Loss of Heterozygosity Affecting the APC and MCC Genetic Loci in Patients with Primary Breast Carcinomas

Arnaldo C. Medeiros,1 Maria Aparecida Nagai, Mario Mourão Neto, and Ricardo R. Brentani

Department of Biologia Molecular/Center of Ciências Exatas and da Natureza/Laboratório de Tecnologia Farmacêutica/Universidade Federal da Paraíba [A.C.M.I., João Pessoa, 58000, PB-Brazil; and Disciplina de Oncologia/Faculdade de Medicina da Universidade de São Paulo 01246-520; Hospital A. C. Camargo (M.M.N.) and Instituto Universitário de Pesquisas sobre o Cancer (M.A.N., R.R.B.), São Paulo, SP, 01509-010-Brazil.

Abstract

Tumor suppressor genes APC and MCC were identified recently, and their chromosomal location was ascribed to chromosome 5q21. Mutations in the APC gene give rise to familial adenomatous polyposis and occur in many, perhaps even the majority, of sporadic colon cancers. Loss of heterozygosity has been described within the chromosomal region 5q21 of MCC(Mutated in Colorectal Carcinoma) (5) and APC (Adenomatous Polyposis Coli) (4-6) and MCC(Mutated in Colorectal Carcinoma) (5) were described within the chromosomal region 5q21. APC is often mutated in the germ line DNA of patients with FAP and/or MCC may be involved in the pathogenesis and/or progression of sporadic colorectal cancer (5).

The region 5q21 has been evaluated not only in FAP or sporadic colorectal cancer, but also in human lung carcinoma with a LOH of 80% (10-11) and esophageal cancer (12). The latter studies reported a LOH of 70% and 63-66%, respectively. It has also been reported that 5q allelic losses occur late during the neoplastic progression of Barrett esophagus (13). Allelic deletions of APC/MCC were likewise involved in the pathogenesis and/or progression of cancerous ulcerative colitis (14). These data suggest that APC and/or MCC may be associated with several human tumors.

We have undertaken here a study about the prevalence of APC and MCC LOH in sporadic breast tumors. DNA extracted from normal and tumor tissue from 86 patients was examined for loss of heterozygosity at the APC and MCC loci using the PCR.

Materials and Methods

Human breast cancer samples were obtained from 85 patients. All tumors were removed surgically, and fresh specimens were frozen in liquid nitrogen until DNA isolation. For every tumor sample obtained, a sample of adjacent normal tissue was collected.

Tissues were pulverized in liquid nitrogen, homogenized in lysis buffer (10 mM Tris-HCl, pH 8.0, containing 5 mM EDTA-0.6% sodium dodecyl sulfate), and digested with proteinase K at 37°C overnight. After phenol/chloroform extraction and sodium acetate-ethanol precipitation, DNA was dissolved in Tris-EDTA buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and incubated with RNase A at 37°C for 30 min to eliminate contaminating RNA. After RNAse treatment, DNA was extracted with phenol/chloroform and precipitated with ethanol (15).

For the PCR, DNA was amplified using primers for exons 11 and 15 of the APC gene and for exon 10 of the MCC gene (Table 1). Primers were synthesized for the regions flanking a RsaI and a MspI restriction fragment length polymorphism in exons 11 and 15 of the APC gene, respectively (12, 16). For MCC, primers amplified a variable insertion region within exon 10 (12). The following ingredients were mixed with H2O in a total volume of 50 μl: 500 ng of DNA; 50 pmol of each primer; 5 μl of reaction buffer (final concentration of 10 mM Tris, pH 8.4, 50 mM KC1, and 1.5 mM MgCl2), 50 μM of each deoxynucleotide triphosphate, and 1.25 units of Taq DNA polymerase. Amplification was carried out with a Thermal Cycler (Perkin Elmer/Cetus). The conditions for PCR were: (a) one cycle of 5 min at 95°C, 1 min at 59°C, and 1 min at 72°C; and 29 cycles of 30 sec at 95°C, 1 min at 59°C, and 1 min at 72°C for exon 11 of the APC gene. The PCR products were digested with 4 units of RsaI at 37°C overnight. (b) One cycle of 5 min at 95°C, 1 min at 60°C, and 1 min at 72°C; followed by 35 cycles of 1 min at 92°C, 1 min at 60°C, and 1 min at 72°C for exon 15 of the APC gene. The products were digested with 4 units of MspI at 37°C overnight. (c) One cycle of 5 min at 95°C, 1.5 min at 55°C, and 2 min at 72°C; followed by 30 cycles of 1.5 min at 95°C, 1.5 min at 55°C, and 2 min at 72°C for the MCC exon 10. PCR

Received 8/10/93; revised 10/29/93; accepted 10/29/93.

1 At Instituto Ludwig de Pesquisa sobre Cancer, R. Prof. Antonio Prudente, 109-40 Andar, 01509-010 São Paulo, Brazil.

2 LOH, loss of heterozygosity; FAP, familial adenomatous polyposis; PCR, polymerase chain reaction.

Downloaded from cebp.aacrjournals.org on August 27, 2017. © 1994 American Association for Cancer Research.
Table 1 Primer sets used in the PCR-LOH assay

<table>
<thead>
<tr>
<th>Amplified region</th>
<th>Product size (bp)</th>
<th>Polymorphism type</th>
<th>Primer sequence (Ref.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC exon 11</td>
<td>133</td>
<td>Rsal RFLP</td>
<td>GGAATACAGGCAATTCAGAA  (12)</td>
</tr>
<tr>
<td>APC exon 15</td>
<td>650</td>
<td>MspI RFLP</td>
<td>GCTATCTTCAGGAGGCAAGC   (16)</td>
</tr>
<tr>
<td>MCC exon 10</td>
<td>79 or 93</td>
<td>VNTR</td>
<td>TACAAATCAATCCCAACA     (12)</td>
</tr>
</tbody>
</table>

$^a$ bp, base pairs.
$^b$ RFLP, restriction fragment length polymorphism.
$^c$ VNTR, variable number of tandem repeats.

