Detailed deletion mapping at chromosome 11q23 in colorectal carcinoma

AS-G Lee^{1,4}, Y-C Seo¹, A Chang¹, S Tohari¹, K-W Eu², F Seow-Choen² and JO'D McGee³

¹Department of Clinical Research; ²Department of Colorectal Surgery, Singapore General Hospital, Outram Road, Republic of Singapore 169608; ³Nuffield Department of Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK; ⁴Division of Medical Sciences, National Cancer Centre, Republic of Singapore 169610

Summary Loss of heterozygosity (LOH) is frequent at the chromosomal region 11q22–q23 in several types of tumours of diverse cell origin. Previous investigations of LOH at this chromosomal region in colorectal carcinoma have been contradictory in their findings, and have only included between 1–4 loci. In order to define any regions of LOH on 11q23, we investigated 16 loci between D11S940 and D11S934 on the long arm of chromosome 11 using microsatellite analysis. Of 57 colorectal carcinomas specimens, 36 (63.2%) demonstrated LOH at one or more marker, with the highest frequencies of LOH at D11S1340 (41.0%), located between 105.13–111.97 Mb from the centromere, and D11S924 (37.1%) and D11S4107 (40.5%), both located approximately 113 Mb from the centromere. No statistically significant associations between LOH and age-of-presentation or Dukes' stage were found. LOH was observed in colorectal tumours of all Dukes' stages, including Dukes' stages A and B, suggesting that the inactivation of a tumour suppressor gene(s) on 11q23 occurs in the early stages of colorectal carcinoma. These results confirm the presence of putative tumour suppressor gene(s) at chromosome 11q23, involved in the carcinogenesis of colorectal carcinoma, and will facilitate future identification of candidate genes. © 2000 Cancer Research Campaign

Keywords: loss of heterozygosity (LOH); chromosome 11q; tumour suppressor genes; colorectal carcinoma

Colorectal carcinoma is one of the leading causes of cancer mortality in the Western world, with an increasing incidence in Singapore (Chia et al, 1996; Wingo et al, 1998). The development of colorectal carcinoma is a multi-step progression with transformation of the normal colonic epithelium to an adenomatous polyp and ultimately an invasive cancer (Gryfe et al, 1997). Genetic alterations that are involved during this progression include the dysregulation of the K-ras gene and LOH of chromosomes 5q, 17p and 18q. These chromosomal regions harbour several tumour suppressor genes: the adenomatous polyposis gene (APC)(5q), TP53 gene (17p), and the deleted in colorectal cancer (DCC) gene (18q) (Fearon and Vogelstein, 1990; Gryfe et al, 1997).

Recent independent analyses of chromosome 11q suggest that a putative tumour suppressor gene or genes located in chromosome 11q22–q24 may be involved in the tumorigenesis of several solid tumours of diverse cell types, such as tumours of the breast (Carter et al, 1994; Negrini et al, 1995; Tomlinson et al, 1995), ovary (Davis et al, 1996; Gabra et al, 1996), stomach (Baffa et al, 1996), lung (Rasio et al, 1995), cervix (Hampton et al, 1994), nasopharynx (Hui et al, 1996) and malignant melanoma (Tomlinson et al, 1993; Herbst et al, 1995; Robertson et al, 1996). The involvement of this chromosomal region in the tumorigenesis of colorectal cancer is unclear, with cytogenetic (Konstantinova et al, 1991; Keldysh et al, 1993) and some molecular studies (Tomlinson and Bodmer, 1996; Connolly et al, 1999) indicating losses in this region but with other molecular studies reporting no significant losses (Koreth et al, 1997). These studies employed four or fewer loci from the 11q22–23 region, an insufficient

number to establish clearly the regions of 11q deletion in colorectal carcinogenesis.

In order to confirm whether the chromosomal region 11q22–23 is indeed lost in colorectal carcinoma and also to identify the regions of loss, we have performed a comprehensive genetic analysis of chromosome 11q22–q23 in 57 colorectal tumours using 16 highly polymorphic microsatellite markers. We have demonstrated that loss of heterozygosity at this chromosomal region is common in colorectal carcinoma. The critical regions of LOH defined by this study will be crucial for future work on the identification of putative tumour suppressor gene(s) by positional cloning techniques. We have also observed LOH on 11q23 in tumours from patients with early Dukes' Stage (A and B), implying that loss of 11q is an early event in colorectal cancer.

