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A Distinct Tumor Suppresser Gene Locus on Chromosome 15q21.1 in Sporadic Form of Colorectal Cancer

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Abstract

The SM1311 family is an Ashkenazi family with dominantly inherited predisposition to colorectal adenomas and carcinomas and has a high-penetrance locus in chromosome 15q, with a multipoint logarithm of the odds score of 3.06 at marker D15S118. In the present study, we performed a high-density loss of heterozygosity study with 13 polymorphic microsatellite markers, including D15S118, spanning 15q15.3-22.1q, on 70 cases of the sporadic form of colorectal tumors. Our deletion mapping data showed a locus at D15S968 in chromosomal sub-band 15q21.1 may harbor a tumor suppressor gene in an area <0.52 Mb in physical map distance defined by markers D15S514 and D15S222. THBS1, 0.185 Mb proximal to D15S968, is the nearest known gene to this specific narrow loss of heterozygosity region. Thus, we speculate that THBS1 might be the most probable candidate gene involved in colorectal cancer carcinogenesis.

Introduction

Colorectal cancer is one of the leading causes of cancer death in the world, and about 15% have a hereditary component. Two major colorectal cancer predisposition syndromes, familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer, account for up to only 5% of the total new cases of colorectal cancer (1). A number of other rare inherited syndromes, including juvenile polyposis (2) and Peutz-Jeghers syndrome (3, 4), are also associated with increased susceptibility to colorectal cancer. Recently, a missense APC polymorphism (I1307K) was reported that indirectly leads to colorectal adenoma and carcinoma predisposition by creating a locus of the gene that is prone to somatic mutations (5). This APC variant allele was observed in 6% of Ashkenazi Jews and about 28% of Ashkenazim with a family history of colorectal cancer. However, the genes responsible for most of the familial cases are unknown as of yet.

Recently, Tomlinson et al. (6) described that the SM1311 family is an Ashkenazi family with dominantly inherited predisposition to colorectal adenomas and carcinomas. This family has no evidence of linkage to known colorectal cancer susceptibility loci (including APC, TP53, PTEN, hMSH2, hMSH6, hMLH1, hPMS1, hPMS2, and SMAD4), no germline mutation of APC, hMSH2, and hMLH1, and no T-to-A I1307K APC polymorphism (6). Furthermore, Tomlinson et al. (6) demonstrated a high-penetrance locus in chromosome 15q with a multipoint logarithm of the odds score of 3.06 at marker D15S118 in the SM1311 family by way of genetic linkage analysis. These results strongly suggested that there might be a new tumor suppressor gene on the chromosomal region 15q for the sporadic form of colorectal cancer.

In the present study, we performed a high-density LOH study with 13 polymorphic microsatellite markers, including D15S118, spanning 15q15.3-22.1q, on 70 cases of the sporadic form of colorectal tumors (26 adenomas and 44 carcinomas). Our results indicate that a locus at D15S968 in chromosomal sub-band 15q21.1 may harbor a new tumor suppressor gene in an area <0.52 Mb in physical map distance defined by markers D15S514 and D15S222. THBS1, 0.185 Mb proximal to D15S968, is the nearest gene to this specific narrow LOH region. Thus, we speculate that THBS1 is the most probable candidate gene involved in colorectal cancer carcinogenesis.

Materials and Methods

Materials. A total of 70 formalin-fixed, paraffin-embedded sporadic colorectal tumor specimens (26 adenomas, 11 right-sided and 15 left-sided; 44 invasive cancers, 16 right-sided and 28 left-sided) were obtained from College of Medicine, The Catholic University of Korea. None of the patients had a family history of Peutz-Jeghers syndrome, familial adenomatous polyposis, or hereditary nonpolyposis colorectal cancer.

