Identification of a Region of Frequent Loss of Heterozygosity at 11q24 in Colorectal Cancer

Kate C. Connolly, Hani Gabra, Christopher J. Millwater, Karen J. Taylor, Genevieve J. Rabiasz, J. E. Vivienne Watson, John F. Smyth, Andrew H. Wyllie, and Duncan I. Jodrell

Imperial Cancer Research Fund Medical Oncology Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, Scotland, United Kingdom.

Abstract

Loss of heterozygosity (LOH) at 11q23–qter occurs frequently in ovarian and other cancers, but for colorectal cancer, the evidence is conflicting. Seven polymorphic loci were analyzed between D11S897 and D11S969 in 50 colorectal tumors. Two distinct LOH regions were detected, suggesting possible sites for tumor-suppressor genes involved in colorectal neoplasia: a large centromeric region between D11S897 and D11S925, and a telomeric 4.9-Mb region between D11S912 and D11S969. There was no correlation with clinicopathological features. This analysis describes a region of LOH in the region 11q23.3–24.3 for the first time in colorectal cancer and provides complementary evidence for the ongoing effort to identify the gene(s) involved.

Introduction

Colorectal carcinogenesis may be explained in terms of activation of oncogenes coupled with inactivation of tumor-suppressor genes. These genetic alterations often occur in a certain sequence such as that proposed by Fearon and Vogelstein (1). The loss of specific chromosomal regions usually involves only one of the two parental chromosomes in tumor cells. These allelic losses have been interpreted as evidence that the regions affected contain tumor-suppressor genes, the products of which normally regulate growth and differentiation in a negative way, hence preventing neoplastic development. Chromosome 11 regional allele loss is frequently involved in many different tumor types; it has therefore been proposed that it may house tumor-suppressor genes.

At present in colorectal cancer, there are known regions of LOH in association with tumor suppressor genes on chromosomes 5q (APC; Ref. 2), 17p (p53; Ref. 3), 8p (4), and 18q (DCC; Ref. 5), which occur frequently in association with somatic mutation of tumor-suppressor genes, but many other chromosomes also show areas of allelic loss. Chromosome 11 was considered a candidate for harboring tumor-suppressor genes because of cytogenetic analyses of colorectal cancers in which have been found frequent deletions of the long arm of chromosome 11 (6, 7). There is evidence (8) that human colon carcinoma cells into which a normal copy of chromosome 11 had been transferred show a reduced tumor growth rate in vivo, although there is no suppression of tumorigenicity. This suggests the presence of a gene on chromosome 11 that affects cell growth, although the position of this gene, if it exists, has not yet been determined. There are several candidate tumor suppressor genes on chromosome 11p that could be responsible for this effect, such as WT1 (11p13; Ref. 9), so-called WT2 (11p15.5; Ref. 10), p57 (11p15.5; Ref. 11), and KAI-1 (11p13; Ref. 12). The existing evidence for LOH2 on chromosome 11q in colorectal cancer is conflicting. Keldysh et al. (13) were able to map frequent LOH to 11q22–23 and correlate the deletions to clinicopathological characteristics. Deletions of this region were apparently associated with rectal rather than colonic sites and with well-differentiated tumors. Gustafson et al. (14) attempted to confirm this result and analyzed 101 samples for allelic loss at the DRD2 gene located at 11q22–23, where they found a lower rate of LOH, and a significant association of LOH of this region with losses on chromosome 14. A region of LOH has been discovered recently (15, 16) at 11q23.3-q24.3 in epithelial ovarian cancer, and this has been associated with poor prognosis (15). The presence of a tumor-suppressor gene in this region, the loss of which encourages tumor progression, has been suggested, although the gene responsible has not yet been cloned. In the light of this new region of LOH which has been mapped and linked to survival in ovarian cancer, the regions 11q23.1–q23.3 and 11q23.3–q24.3 were examined in DNA derived from colorectal tumor samples. The aim of this study was to map the above region using oligonucleotide primers in a series of blood/tumor pairs from a population of patients with colorectal cancer.

Materials and Methods

Patient Population and Tumor Samples. Fresh primary colorectal tumor tissue from 50 patients was obtained, and DNA was extracted according to standard methods as described previously (17). Patient characteristics are outlined in Table 1. Patients were on continuing follow-up, which ranged up to 2492 days.

LOH Analysis. DNA samples were analyzed as normal/tumor pairs by PCR using primers for the seven CA repeat polymorphic microsatellites in the region 11q23.1 to 11q24.3 (Fig. 1). Primer sequences were obtained from the Genome Database, and the location was determined from the radiation hybrid map produced by James et al. (18). Each primer was optimized with HeLa DNA to determine the ideal PCR reaction conditions. The PCR products were resolved by electrophoresis on a 6% denaturing urea/polyacrylamide gel, passively transferred to Hybond nylon membrane, and exposed to UV light to cross-link the DNA to the filter. The products were probed with γ-32P end-labeled (CA)35 oligonucleotide and exposed to film.

