36 mo	DOUC ^{a,f} —36 mo
16	F/23	Perforation	Appendectomy	0.4 cm	Muscularis	NA	0	36 mo	ANED—36 mo

^a Abbreviations: alive with no evidence of disease, ANED; alive with disease, AWD; dead of disease, DOD; dead of unrelated cause, DOUC; follow-up, F/U; not available, NA; total abdominal hysterectomy and bilateral salpingo-oophorectomy, TAHBSO.

^b Metastasis to omentum.

^c Right ileocectomy had minimal residual disease (patient #2) and no residual disease (patient #3).

^d Received chemotherapy with 5-fluorouracil, leucovorin and CPT-11.

^e Detected during laparotomy for undifferentiated ovarian carcinoma (patient #14) and during follow-up imaging for transitional cell carcinoma of urinary bladder (patient #15).

^f Died of glioblastoma multiforme.

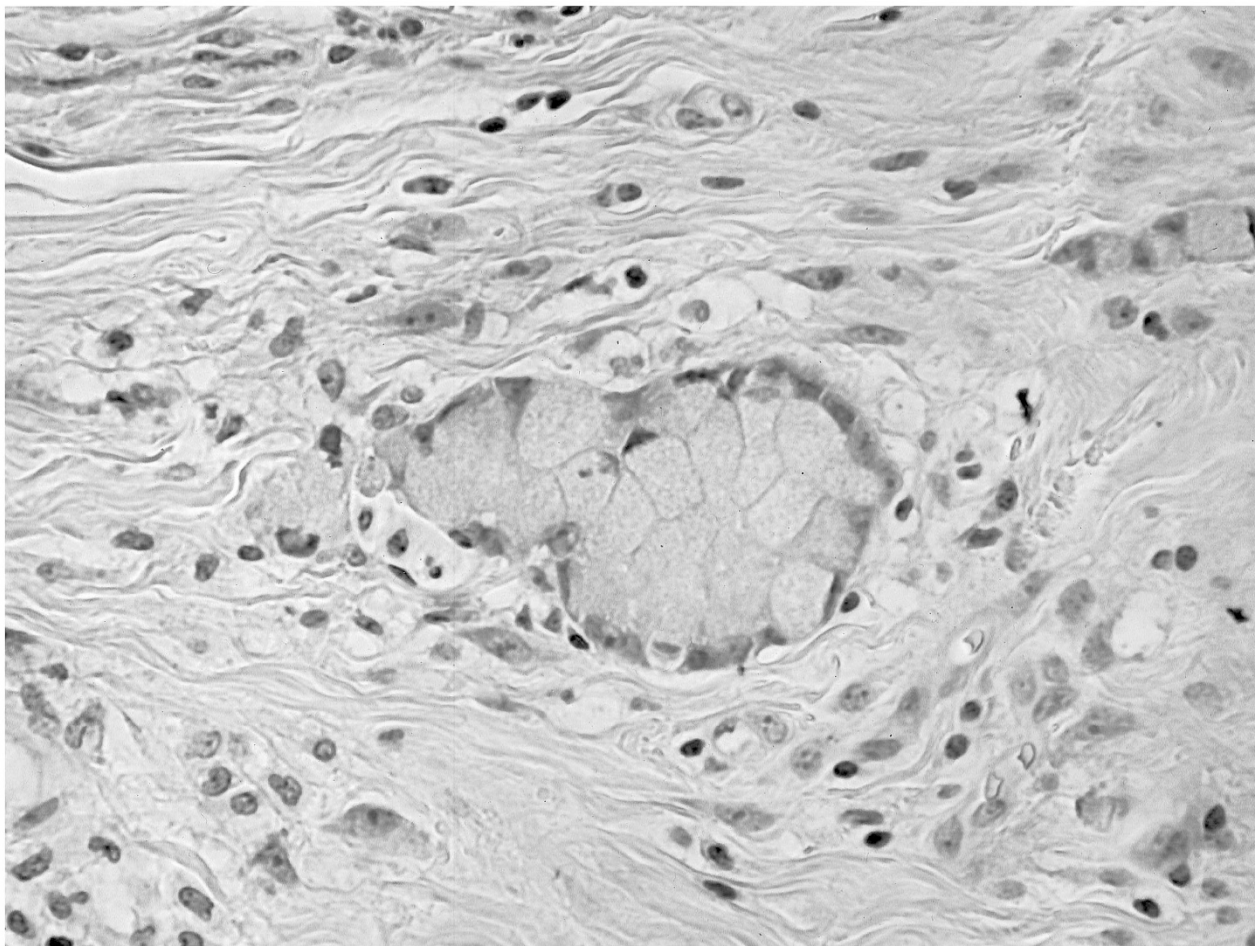


FIGURE 1. Goblet cell carcinoid of the appendix. (Original magnification 200×).

and standard techniques including antigen retrieval as described previously (31). Overexpression for p53 gene product was considered when >50% of the tumor cells demonstrated nuclear staining by immunohistochemistry.

DPC4 protein staining was assessed by using mouse monoclonal antibody to DPC4 (clone B8, 1:250 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) and standard techniques including antigen retrieval as described previously (32). Positive staining for DPC4 protein was scored as diffusely positive if >50% of tumor cells were staining, focally positive if <50% of cells were positive, or negative if no tumor cells were positive. Normal epithelium, fibroblasts, and lymphocytes served as positive internal controls in each of the sections.

Statistical Analysis

The differences in frequencies among clinicopathological factors and genetic alterations, and between genetic alterations of goblet cell carcinoids and carcinoid tumors were analyzed by Fisher's exact test. All statistical analyses were performed

using the SPSS statistical program (SPSS Inc., Chicago, IL).

RESULTS

Clinicopathologic Features

The clinicopathological features of the 16 patients with goblet cell carcinoid are summarized in Table 1. There were 8 male and 8 female patients, ranging from 23 to 74 years in age (mean, 47 ± 16 y). Perforated appendix was the most common clinical presentation and was present in 70% (7 of 10) patients. In two patients the tumor was detected incidentally.

