Distribution of Ha-ras alleles in patients with colorectal cancer and Crohn's disease

R Klingel, P Mittelstaedt, W G Dippold, K-H Meyer zum Büschenfelde

Abstract

The allele distribution of the Ha-ras gene on chromosome 11p was analysed by the restriction fragment length polymorphism of the enzymes MspI/HpaII in 238 individuals. The investigation covered 116 patients with colorectal carcinoma and 122 patients with Crohn's disease, representing two patient populations with the same ethnic origin, one with a malignant and the other a benign disease of the same organ system. A total of 17 different alleles were detected belonging to the common, intermediate, and rare classes according to the original nomenclature of Ha-ras alleles. Patients with Crohn's disease showed no difference in the distribution of Ha-ras alleles when compared with expected frequencies. In patients with colorectal carcinoma, the frequency of rare alleles was significantly increased compared with the patients with Crohn's disease ($\chi^2=8.166$; Fisher's exact test=0.005) and with a reference population of 424 cancer free individuals ($\chi^2=49.312$; Fisher's exact test=0.000). Homozygosity was not detected for any rare allele. The occurrence of a rare Ha-ras allele was not linked to the location of the colorectal tumour. These results confirm the hypothesis that unique Ha-ras alleles represent an inherited factor which predisposes the development of colorectal cancer.

A polymorphism is observed at the human Ha-ras locus on chromosome 11p based on a variable nuclear tandem repeat. The restriction fragment length polymorphism (RFLP) of the Ha-ras gene arises from changes in the number of this tandemly repeated 28 bp consensus sequence located at the 3' flanking region of the gene.¹ The polymorphic DNA segments segregate as codominant markers in a Mendelian fashion and can be used to define classes of alleles of this oncogene: common, intermediate, and rare alleles.²⁻⁴ Especially rare alleles were hypothesised to confer susceptibility to develop particular types of malignant tumours.^{3,4} Two recent studies investigating the Ha-ras allele distribution in patients with colorectal cancer were controversial in their results, but did not use the original definition of alleles.^{5,6}

A conclusive model which summarises several molecular genetic events associated with the known sequence leading from normal colonic mucosa to the hyperproliferative colonic polyps and finally to metastatic colorectal carcinoma was recently presented by Vogelstein.^{7,8} The ras oncogenes contribute to this hypothesised multi-

step process of colorectal cancer development at early stages by hypomethylation and mutations when hyperproliferation has developed.⁷⁻¹⁰ Besides this general hypothesis, there are two hereditary syndromes which predispose to colorectal cancer: familial adenomatous polyposis with the responsible gene located on chromosome 5q¹¹ and hereditary non-polyposis colorectal cancer, which has not as yet been linked to any genetic locus (Lynch syndromes I and II).¹²

Two disease entities of chronic inflammatory bowel disorders exhibit a quite different potential for the development of colorectal carcinoma. The observed number of extraintestinal cancers in both Crohn's disease and ulcerative colitis is in the range normally expected in the standard population. Ulcerative colitis represents a highly premalignant condition for colorectal carcinoma, particularly when the total colon is involved in combination with a long duration of disease.¹³ The risk of cancer of the colon and intestine in Crohn's disease is significantly less increased, estimated as about one third the rate found in ulcerative colitis.^{13,14} In general the risk of gastrointestinal cancer in chronic inflammatory bowel disease increases with the duration of the disease.¹³ Our aim was to analyse allele frequencies in patients with Crohn's disease and colorectal carcinoma and compare these with the known distribution of alleles.