The autoradiographs were analyzed by visual reporting and by computer densitometric analysis. Autoradiographic data were acquired using GDS 7500 Gel Documentation System (UVP) and analyzed using GelBase Pro software V3.11 (Synoptics, Ltd.). Each pair of samples was assigned to one of four groups; heterozygous with LOH, heterozygous without LOH, uninformative (homozygous), or not determinable. The relative ratio of alleles was determined, normalized, and compared. Where the tumor allele ratio differed from the normal allele by 30% or more (r < 0.7), LOH was assigned as described previously (19).

Statistical Analysis. Fisher’s exact test was used to look for associations between LOH regions and clinicopathological parameters.

Received 12/17/98; accepted 4/28/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Imperial Cancer Research Fund, Medical Oncology Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, Scotland, United Kingdom.
2 The abbreviation used is: LOH, loss of heterozygosity.
Results

Clinical and pathological characteristics of the cohort are outlined in Table 1.

LOH was detected somewhere on chromosome 11 in 47 (94%) of 50 tumors in this series. Fig. 1 shows the regions of LOH in all of the tumor samples. A centromeric region of loss was identified from D11S897 and D11S925. There is a secure area of loss bordered by these two markers in seven tumors. This area of loss may also be present in an additional 26 tumors, where only one or other of the markers is unambiguously deleted. This suggests a maximum LOH rate for this centromeric region of 66%.

A distal region defined by the three telomeric markers (D11S912, D11S1320, and D11S969) was deleted in 35 of 50 tumors (70%). Of tumors with this deletion, several (11 of 35; 31%) showed loss of D11S1320 only with retention of the adjacent centromeric and telomeric markers. D11S1320 demonstrated LOH in 30 of 44 (68%) of cases. Clear examples of LOH selectively at this locus are shown in Fig. 2.

There was no significant correlation between regional losses and any recorded clinicopathological features (survival, sex, site of tumor, differentiation, vascular invasion, perineural invasion, or mucin production).

Discussion

The above data map a region of LOH to 11q23.3–q24.3 for the first time in colorectal cancer. The data presented here not only confirm the presence of this region in colorectal cancer but also reduce the likely region housing a tumor-suppressor gene from 8.5 to 4.9 Mb lying...
between D11S912 and D11S969. In this colorectal study, we found no associations with clinicopathological factors. This is consistent with the findings of Keldysh et al. (13), which also did not associate LOH on 11q with adverse clinicopathological features. Recent evidence has been published that the 11q23.3–q24.3 region may contain a late-acting suppressor that is prognostically important for ovarian cancer (15, 20). However, this evidence is not unequivocal because another ovarian cancer study (16) found a relatively high rate of LOH in early Fédération Internationale des Gynécologues et Obstétristes stage tumors, suggesting that LOH in this region may represent an early rather than a late event. The findings in our colorectal study appear to concur with the latter view.

A recent study by Koreth et al. (21) concluded that distal loss on chromosome 11 was not frequent in colorectal tumors, in contradistinction to a parallel series of breast cancer cases reported in the same study. The regions examined by Koreth et al. were 11q22–q23.1 and 11q25–qter; in particular, the telomeric marker D11S969 was lost at a low rate (15%; 3 of 20 cases). The authors used 50% as the cutoff value (<0.5), as compared with 30% used in this study (see “Materials and Methods” above). Our study, in contrast, clearly demonstrates that the 11q23.3 region is frequently subject to LOH but is much larger. This region overlaps with others that have been defined previously in colorectal (13), breast (21, 22), ovarian (15, 16, 20), cervical (23), lung (24), and nasopharyngeal (25) carcinoma.

This overlap is demonstrated in Fig. 3. Three consensus regions are defined by previous analyses. The most centromeric of these is located centromeric of D11S897, our most centromeric marker. Our study does support a second consensus region located telomeric of this, defined by CD53 and D11S925 (representing the telomeric half of the centromeric region presented in this report). A third consensus region of deletion defined in other studies lying between D11S1345 and D11S328 was not identified in our study, although there were three tumors in our series demonstrating secure LOH between D11S295 and D11S912 and could represent this region.

To these three consensus regions of deletion, we now add a fourth, defined by four separate series (including this one). The consensus for this region lies between D11S912 and D11S969 and has been observed in two separate ovarian cancer series (15, 16) and a breast cancer series (21). Several candidate genes are located in the 11q23–qter region that warrant further investigation. These include the ATM gene (26) PGL-1 (27), LOHI1CCR2A (28), and CHK-1 (29) and ALL-1 (30). The distal deletion interval at the time of this report contains no plausible identified candidate genes according to the Gene Map '98 web site (http://www.ncbi.nlm.nih.gov/genemap/). It is proposed that further refinement of the region will be conducted using more microsatellites. Candidate genes from this region will be examined and analyzed for mutations. Additional studies will be required to delineate the locations of these putative tumor-suppressors, but this study has already contributed to the cloning effort by reducing the minimum size of the most telomeric region. The accumulating evidence for frequent LOH in the distal deletion unit in many tumor types suggests that the proposed tumor-suppressor gene from this region may be fundamental to the process of neoplasia.
References

Identification of a Region of Frequent Loss of Heterozygosity at 11q24 in Colorectal Cancer

Kate C. Connolly, Hani Gabra, Christopher J. Millwater, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/12/2806

Cited articles
This article cites 29 articles, 14 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/12/2806.full#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/12/2806.full#related-urls

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

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

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