Ten patients had appendectomy, including 1 patient who had total abdominal hysterectomy with bilateral salpingo-oophorectomy, 1 patient had appendectomy and regional lymph node dissection, 2 patients had appendectomy followed by right ileocollectomy, and 3 patients had right ileocollectomy. The mean size of tumor was 2.0 ± 1.5 cm (range, 0.4 to 4.5 cm). Histologically, all tumors were composed of scattered nests and tubules of small-to-

intermediate size cells with monomorphic nuclei and scant eosinophilic cytoplasm, admixed with goblet cells (Fig. 1). In all cases, an infiltrative single-file growth pattern of bland goblet cells comprised 25% or less of the dominant (nested) tumor growth pattern. At least focal immunohistochemical staining for chromogranin A and synaptophysin was detected in all tumors. The tumor invaded the serosa of the appendix in 50% (8/16) of patients, the subserosal connective tissue of appendix or mesoappendix in 31% (5 of 16) patients, or the muscularis propria of appendix in 13% (2 of 16) patients. Two patients had regional lymph nodes metastases (Patients 1 and 4), and one (Patient 1) also had omental metastases.

A mean clinical follow-up of 24 ± 14 months (median, 23 mo) was available for 10 patients. All but 2 patients were alive. One patient with regional tumor spread and lymph node metastasis (Patient 1) died of goblet cell carcinoid 24 months after diagnosis. The second patient (Patient 15) died of an unrelated malignancy 36 months after diagnosis. Two other patients had recurrences: in the small bowel, sigmoid colon, peritoneum, and abdominal wall (Patient 2) at 20 months after diagnosis or in omentum (Patient 3) at 32 months after diagnosis. All four patients with lymph node metastases, recurrence and/or death due to goblet cell carcinoid had serosal involvement of the appendix at presentation (4/8 versus 0/8, $P = .02$). Two patients had appendectomy followed by right ileocelectomy, and minimal residual disease (small subserosal tumor deposit in the cecum adjacent to appendectomy site) was found in one of these (Patient 2). This patient received postoperative chemotherapy with 5-fluorouracil, leucovorin, and CPT-11 and was alive with disease 34 months after diagnosis.

