8. De Bono DP, Samani NJ, Spyt TJ, Harsthorne T, Thrush AJ, Evans DH. Transcutaneous ultrasound measurement of blood flow in internal mammary artery to coronary artery grafts. *Lancet* 1992, **339**, 379–381.
9. Ranke C, Hendrickx P, Roth U, Brassel F, Creutzig A, Alexander K. Colour and conventional image-directed Doppler ultrasonography: accuracy and sources of error in quantitative blood flow measurements. *J Clin Ultrasound* 1992, **20**, 187–193.
10. Gill RW. Measurement of blood flow by ultrasound: accuracy and sources of error. *Ultrasound Med Biol* 1985, **11**, 625–641.
11. Stoll BA, Andrews JJ, Radiation induced peripheral neuropathy. *Br Med J* 1966, **1**, 834–837.
12. Treves N. An evaluation of the etiological factors of lymphedema following mastectomy. *Cancer* 1957, **10**, 444–459.
13. Kori SH, Foley KM, Posner JB. Brachial plexus lesions in patients with cancer: 100 cases. *Neurology* 1981, **31**, 45–50.
14. Thomas JE, Colby MY. Radiation induced or metastatic brachial plexopathy? A diagnostic dilemma. *JAMA* 1981, **222**, 1392–1395.
15. Partanen VS, Nikkanen TA. Electromyography in the estimation of nerve lesions after surgical and radiation therapy for breast cancer. *Strahlentherapie* 1978, **154**, 489–494.
16. Homans J. Lymphedema of the limbs. *Arch Surg* 1940, **40**, 232–252.
17. Brunning J, Gibson AG, Perry M. Oedeme bleu: a reappraisal. *Lancet* 1980, **1**, 810–812.
18. Cooke ED, Ward C. Vicious circles in reflex sympathetic dystrophy—a hypothesis: a discussion paper. *J R Soc Med* 1990, **83**, 96–99.
19. Blockx P, Driessens M. The use of $^{99}Tc^{m}$-HSA dynamic vascular examination in the staging and therapy monitoring of reflex sympathetic dystrophy. *Nuc Med Commun* 1991, **12**, 725–731.

Acknowledgements—We thank Deborah Price, Medical Statistician in the Department of Medical Physics, the Royal Post-graduate Medical School, Hammersmith Hospital, for help and advice for the statistical analysis. We thank the Breast Unit of the Department of Medicine, the Royal Marsden Hospital, for helping enrol patients for the control group.

Pergamon

European Journal of Cancer Vol. 30A, No. 5, pp. 664–670, 1994

0959-8049/94 $7.00+0.00

0959-8049(94)E0070-K

Loss of Heterozygosity of Tumour Suppressor Gene Loci in Human Colorectal Carcinoma

B. Iacopetta, S. DiGrandi, B. Dix, C. Haig, R. Soong and A. House

We used Southern blot analysis and polymerase chain reaction-based techniques to examine deletions of tumour suppressor gene loci in 91 primary colorectal tumours. The tumour suppressor genes studied were MCC and APC on chromosome 5q, p53 on chromosome 17p, DCC on chromosome 18q, and the putative suppressor gene nm23-H1 on chromosome 17q. The most frequent allelic loss observed was in chromosome 17p with 76% (68/89) of informative tumours showing loss of heterozygosity at this locus, followed by 34% (19/55) for DCC, 31% (12/39) for MCC, 17% (9/53) for APC and 16% (3/19) for nm23. No significant differences in the frequency of these suppressor gene allelic losses were observed between Dukes B and C stage adenocarcinomas.

Key words: tumour suppressor genes, colorectal carcinoma, p53, MCC, APC, DCC
Eur J Cancer, Vol. 30A, No. 5, pp. 664–670, 1994

INTRODUCTION

THE MOLECULAR genetic alterations in colorectal cancer are the best understood of all human cancers [1]. It appears this disease arises from the accumulation of mutations during progression from normal epithelial mucosa to adenoma and carcinoma. In addition to the activation of oncogenes such as Ki-*ras*, there is a loss of chromosomal segments at specific loci which may indicate the deletion of tumour suppressor genes. Four such genes associated with colorectal carcinoma have now been identified and cloned: MCC (mutated in colon cancer) and APC (adenomatous polyposis coli) on chromosome 5q [2–4], p53 on chromosome 17p [5], and DCC (deleted in colon cancer) on chromosome 18q [6]. The high frequency of mutations and loss of heterozygosity of these genes in colorectal tumours suggest that their normal gene products have a tumour suppressor function. Loss of heterozygosity of suppressor gene loci has traditionally been studied by Southern blot analysis and more recently using polymerase chain reaction (PCR)-based techniques, while gene mutation has been studied by direct sequencing, RNase mismatch cleavage and single strand conformation polymorphism (SSCP) analysis.