After the initial report of Krontiris *et al.*,³ several groups investigated the Ha-ras RFLP in a variety of malignancies. The hypothesis of a different distribution of Ha-ras alleles in patients with malignant diseases was confirmed in lung cancer,^{15,16} acute myeloid leukaemia,¹⁷ a mixed group of Japanese cancer patients,¹⁸ and miscellaneous intracranial tumours.¹⁹ No difference was detected for myelodysplasia,^{17,20} ovarian carcinoma,²¹ urothelial cancer²²⁻²⁴ and Wilms' tumour.²⁴ As with colonic carcinoma, results are contradictory for malignant melanoma²⁴⁻²⁷ and breast cancer.^{21,28-30} It must be noted, however, that most of these studies did not use MspI/HpaII digested DNA to define allele classes and designate exactly individual fragments according to the original nomenclature with the highest resolution.⁴

Because the distribution of Ha-ras alleles varies in different ethnic groups,² we analysed two clinically well defined patient groups of homogenous ethnic origin, one with a malignant and the others a benign disease of the same organ system. We adhered strictly to the original nomenclature of alleles after digestion of DNA with MspI/HpaII because they enable clear definition of the allele size and avoid underestimation of the total number of alleles.^{16,31}

First Department of Internal Medicine, University of Mainz, Federal Republic of Germany
R Klingel
P Mittelstaedt
W G Dippold
K-H Meyer zum Büschenfelde

Correspondence to:
Dr Reinhard Klingel, First Department of Internal Medicine, University of Mainz, Langenbeckstr 1, D-6500 Mainz, Federal Republic of Germany.

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Figure 1: Resolution of Ha-ras allelic fragments. Leukocyte DNAs representing Ha-ras genotypes with the four common alleles: (a) BamHI digestion of genotype a1 (6.9 kb)/a4 (8.3 kb); (b) BamHI digestion of genotype a2 (7.5 kb)/a3 (8.0 kb); (c) corresponding MspI/HpaII digestion of genotype a1 (1000 bp)/a4 (2560 bp), and (d) corresponding MspI/HpaII digestion of genotype a2 (1500 bp)/a3 (2160 bp).

Materials and methods

STUDY POPULATION

White blood cells were collected consecutively from 122 unrelated white patients with Crohn's disease proved by clinical examination, endoscopy, and biopsy, and with no evidence of malignancy (76 female/46 male) and 116 unrelated white patients with colorectal carcinoma (58 female/58 male). All patients were of German origin. In the group with tumours 49 (42.2%) had carcinomas of the rectum, 28 (24.1%) of the sigmoid colon, two (1.7%) of the descending colon, 14 (12.1%) of the transverse colon (including hepatic and splenic flexures), 18 (15.5%) of the ascending colon (including caecum) and five (4.3%) patients had familial adenomatous polyposis.

SOUTHERN ANALYSIS

Leukocyte DNAs were prepared, as described previously³² and digested with BamHI and MspI/HpaII respectively under the conditions recommended by the manufacturers. Samples (10 µg) were subjected to electrophoresis (16–18 hours, constant voltage 40V) through 0.7% agarose gels for BamHI and 1% agarose gels for MspI/HpaII. Southern blotting; ³²P-labelling of DNA; and conditions for stringent hybridisation to a complete c-Ha-ras-1 probe (clone pEJ containing the 6.6 kb BamHI c-Ha-ras-1 fragment including the VTR region); and uncut lambda DNA were performed as described previously.³²

DETERMINATION OF HA-RAS GENOTYPES AND ALLELE SIZES

Alleles were designated according to the nomenclature of Krontiris.⁴ Reference DNAs of frequent alleles a1, a2, a3, and a4 were obtained from Krontiris, Boston. Intermediate and rare alleles were measured as relative mobility by co-electrophoresis to the frequent alleles and already known fragments of similar size in adjacent tracks. Fragment sizes were then determined by linear regression analysis of the rate of migration, which is inversely proportional to the logarithm of their length in base pairs. At least two independent experiments which resulted in regression lines with $r > 0.99$ for the frequent alleles were used for calculation. This procedure permits size measurements with an SD of 0.6% of the size of the individual allele.² HindIII and BstEII digested lambda DNAs were used as size markers. BamHI mapping was useful to indicate homozygosity or heterozygosity of the genotypes in case of some partial digestions by MspI/HpaII. Calculation of identical fragment sizes was tested by subsequent co-electrophoresis of DNA samples.