Microdissection and DNA Extraction. Tumor cells were selectively procured from H&E-stained slides using a 30 G1/2 hypodermic needle (Becton Dickinson, Franklin Lake, NJ) affixed to a microdissection device [SPEM II (Simple, Precise, and Economical Microdissection device); BM Korea Co., Seoul, Korea], as described previously (7). We also obtained inflammatory or surrounding normal mucosa cells for corresponding normal DNAs from the same slides in all cases. DNA extraction was performed by a modified single-step DNA extraction method, as described previously (8).

LOH Analysis. Tumor DNA and corresponding normal DNA from each slide were amplified by thermal cycler (MJ Research, Inc., Watertown, MA) with 13 microsatellite markers (Research Genetic, Huntsville, AL), including D15S1040, D15S971, and D15S118 in the 15q15.3 region; D15S129, D15S114, D15S968, D15S222, D15S132, and D15S209 in the 15q21.1 region; D15S117 and D15S195 in the 15q21.2 region; D15S1036 in the 15q22.1 region. Each PCR reaction was generally performed under standard conditions in a 10-μl reaction mixture containing 1 μl of template DNA, 0.4 μM each primer, 125 μM each dNTP, 1.5 mM MgCl2, 0.4 unit of Taq polymerase, 0.5 μCi of [α-32P]dCTP (American, Buckinghamshire, United Kingdom), and 1 μl of 10× buffer. The reaction mixture was denatured for 5 min at 95°C and incubated for 35 cycles (denaturing at 95°C for 30 s, annealing at 57°C for 90 s, and extending at 72°C for 90 s), with variations in the annealing

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3 The abbreviations used are: APC, adenomatous polyposis coli; LOH, loss of heterozygosity; THBS1, thrombospondin 1.

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observed no LOH at all in 24 informative adenomas (10 right-sided, 14 left-sided). In invasive carcinomas, 14 of 40 informative cases (35%) showed allelic loss of at least one informative marker within 15q15.3–22.1. Among these 14 cases, 7 (case no. 4, 12, 28, 48, 54, 69, and 71) showed allelic loss at every informative marker, suggesting a whole chromosome loss in each case (Figs. 2 and 3). The remaining seven tumors showed interstitial deletions. Among these cases 8 and 23 revealed allelic loss at only one marker, D15S968; this single region, marked distally by D15S222 and proximally by D15S514, revealed allelic loss in all 14 tumor cases that showed allelic loss of at least one informative marker (Figs. 2 and 3). Constitutional heterozygosity at D15S514, 0.439 Mb proximal to D15S968, was retained in case 23 (Fig. 2), and two cases (6 and 43) retained heterozygosity at D15S222, which is about 0.082 Mb distal to D15S968 (Fig. 3). These results indicate that a locus at D15S968 in chromosomal sub-band 15q21.1 may harbor a tumor suppressor gene in an area <0.521 Mb in physical map distance defined by markers D15S514 and D15S222. There was no significant difference in allelic loss frequencies between the left-sided and right-sided colon cancers.

The autoradiograms of three selected cases showing LOH are displayed in Fig. 2, and a fine deletion map of chromosome 15q15.3-q21.1 is shown in Fig. 3. The almost complete absence of a signal in deleted alleles of tumor DNA (Fig. 2, arrowheads) suggests that tumor samples are nearly devoid of normal cell contamination.

Discussion

Colorectal cancer carcinogenesis is a multistep process and seems to require at least seven genetic events for completion (1, 9). However, the whole process of colorectal cancer carcinogenesis is still poorly understood. Recently, Tomlinson et al. (6) described a high-penetration locus in chromosome 15q with a multipoint logarithm of odds score of 3.06 at marker D15S118 in the SM1311 Ashkenazi family, which has dominantly inherited predisposition to colorectal adenomas and carcinomas. These results strongly suggest that there might be a new tumor suppressor gene near the D15S118 marker on chromosomal region 15q, however, specific narrow regions of LOH in this area on colorectal cancer have not yet been defined. This led us to focus our attention on chromosomal region 15q15.3-q22.1, including marker D15S118 in the sporadic form of colorectal cancer.