So far, identification of the molecular genetic alterations useful for the clinical diagnosis of colorectal cancer is still in the early stages. Recent work has indicated that deletions within the 17p and 18q chromosomal arms may be of prognostic value [7–9], but not 5q deletions or *ras* oncogene mutations. In the present study we have examined loss of heterozygosity (LOH) of the MCC, APC, p53 and DCC genes in a panel of 91 colorectal tumour specimens, 81 of which were Dukes stage B or C adenocarcinomas. Allelic deletion of the NM23-H1 gene located at chromosome 17q and thought to be involved in suppression

Correspondence to B. Iacopetta.
The authors are at the University Department of Surgery, Queen Elizabeth II Medical Centre, Nedlands 6009, Australia.
Revised and accepted 7 Feb. 1994.

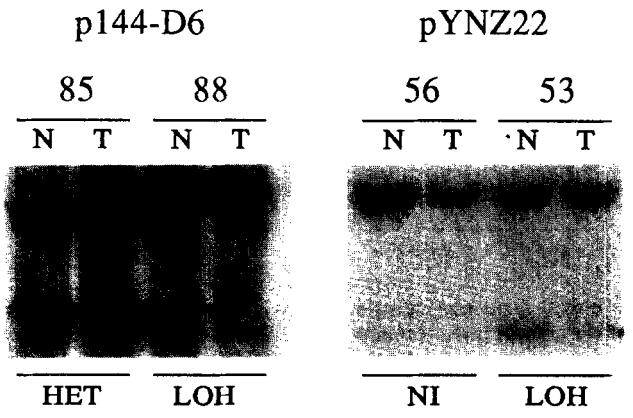

Figure 1. Examples of Southern blots used to detect loss of heterozygosity of chromosome 17p in DNA from colorectal tumours of patients 85, 88, 56 and 53. The probes p144–D6 and pYNZ22 identify VNTR polymorphisms in the chromosomal region flanking the p53 gene. N, normal germ-line DNA; T, tumour DNA; NI, non-informative; HET, heterozygous, no loss; LOH, loss of heterozygosity.

of tumour metastasis [10, 11] was also examined. We used Southern blot analysis to detect allelic loss of the p53, nm23 and DCC genes and PCR-based techniques to detect LOH (PCR-LOH) of the MCC, APC and p53 genes. Our results show that the frequency of suppressor gene allelic loss is the same in Dukes B and C adenocarcinomas.

MATERIALS AND METHODS

Tissue and DNA preparation

Resected colorectal tumours were obtained from 91 patients (35 male, 56 female) at the Queen Elizabeth II Medical Centre (Nedlands, Australia) during 1990–1992. All had been diagnosed as having primary colorectal carcinomas. The average age of patients at operation was 70 years. Ten of the tumours were classified as adenomas, 47 as Dukes B adenocarcinomas and 34 as Dukes C adenocarcinomas [12]. Macroscopically normal colonic mucosae from the same patients was also obtained for the preparation of germ-line DNA. All tissue was snap frozen in liquid nitrogen and stored at −80°C. High molecular weight DNA was prepared from each tumour specimen by means of SDS–proteinase K digestion and phenol/chloroform extraction. DNA concentration was estimated by spectrophotometric absorption at 260/280 nm.

Analysis of LOH in p53, nm23 and DCC genes by Southern blot

Ten micrograms of extracted DNA were digested with an appropriate restriction enzyme and the resulting DNA fragments resolved by 0.8% agarose gel electrophoresis and vacuum transferred to nylon membranes (Hybond N, Amersham, U.K.). The probes pYNZ22 [13] and p144-D6 [14] were used to detect allelic losses on chromosome 17p13 flanking the p53 gene. Each identifies a more than 10 allele VNTR (variable number of tandem repeat) polymorphism with MspI. To detect LOH of the nm23 gene, a probe from the pNM23-H1 clone [15] was used. It identifies a two allele polymorphism with BglII digestion. For loss of the DCC gene, a probe from the p15-65 plasmid [6] which detects four alleles with MspI was used. Hybridisation and washing procedures were carried out according to conditions specified by the suppliers of each probe.

Where two different alleles were distinguishable in the germ-line DNA, the patient was referred to as being heterozygous or informative, for the particular locus being examined. If the matching tumour DNA showed decreased intensity of one allele relative to the other this was considered to represent loss of heterozygosity. In most cases loss was not complete, presumably due to contaminating normal tissue and/or tumour heterogeneity.