STATISTICAL ANALYSIS

Alleles were grouped into three sets: common (expected frequency 0.07 to 0.65); intermediate (expected frequency 0.025 to 0.004); and rare alleles (expected frequency less 0.004) as defined by Krontiris in the largest survey (796 individuals/1592 haplotypes) of allelic distribution.⁴

Comparative analysis of the genotypic or allelic distributions were performed by 2×2 contingency tables with no a priori assumption about deviations of individual alleles from the expected frequencies. Patients with colorectal carcinoma and Crohn's disease were compared with each other and with two control groups published by Krontiris,⁴ consisting of 424 cancer-free persons (abbreviated and referred to in the text as 'Boston cancer free group') and 372 cancer patients with a wide variety of tumours ('Boston cancer group'; the tumours were: carcinomas of head and neck 1%; lung 5%; breast 16%; gastrointestinal tract 5% including oesophagus, stomach, pancreas, liver, colorectum; genitourinary tract 22% including kidney, bladder, prostate, testis; female reproductive tract 8% including ovary, uterus, cervix, endometrium; lymphoma 18%; melanoma 4%; sarcoma 2%; myeloma 1%; acute and chronic leukaemia 11%; brain tumours 2%; non-melanoma skin cancers 3%; tumours of unknown primary site 2%).⁴ To test differences for their significance χ^2 test (df 1) with Yates's correction for continuity and Fisher's exact test for the two tailed significance level were used, the latter because the χ^2 test alone is not valid for the expected low range of relative frequencies of intermediate and rare alleles (0.056 and 0.024 respectively³⁴).

Results

The analysis of 238 DNA samples resulted in the identification of 17 distinct types of the 32 known allelic Ha-ras haplotypes defined by the MspI/HpaII restriction fragment length polymorphism³⁴ (Table I) in the 116 patients with colorectal cancer and 122 with Crohn's disease. The four most frequent alleles in our patient groups proved identical to the common alleles a1, a2, a3, and a4 obtained as reference probes from Krontiris (Fig 1). Linear regression analysis ($r > 0.99$) of the mobility of MspI/HpaII fragments in 1% agarose gels were used to calculate intermediate and rare fragments (Fig 2). DNAs with accurately determined fragment sizes were incorporated in the further measurement of unknown fragment lengths. BamHI digested DNA samples were helpful in case of partial digestions by MspI/HpaII but were actually very limited in the discrimination of small size differences (Fig 3).

GENOTYPE AND ALLELE DISTRIBUTION OF PATIENTS WITH COLORECTAL CARCINOMA

In the 232 allelic fragments of the Ha-ras gene of 116 patients with colorectal carcinoma, 207 common (frequency 0.892), nine intermediate (frequency 0.039), and 16 rare alleles (frequency 0.069) were detected. All four of the known common alleles but only three of the seven intermediate alleles and eight of the 21 known rare alleles were detected (Table I). There was no significant preference of rare alleles when arranged by the anatomical site of the colorectal carcinomas (Fig 4). In the common group, 46 patients were homozygous for a1, two for a2, none for a3, and two for a4. In the intermediate group homozygosity was observed only once for

TABLE I Ha-ras allelic frequencies in 116 patients with colorectal carcinoma and 122 patients with Crohn's disease*