The clinicopathological features of carcinoid tumors are summarized in Table 2.

Allelic Loss in Goblet Cell Carcinoids

LOH of chromosome 11q was present in 27% (4/15) of goblet cell carcinoids (examples in Fig. 2 and summarized in Table 3). The markers D11S937 and D11S968 showed loss in 8% and 50% of informative loci, respectively, but adjoining centromeric or telomeric markers D11S4191, D11S4127, and D11S912 did not show any loss. LOH of chromosome 16q was present in 37% (6/16) of goblet cell carcinoids. The telomeric markers D16S3066, D16S505, and D16S520 showed loss in 37%, 20%, and 9% of informative loci, respectively, but centromeric markers D16S3136 and D16S514 did not show any loss. LOH of chromosome 18q was present in 56% (9/16) of goblet cell carcinoids. The telomeric markers D18S55, D18S61, D18S58, and D18S70 showed loss in 66%, 30%, 50%, and 25% of informative loci, respectively. Five goblet cell carcinoids showed loss of centromeric markers D18S474, D18S69, and D18S64, and two of these tumors had no loss of telomeric markers and one had retention of an intervening marker. Fraction allelic loss (LOH of polymorphic markers/number of polymorphic markers for each tumor) ranged from 0 to 62% (mean, 27.8%). No correlation was found between LOH status and the clinicopathological features of goblet cell carcinoids.

Comparison of Allelic Loss in Goblet Cell Carcinoids with Carcinoid Tumors

LOH of chromosomes 11q, 16q, and 18q was present in 11% (2/18), 11% (2/18), and 39% (7/18)

TABLE 2. Clinical and Histopathological Characteristics of Patients with Gastrointestinal Carcinoid Tumors

Number	Sex/Age (years)	Site of Primary Tumor	Tumor Size	Liver Metastasis	Lymph Node Metastases	Status—Survival
1	M/81	Stomach	0.8 cm	Present	Absent	ANED ^a —134 mo
2	F/64	Stomach	1.5 cm	Absent	Absent	ANED—12 mo
3	M/68	Duodenum	1.2 cm	Absent	Present	ANED—16
4	F/67	Duodenum	3.5 cm	Absent	Present	ANED—23 mo
5	F/75	Ileum	1.8 cm	Absent	Absent	ANED—14 mo
6	M/78	Ileum	2.4 cm	Present	Present	ANED—16 mo
7	M/73	Ileum	2.0 cm	Present	Absent	ANED—21 mo
8	F/55	Ileum	NA ^a	Present	Absent	AWD ^a —108 mo
9	M/74	Ileum	2.0	Present	Absent	ANED—168 mo
10	F/68	Ileum	1.5 cm	Present	Present	AWD—59 mo
11	M/59	Ileum	3.0 cm	Present	Present	AWD—18 mo
12	F/62	Appendix	0.8 cm	Absent	Absent	ANED—4 mo
13	F/40	Appendix	3.0 cm	Absent	Present	ANED—15 mo
14	M/52	Appendix ^b	2.0 cm	Absent	Absent	ANED ^b —17 mo
15	F/41	Colon (cecum)	2.0 cm	Absent	Present	ANED—26 mo
16	F/63	Colon (cecum)	2.3 cm	Present	Present	AWD—51 mo
17	M/73	Colon (cecum)	1.0 cm	Present	Present	AWD—26 mo
18	F/43	Rectum	2.0 cm	Absent	Absent	ANED—12 mo

^a Abbreviations: alive with no evidence of disease, ANED; alive with disease, AWD; not available, NA.

^b Incidentally found during work-up for an ileal leiomyosarcoma, has recurrent leiomyosarcoma but no recurrent carcinoid tumor.