Analysis of LOH in MCC, APC and p53 by PCR

Polymorphisms in the nucleotide sequence of the p53 [16], APC [4, 17] and MCC [2] genes have been described. PCR-based techniques were used to distinguish between polymorphic alleles in heterozygous individuals and thus determine whether

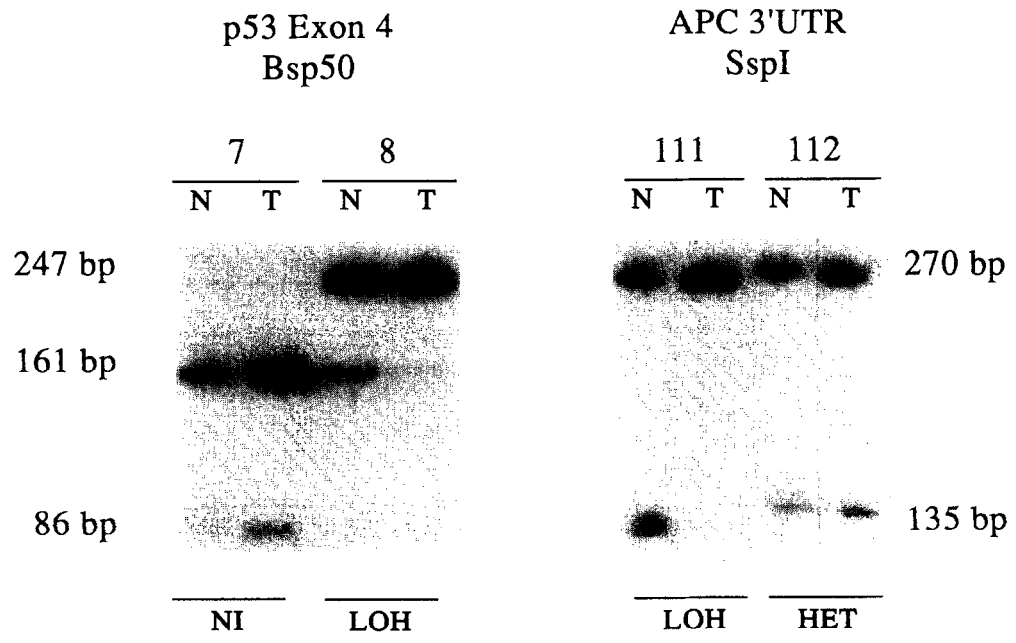

Figure 2. Examples of loss of heterozygosity in the p53 and APC genes detected by PCR and restriction enzyme digestion. Bsp50 enzyme digestion reveals the polymorphism in exon 4 of p53 and SspI recognizes the polymorphism in the 3′ untranslated region of the APC gene. The size of the restriction fragments in bp is indicated. Patients 8 and 111 each show loss of the digested allele of the p53 and APC genes respectively. N, normal DNA; T, tumour DNA; NI, non-informative; HET, heterozygous, no loss; LOH, loss of heterozygosity.

a loss had occurred in tumour DNA. The primer sequences (Biotech Intl, Perth, Australia) used to amplify the polymorphic regions were: for p53 exon 4 (p53E4) GATGCTGTCCCCGGACGATATT (5′) and GCCAAGTCTGTGACTTGCACG (3′), amplicon size 247 bp; for APC exon 11 (APCE11) GGACTACAGGCCATTGCAGA (5′) and GGCTACATCTCCAAAAGTCA (3′), amplicon size 133 bp; for APC 3′ untranslated region (APC3′UT) GCATTAAGAGTAAAATTCCTCT (5′) and ATGACCACCAGGTAGGTGTATT (3′), amplicon size 850 bp; and for MCC exon 15 (MCCE15) GGCCTAACTGGAATGTGT (5′) and GCCCAGATAAACACCAGC (3′), amplicon size 180 bp. The annealing temperature was 60°C except for APC3′UT which was 55°C. Extension was at 70°C for 2 min. Thirty-five amplification cycles were carried out in a Perkin-Elmer Cetus (Norwalk, Connecticut, U.S.A.) thermocycler. Each PCR amplification was in a 25-μl volume and included 100 ng of genomic DNA, 60 ng of each primer, 5 × reaction mix (Biotech Intl), and 0.5 μCi of [α-^{32}P]deoxycytosine triphosphate (>3000 Ci/mmol; Bresatec, Adelaide, Australia) and 0.2 U of *Taq* polymerase.

PCR products were digested overnight with an appropriate restriction enzyme in order to reveal the restriction fragment length polymorphism (RFLP): p53E4 with Bsp50 (Stratagene, La Jolla, California, U.S.A.), APCE11 with RsaI (Promega) and APC3′UT with SspI (Promega, Madison, Wisconsin, U.S.A.). Radiolabelled digestion products (usually 1–5 μl) were resolved on 10% polyacrylamide gels which were then dried and exposed to X-ray film for 24 h. To determine LOH, densitometry was performed as described by Ganly and colleagues [18] using a Molecular Dynamics scanning densitometer.

The MCCE15 PCR product was added 1:1 to 95% formamide loading buffer, heated to 95°C for 5 min, cooled on ice and run on a non-denaturing 10% polyacrylamide/10% glycerol SSCP gel [19] in order to reveal the polymorphism and any possible allelic loss in the tumour DNA [20]. The gel was run overnight at 1800 V in a sequencing gel apparatus (BioRad, North Ryde, Australia), dried and exposed to X-ray film at −80°C for 4 days.