Fig. 1. LOH at the APC exon 11 locus. DNA band sizes are indicated in base pairs (bp). N, normal tissue; T, tumor. Remaining signal at 85 base pairs is probably due to contaminating normal cells in the tumor.

Fig. 2. LOH at the APC exon 15 locus. DNA band sizes are indicated in base pairs (bp). N, normal tissue; T, tumor.

Table 2 Results of the PCR-LOH assay for region 5q21

<table>
<thead>
<tr>
<th>Region</th>
<th>% of informativity$^a$</th>
<th>% of LOH$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC exon 11</td>
<td>40 (35/87)</td>
<td>25 (9/35)</td>
</tr>
<tr>
<td>APC exon 15</td>
<td>41 (34/83)</td>
<td>11 (4/34)</td>
</tr>
<tr>
<td>MCC exon 10</td>
<td>48 (40/83)</td>
<td>17 (7/40)</td>
</tr>
<tr>
<td>Region 5q21</td>
<td>71 (62/87)</td>
<td>29 (18/62)</td>
</tr>
</tbody>
</table>

$^a$ (Heterozygosity/total number of cases).
$^b$ (LOH cases/informative cases).

Results

Loss of heterozygosity at exons 11 and 15 of the APC gene and exon 10 of the MCC gene was demonstrated when tumor DNA showed the loss of one allele as compared with normal DNA. For exon 11, three bands were visualized in the normal DNA from heterozygous patients: a 133-base pair uncut band from the allele lacking the Rsal restriction enzyme site and two other bands (85 and 48 base pairs) representing the allele that contains the site (Fig. 1). At the APC exon 11 locus, 35 of 87 patients were informative or heterozygous (40%), of which (25%) showed LOH. For exon 15, two bands were detected: a 650- and a 350-base pair band from alleles with or without the presence of the MspI restriction enzyme site (Fig. 2). At the APC exon 15 locus, 34 of 83 (41%) patients were informative and 4 of 34 (11%) showed LOH. Heterozygous patients showed two bands at the MCC exon 10 locus. There is a variable insertion region within this exon, and depending on the absence or presence of the insertion, a product with 79 or 93 base pairs is generated. At the MCC exon 10 locus, 40 of 83 patients assayed were informative (48%), 7 of which (17%) demonstrated LOH (Table 2).

Sixty-two of 87 patients were informative at one or more loci analyzed with a frequency of 71%. Eighteen of 62 (29%) showed loss of heterozygosity when we considered the region 5q21 analyzed with three loci as markers. One patient showed LOH at APC exons 11 and 15 and another one at APC exon 11 and MCC exon 10. All others showed LOH at only one marker site.
Discussion

Human breast cancer is a disease that shows a broad spectrum of clinical and pathological characteristics. These diverse characteristics may be a reflection of the multiplicity and heterogeneity of the molecular mechanisms involved in its genesis and progression. From the point of view of genetic alterations in breast cancer, several studies have already been done analyzing loss of heterozygosity, and these have also shown the involvement of several chromosome regions, such as 1q, 3p, 11p, 13q, and 17q (1-3, 17). These results suggest the possible involvement of tumor suppressor genes during breast tumorigenesis. On the other hand, other studies have also suggested oncogene amplification like c-erb-2, c-myc, and int-2 (2). Sato et al. (18), in an allelotype study, showed that at least 4 tumor suppressor genes would be involved in breast tumorigenesis. In their study, they analyzed region 5q and found a LOH of 18% using polymorphic probes by Southern blots. APC and MCC genes have been identified subsequently; thus, these authors could not correlate their results within region 5q with these two genes. Our results show that loss of heterozygosity on chromosome 5q21 in informative primary breast carcinomas is 29%. These allelic losses were analyzed for two exons of the APC gene and one exon of the MCC gene using a LOH-PCR assay.

Previous studies have reported significant associations between specific tumor mutations and particular clinical parameters of the patient’s history, characteristics of the tumor, or the patient’s prognosis. In our study, however, we tried to see the correlation between LOH on chromosome 5q and some clinical parameters, and the results do not show any significance (data not shown), possibly due to insufficient sample size.

These findings implicate LOH involving APC or MCC or both, as well as other tumor suppressor genes, in the genesis of several human carcinomas. LOH on chromosome 5q, besides its clear involvement with FAP or sporadic colorectal cancer (19), has also shown that these tumor suppressor genes have an important role in other human tumors as was shown for the loss of a normal allele of the APC gene in adenocarcinoma from a patient with FAP (20). The APC gene is also mutated in patients with gastric (21) and pancreatic (22) cancers. These results suggest that the deletion and/or heterozygous mutant/wild-type condition of the APC gene may confirm the hypothesis that this gene functions as a tumor suppressor gene in several tissues. The finding of 29% LOH on chromosome 5q in primary breast cancer obtained in our study provides the basis for a working hypothesis, which states that different subsets of mutations may make comparable contributions to the malignant process. This concept of different subsets of mutations possibly acting in a complementary fashion is consistent with the heterogeneous nature of etiological factors that provide the selective pressure for mutations during breast carcinogenesis.

Acknowledgments

We thank Dr. Luisa Lina Villa for valuable suggestions during the manuscript preparation.

References

Loss of heterozygosity affecting the APC and MCC genetic loci in patients with primary breast carcinomas.

A C Medeiros, M A Nagai, M M Neto, et al.


Access the most recent version of this article at: http://cebp.aacrjournals.org/content/3/4/331

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.