MATERIALS AND METHODS

Samples

Fifty-seven colorectal carcinoma samples and corresponding normal colonic mucosa were snap-frozen in liquid nitrogen upon resection and stored at –70°C. Peripheral blood samples were collected from each of the patients in EDTA tubes and stored at –70°C. Fifty-five cases were identified histopathologically as adenocarcinoma, and two cases were mucinous adenocarcinoma. Four (7.1%) of the patients presented at Dukes' stage A, 15 (26.8%) were Stage B, 17 (30.4%) were Stage C and 20 (35.7%) were stage D. The Dukes' Stage for one patient was unknown.

DNA extraction

Cryostat sections were cut from each of the tumours, stained with H&E and viewed. The tumours were then trimmed to exclude

Received 6 December 1999

Revised 11 May 2000

Accepted 8 June 2000

Correspondence to: A S-G Lee

normal cells and for the enrichment of neoplastic cells. Cryostat sections of normal colonic mucosa were also viewed to confirm the exclusion of neoplastic cells. DNA was extracted from tissues using DNAzol (GIBCO/BRL, USA) according to the manufacturer's instructions. DNA was extracted from frozen whole blood using sucrose lysis buffer and proteinase K digestion. The extracted DNA was quantified by spectrophotometry.

Microsatellite analysis

Sixteen microsatellite markers from the chromosomal region 11q22–23 were selected: D11S940, D11S1778, D11S1818, D11S1340, D11S29, D11S1356, D11S4104, D11S924, D11S4171, D11S4132, D11S925, D11S4107, D11S1345, D11S1328, D11S933 and D11S934. Two microsatellite markers from chromosome 11p, D11S929 and D11S1344 were selected as controls. The primer sequences, chromosome localization and frequency of heterozygosity were obtained electronically from the Genome Database and the US National Center for Biotechnology Information (NCBI) database. Polymerase chain reactions (PCR) were performed in a 10 µl volume with 100–400 ng of DNA, 125 µM of each dNTP, 0.5 µM of each primer, 1.5 mM MgCl₂ and 0.2 units of *Taq* DNA polymerase (Promega, USA). The sense primer was end-labelled with [γ -³³P]dATP (Amersham, USA or NEN, USA). The PCR conditions were an initial denaturation step at 94°C for 3 min, 25 cycles of denaturation (1 min at 94°C), annealing (1 min at 60–67°C), and extension (1 min at 72°C), and a final extension step (72°C, 7–15 min). Annealing temperatures were optimized for each primer pair. The PCR products were separated on an 8% polyacrylamide sequencing gel and exposed to

X-ray film overnight and also exposed to the CS phosphor screens (Biorad, USA) for 4–6 h.

Assessment of LOH

DNA quantitation was performed by scanning the exposed CS phosphor screens with the Molecular Imager (Model GS-250, Biorad, USA). LOH was determined by quantitation of the signal intensity of each allele, and comparing the ratios of the intensity of the alleles from the tumour DNA with that of the constitutional (normal mucosa or blood) DNA. The difference in allele ratios between normal and tumour samples was divided by the allele ratio for the normal sample and a value of over 0.3 was scored as LOH, as has been previously described by other investigators studying LOH in this region (Hampton et al, 1994; Negrini et al, 1995; Connolly et al, 1999).

Calculation of allele ratios were determined by the allelic ratio method as well (Cawkwell et al, 1993), to allow for the comparison of our data with that of Koreth et al (1997). Briefly, the ratio of alleles was calculated for each normal and tumour sample and then the tumour ratio was divided by the normal ratio, i.e. T1:T2/N1:N2, and with a cut-off ratio of 1.5. All samples with LOH were repeated to confirm the result.