RESULTS

The two VNTR polymorphisms flanking the p53 gene on chromosome 17p and identified by Southern blot analysis were highly informative, with 94% (68/72) and 85% (74/87), respectively, of patients being heterozygous. The probes identifying the RFLPs in the DCC and nm23 genes were informative for 64% (55/86) and 39% (19/49) of patients, respectively. In the PCR-LOH studies, 46% (39/84) of patients were heterozygous for the MCC exon 15 polymorphism, 45% (39/87) for the APC exon 11 polymorphism, 52% (45/87) for the APC 3′ untranslated region polymorphism, and 27% (25/91) for the p53 exon 4 polymorphism. Other studies have reported similar frequencies of heterozygosity for these loci [13, 14, 20–23]. Examples of LOH detected by Southern blot analysis are shown in Figure 1, by PCR restriction enzyme digestion in Figure 2, and by PCR-SSCP in Figure 3. Results of scanning densitometry used to quantitate allelic loss for the p53 and APC genes are shown in Figure 4. This quantitative approach was especially useful for determining loss in cases where partial allelic loss due to tumour heterogeneity and/or the presence of contaminating normal tissue made objective assessment difficult. As discussed by Ganly and colleagues [18], the possibility of heterodimer formation during the PCR of polymorphic alleles and the refraction of these to restriction enzyme digestion must be taken into account when examining loss by PCR. Depending on the extent of

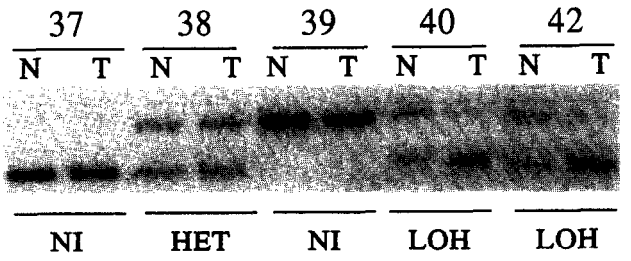

Figure 3. Detection of loss of heterozygosity by PCR-SSCP analysis of the polymorphic region of exon 15 of the MCC gene. The two bands evident in the normal DNA of patients 38, 40 and 42 represent the two alternative alleles. Patients 37 and 39 are homozygous (NI) for the two different alleles, while patients 40 and 42 show a partial loss (LOH) of the upper allele in their tumour DNA., N, normal DNA; T, tumour DNA.

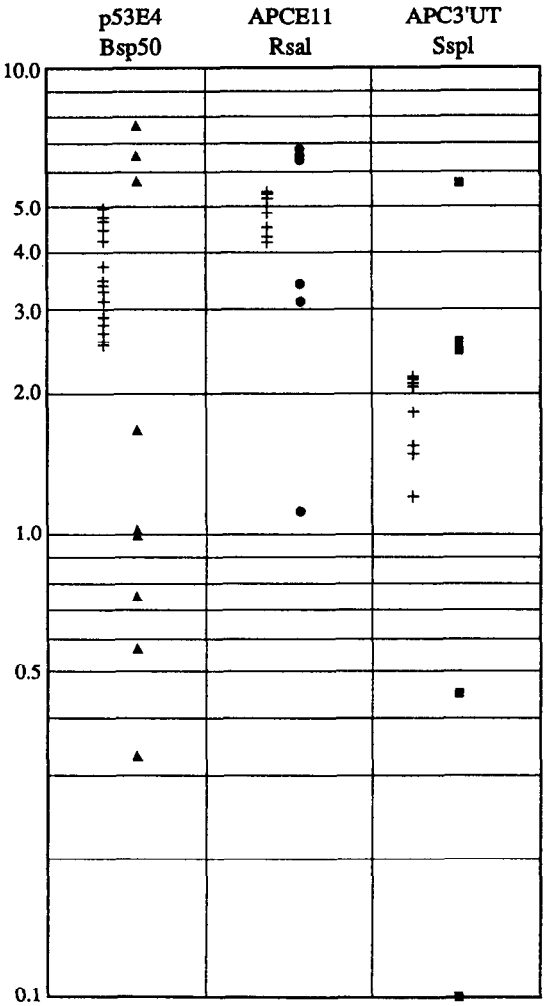

Figure 4. Densitometric analysis of loss of heterozygosity of the p53E4 (Bsp50), APCE11 (RsaI) and APC3′UT (SspI) polymorphisms. Allele ratio values (undigested allele/digested allele) for normal DNA from heterozygous patients are shown as + for each polymorphism. Tumours with allele ratios falling outside this normal range were considered to show loss. Examples are given for p53E4 (▲), APCE11 (●) and APC3′UT (■). Several tumours having almost total loss of one of the two alleles have ratios which are beyond scale and are not shown.