TABLE 3. Summary of Loss of Heterozygosity of Chromosomes 11q, 16q, and 18q in Goblet Cell Carcinoids

Microsatellite Marker	Chromosomal Location	Goblet Cell Carcinoids #																LOH Frequency ^a % (fraction)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Chromosome 11q																		27 (4/15)
D11S4191	11q12.1-12.3	NI	○	NI	○	○	○	NI	○	○	○	○	○	○	○	○	○	0
D11S937	11q13.2-13.5	NI	○	NI	NI	○	NI	○	●	○	○	○	○	○	○	○	○	8
D11S4127	11q22.2-23.3	NI	○	NI	○	○	NI	○	○	○	○	○	NI	○	NI	NI	○	0
D11S912	11q23.3-24.3	NI	○	○	NI	NI	NI	NI	NI	NI	○	○	NI	○	○	○	○	0
D11S968	11q25	NI	○	NI	NI	○	●	NI	NI	NI	●	NI	NI	NI	○	●	NI	50
Chromosome 16q																		37 (6/16)
D16S3136	16q11.2-12.1	NI	○	○	NI	NI	NI	NI	NI	○	NI	NI	NI	NI	NI	NI	NI	0
D16S514	16q12.2-22.2	○	○	NI	○	NI	○	○	○	○	○	○	NI	○	○	NI	○	0
D16S3066	16q21-22.2	●	NI	●	NI	○	NI	○	NI	NI	○	NI	NI	○	○	●	NI	37
D16S505	16q22.3-23.2	○	○	○	○	NI	●	○	●	NI	NI	NI	○	○	○	NI	NI	20
D16S520	16q24.1-24.2	○	○	○	NI	○	○	NI	NI	○	●	○	○	○	NI	NI	○	9
Chromosome 18q																		56 (9/16)
D18S474	18q21.1-21.2	NI	NI	○	NI	○	NI	○	○	○	○	○	○	○	NI	●	○	18
D18S69	18q21.1-21.31	NI	NI	○	●	○	NI	NI	○	○	○	○	○	○	○	NI	○	9
D18S64	18q21.32	●	○	○	●	○	●	NI	○	○	NI	○	NI	NI	NI	NI	○	30
D18S1147	18q21.32-21.33	○	○	○	○	○	NI	○	NI	NI	●	○	NI	○	○	NI	○	9
D18S55	18q22.1	NI	NI	NI	●	NI	NI	NI	NI	NI	●	NI	○	NI	NI	NI	NI	66
D18S61	18q22.3-22.3	NI	○	○	NI	NI	●	○	●	NI	○	○	NI	○	○	●	NI	30
D18S58	18q22.3-23	NI	NI	NI	NI	○	NI	NI	●	○	NI	●	NI	NI	○	●	NI	50
D18S70	18q23	○	NI	NI	○	○	●	●	NI	○	NI	○	NI	NI	NI	○	NI	25
FAL ^b (%)		28	0	10	33	0	62	10	36	0	30	7	12	0	0	50	0	

● - polymorphic, loss of heterozygosity; ○ - polymorphic, no loss; NI - monomorphic, not informative.

^a Loss of heterozygosity frequency = number of tumors with loss of heterozygosity/number of tumors with polymorphic markers.

^b Fraction allelic loss = loss of heterozygosity of polymorphic markers/number of polymorphic markers for each tumor.

of carcinoid tumors (examples in Fig. 2 and summarized in Table 4). This was predominantly due to frequent LOH in ileal carcinoid tumors compared with nonileal carcinoid tumors (Table 5): loss of

chromosomes 11q was present in 14% of ileal carcinoid *versus* 9% of nonileal carcinoids, loss of chromosome 16q in 29% *versus* 0% ($P = .04$), and loss of chromosome 18q in 86% *versus* 9% ($P =$