DNA fingerprinting

All samples with microsatellite instability (MI) were subjected to DNA fingerprinting using the D1S80 microsatellite marker in order to verify that the normal and tumour samples were from the same patient and not from unrelated patients. The extracted DNA

Table 1 The frequency of LOH at 16 microsatellite markers on chromosome 11q22–q23 and two microsatellite markers on chromosome 11p in colorectal carcinoma

Marker	Distance from centromere in megabases (min/max) ^a	No. with LOH/No. of informative cases (%LOH) ^b	No. with LOH/No. of informative cases (%LOH) ^c
D11S929	19.38/19.79 ^d	4/33 (12.1)	1/33 (3.0)
D11S1344	35.07/53.57 ^d	8/32 (25.0)	5/32 (15.6)
D11S940	91.97/97.42	9/41 (22.0)	6/41 (14.6)
D11S1778	101.56/105.43	12/44 (27.3)	11/44 (25.0)
D11S1818	104.23/110.40	16/46 (34.8)	15/46 (32.6)
D11S1340	105.13/111.97	16/39 (41.0)	14/39 (35.9)
D11S29	110.64/120.93	15/46 (32.6)	14/46 (30.4)
D11S1356	106.59/108.87, 111.30/111.97	14/46 (30.4)	14/46 (30.4)
D11S4104	112.75/112.75	9/38 (23.7)	7/38 (18.4)
D11S924	113.02/113.02	13/35 (37.1)	12/35 (34.3)
D11S4171	113.14/113.14	13/41 (31.7)	13/41 (31.7)
D11S4132	113.28/113.28	7/37 (18.9)	6/37 (16.2)
D11S925	113.41/115.23, 115.62/115.62	14/43 (32.6)	13/43 (30.2)
D11S4107	113.54/113.60	17/42 (40.5)	15/42 (35.7)
D11S1345	117.40/117.40, 118.32/118.60	6/43 (14.0)	5/43 (11.6)
D11S1328	118.92/119.09, 119.58/119.58	9/41 (22.0)	8/41 (19.5)
D11S933	119.19/121.31	7/27 (25.9)	6/27 (22.2)
D11S934	120.21/121.31	11/33 (33.3)	10/33 (30.3)

^aThe distance from the centromere was obtained from the Genome Database (<http://gdbwww.gdb.org>);

^bAssessment of LOH using the 30% cutoff as previously described (Hampton et al, 1994; Negrini et al, 1995; Koreth et al, 1997); ^cAssessment of LOH using the 50% cutoff as previously described (Cawkwell et al, 1993; Connolly et al, 1999); ^dDistance from the centromere on chromosome 11p

© 2000 Cancer Research Campaign

Table 3 LOH and clinicopathological parameters

	LOH (%)	No LOH (%)	Statistical test	P
Age at presentation			Fisher's exact test	0.163
≤ 50 years	10 (79.9)	3 (23.1)		
> 50 years	25 (56.8)	19 (43.2)		
Dukes' Stage			Chi-squared test by exact method	0.156
A	1 (25.0)	3 (75.0)		
B	10 (66.7)	5 (33.3)		
C	13 (76.5)	4 (23.5)		
D	10 (50.0)	10 (50.0)

Figure 1 Examples of LOH on chromosome 11 in representative colorectal carcinoma samples. *Top* = case numbers; *Left* = microsatellite markers listed from the most centromeric to the most telomeric; *N* = normal; *T* = tumour. Shown at the *bottom* of each autoradiograph is *D*, the difference in allele ratios between normal and tumour samples, divided by the allele ratio for the normal sample. When the allele ratio in tumour DNA differed more than 30% from the ratio of alleles in normal DNA, LOH was scored. *Arrowheads* indicate the allele lost in tumour DNA

spanning 4.9 Mb, between D11S897 and D11S925 as reported by Connolly et al (1999).

Previous RFLP and microsatellite analyses of the chromosomal region 11q22–q23 in colorectal carcinoma have only utilized between one to four loci, with conflicting data on the frequency of loss of heterozygosity (LOH). Koreth et al (1997) reported a low

frequency of LOH of 11–12% in this area, utilizing two microsatellite markers to this region, D11S35 and D11S29. Moderate levels of LOH were obtained at the D11S29 locus (29%) and the dopamine D2 receptor locus (33.8%) in two other studies (Gustafson et al, 1994; Tomlinson and Bodmer, 1996). By employing RFLP methods and studying only four loci at this chromosomal region, Keldysh et al (1993) detected LOH at a high frequency of 50–60% in the same chromosomal region. Recently, high frequencies of LOH of 40% and 47.6% were reported by Connolly et al (1999), in their study employing two microsatellite markers in this region, D11S897 and D11S925. Our findings of high frequencies of LOH provides further evidence that the chromosomal region 11q23 is indeed lost during colorectal carcinogenesis.