Alleles	Size of MspI/HpaII fragments*	Colorectal carcinoma (n=116) Frequencies:		Crohn's disease (n=122) Frequencies:		Expected relative frequencies
		Absolute	Relative	Absolute	Relative	
Common:						
a1	1000	207	0.892	230	0.943	0.918
a2	1500	140	0.608	161	0.660	0.612
a3	2160	24	0.103	31	0.127	0.121
a4	2560	26	0.108	23	0.094	0.097
		17	0.073	15	0.062	0.088
Intermediate:						
a1.2	1110	9	0.039	10	0.041	0.056
a1.1	1060	4	0.017	6	0.025	0.021
a4.1	2610			2	0.008	0.010
a0.1	980	2	0.009	2	0.008	0.007
a1.3	1230					0.005
a1.4	1450	3	0.013			0.004
a5	2800					0.004
Rare:						
a3.2	2280	16	0.069	4	0.016	0.024
a2.2	1820	1	0.004			0.002
a2.3	1880	1	0.004			0.002
a2.4	2100					0.002
a3.1	2220	2	0.009	2	0.008	0.002
a3.3	2330					0.002
a3.4	2410					0.002
a1.3	1280					0.001
a2.0	1560	1	0.004	1	0.004	0.001
a3.5	2480	5	0.002			0.001
a4.2	2710	2	0.009			0.001
a0.1	880					0.0006
a0.2	810					0.0006
a1.25	1180	3	0.013	1	0.004	0.0006
a2.015	1590	1	0.004			0.0006
a2.02	1650					0.0006
a2.025	1680					0.0006
a2.1	1710					0.0006
a2.11	1750					0.0006
a2.12	1790					0.0006
a5.2	3080					0.0006

*Nomenclature and estimated frequencies of alleles represented by the MspI/HpaII restriction fragments according to Krontiris.⁴

the allele a0.1. Homozygosity was not observed for rare alleles. Apart from one patient with a0.1, a combination of either an intermediate or a rare allele with a common one was generally found. Separation by sex showed a nearly identical frequency pattern of common (female/male=0.871/0.914) and rare (female/male=0.060/0.078) alleles, but eight of nine intermediate alleles occurred in women (female/male=0.069/0.009). In addition, the patient who was homozygous for a0.1 was female.

GENOTYPE AND ALLELE DISTRIBUTION OF PATIENTS WITH CROHN'S DISEASE
In 244 allelic fragments of 122 patients with Crohn's disease, 230 common (frequency 0.943),

Figure 2: Examples of identified intermediate and rare Ha-ras alleles; co-electrophoresis with common alleles a1, a2, a3, and a4: (a) a1/a2; (b) a1/a2.2, weak extra bands are due to partial digestion; (c) a1/a3; (d) a2/a4.1; (e) a1/a4; (f) a1.2/a4; (g) a1/a2; (h) a2/a2.01; (i) a1/a2, and (j) a1.25/a2.

10 intermediate (frequency: 0.041), and four rare alleles (frequency 0.016) were detected. All four common alleles, three intermediate, and three different rare alleles were detected (Table I). Homozygosity was only seen in the common group: 53 patients showed the genotype a1/a1, one patient a2/a2, two had a3/a3, and none was homozygous for a4. Intermediate and rare alleles were always detected in combination with the common group. Separation by sex showed a similar frequency of distribution of common alleles (female/male=0.921/0.978). In contrast, intermediate (female/male=0.053/0.022) and rare (female/male=0.026/0.000) alleles occurred preferentially in women.

COMPARATIVE ANALYSIS OF THE ALLELE DISTRIBUTION

The statistical analysis of the distribution of 232 Ha-ras haplotypes in patients with colorectal carcinoma and 244 haplotypes in patients with Crohn's disease was carried out by the χ^2 test and Fisher's exact test (Table II). The three classes of Ha-ras alleles in the two populations investigated in this study were compared with the 'Boston cancer free group' and the 'Boston cancer group' and significant differences in distribution were shown. The frequency of rare alleles in the patients with colorectal carcinoma was significantly increased. It was higher than in the patients with Crohn's disease ($\chi^2=8.166$; Fisher's exact test=0.005) and the 'Boston cancer free group' ($\chi^2=49.312$; Fisher's exact test=0.000). Intermediate alleles were equally distributed in each population. Eight rare alleles were detected in 16 patients with colorectal carcinoma, whereas only three were detected in four patients with Crohn's disease. The increase in rare alleles found in colorectal carcinoma patients was at the expense of the common alleles. The decrease of common alleles was essentially identical ($\chi^2=10.615$; Fisher's exact test=0.002) to that found when the Boston cancer group and the Boston cancer free group