Table 1. Loss of heterozygosity of tumour suppressor genes in human colorectal cancers

Patient no.	MCC	5q APC a	b	c	17p p53 d	e	17q nm23	18q DCC
Adenoma								
3	○	○	○	●	●	NI	—	○
7	○	—	—	NI	—	○	—	●
27	NI	NI	NI	NI	○	○	—	○
50	NI	○	○	NI	●	NI	○	○
57	NI	○	NI	NI	●	○	—	○
74	NI	○	○	NI	○	●	NI	NI
89	○	NI	NI	NI	○	●	—	○
90	○	○	○	NI	●	NI	NI	NI
102	NI	NI	○	●	○	—	○	○
108	○	○	○	NI	NI	○	—	NI
Dukes B adenocarcinoma								
8	●	○	○	●	○	○	—	—
11	○	NI	NI	NI	○	○	—	○
12	NI	○	NI	NI	—	●	—	○
16	○	NI	NI	NI	●	●	—	—
17	NI	NI	○	NI	○	NI	—	NI
19	○	○	○	NI	○	●	—	○
20	NI	NI	NI	●	○	●	—	●
21	NI	NI	NI	NI	●	●	—	●
23	○	NI	NI	NI	○	○	—	○
24	NI	NI	NI	NI	—	—	—	○
25	○	NI	○	NI	○	○	—	○
29	NI	NI	NI	○	○	○	NI	NI
30	NI	○	○	●	●	●	●	●
31	NI	○	NI	●	●	○	○	●
32	—	—	—	NI	—	●	NI	●
33	NI	○	○	NI	○	●	—	●
35	NI	NI	NI	NI	●	○	○	○
37	NI	NI	○	NI	○	●	NI	○
38	○	NI	NI	NI	●	NI	NI	NI
42	●	●	○	NI	●	●	NI	○
45	NI	NI	NI	NI	●	NI	NI	○
47	NI	○	○	○	○	○	NI	○
49	NI	●	●	NI	●	○	NI	NI
51	NI	NI	○	●	●	○	○	NI
53	NI	○	○	NI	●	●	—	○
54	●	●	●	NI	●	●	—	●
56	NI	○	NI	●	NI	NI	—	●
58	NI	NI	NI	●	●	○	—	●
60	NI	○	○	NI	●	●	—	●
61	○	NI	NI	NI	●	○	—	○
62	NI	NI	NI	●	●	○	—	●
64	○	NI	NI	●	NI	○	—	NI
66	—	○	○	NI	—	●	—	○
67	NI	NI	NI	NI	○	○	—	○
71	○	NI	NI	NI	●	●	●	NI
72	●	NI	NI	NI	●	●	NI	●
85	○	○	NI	○	○	○	NI	○
88	NI	NI	○	NI	—	●	—	NI
92	—	—	—	NI	●	●	NI	NI
93	NI	NI	NI	NI	●	—	NI	○
96	NI	○	○	NI	○	●	NI	NI
97	○	○	○	NI	●	—	NI	○
103	NI	NI	○	NI	—	●	NI	○
105	●	NI	NI	NI	●	NI	○	NI
106	●	NI	NI	NI	—	●	NI	○
112	NI	NI	○	●	●	●	○	NI
116	NI	NI	NI	NI	○	○	○	NI

Continued overleaf

Table 1. Continued

Patient no.	MCC	5q APC a	b	c	17p p53 d	e	17q nm23	18q DCC
Dukes C adenocarcinoma								
5	NI	○	○	●	—	○	—	●
6	●	○	NI	●	—	NI	—	NI
13	NI	○	○	●	—	●	—	○
14	NI	NI	NI	NI	—	○	—	NI
22	—	●	NI	NI	●	●	—	○
26	●	NI	○	NI	○	●	—	—
28	●	NI	NI	●	●	○	NI	●
36	○	○	○	NI	○	○	NI	○
39	NI	NI	NI	NI	●	●	NI	NI
40	●	NI	NI	NI	●	●	NI	NI
43	NI	NI	NI	○	○	NI	○	○
44	○	○	○	NI	●	●	NI	●
52	NI	NI	●	NI	●	NI	NI	NI
59	○	NI	NI	○	○	NI	—	○
63	○	NI	NI	NI	●	○	—	○
69	○	●	○	NI	●	●	—	○
73	—	—	—	NI	—	NI	NI	NI
76	NI	NI	○	●	○	○	○	NI
79	NI	○	○	○	○	○	○	—
80	NI	NI	NI	NI	●	○	○	●
81	●	NI	NI	NI	●	○	NI	NI
82	○	NI	○	○	—	○	NI	—
84	NI	○	○	NI	●	○	○	NI
95	○	○	○	NI	●	●	○	NI
98	○	NI	○	NI	●	○	NI	○
99	○	○	○	NI	NI	○	○	NI
100	NI	○	NI	○	—	○	NI	NI
104	NI	○	○	NI	—	●	—	○
107	○	○	○	NI	●	○	●	○
109	○	NI	●	NI	●	○	—	NI
110	●	●	○	NI	○	○	NI	NI
111	NI	○	●	NI	—	●	○	●
114	—	NI	NI	NI	—	●	—	NI
115	—	NI	NI	NI	—	●	—	●