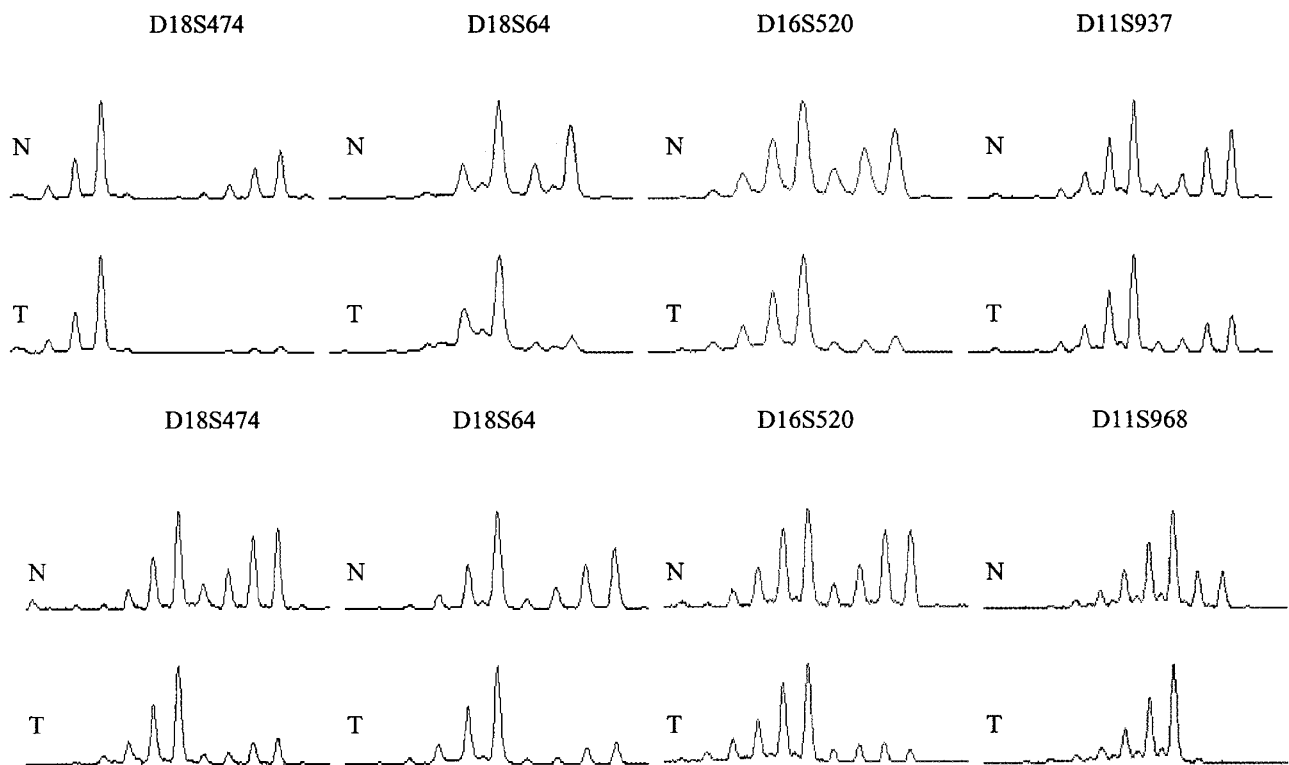


FIGURE 2. Chromosome 11, 16q and 18q loss by PCR amplification using fluorescent primers in goblet cell carcinoids and gastrointestinal carcinoid tumors. The markers are indicated at the top. PCR was performed using nonneoplastic DNA (N) and tumor DNA (T). Examples of allelic loss in goblet cell carcinoids (*top panels*) and gastrointestinal carcinoid tumors (*bottom panels*) are shown in representative tumors for each marker.

TABLE 4. Summary of Loss of Heterozygosity of Chromosomes 11q, 16q, and 18q in Gastrointestinal Carcinoid Tumors