Figure 3: Comparison between BamHI (a,b,c) and MspI/HpaII (d,e,f) digested DNAs (arrows on the left side of lane a represent BstEII digested lambda-DNA used as marker for the BamHI gel): genotype a1/a4 (a,d); genotype a1/a3 (b,e), partial digestion of MspI/HpaII was confirmed by BamHI; genotype a1/a1.25 (c,f), BamHI did not discriminate double band, indicating heterozygosity.

TABLE II Statistical analysis of the distribution of Ha-ras allelic classes in patients with colorectal carcinoma (116 patients/232 haplotypes) or Crohn's disease (122 patients/244 haplotypes) compared with the Boston survey*

Case group	Comparison group	χ^2	p value	Fisher's exact test (2-tail)
Common alleles:				
Boston cancer group	Boston cancer free group	25.785	0.000	0.000
Colorectal carcinoma	Boston cancer free group	10.615	0.001	0.002
Crohn's disease	Boston cancer free group	0.240	0.624	0.622
Colorectal carcinoma	Boston cancer group	0.241	0.623	0.725
Crohn's disease	Boston cancer group	7.624	0.006	0.005
Colorectal carcinoma	Crohn's disease	4.013	0.045	0.065
Intermediate alleles:				
Boston cancer group	Boston cancer free group	3.361	0.067	0.083
Colorectal carcinoma	Boston cancer free group	0.295	0.587	0.722
Crohn's disease	Boston cancer free group	0.166	0.684	0.862
Colorectal carcinoma	Boston cancer group	2.714	0.099	0.177
Crohn's disease	Boston cancer group	2.410	0.121	0.128
Colorectal carcinoma	Crohn's disease	0.015	0.903	1.000
Rare alleles:				
Boston cancer group	Boston cancer free group	38.399	0.000	0.000
Colorectal carcinoma	Boston cancer free group	49.312	0.000	0.000
Crohn's disease	Boston cancer free group	6.830	0.009	0.025
Colorectal carcinoma	Boston cancer group	1.083	0.298	0.324
Crohn's disease	Boston cancer group	5.430	0.020	0.017
Colorectal carcinoma	Crohn's disease	8.166	0.004	0.005

*Calculations for Boston cancer free group (424 individuals/848 haplotypes) and Boston cancer group (372 patients/744 haplotypes) were based on the data of allele distributions in these two populations reported by Krontiris and co-workers.⁴ For each class of alleles, groups were compared by pairs in 2×2 contingency tables using χ^2 test (df 1) and Fisher's exact test.

were compared ($\chi^2=25.785$; Fisher's exact test=0.000). Although the frequency of rare Ha-ras alleles in the Crohn's group was significantly lower than in the colorectal carcinoma group, the allele distribution was not identical to that of the Boston cancer free group. When our patients with Crohn's disease were compared with the Boston cancer group for common alleles ($\chi^2=7.624$; Fisher's exact test=0.005) and the Boston cancer free and cancer groups for rare alleles ($\chi^2=6.830$; Fisher's exact test=0.025 and $\chi^2=5.430$; Fisher's exact test=0.017, respectively) significant differences were seen (Table II). These differences were not as great as those for colorectal carcinoma but were placed between Boston cancer free group and Boston cancer group (Table II).