NI, non-informative; —,no results; ○, heterozygous, no loss; ●,loss of heterozygosity; a, APC exon 11 polymorphism (RsaI); b, APC 3′ untranslated region polymorphism (SspI); c, p53 exon 4 polymorphism (Bsp50); d, chromosome 17p VNTR polymorphism (pYNZ22); e, chromosome 17p VNTR polymorphism (p144-D6)

Table 2. Tumour suppressor gene loss of heterozygosity in Dukes B and Dukes C adenocarcinomas

	Dukes B	Dukes C
MCC	35% (6/17)	35% (6/17)
APC	13% (3/24)	27% (6/22)
p53	80% (37/46)	73% (24/33)
nm23	25% (2/8)	11% (1/9)
DCC	38% (12/32)	38% (6/16)

Loss of heterozygosity data from the two polymorphic APC loci were combined. Similarly, data from the two 17p VNTR polymorphic loci and from the intragenic p53 polymorphic locus were combined to give one value for p53 loss.

heterodimer formation the allele*1*/allele*2* ratio (allele*1* is the undigested band and allele*2* the larger of the two digestion products) varies within a defined range for heterozygous individuals. For the p53E4 (Bsp50) polymorphism, this range was 2.5–5.0, for the APCE11 (RsaI) polymorphism it was 4–6, and for the APC3′UT (SspI) polymorphism it was 1–2.5 (Figure 4). Allele ratios falling above or below these ranges in tumour DNA indicated a loss of either one or the other allele. Digested and undigested alleles were lost with equal frequency.

Individual results for suppressor gene LOH in all tumours and at each polymorphic locus examined are listed in Table 1. These results are summarised in Table 2 for the Dukes B and Dukes C adenocarcinomas. For the APC gene, results from the two intragenic polymorphic sites were combined. Allelic loss of either of the two 17p VNTR regions was assumed to include deletion of the p53 gene.

The highest frequency of allelic loss observed was at the

three chromosome 17p polymorphic loci, with 76% (68/89) of informative tumours showing loss of at least one locus. The pYNZ22 probe detected loss in 62% (42/68) of tumours, the p144-D6 probe in 49% (36/74), and the p53 exon 4 polymorphism revealed LOH in 68% (17/25). Interestingly, of the eight informative tumours which showed no loss at the intragenic p53 exon 4 polymorphism (tumours 29, 47, 85, 43, 59, 79, 82, 100), none showed deletions at either of the two 17p VNTR polymorphic loci (Table 1). This finding provides indirect evidence that the large majority of 17p allelic losses are centred on the p53 gene.

Of the adenomas, 70% (7/10) showed LOH of p53 (Table 1), while 80% (37/46) and 73% (24/33) of Dukes B and C adenocarcinomas, respectively, showed loss of this gene (Table 2). The DCC gene showed loss in 34% (19/55) of informative tumours, including one of the seven informative adenomas. No difference in the frequency of DCC gene loss was apparent between Dukes B and C adenocarcinomas (Table 2). Only 16% (3/19) of informative tumours showed loss of the nm23 gene. The incidence of MCC gene deletion was 35% (6/17) in both Dukes B and C tumours, while that of the APC gene was 13% (3/24) and 27% (6/22), respectively (Table 2). In agreement with previous observations [2, 3, 24], loss of the MCC and APC genes occurred independently in some cases (tumours 6, 8, 69, 109). Four tumours (42, 69, 110, 111) also showed loss at one polymorphic APC site but not the other, suggesting the occurrence of partial intragenic deletions.

DISCUSSION

We have used Southern blot analysis and PCR techniques to detect LOH of five different tumour suppressor genes thought to be involved in the pathogenesis of colorectal cancers. PCR techniques have obvious advantages over Southern blot analysis in the minimal tissue requirement and in the relative ease and rapidity of diagnosis. One major disadvantage, however, is that the maximum heterozygosity of intragenic sequence polymorphisms is usually 50%. Oligonucleotide primer sets for the PCR amplification of several different polymorphic loci within the p53, MCC, APC, and DCC genes have recently been described [21, 24] and should prove useful for the detection of gene deletions.

Despite taking no special precautions to minimise the amount of extraneous non-tumoral tissue in our specimens, we are confident this did not significantly mask detection of loss at the various alleles. Other workers have reported similar frequencies of allelic loss in the 17p [7, 8, 23, 25–30] and 5q [7, 9, 25, 31, 32] chromosomal arms in colorectal cancers, being 75 and 35%, respectively. Most of these studies were performed using tumour DNA isolated from cryostat sections in which the amount of tumour tissue had been maximised. We cannot explain, however, why the frequency of DCC gene loss we observed (34%) is only half that reported by others for the 18q chromosomal region [6, 25].