For finely detailed deletion mapping, we performed a high-density LOH study with 13 microsatellite markers, including D15S118 in 70 colorectal tumors; thirteen of these markers were positioned approximately every 1 Mb or less throughout chromosomal band 15q15.3-q22.1 (physical map distance from 48.850–64.265 Mb). Our deletion mapping data revealed a common region of deletion at D15S968, 2.62 Mb distal to D15S118, which indicated the presence of a potential tumor suppressor gene in an area <0.521 Mb in physical map distance defined by markers D15S514 and D15S222. This is the first study documenting the observation of specific allele loss of chromosome 15 in the sporadic form of colorectal cancer and is an important step toward the eventual isolation of a putative tumor suppressor gene associated with colorectal cancer by positional cloning. Up to date, however, five genes are known to be located in this narrow LOH region5; solute carrier family 12 member 1 (SLC12A1), microfibrillar-associated protein 1 (MFAP1), cholesterol repressible protein 39B (CHR39B), calpain large polypeptide L3 (CAPN3), and THBS1. Of these genes, THBS1 is the nearest gene to marker D15S968, 0.185 Mb proximal to D15S968. Although we did not perform positional cloning in this region, we suspect that THBS1 is the most probable candidate

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4 http://bioinformatics.weizmann.ac.il/databases/lldb/chrom15/gmap.

tumor suppressor gene involving colorectal cancer at present because it is already known that THBS1 has tumor suppressor properties.

THBS1 is a multifunctional glycoprotein with p53- and retinoblastoma-regulated angiogenesis inhibition property and is found in platelets and secreted by a wide range of tissues, where it is incorporated into the extracellular matrix. THBS1 has been reported to modulate platelet aggregation, wound healing, protease activity, and cellular functions such as adherence, motility, and growth (10, 11). In addition to these diverse functions, it is a potent antiangiogenic factor (12). It plays a role in several other tumor types, including mesothelioma (21), prostate cancer (22), and ovarian cancer (23). Furthermore, Wick et al. (24) demonstrated a significant difference in LOH frequency in this area between nonmetastatic primary breast cancer (11%) and metastatic cancer (22), and ovarian cancer (23).

Losses overlapping this same region have also been observed in several other tumor types, including glioblastoma multiforme and in a subset of colorectal cancer (19, 20). These results are in agreement with our data that the D15S968 area might harbor a tumor suppressor gene involved in colorectal cancer progression but not in tumor initiation.

In the present study, the frequency of LOH at D15S968 was 44.4% (8 of 18 informative cancer cases; Fig. 3). Although the frequency of LOH at this marker is not that high, because it was detected only in invasive cancers, we speculate that allelic loss of this area might be involved in colorectal cancer progression but not in tumor initiation. Losses overlapping this same region have also been observed in several other tumor types, including glioblastoma multiforme and in a subset of colorectal cancer (11%) and metastatic cancer to the brain (70%). These results are in agreement with our data that the D15S968 area might harbor a tumor suppressor gene involving colorectal cancer cell lines (15); (d) transfection of a plasmid containing the THBS1 gene resulted in reduced THBS1 expression was shut off in rapidly growing endothelial cells from mouse hemangiomas induced by the polyoma virus middle 1 antigen (14); (e) THBS1 production correlated inversely with tumorigenicity and/or metastatic potential in breast, melanoma, and lung cancer cell lines (15); (d) transfection of THBS1 resulted in reduced size of the heterotransplanted primary tumors, a decrease in spontaneous pulmonary metastasis of human breast carcinoma cells, and suppressed tumorigenicity of transformed NIH3T3 cells (12, 15); and (e) reintroduction of an extra copy of chromosome 15 into human skin cancer cells lacking one copy of chromosome 15 resulted in tumor suppression (16). Other known functions of THBS1 in addition to angiogenesis may also contribute to tumor progression. For instance, THBS1 is a tight-binding competitive inhibitor of several proteases. Thus, low expression of THBS1 in cancer cells could enhance invasion through the matrix by uninhibited protease activity (17). Therefore, the THBS1 gene seems to be a new tumor suppressor gene involved in a late event, including tumor progression and/or metastasis in colorectal cancer carcinogenesis, as well as in other human cancers.