Variations in the frequencies of LOH between studies of the same tumour type and using the same microsatellite marker may be due to the different cutoff criteria used for the assessment of LOH. In order to compare our microsatellite analysis with those from other studies, we have reported the LOH frequency using the 30% cutoff value (Hampton et al, 1994; Negrini et al, 1995; Connolly et al, 1999) and the 50% cutoff value (Cawkwell et al, 1993; Koreth et al, 1997) (Table 1). We note a general reduction of %LOH using the 50% cutoff criteria, described by Cawkwell et al (1993). Using the 30% cutoff, LOH at D11S925 was observed in 47.6% of cases analysed by Connolly et al (1999) but in only 32.6% of our cases. Nevertheless, these values are higher than that reported by Koreth et al (1997) at D11S29 of 12%, which was assessed using the 50% cutoff. A similar analysis of this same marker, D11S29, in our case showed LOH of 30.4%. However, we conclude that either criteria may be used to define the frequencies of LOH within a study, as both criteria allow the identification of high LOH rates in relation to LOH rates observed at other markers in that study. The highest rates of LOH in this study, at D11S1340, D11S924 and D11S4107, were observed at the same markers, for both cutoff values. Reporting of LOH frequency using both criteria may be helpful for comparisons between studies.

By utilizing an extensive panel of microsatellite markers in this study, we have excluded the possibility of these losses being a reflection of generalized chromosomal instability. The low frequency of LOH on the short arm of chromosome 11 at D11S929 was similar in frequency to that at D11S1345, on 11q. In contrast, higher frequencies of LOH were observed at the D11S1340, D11S924 and D11S4107 on 11q23.

The D11S1340 locus is located approximately 105.13–111.97 Mb from the centromere. Also located in this region is the recently identified putative tumour suppressor gene, PPP2R1B, which encodes the β isoform of the A subunit of the serine/threonine protein phosphatase (PP2A). PP2A has been linked to

Figure 2 Examples of microsatellite instability (MI) in representative colorectal carcinoma samples. Top = case numbers and microsatellite markers; N = normal; T = tumour. MI as depicted by additional bands in the tumour DNA are shown arrowed

carcinogenesis, is involved in the down-regulation of the mitogen-activated protein kinase cascade and relays signals for cell proliferation. Alterations of the PPP2R1B gene were detected in human lung and colon cancer (Wang et al, 1998). This gene was identified from a region exhibiting high frequency of LOH of 42.9% and 46.2% respectively, using the D11S1647 and D11S1987 loci (Wang et al, 1998).

Both the loci D11S924 and D11S4107 are located approximately 113 Mb from the centromere. Deletions in this region have been detected in tumours of the nasopharynx (Hui et al, 1996), cervix (Hampton et al, 1994), ovary (Gabra et al, 1996), breast (Negrini et al, 1995) and lung (Rasio et al, 1995). Other genes located in this region include the CBL gene which participates in the signal transduction of haematopoietic cells (Blake et al, 1991) and the MLL gene which is translocated in acute leukaemias and possibly acts as a transcriptional regulatory factor (Gu et al, 1994). Other possible candidate genes include the LOH11CR2A gene that has been molecularly cloned (Monaco et al, 1997) and the BRCA3 gene which has been associated with the constitutional translocation 11q;22q (Iselius et al, 1983; Lindblom et al, 1994).

We have observed LOH on 11q23 in colorectal tumours from patients with Dukes' stage A (25%) and B (67%), suggesting that loss of function of a tumour suppressor gene on 11q23 occurs in the early stages of colorectal cancer. This finding is consistent with those of Evans et al (1998) and Davis et al (1996) who have reported that 11q deletion occurs early in cervical and ovarian neoplasia.