The major finding of the present study is that Dukes B and C stage adenocarcinomas have no significant differences in the frequency of known tumour suppressor gene deletions (Table 2). The additional genetic alterations leading to spread of tumours to lymph nodes are not known. The low frequency of nm23 allelic deletion observed in this study (three out of 19 tumours) suggests that this gene does not play a role. It may be that no further suppressor gene alterations are required and that with time all Dukes B tumours eventually grow and spread to pericolic lymph nodes. The number of adenomas examined in this study was too low to allow any conclusions to be made concerning the timing of allelic losses, except perhaps that 17p deletions appear to be relatively early events.

The short post operative follow-up time of patients in this study (mean of 26 months) does not yet allow us to draw any conclusions regarding the prognostic significance of the various genetic alterations observed. Current information suggests that deletion of the 5q region containing the MCC/APC genes is not of prognostic importance in colorectal cancers [7–9]. Deletion of the 17p region has been reported to be associated with shorter survival [7, 9]. The importance of 18q deletions is unclear, with one study reporting significant association with poorer survival [7], one reporting no significant association [9], and another suggestive of increased tumour recurrence [8].

Additional studies involving larger numbers of Dukes B and C adenocarcinomas will be required in order to more accurately identify those patients with aggressive tumours. These patients could benefit from recent clinical advances in adjuvant chemotherapy regimes following surgical resection of colorectal tumours. The application of PCR-LOH techniques as described in this paper and others [21, 24] using intragenic polymorphic loci should greatly facilitate the detection of p53 and DCC gene alterations. Large retrospective studies of archival paraffin-embedded specimens where patient outcome is known can also be envisaged using PCR-LOH analysis.

1. Fearon ER, Vogelstein B. A genetic model for colorectal tumourigenesis. *Cell* 1990, **61**, 759–767.
2. Kinzler KW, Nilbert MC, Vogelstein B, *et al.* Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. *Science* 1991, **251**, 1366–1370.
3. Nishisho I, Nakamura Y, Miyoshi Y, *et al.* Mutations of chromosome 5q21 genes in FAP patients and colorectal cancer patients. *Science* 1991, **253**, 665–669.
4. Groden J, Thliveris A, Samowitz W, *et al.* Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 1991, **66**, 589–600.
5. Baker SJ, Fearon ER, Nigro JM, *et al.* Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science* 1989, **244**, 217–221.
6. Fearon ER, Cho KR, Nigro JM, *et al.* Identification of a chromosome 18q gene that is altered in colorectal cancers. *Science* 1990, **247**, 49–56.
7. Kern SE, Fearon ER, Kasper WF, *et al.* Allelic loss in colorectal carcinoma. *JAMA* 1989, **261**, 3099–3103.
8. O'Connell MJ, Schaid DJ, Ganju V, Cunningham J, Kovach JS, Thibodeau SN. Current status of adjuvant chemotherapy for colorectal cancer: can molecular markers play a role in predicting prognosis? *Cancer* 1992, **70**, 1732–1739.
9. Laurent-Puig P, Olschwang S, Delattre O, *et al.* Survival and acquired genetic alterations in colorectal cancer. *Gastroenterology* 1992, **102**, 1136–1141.
10. Leone A, McBride OW, Weston A, *et al.* Somatic allelic deletion of nm23 in human cancer. *Cancer Res* 1991, **51**, 2490–2493.
11. Cohn KH, Wang F, DeSoto-LaPaix F, *et al.* Association of nm23-H1 allelic deletions with distant metastases in colorectal carcinoma. *Lancet* 1991, **338**, 722–724.
12. Dukes CE. The classification of cancer of the rectum. *J Path Bact* 1932, **35**, 323–332.
13. Nakamura Y, Ballard L, Leppert M, *et al.* Isolation and mapping of a polymorphic DNA sequence (pYNZ22) on chromosome 17p. *Nucleic Acids Res* 1988, **16**, 5707.
14. Kondoleon S, Vissing H, Luo XY, Magenis RE, Kellogg J, Litt M. A hypervariable RFLP on chromosome 17p13 is defined by an arbitrary single copy probe p144D6. *Nucleic Acids Res* 1987, **15**, 10605.
15. Yague J, Juan M, Leone A, *et al.* BglIII and EcoR1 polymorphism of the human nm23-H1 gene (NME1). *Nucleic Acids Res* 1991, **19**, 6663.
16. de la Calle-Martin O, Fabregat V, Romero M, Soler J, Vives J,