DNA methylation of promoter-associated CpG islands, which results in the transcriptional inactivation of selected genes in cancer, is one of the alternative mechanisms of silencing tumor suppressor genes (18). The THBS1 promoter also contains a typical CpG island that starts 1.3 kb upstream of exon 1 at the ends of intron 1 (19). Although the cause of THBS1 methylation in cancers remains to be defined, methylation of the THBS1 gene has also been reported in glioblastoma multiforme and in a subset of colorectal cancer (19, 20).

These observations also strongly suggest that THBS1 is a tumor suppressor gene.

In the present study, the frequency of LOH at D15S968 was 44.4% (8 of 18 informative cancer cases; Fig. 3). Although the frequency of LOH at this marker is not that high, because it was detected only in invasive cancers, we speculate that allelic loss of this area might be involved in colorectal cancer progression but not in tumor initiation. Losses overlapping this same region have also been observed in several other tumor types, including mesothelioma (21), prostate cancer (22), and ovarian cancer (23). Furthermore, Wick et al. (24) demonstrated a significant difference in LOH frequency in this area between nonmetastatic primary breast cancer (11%) and metastatic cancer to the brain (70%). These results are in agreement with our data that the D15S968 area might harbor a tumor suppressor gene involving colorectal cancers.

Fig. 2. For each of the 13 polymorphic markers, autoradiograms of LOH analysis for three selected cases are shown. Case 23 exhibits LOH at only one locus (D15S955), the D15S118 marker shows microsatellite instability, and heterozygosity is retained at four loci, including D15S514, which is the proximal border of D15S968. Case 13 reveals allelic loss at seven loci, and heterozygosity is retained at the five most distal markers, except D15S95 (noninformative). Case 69 shows allelic loss at 10 loci, with three noninformative markers. Arrowheads indicate allelic loss in tumor DNA. The numbers at the top represent the microsatellite markers. N, normal DNA; T, tumor DNA.

Fig. 3. Deletion map of chromosome 15q15.3-q22.1 with 14 cases of the sporadic form of colorectal cancers. The smallest region of overlap of deletion spans marker D15S968. Mb, physical map distance; sub-band location; , LOH; , noninformative. The numbers at the top represent the case number of colorectal cancers. The percentage numbers in the right column represent the overall frequency of LOH in colorectal cancers.
ing tumor progression and/or metastasis in colorectal cancers and strongly suggest that it requires mutational, methylation studies, as well as LOH analysis in primary and metastatic colorectal cancers.

Tomlinson et al. (6) pinpointed the locus of the responsible gene of the SM1311 family to D15S118, which lies 2.62 Mb proximal to marker D15S968. In the present study, eight cases showed LOH at D15S118, but one case (case 27) retained heterozygosity at this locus. The question of whether the responsible gene loci for the SM1311 family and D15S968 or THBS1 loci are the same was unclear at the time of this study, however, THBS1 may possibly be the responsible gene for the SM1311 family because the physical map distance between THBS1 and D15S118 is only 2.345 Mb apart (Fig. 3).

In summary, we have been able to detect a distinct region for a putative tumor suppressor gene of colorectal cancer with 44.4% of LOH frequency at marker D15S968, measuring 0.521 Mb in physical distance by way of high-density LOH analysis. We concluded that THBS1, 0.185 Mb proximal to D15S118, is the most probable candidate gene involving tumor progression in colorectal cancer as well as other human malignancies. Mutational and methylation studies of THBS1 in primary and metastatic colorectal cancers are currently in progress.

References
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