Discussion

Three classes of alleles have been defined for the Ha-ras oncogene by a RFLP caused by a tandem repeat unit located at the 3' flanking region of the gene. At least 34 allelic fragments are known so far, based on the detailed nomenclature of Krontiris *et al.*^{3,4} The distribution of alleles may

reflect an inherited predisposition to develop certain types of malignant diseases.³ The aim of this study was to test the hypothesis that the Ha-ras gene locus is informative for an inherited predisposition to develop colorectal cancer. To compare two well defined patient populations of identical ethnic origin, patients with colorectal carcinoma or Crohn's disease were studied. This was the first time that a distinct patient population with a benign disease of the same organ had been used as a control group. Calculation of Ha-ras alleles adhered to the definitions of MspI/HpaII restriction fragments in the largest comparable survey of subjects.^{3,4}

Two previous studies investigating colorectal carcinoma are not comparable because of the methodology used. In one study, 62 patients who were categorised into only eight different TaqI defined alleles did not exhibit a cancer related allele distribution.⁵ In contrast, a significant increase in the frequency of two alleles was shown in 46 patients classified by nine AuaI defined allelic fragments.⁶ The conflicting results concerning several other tumour types are the result of differences in methodology which lead to an underestimation of the total number of alleles. Furthermore, site polymorphisms for AuaI in the frequent allele a2 and for TaqI in a4 exist.¹⁷ The results indicate that each tumour type must be examined separately for an association with a group of Ha-ras alleles; these need not be the rare alleles. In lung cancer patients in the United Kingdom, a difference in distribution of one of the common alleles was observed between small cell lung cancer and non-small cell lung cancer.¹⁵ But this study also used a different designation of alleles after digestion of DNA with PvuII.

By comparing the distribution of Ha-ras alleles of patients with Crohn's disease and those with colorectal carcinoma, we could confirm the hypothesis that colorectal carcinoma belongs to the group of malignant tumours associated with rare Ha-ras alleles.³ A statistically significant greater frequency of rare Ha-ras alleles occurred in patients with colorectal cancer than in Crohn's disease patients. This difference was also significant when both groups were compared with two control populations analysed by identical methods: 424 persons without a malignant disease (Boston cancer free group) and 372 patients with a wide variety of malignant diseases (Boston cancer group).⁴ Like the colorectal

Figure 4: Anatomical sites of tumours of the 116 patients with colorectal cancer compared with the distribution of detected rare alleles in these sites. No significant preference could be detected.

cancer patients, the Boston cancer group showed a significantly increased frequency of rare alleles. Alleles of the rare group were not found exclusively in the cancer patients. Statistical analysis placed the patients with Crohn's disease between the Boston cancer free group and colorectal carcinoma patients. The finding of a different distribution of the Ha-ras genotype in colorectal carcinoma and in Crohn's disease must be considered in parallel with the observation that ulcerative colitis is a highly premalignant risk factor while Crohn's disease is not.¹³ But the incidence of colorectal cancer in patients with Crohn's disease is not identical to that of the general population.^{13,14} Our results could mean that the malignant potential of Crohn's disease is reflected by an increased rate of rare Ha-ras alleles which were correlated to colorectal carcinoma.

The observation that the allele distribution showed certain differences in the two disease populations when the sexes were separated should not lead to speculation but must be noted. Intermediate alleles occurred predominantly in women in both groups and the rare allele group consisted exclusively of women with Crohn's disease. In 100 Japanese women with breast carcinoma, there was no significant difference in allele distribution compared with the normal population.¹⁸

The relevance of the inheritance of different Ha-ras alleles with regard to susceptibility to other types of cancer has to be elucidated. The functional explanation could be found in the enhancer activity of the repetitive sequence element 3' of the human Ha-ras gene. Based on the polymorphism, the activity of this enhancer is variable within the human population.^{33,34} In addition, allele specific methylation patterns have been observed.^{35,36} Within the scope of the multistep model of colorectal tumorigenesis, hypomethylation and mutations of the ras oncogenes occur in the early neoplastic stages.^{7,8} This could be part of a mechanism whereby individual Ha-ras alleles promote colorectal cancer. Only indirect evidence exists for an influence of Ha-ras alleles over the expression of the gene. In lung squamous cell carcinomas, Krontiris *et al* recently showed an increased frequency of rare Ha-ras alleles,¹⁶ and these tumours frequently exhibit raised Ha-ras expression.³⁷ Increased expression of the Ha-ras gene was also shown for colorectal carcinoma^{38,39} and has to be considered as an early phenomenon of colorectal tumorigenesis.³⁹