Microsatellite Marker	Chromosomal Location	Gastrointestinal Carcinoid Tumors #																		LOH Frequency ^a % (fraction)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Chromosome 11q																				11 (2/18)
D11S4191	11q12.1-12.3	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	0
D11S937	11q13.2-13.5	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	0
D11S4127	11q22.2-23.3	NI	○	NI	○	NI	○	○	NI	●	○	○	○	○	○	○	○	○	○	9
D11S912	11q23.3-24.3	○	NI	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	0
D11S968	11q25	○	NI	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	6
Chromosome 16q																				11 (2/18)
D16S3136	16q11.2-12.1	NI	○	NI	NI	●	○	○	○	○	○	○	○	○	○	○	○	○	○	7
D16S514	16q12.2-22.2	NI	○	○	○	●	○	○	○	○	○	○	○	○	○	○	○	○	○	7
D16S3066	16q21-22.2	○	NI	○	NI	●	NI	○	○	○	○	○	○	○	○	○	○	○	○	7
D16S505	16q22.3-23.2	NI	NI	○	○	●	○	○	○	○	○	○	○	○	○	○	○	○	○	13
D16S520	16q24.1-24.2	○	○	NI	○	●	○	○	○	○	○	○	○	○	○	○	○	○	○	7
Chromosome 18q																				39 (7/18)
D18S474	18q21.1-21.2	NI	○	NI	○	●	●	●	●	●	●	○	○	○	○	○	○	○	○	50
D18S69	18q21.1-21.31	○	○	○	○	NI	●	○	○	○	○	○	○	○	○	○	○	○	○	12
D18S64	18q21.32	○	○	○	○	●	●	NI	○	○	○	○	○	○	○	○	○	○	○	15
D18S1147	18q21.32-21.33	○	○	○	○	●	●	●	●	●	○	○	○	○	○	○	○	○	○	36
D18S55	18q22.1	○	○	NI	○	●	NI	○	○	○	○	○	○	○	○	○	○	○	○	23
D18S61	18q22.3-22.3	○	○	○	○	NI	○	○	○	○	○	○	○	○	○	○	○	○	○	31
D18S58	18q22.3-23	○	○	○	○	●	●	●	●	●	○	○	○	○	○	○	○	○	○	29
D18S70	18q23	●	○	○	○	●	●	●	NI	●	○	○	○	○	○	○	○	○	○	31
FAL ^b (%)		7	0	0	0	73	47	29	25	53	20	0	0	8	0	0	0	0	0	

● - polymorphic, loss of heterozygosity; ○ - polymorphic, no loss; NI - monomorphic, not informative.

^a Loss of heterozygosity frequency = number of tumors with loss of heterozygosity/number of tumors with polymorphic markers.

^b Fraction allelic loss = loss of heterozygosity of polymorphic markers/number of polymorphic markers for each tumor.

.0007). Fraction allelic loss ranged from 0 to 73% (mean, 41.2%) in ileal carcinoid tumors and ranged from 0 to 8% (mean, 1.2%) in carcinoid tumors of other sites.

Allelic loss of chromosomes 11q, 16q, and 18q was as frequent in goblet cell carcinoids as in ileal carcinoid tumors but was less frequent in nonileal carcinoid tumors (Table 5): LOH of chromosome 11q was present in 25% of goblet cell carcinoids, 14% of ileal carcinoid tumors, and 9% of nonileal carcinoid tumors; of chromosome 16q in 38%, 29%, and 0 ($P = .02$); and of chromosome 18q in 56%, 86%, and 9% ($P = .002$), respectively.

Other Genetic Alterations of Goblet Cell Carcinoids

No *K-ras* or *β-catenin* mutations in 16 tumors, *DPC4* mutations in 15 tumors, p53 overexpression in 12 tumors, or loss of staining for *DPC4* in 14 tumors were identified.

DISCUSSION

In our study, we found infrequent allelic loss of chromosome 11q but frequent allelic loss of chromosomes 16q and 18q in goblet cell carcinoids and carcinoid tumors. The long arm of chromosome 11 is a gene-rich region (33) but contains only a few known or putative tumor suppressor genes mapped to 11q13 (*MEN 1*, *SDHD* and *PGL2* genes) and 11q22-q23 (*PGL1* and *PPP2R1B* genes). Allelic loss of more distal chromosomal regions located at 11q25 were identified in breast and ovarian cancer (33). Similar to our study, in a recent report, allelic loss of 11q25 was identified in 40% of classical ileal carcinoids and was not found in any of the seven classical appendiceal carcinoids (34). Another study reported a high frequency of allelic loss at 11q13 locus (62%) in classical midgut carcinoids, but a low frequency of *SDHD* gene mutations, suggesting that other tumor suppressor genes may be targets for allelic loss at this region (35). In our study, allelic

