Two Distinct Regions of Deletion on the Long Arm of Chromosome 5 in Differentiated Adenocarcinomas of the Stomach

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ABSTRACT

Frequent loss of heterozygosity (LOH) on the long arm of chromosome 5 (5q) has been reported in many types of human malignancies, including gastric carcinoma. One of the targets of 5q-LOH in colorectal carcinoma is certainly the adenomatous polyposis coli (APC) gene on 5q21. However, other evidence has suggested the presence of another tumor suppressor gene in this region which may be inactivated in gastric carcinoma. In the present study, to determine the location of the putative tumor suppressor gene on 5q, LOH at nine microsatellite loci on 5q were investigated in 38 differentiated adenocarcinomas of the stomach that probably did not carry APC mutations. LOH at any locus on 5q occurred in 37% (14 of 38) of the tumors. Although many tumors exhibited large interstitial deletions on 5q that included the APC locus (5q21), we have identified minimum regions of deletion as the D5S428 locus and the interferon regulatory factor-1 (IRF-1) locus. Thus, at least two putative tumor suppressor genes, which play a crucial role in the genesis of differentiated adenocarcinoma of the stomach and are distinct from the APC gene, lie on 5q.

INTRODUCTION

The frequent LOH is a hallmark of the existence of a tumor suppressor gene that plays a crucial role in the genesis and/or progression of human malignancies. Frequent LOH on 5q has been reported in a wide variety of human malignancies including carcinomas arising in the colorectum (1), stomach (2, 3), esophagus (4, 5), oral cavity (6), lung (7), pancreas (8), liver (9), kidney (10), breast (11), ovary (12), and leukemias and preleukemic disorders (13). These findings suggest that one or more tumor suppressor gene(s) that play(s) an important role in the genesis of many types of human malignancies may exist on 5q, similar to the previous description of the p53 tumor suppressor gene on 17p (14).

The APC tumor suppressor gene, which is responsible for FAP, has been cloned and mapped to 5q21 (15, 16). In colorectal tumorigenesis, mutations of the APC gene are early steps in adenoma development, not only in FAP but also in sporadic forms of colorectal tumors (17). Concordant LOH on 5q and APC mutations in the remaining allele, a mechanism of inactivating a tumor suppressor gene originally postulated by Knudson (18), have been found frequently in colorectal adenomas even during their early stages (19). Analysis of the amino acid sequence of the APC gene revealed several regions with a high probability of forming coiled-coil structures, which have been implicated in protein-protein interactions (16, 20). It has been reported that catenins, which bind to E-cadherin, are associated with the APC gene product; therefore, APC has been postulated to be involved in cell adhesion (21, 22).

Other investigators and ourselves have attempted to clarify whether the APC gene is the true target of frequent 5q-LOH and have found that there are considerable differences in the incidences between 5q-LOH and mutations of the APC gene. The incidence of 5q-LOH is much higher than that of mutations in the APC gene in carcinomas other than colorectal carcinoma, such as those of the stomach (23, 24), esophagus (24, 25), oral cavity (6), lung (8), pancreas (8), liver (8), kidney (8), breast (11), and ovary (12). These observations suggest that tumor suppressor gene(s) other than the APC gene may exist elsewhere on 5q.

In the present study, we attempted to determine the location of a putative tumor suppressor gene on 5q for differentiated adenocarcinoma of the stomach by investigating LOH in 38 differentiated adenocarcinomas of the stomach using 9 microsatellite markers on 5q.

MATERIALS AND METHODS

Samples. Thirty-eight carcinoma tissues and corresponding normal tissues were obtained surgically from 38 patients. A part of these tissues were frozen and stored at −80°C for DNA extraction, and the remaining tissues were fixed in 10% buffered formalin for histological examination. The carcinomas studied were histologically differentiated adenocarcinomas and consisted of 19 early (depth of invasion was limited to the mucosa or submucosa) and 19 advanced carcinomas.

DNA Extraction. DNA was isolated by a standard proteinase K digestion and phenol/chloroform extraction procedure.

