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ORIGINAL RESEARCH ARTICLE

Absence of *GNAS* mutation in colorectal carcinogenesis

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ABSTRACT

Aims and background: The incidence rate of colorectal cancer (CRC) increases every year in Korean populations. However, association between the *GNAS* mutation and colorectal precancerous lesions has not been studied in in Korean populations. To contribute to better understanding of colorectal carcinogenesis, we analyzed *GNAS* mutation in 100 cancerous and 96 precancerous colorectal lesions.
Methods: The records of colonoscopic polypectomy performed at Dongsan Medical Center between 1999 and 2003 were reviewed retrospectively. Precancerous lesions included 7 villous adenomas, 59 tubular adenomas, and 18 sessile serrated adenomas, and 12 hyperplastic polyps. Keimyung Human Bio-Resource Bank at Dongsan Medical Center provided 100 CRC samples.
Results: *GNAS* mutation was not found in any colorectal cancer or any precancerous colorectal lesions, including villous adenoma, which is thought to harbor the mutation.
Conclusions: The role of *GNAS* mutation might be limited in colorectal neoplasms of the Korean population.

Keywords: Colorectal cancer, *GNAS*, Hyperplastic polyps, Sessile serrated adenomas, Tubular adenoma, Villous adenoma

Introduction

Colorectal cancer (CRC) is the third most common cancer in the world and its incidence rate has increased every year in Korean populations (1, 2). Recently, not only tubular adenomas but also serrated lesions increasingly have been recognized as potential precancerous lesions (3). Each of these distinct types harbors different pathways of carcinogenesis and various alterations in many genes were suggested to be the drivers of these processes. One of these genes was *GNAS* (guanine nucleotide binding protein, α-stimulating), which encodes the stimulatory G-protein s-subunit (4). *GNAS* mutation was first found in pituitary adenomas and pancreatic tumors at codon 201 hotspot (5). This mutation leads to the constitutive activation of adenylate cyclase, which increases cAMP production. Interestingly, *GNAS* mutation may be a characteristic genetic feature of colorectal villous adenomas in association with *KRAS* or *BRAF* mutation (6, 7). Although it was rare in CRC, it was associated with right-side and *KRAS* or *BRAF* mutations (8). According to a study of *GNAS* mutation in Korean patients with advanced CRC, *GNAS* mutation was extremely rare (0.47%, 1/215) (9). However, it has not been studied in colorectal precancerous lesions in Korean populations.

We analyzed *GNAS* mutation in 100 cancerous and 96 precancerous colorectal lesions. To contribute to better understanding of colorectal carcinogenesis, *KRAS* and *BRAF* mutations and microsatellite instability (MSI), as key markers in CRCs, were also studied in these lesions.

Materials and methods

Patients and DNA extraction

All patients who underwent surgical resection for CRCs at Dongsan Medical Center between 1999 and 2003 were initially considered for enrollment in this study. The exclusion criteria included preoperative chemoradiotherapy, history of surgical resection for CRCs, death within 30 postoperative days, and evidence of hereditary nonpolyposis colorectal cancer (Amsterdam criteria) or familial adenomatous polyposis. Keimyung Human Bio-Resource Bank at Dongsan Medical Center provided 100 CRC samples.

The records of colonoscopic polypectomy performed at Dongsan Medical Center between 1999 and 2003 were reviewed retrospectively. Precancerous lesions were diagnosed by their microscopic appearance histomorphologically, and included 7 villous adenomas, 59 tubular adenomas, and 18 sessile serrated adenomas, and 12 hyperplastic polyps. Keimyung Human Bio-Resource Bank at Dongsan Medical Center provided 100 CRC samples. All cases were reviewed by 2 gastrointestinal

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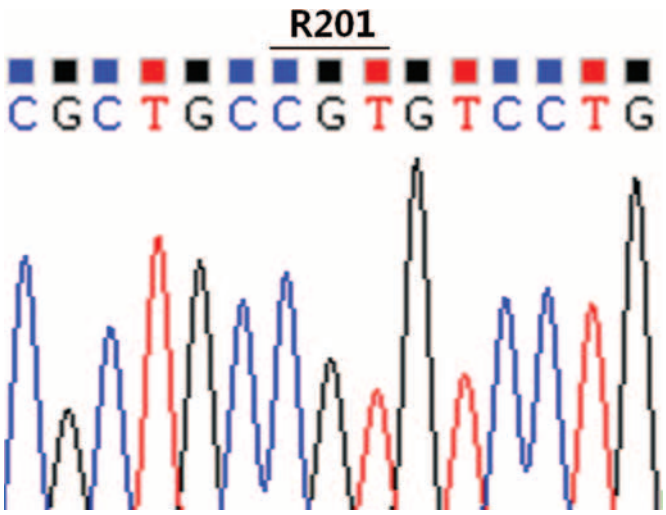

Fig. 1 - Sequencing chromatogram of patient colorectal cancer DNA shows no mutation at codon 201.

pathologists (I.H., Y.-N.K.). The study was approved by the Institutional Regional Review Board at Dongsan Medical Center (IRB no. 10-157).