TABLE 5. Summary of Loss of Heterozygosity of Chromosome 11q, 16q, and 18q in Goblet Cell Carcinoids, Ileal Carcinoid Tumors, and Non-Ileal Carcinoid Tumors

Chromosomal Arm	Goblet Cell Carcinoids	Ileal Carcinoid Tumors	Non-Ileal Carcinoid Tumors	P Value ^a
	Frequency % (fraction)			
11q	25 (4/16)	14 (1/7)	9 (1/11)	NS ^b
16q	38 (6/16)	29 (2/7)	0 (0/11)	0.02 ^{c,d}
18q	56 (9/16)	86 (6/7)	9 (1/11)	0.002 ^{c,d}

^a Comparison among all three groups.

^b NS, not significant.

^c Goblet cell carcinoids versus non-ileal carcinoid tumors ($P = 0.006$ for chromosome 16q and $P = 0.008$ for chromosome 18q).

^d Ileal carcinoid tumors versus non-ileal carcinoid tumors ($P = 0.04$ for chromosome 16q and $P = 0.0007$ for chromosome 18q).

loss of chromosome 11q in goblet cell carcinoids was present at locus 11q25, and only one GCC and one ileal carcinoid tumor had loss of 11q13 locus, suggesting that *MEN 1* or *SDHD* genes are not involved in the tumorigenesis of appendiceal goblet cell carcinoids. These findings suggest that an unknown tumor suppressor gene located on 11qter may have a role in tumorigenesis of some of the appendiceal goblet cell carcinoid and in ileal carcinoid tumors.

More than a third of goblet cell carcinoids and ileal carcinoid tumors in our study demonstrated LOH of 16q with loss of loci 16q21-q24.2. This chromosomal region harbors two putative tumor suppressor genes: *CTCF* gene (CCCTC-binding factor), a transcriptional repressor of c-myc, and the *E-cadherin/CDH1* gene involved in cell adhesion. Homozygous loss of function of *CTCF* gene by tumor-specific rearrangements has been identified in breast carcinomas (36), whereas mutations in *E-cadherin* have been reported in invasive lobular carcinoma of breast (37). Interestingly, Kytola *et al.* (22) reported allelic loss of 16q21-qter in metastatic midgut carcinoids but not in primary carcinoid tumors, suggesting that putative tumor suppressor genes mapped at this locus may be involved in tumor progression.

In our study we identified frequent allelic loss of chromosome 18q in goblet cell carcinoids and ileal carcinoid tumors, and no loss of 18p in goblet cell carcinoids. The high frequency of allelic loss of 18q in our cases indicates that the genetic alterations (allelic loss or mutational inactivation) of tumor suppressor genes mapped to the telomeric 18q play an important role in the carcinogenesis of appendiceal goblet cell carcinoids and ileal carcinoid tumors. The most frequent chromosomal loss was on the long arm of chromosome 18 at the locus 18q21–23 in goblet cell carcinoids that is frequently deleted in different types of tumors including pancreatic, colorectal, and breast carcinomas (for review, see 38). In contrast, the ileal carcinoid tumors had allelic loss of all the markers on chromosome 18q or loss of two or three loci suggesting involvement of more than one gene on chromosome 18q. Among the tumor suppressor genes harbored on 18q, the most extensively studied are the *DCC* gene localized at 18q21.2 and the *DPC4 (SMAD4)* gene localized at 18q21.1. Although inactivation of *DCC* is identified not only in colorectal carcinoma (39), but also in pancreatic, gastric, mammary, prostatic carcinomas (40–43), *DPC4* gene inactivation occurs in almost half of the pancreatic carcinomas (24) but is uncommon in other malignancies (44, 45). We ruled out involvement of *DPC4* gene by demonstrating no *DPC4* mutations or loss of staining of *DPC4* protein by immunohistochemistry. However, the role of *DCC* gene in GCCs remains unclear.