Jague J. AccII polymorphism of the p53 gene. *Nucleic Acids Res* 1990, **18**, 4963.
17. Heighway J, Hoban PR, Wyllie AH. Ssp1 polymorphism in sequence encoding 3′ untranslated region of the APC gene. *Nucleic Acids Res* 1991, **19**, 6966.
18. Ganly PS, Jarad N, Rudd RM, Rabbits PH. PCR-based RFLP analysis allows genotyping of the short arm of chromosome 3 in small biopsies from patients with lung cancer. *Genomics* 1992, **12**, 221–228.
19. Orita M, Suzuki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 1989, **5**, 874–879.
20. D'Amico D, Carbone DP, Johnson BE, Meltzer SJ, Minna JD. Polymorphic sites within the MCC and APC loci reveal very frequent loss of heterozygosity in human small cell lung cancer. *Cancer Res* 1992, **52**, 1996–1999.
21. Greenwald BD, Harpaz N, Yin J, *et al.* Loss of heterozygosity affecting the p53, Rb, and mcc/apc tumour suppressor gene loci in dysplastic and cancerous ulcerative colitis. *Cancer Res* 1992, **52**, 741–745.
22. Oka K, Ishikawa J, Bruner JM, Takahashi R, Saya H. Detection of loss of heterozygosity in the p53 gene in renal cell carcinoma and bladder cancer using the polymerase chain reaction. *Molec Carcinogen* 1991, **4**, 10–13.
23. Campo E, de la Calle-Martin O, Miquel R, *et al.* Loss of heterozygosity of p53 gene and p53 protein expression in human colorectal carcinomas. *Cancer Res* 1991, **51**, 4436–4442.
24. Huang Y, Boynton RF, Blount PL, *et al.* Loss of heterozygosity involves multiple tumour suppressor genes in human esophageal cancers. *Cancer Res* 1992, **52**, 6525–6530.
25. Vogelstein B, Fearon ER, Kern SE, *et al.* Allelotype of colorectal carcinomas. *Science* 1989, **244**, 207–211.
26. Shaw P, Tardy S, Benito E, Obrador A, Costa J. Occurrence of Ki-*ras* and p53 mutations in primary colorectal tumours. *Oncogene* 1991, **6**, 2121–2128.
27. Baker SJ, Preisinger AC, Milburn-Jessup J, *et al.* p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. *Cancer Res* 1990, **50**, 7717–7722.
28. Kikuchi-Yanoshita R, Konishi M, Ito S, *et al.* Genetic changes of both p53 alleles associated with the conversion from colorectal adenoma to early carcinoma in familial adenomatous polyposis and non-familial adenomatous polyposis patients. *Cancer Res* 1992, **52**, 3965–3971.
29. Lothe RA, Fossli T, Danielsen HE, *et al.* Molecular genetic studies of tumor suppressor gene regions on chromosomes 13 and 17 in colorectal tumors. *J Natl Cancer Inst* 1992, **84**, 1100–1108.
30. Cunningham J, Lust JA, Schaid DJ, *et al.* Expression of p53 and 17p allelic loss in colorectal carcinoma. *Cancer Res* 1992, **52**, 1974–1980.
31. Ashton-Rickardt PG, Wyllie AH, Bird CC, *et al.* MCC, a candidate familial polyposis gene in 5q.21, shows frequent allele loss in colorectal and lung cancer. *Oncogene* 1991, **6**, 1881–1886.
32. Solomon E, Voss R, Hall V, *et al.* Chromosome 5 allele loss in human colorectal carcinomas. *Nature* 1987, **328**, 616–619.

Acknowledgements—This work was supported by a grant from the Cancer Foundation of Western Australia.
The authors wish to thank the following people for the provision of probes: R. White, Y. Nakamura, B. Vogelstein and P. Steeg. We also thank Dr Tom Ratajczak for his involvement in the early part of this work.

Pergamon

European Journal of Cancer Vol. 30A, No. 5, pp. 670–674, 1994
Elsevier Science Ltd
Printed in Great Britain
0959–8049/94 $7.00+0.00

0959-8049(94)E0064-B

A Small GTP-binding Protein is Frequently Overexpressed in Peripheral Blood Mononuclear Cells From Patients With Solid Tumours

S. Culine, N. Honore, V. Closson, J.-P. Droz, J.-M. Extra, M. Marty, A. Tavitian and B. Olofsson

***ras* oncoproteins and *ras*-related proteins constitute a large family of the small GTP-binding protein family. The *rab* branch of the *ras* superfamily is involved in the intracellular transport along the secretory and endocytic pathway in eukaryotic cells. We here demonstrate that a member of the *rab* branch, the *rab2* protein, is frequently overexpressed in peripheral blood mononuclear cells from patients with solid neoplasms. Moreover, this expression is shown to be greatly modified during the course of therapy. Our results provide strong evidence for the implication of a small GTP-binding protein in immunological events associated with neoplastic diseases. The precise cellular population involved as well as the potential prognostic value of this process remains to be determined.**

Key words: *rab* proteins, GTP-binding proteins, peripheral blood cells, solid tumours
Eur J Cancer, Vol. 30A, No. 5, pp. 670–674, 1994

INTRODUCTION

RECENT DISCOVERIES indicate that the *ras* oncoproteins belong to a large family of low molecular weight (21–27 kD) monomeric proteins capable of binding and hydrolysing GTP [1, 2]. All members of the so-called *ras* superfamily contain highly conserved domains required for guanine nucleotide binding, GDP/GTP exchange and GTP hydrolysis [3]. These domains interact with regulatory proteins which stimulate guanine nucleotide dissociation, inhibit GDP exchange or promote GTP hydrolysis [4]. According to the sequence homologies of the proteins,