Our study has confirmed that LOH on chromosome 11q23 plays an important role in the pathogenesis of colorectal cancer. The two regions of deletion defined here suggest the presence of at least two putative tumour suppressor genes on 11q23 and will facilitate the identification of candidate tumour suppressor genes from this region.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Medical Research Council (NMRC) of Singapore, The Singapore Cancer Society and the Singapore General Hospital (SGH) Research Fund. We thank Ms Stephanie Fook Chong for statistical assistance, Dr PY Cheah for critical reading of the manuscript and

A/Prof KC Soo for use of laboratory facilities at the Department of General Surgery, Singapore General Hospital.

REFERENCES

- Baffa R, Negrini M, Mandes B, Rugge M, Ranzani GN, Hirohashi S and Croce CM (1996) Loss of heterozygosity of chromosome 11 in adenocarcinoma of the stomach. *Cancer Res* **56**: 268–272
- Blake TJ, Shapiro M, Morse HC III and Langdon WY (1991) The sequences of the human and mouse c-cbl proto-oncogenes show v-cbl was generated by a large truncation encompassing a proline-rich domain and a leucine zipper-like motif. *Oncogene* **6**: 653–657
- Carter SL, Negrini M, Baffa R, Gillum DR, Rosenberg AL, Schwartz GF and Croce CM (1994) Loss of heterozygosity at 11q22–q23 in breast cancer. *Cancer Res* **54**: 6270–6274
- Cawtkwell L, Bell SM, Lewis FA, Dixon MF, Taylor GR and Quirke P (1993) Rapid detection of allele loss in colorectal tumours using microsatellites and fluorescent DNA technology. *Br J Cancer* **67**: 1262–1267
- Chia KS, Lee HP, Seow A and Shanmugaratnam K (1996) Trends in cancer incidence in Singapore 1968–1992. In *Singapore Cancer Registry Report No. 4*, pp. 13–15. Singapore Cancer Registry
- Connolly KC, Gabra H, Millwater CJ, Taylor KJ, Rabiasz GJ, Watson JEV, Smyth JF, Wyllie AH and Jodrell DI (1999) Identification of a region of frequent loss of heterozygosity at 11q24 in colorectal cancer. *Cancer Res* **59**: 2806–2809
- Davis M, Hitchcock A, Foulkes WD and Campbell IG (1996) Refinement of two chromosome 11q regions of loss of heterozygosity in ovarian cancer. *Cancer Res* **56**: 741–744
- Evans MF, Koreth J, Bakkenist CJ, Herrington CS and McGee JO'D (1998) Allelic deletion at 11q23.3–q25 is an early event in cervical neoplasia. *Oncogene* **16**: 2557–2564
- Fearon ER and Vogelstein B (1990) A genetic model for colorectal tumorigenesis. *Cell* **61**: 759–767
- Gabra H, Watson JEV, Taylor KJ, Mackay J, Leonard RCF, Steel CM, Porteous DJ and Smyth JF (1996) Definition and refinement of a region of loss of heterozygosity at 11q23.3–q24.3 in epithelial ovarian cancer associated with poor prognosis. *Cancer Res* **56**: 950–954
- Gryfe R, Swallow C, Bapat B, Redston M, Gallinger S and Couture J (1997) Molecular biology of colorectal cancer. *Curr Probl Cancer* **21**: 233–300
- Gu Y, Alder H, Nakamura T, Schichman SA, Prasad R, Canaani O, Saito H, Croce CM and Canaani E (1994) Sequence analysis of the breakpoint cluster region in the ALL-1 gene involved in acute leukemia. *Cancer Res* **54**: 2326–2330
- Gustafson CE, Young J, Leggett B, Searle J and Chenevix-Trench G (1994) Loss of heterozygosity on the long arm of chromosome 11 in colorectal tumours. *Br J Cancer* **70**: 395–397
- Hampton GM, Penny LA, Baergen RN, Larson A, Brewer C, Liao S, Busby-Earle RMC, Williams AWR, Steel CM, Bird CC, Stanbridge EJ and Evans GA (1994) Loss of heterozygosity in cervical carcinoma: Subchromosomal localization of a putative tumour-suppressor gene to chromosome 11q22–q24. *Proc Natl Acad Sci USA* **91**: 6953–6957