Other putative tumor suppressor genes localized immediately telomeric to *DCC* and *DPC4* at microsatellite locus 18q21.3 (D18S55) are serpins (serine proteinase inhibitors), some of which (maspin) reportedly can suppress tumor growth and spread in mammary carcinoma (46). In our study, allelic loss at loci 18q21–23 that are telomeric to *DCC* and *DPC4* genes suggests loss of a region that harbors some of the *cadherin* genes that are considered tumor suppressor genes because of their role in cell adhesion (47). Interestingly, a similar frequency of allelic loss of 18q was reported for classical midgut carcinoids (22, 48) and for sporadic adenocarcinomas of the small intestine (49) but not in two studies of lung carcinoids (50, 51). However, ileal carcinoids lack *DPC4* mutations as compared with small intestinal carcinomas (48, 49).

In our study, there were no *K-ras*, *β -catenin*, or *DPC4* mutations; p53 overexpression; or loss of staining for *DPC4* in goblet cell carcinoids. *K-ras* oncogene was shown to have a major role in carcinogenesis and/or tumor progression in a wide variety of human neoplasms including colorectal carcinomas, pancreatic adenocarcinomas, cholangiocarcinomas, and lung adenocarcinomas (52). Recently, Kabbani *et al.* (12) reported frequent *K-ras* mutations in appendiceal adenocarcinomas. In contrast, we and other investigators (18) did not find *K-ras* mutations in goblet cell carcinoids. Furthermore, *K-ras* mutations are not present in foregut or midgut carcinoid tumors or pancreatic endocrine tumors (15, 17, 18, 53).

A recent study reported abnormal nuclear accumulation of *β -catenin* in 36% digestive tract carcinoids and 27% of islet cell tumors (54), but no genetic alteration of *β -catenin (CTNNB1)* gene (26, 54). Li *et al.* (55) found that the majority of the goblet cell carcinoids of the appendix studied demonstrated strong membranous staining for *β -catenin*, similar to normal appendiceal mucosa, and no nuclear or cytoplasmic staining. In our study, none of the appendiceal goblet cell carcinoid demonstrated mutations of *β -catenin* gene.

p53 overexpression was not identified in goblet cell carcinoids in our study, a finding that is in agreement with previous reports of infrequent *p53* gene mutations in goblet cell carcinoid and adenocarcinomas of appendix (2, 12, 17). In contrast, Ramnani *et al.* (18) reported *p53* mutations in 25% and 44% of appendiceal goblet cell and classical carcinoids, respectively.

Overall, these findings suggest that alterations of oncogenes and tumor suppressor genes, frequently altered in adenocarcinomas of gastrointestinal tract, do not play a role in the pathogenesis of appendiceal goblet cell carcinoid.

Goblet cell carcinoid is considered a malignancy that is more aggressive than carcinoid tumor of the

appendix (2, 8, 9, 56–58), with 5-year survival rate ranging from 73 to 84% (8, 9). In our study, serosal involvement by the tumor at presentation was associated with adverse outcome, similar to the case in previously reported studies (8, 18, 56), suggesting that tumor stage is the most important prognostic factor in appendiceal goblet cell carcinoid. Another possible unfavorable prognostic factor reported by Burke and co-workers (59) is the percentage of carcinomatous growth defined as fused or cribriform glands, single-file structures, diffusely infiltrating signet-ring cells, or solid sheets of cells. According to this study, all 25 patients with appendiceal goblet cell carcinoids with no or <25% carcinomatous growth were alive an average of 19 months after diagnosis, regardless of the treatment modality. However, this finding may not be conclusive because goblet cell carcinoid of the appendix may recur and metastasize >15 years after diagnosis (2, 58).

In conclusion, our study provides evidence that goblet cell carcinoids of the appendix are neuroendocrine tumors with genetic alterations similar to those of ileal carcinoid tumors and different from those of appendiceal adenocarcinomas. The loss of chromosomes 11q, 16q, and 18q identified in the majority of these tumors suggests that putative or yet unknown tumor suppressor genes may have a major role in the pathogenesis of goblet cell carcinoids and ileal carcinoids.

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