The putative influence of Ha-ras alleles on cancer development must be based on independent alleles. Homozygosity was not seen for any rare allele and no significant differences between colorectal cancer and Crohn's disease patients were detected at the level of the complete Ha-ras genotype. The occurrence of an individual Ha-ras allele cannot be considered a general predisposition to malignancy – for example, to identify affected people – but could help define subgroups of patients with a special tumour, which could be important in determining the best treatment strategies. Other benign diseases could also be tested for a malignant potential, in cases where the malignant tumour of the

involved organ system shows a distribution of Ha-ras alleles that is different to that found normally. Attempts to correlate the Ha-ras allele distribution to clinical or biological characteristics of a particular type of tumour or disease need larger surveys to resolve these issues.

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- 1 Capon DJ, Chen EY, Levinson AD, Seeburg PH, Goeddel DV. Complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue. *Nature* 1983; 302: 33–7.
- 2 Baird M, Balazs I, Giusti A, *et al*. Allele frequency distribution of two highly polymorphic DNA sequences in three ethnic groups and its application to the determination of paternity. *Am J Hum Genet* 1986; 39: 489–501.
- 3 Krontiris TG, DiMartino NA, Colb M, Parkinson DR. Unique allelic restriction fragments of the human Ha-ras locus in leukocyte and tumour DNAs of cancer patients. *Nature* 1985; 313: 369–74.
- 4 Krontiris TG, DiMartino NA, Mitcheson HD, Loneragan JA, Begg C, Parkinson DR. Human hypervariable sequences in risk assessment: rare Ha-ras alleles in cancer patients. *Environ Health Perspect* 1987; 76: 147–53.
- 5 Ceccherini-Nelli L, De Re V, Viel A, Molaro G, Zilli L, Clemente C, *et al*. Ha-ras-1 restriction fragment length polymorphism and susceptibility to colon adenocarcinoma. *Br J Cancer* 1987; 56: 1–5.
- 6 Wyllie FS, Wynford-Thomas V, Lemoine NR, Williams GT, Williams ED, Wynford-Thomas D. Ha-ras restriction fragment length polymorphisms in colorectal cancer. *Br J Cancer* 1988; 57: 135–8.
- 7 Stanbridge EJ. Identifying tumor suppressor genes in human colorectal cancer. *Science* 1990; 247: 12–3.
- 8 Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; 61: 759–67.
- 9 Feinberg AP, Vogelstein B. Hypomethylation of ras oncogenes in primary human cancers. *Biochem Biophys Res Comm* 1983; 111: 47–54.
- 10 Vogelstein B, Fearon ER, Hamilton SR, *et al*. Genetic alterations during colorectal tumor development. *N Engl J Med* 1988; 319: 525–32.
- 11 Bodmer WF, Bailey CJ, Bodmer J, *et al*. Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 1987; 328: 614–6.
- 12 Lynch PM, Lynch HT, Lynch JF. Hereditary nonpolyposis colon cancer: epidemiologic and clinical-genetic features. In: Lynch PM, Lynch HT, eds. *Colon cancer genetics*. New York: Van Nostrand Reinhold, 1985: 52–98.
- 13 Dobbins WO. Dysplasia and malignancy in inflammatory bowel disease. *Ann Rev Med* 1984; 35: 33–48.