PCR and Microsatellite Analysis. Nine microsatellite markers, D5S107, D5S428, D5S409, D5S346, D5S210, FBN2, IRF1, D5S178, and D5S209 were investigated. Primers for PCR were obtained from MapPairs (Research Genetics, Huntsville, AL). These markers were mapped by Weber et al. (26) and Gyapay et al. (27). The extracted DNA was amplified by PCR with 35 cycles of a denaturation step of 94°C for 30 s, an annealing step of 55°C for 30 s, and an elongation step of 72°C for 1 min. PCR was performed in a total volume of 10 μl in 1× PCR buffer (50 mM KCl, 0.01% gelatin, and 10 mM Tris buffer, pH 8.3) containing 20 pm of each primer, 1 mM MgCl₂, 0.2 mM of each deoxynucleotide triphosphate, 0.5 units of AmpliTaq DNA polymerase (Perkin Elmer Cetus Corp., Norwalk, CT), 0.5 μl of [α-32P]dCTP (3000 Ci/mmol, 10 Ci/μl), and 100 ng of genomic DNA. Five μl of the PCR product were diluted with 45 μl of a gel-loading buffer [98% formamide, 10 mM ethylenedinitrilotetraacetic acid (pH 8.0), 0.025% xylene cyanol, and 0.025% bromophenol blue], heated at 94°C for 2 min, and stored on ice until use. Electrophoresis was performed on a 6% polyacrylamide gel containing 7 M urea at 60 W for 2–2.5 h. The gel was fixed to Seq gel filter paper (Bio-Rad, Hercules, CA), dried on a vacuum slab gel dryer, and exposed to X-ray film at −80°C for 12–24 h.

Assessment of Microsatellite Alterations. LOH was defined as a visible change in allele:allele ratio in the tumor DNA relative to the ratio in corresponding normal DNA. Alterations were judged as RER when additional bands which were not seen in the corresponding normal DNA appeared in the tumor DNA. A second PCR-microsatellite analysis was performed to ensure that the results were reproducible in each case that showed LOH or RER.

PCR-SSCP Analysis of the APC Gene. The absence of APC gene mutations was confirmed in all of the 38 differentiated adenocarcinomas of the stomach. This was accomplished by performing PCR-SSCP analysis in codons 1125 to 1547 of the APC gene in which 74% (20 of 27) of the somatic mutations in gastric adenomas and adenocarcinomas (28–30) and the location of the mutation cluster region of colorectal carcinoma (31) have been reported.
The primer sequences are listed in Table 1. The procedures of PCR-SSCP analysis have been described previously (24, 30).

RESULTS

LOH at any locus on 5q occurred in 37% (14 of 38) of the tumors (Fig. 1). The incidences of LOH at each locus were 6% (2 of 33) at DSS107, 35% (7 of 20) at DSS428, 35% (6 of 17) at DSS409, 11% (2 of 18) at DSS346, 26% (5 of 19) at DSS210, 27% (4 of 15) at FBN2, 27% (6 of 22) at IRF-I, 11% (2 of 19) at DSS178, and 9% (2 of 23) at DSS209. Although many tumors (DC 16, DC 13, DC 2, DC 17, DC 11, DC 12, and DC 25) exhibited large interstitial or total deletions on 5q, some tumors showed LOH at restricted loci (Fig. 2). We have found two distinct regions of deletion on 5q. Three tumors (DC 10, DC 31, and DC 36) exhibited LOH at DSS428 and retained heterozygosity (or not informative) at other loci (Fig. 2). Two tumors (DC 35 and DC 37) retained heterozygosity at DSS428 and exhibited LOH at a more telomeric region, which was restricted at IRF-I in DC 37 (Fig. 2). The majority of examined loci were not informative in DC 38, and LOH was detected only at the telomeric region (DSS209) in DC 6 (Fig. 2). Finally, we have identified minimum regions of deletion as the DSS428 locus and the IRF-I locus. LOH on 5q at any locus occurred in both early (33%; 6 of 18) and advanced (47%; 8 of 17) carcinomas. RER was present at multiple loci in 4 (11%) tumors (DC 7, Fig. 1; DC 9, DC 32, and DC 38). PCR-SSCP analysis of the APC gene revealed no mobility shift bands in all of the 38 differentiated adenocarcinomas of the stomach (Fig. 3). Therefore, these tumors probably did not carry APC gene mutations.