Tumor areas and adjacent normal mucosa were selected from slides of hematoxylin & eosin-stained sections. Subsequently, the selected areas from paraffin-embedded tissues were used for DNA extraction using a DNA extraction kit (Absolute™ DNA Extraction Kit, BioSewoom, Seoul, South Korea).

Mutation analysis

Polymerase chain reaction (PCR) amplification of exon 8 of *GNAS* was performed as described previously with minor modification (6-8). The primer sequences for amplification were as follows (forward and reverse, respectively): 5'-ACT GTT TCG GTT GGC TTT GGT GA-3' and 5'-AGG GAC TGG GGT GAA TGT CAA GA-3'. Then, direct DNA sequencing was performed using the ABI 3730 DNA sequencer by Bionics Inc. (Daejeon, Korea) (Fig. 1).

KRAS mutations in codons 12 and 13, as well as *BRAF* V600E mutations, were analyzed by pyrosequencing (PyroMark Q24, Qiagen, Sollentuna, Sweden). Primers for amplification and pyrosequencing were designed as described previously (10). The pyrosequencing reaction was performed on a PyroMark Q24 instrument using Pyro Gold Q24 Reagents (Qiagen, Venlo, Netherlands). The pyrosequencing primers were used in a final concentration of 0.3 µmol/L. Resulting data were analyzed and quantified with PyroMark Q24 software, version 2.0.6 (Qiagen, Venlo, Netherlands).

Microsatellite instability analysis

Recent studies have demonstrated that *BAT25* and *BAT26* analysis can accurately detect MSI without the need for additional markers; therefore, MSI was analyzed with 2 microsatellite markers, *BAT25* and *BAT26*. Polymerase chain reaction-single strand conformation polymorphism assay were performed using polyacrylamide gels and silver stain. Direct DNA sequencing was performed on those PCR products that showed altered band mobility in the above analysis.

TABLE I - Genetic alterations in colorectal cancerous and precancerous lesions

	CRC (n = 100)	VA (n = 7)	TA (n = 59)	SSA (n = 18)	HP (n = 12)
GNAS mutation	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
KRAS mutation	26.0 (26)	28.6 (2)	23.7 (14)	5.6 (1)	0 (0)
BRAF mutation	7.0 (7)	0 (0)	0 (0)	11.1 (2)	16.7 (2)
MSI	16.0 (16)	14.3 (1)	6.8 (4)	11.1 (2)	0 (0)

CRC = colorectal cancer; HP = hyperplastic polyp; MSI = microsatellite instability; SSA = sessile serrated adenoma; TA = tubular adenoma; VA = villous adenoma.
Values are % (n).

Results

GNAS mutation was not found in any colorectal lesions, including villous adenoma, which is thought to harbor the mutation (6, 7). The frequency of other genetic alterations was in agreement with previous studies (Tab. I). *KRAS* and *BRAF* mutations were always mutually exclusive in precancerous lesions. *KRAS* mutations were frequent in villous (28.6%) and tubular (23.7%) adenomas but were rare or absent in serrated adenomas (5.6%) and hyperplastic polyps (0%). *BRAF* mutations were found only in serrated adenomas (11.1%) and hyperplastic polyps (16.7%), but were absent in tubular and villous adenomas. These lesions harbored similar frequency of MSI (6.8%-16.0%) except in hyperplastic polyps (0%). These mutations did not show an association with each other.

Discussion

To clarify the role of *GNAS* mutations in colorectal carcinogenesis, we performed a mutation analysis in colorectal lesions. Since the *GNAS* mutation was found in pituitary adenoma and pancreatic cancer, it was revealed that other types of cancer including CRC also harbor the mutation (5, 11). However, the mutation in CRC was very rarely found in the following studies. Two studies reported that the frequency of *GNAS* mutation in CRC was 2.3% (10/428) in the American population and 3% (2/76) in the Japanese population (6, 8). Furthermore, Idziaszczyk and colleagues (12) detected only one mutated CRC among 215 cases. In the Korean population, it has been reported recently that *GNAS* mutation was not found in 1,126 cases of various types of cancer (13), which is compatible with our study. In contrast to the consistent rarity of *GNAS* mutation in CRC, the reported frequency of mutation in villous adenoma of the colorectal region is widely variable, ranging from 46% (6/13) to 83% (20/24) (6, 8). However, we found no *GNAS* mutation in any precancerous lesion and CRC. Based on the rarity of *GNAS* mutation in CRC, one of the potential reasons might be selection bias caused by our small sample size. Further studies are needed that include

more cases of CRC and precancerous lesions, especially villous adenoma. Regarding the relatively high prevalence of *GNAS* mutation in villous adenoma in previous studies, however, selection bias is not sufficient to explain the disagreement between our data and previous studies. In an *in vivo* animal model study, using transgenic mice, *GNAS* mutation alone was not sufficient to drive tumorigenesis and adenoma formation because of compensatory upregulation of cAMP-specific phosphodiesterases (14). The role of *GNAS* mutation might be limited in colorectal neoplasms.

Disclosures

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Conflict of interest: None.

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