- Herbst RA, Larson A, Weiss J, Cavenee WK, Hampton GM and Arden KC (1995) A defined region of loss of heterozygosity at 11q23 in cutaneous malignant melanoma. *Cancer Res* **55**: 2494–2496
- Hui ABY, Lo KW, Leung SF, Choi PHK, Fong Y, Lee JCK and Huang DP (1996) Loss of heterozygosity on the long arm of chromosome 11 in nasopharyngeal carcinoma. *Cancer Res* **56**: 3225–3229
- Iselius L, Lindsten J, Aurias A, Fraccaro M, Bastard C, Bottelli AM, Bui TH, Caufin D, Dalpra L, Delendi N, et al (1983) The 11q:22q translocation: a collaborative study of 20 new cases and analysis of 110 families. *Hum Genet* **64**: 343–355
- Keldysh PL, Dragani TA, Fleischman EW, Konstantinova LN, Perevoschikov AG, Pierotti MA, Della Porta G and Kopnin BP (1993) 11q deletions in human colorectal carcinomas: cytogenetics and restriction fragment length polymorphism analysis. *Genes, Chromosomes & Cancer* **6**: 45–50
- Konstantinova LN, Fleischman EW, Knisch VI, Perevozchikov AG and Kopnin BP (1991) Karyotype peculiarities of human colorectal adenocarcinomas. *Hum Genet* **86**: 491–496
- Koreth J, Bakkenist CJ and McGee JO'D (1997) Allelic deletions at chromosome 11q22–q23.1 and 11q25–qter are frequent in sporadic breast but not colorectal cancers. *Oncogene* **14**: 431–437
- Lindblom A, Sandelin K, Iselius L, Dumanski J, White I, Nordenskjold M and Larsson C (1994) Predisposition for breast cancer in carriers of constitutional translocation 11q:22q. *Am J Hum Genet* **54**: 871–876
- Monaco C, Negrini M, Sozzi G, Veronese ML, Vorechovsky I, Godwin AK and Croce CM (1997) Molecular cloning and characterization of LOH11CR2A, a new gene within a refined minimal region of LOH at 11q23. *Genomics* **46**: 217–222
- Negrini M, Rasio D, Hampton GM, Sabbioni S, Rattan S, Carter SL, Rosenberg AL, Schwartz GF, Shiloh Y, Cavenee WK and Croce CM (1995) Definition and refinement of chromosome 11 regions of loss of heterozygosity in breast cancer: identification of a new region at 11q23.3. *Cancer Res* **55**: 3003–3007
- Rasio D, Negrini N, Manenti G, Dragani TA and Croce CM (1995) Loss of heterozygosity at chromosome 11q in lung adenocarcinoma: Identification of three independent regions. *Cancer Res* **55**: 3988–3991
- Risio M, Reato G, di Celle PF, Fizzotti M, Rossini FP and Foa R (1996) Microsatellite instability is associated with the histological features of the tumour in nonfamilial colorectal cancer. *Cancer Res* **56**: 5470–5474
- Robertson G, Coleman A and Lugo TG (1996) A malignant melanoma tumour suppressor on human chromosome 11. *Cancer Res* **56**: 4487–4492
- Tomlinson IPM and Bodmer WF (1996) Chromosome 11q in sporadic colorectal carcinoma: patterns of allele loss and their significance for tumorigenesis. *J Clin Pathol* **49**: 386–390
- Tomlinson IPM, Gammack AJ, Stickland JE, Mann GJ, Mackie RM, Kefford RF and McGee JO'D (1993) Loss of heterozygosity in malignant melanoma at loci on chromosomes 11 and 17 implicated in the pathogenesis of other cancers. *Genes, Chromosomes & Cancer* **7**: 169–172
- Tomlinson IPM, Stickland JE, Lee ASG, Bromley L, Evans MF, Morton J and McGee JO'D (1995) Loss of heterozygosity on chromosome 11q in breast cancer. *J Clin Pathol* **48**: 424–428
- Wang SS, Esplin ED, Li JL, Huang L, Gazdar A, Minna J and Evans GA (1998) Alterations of the PPP2R1B gene in human lung and colon cancer. *Science* **282**: 284–287
- Wingo PA, Ries LAG, Rosenberg HM, Miller DS and Edwards BK (1998) Cancer incidence and mortality, 1973–1995. *Cancer* **82**: 1197–1207