DISCUSSION

It is widely accepted that carcinogenesis is a multistep process in which several genetic alterations occur. During colorectal tumorigenesis, mutations in APC, K-ras, and p53 and deletion of the DCC (deleted in colorectal carcinoma) genes accumulate sequentially, corresponding to the stages of the adenoma-carcinoma sequence (1, 17, 32, 33). In gastric carcinoma, especially in differentiated ones, similar genetic alterations have been detected (28, 34-36). Therefore, colorectal carcinoma and differentiated adenocarcinoma of the stomach may share a similar genetic pathway in their genesis and progression. However, we have reported recently that the K-ras oncogene was not mutated in either adenomas or adenocarcinomas of the stomach and that the APC gene was mutated in 20% of the adenomas but in only 1.4% of the adenocarcinomas (37). In addition, microsatellite alterations (LOH and RER) on several chromosomes were frequent in adenocarcinomas, even during their early stages, but were quite infrequent in adenomas of the stomach (38). From these observations, we have concluded that the sequential accumulation of genetic alterations characteristic of the adenoma-carcinoma sequence of the colorectum does not occur between gastric adenoma and adenocarcinoma and that the majority of gastric adenocarcinomas develop through a de novo pathway, although p53 mutations and DCC-LOH are highly prevalent in gastric adenocarcinomas. The APC gene is frequently mutated specifically in adenomas (29, 30) and very well-differentiated adenocarcinomas of the stomach (28). These facts have led us to postulate that very well-differentiated adenocarcinomas may arise from adenomas of the stomach. Alternatively, tumor suppressor gene(s) other than the APC gene, which is the true target of the frequent 5q-LOH observed in gastric adenocarcinomas, should exist elsewhere on 5q. In the present study, we have analyzed LOH on 5q using nine microsatellite markers to determine the probable location of the putative tumor suppressor gene. Although we have found that many of the differentiated adenocarcinomas of the stomach exhibited large interstitial deletions on 5q and included the APC gene locus at 5q21, the minimum regions of deletion were identified as the DSS428 locus and the interferon regulatory factor-1 (IRF-I) locus.

In a separate study, we have found that the minimum region of deletion on 5q in esophageal carcinomas was located at the IRF-I locus (39). Esophageal carcinoma is another digestive tract malignancy in which significant differences in incidences of 5q-LOH and APC mutations have been reported (24, 25). This and our present observations suggest that two additional and different tumor suppressor genes exist on 5q, and one of them, which locates near the IRF-I, may play a role in both esophageal and gastric carcinomas. It has been reported recently that the IRF-I and IRF-2 genes show antioncogenic and oncogenic potentials, respectively, because NIH3T3 cells became...
transformed and displayed enhanced tumorigenicity when the IRF-2 gene was overexpressed (40). Furthermore, this effect was reversed by the concomitant overexpression of the IRF-1 gene (40). Although it should be realized that other genes which are located near the IRF-1 gene, such as the cytokine genes IL-3, IL-4, IL-5, GM-CSF, and the mitotic inducer CDC25C gene (41), are the targets of 5q-LOH, it is also possible that the IRF-1 gene itself is the target because of its antioncogenic potential described above. In the analyses of 5q-LOH in other carcinomas, common deleted regions were identified at 5q35–qter in hepatocellular carcinoma (42) and at 5p13–5q14 in lung carcinoma (43).

RER, which might result from aberrations in mismatch repair genes such as the hMSH2 gene (44), was consistently present at multiple loci on 5q in 11% (4 of 38) of the tumors examined in the present study. Han et al. (45) have reported that RER occurred in 17% (3 of 18) of differentiated adenocarcinomas of the stomach. According to the report by Chong et al. (46), RER was more frequently detected in advanced gastric carcinomas than in early ones. In the present study, RER phenotype was observed in 5% (1 of 19) of early and 16% (3 of 19) of advanced carcinomas.

Further work will be required to prove that these loci really do contain putative tumor suppressor genes which themselves play a crucial role in the genesis of differentiated adenocarcinoma of the stomach.

REFERENCES


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