- 14 Greenstein AJ, Sachar DB, Smith H, Janowitz HD, Aufses AH. A comparison of cancer risk in Crohn's disease and ulcerative colitis. *Cancer* 1981; 48: 2742–5.
- 15 Heighway J, Thatcher N, Cerny T, Hasleton PS. Genetic predisposition to human lung cancer. *Br J Cancer* 1986; 53: 453–7.
- 16 Sugimura H, Caporaso NE, Modali RV, *et al*. Association of rare alleles of the Harvey ras protooncogene locus with lung cancer. *Cancer Res* 1990; 50: 1857–62.
- 17 Carter G, Worwood M, Jacobs A. The Ha-ras polymorphism in myelodysplasia and acute myeloid leukaemia. *Leukemia Research* 1988; 12: 385–91.
- 18 Honda K, Ishizaki K, Ikenaga M, Toguchida J, Tanaka K, Ozawa K. Increased frequency of specific alleles of the c-Ha-ras gene in Japanese cancer patients. *Hum Genet* 1988; 79: 297–300.
- 19 Diedrich U, Eckermann O, Schmidtke J. Rare Ha-ras and c-mos alleles in patients with intracranial tumors. *Neurology* 1988; 38: 587–9.
- 20 Thein SL, Oscier DG, Flint J, Wainscoat JS. Ha-ras hyper-variable alleles in myelodysplasia. *Nature* 1986; 321: 84–5.
- 21 Corell B, Zoll B. Comparison between the allelic frequency distribution of the Ha-ras1 locus in normal individuals and patients with lymphoma, breast and ovarian cancer. *Hum Genet* 1988; 79: 255–9.
- 22 Ishikawa J, Maeda S, Kamidono S, Sugiyama T. Restriction fragment length polymorphism and activation of c-Ha-ras gene in urothelial cancer. *Anticancer Res* 1988; 8: 915–24.
- 23 Ishikawa J, Maeda S, Takahashi R, Kamidono S, Sugiyama T. Lack of correlation between rare Ha-ras alleles and urothelial cancer in Japan. *Int J Cancer* 1987; 40: 474–8.
- 24 Hayward NK, Keegan R, Nancarrow DJ, *et al*. C-Ha-ras-1 alleles in bladder cancer, Wilms' tumour and malignant melanoma. *Hum Genet* 1988; 78: 115–20.
- 25 Gerhard DS, Dracopoli NC, Bale SJ, *et al*. Evidence against Ha-ras-1 involvement in sporadic and familial melanoma. *Nature* 1987; 325: 73–5.
- 26 Radice P, Pierotti MA, Borrello MG, Illeni MT, Rovini D, Porta GD. HRAS1 proto-oncogene polymorphisms in human malignant melanoma: TaqI defined alleles significantly associated with the disease. *Oncogene* 1987; 2: 91–5.
- 27 Sutherland C, Shaw HM, Roberts C, *et al*. Harvey-ras oncogene restriction fragment alleles in familial melanoma kindreds. *Br J Cancer* 1986; 54: 787–90.
- 28 Barkardottir RB, Johannsson OT, Arason A, Gudnason V, Egilsson V. Polymorphism of the c-Ha-ras-1 proto-oncogene

- in sporadic and familial breast cancer. *Int J Cancer* 1989; **44**: 251-5.
- 29 Lidereau R, Escot C, Theillet C, *et al*. High frequency of rare alleles of the human c-Ha-ras-1 proto-oncogene in breast cancer patients. *JNCI* 1986; **77**: 697-701.
 - 30 White GRM, Heighway J, Williams GT, Scott D. Constitutional frequency of rare alleles of c-Ha-ras in breast cancer patients. *Br J Cancer* 1988; **57**: 526.
 - 31 Krontiris TG, DiMartino NA, Colb M, Mitcheson HD, Parkinson DR. Human restriction fragment length polymorphisms and cancer risk assessment. *J Cell Biochem* 1986; **30**: 319-29.
 - 32 Klingel R, Mincheva A, Gissmann L, Dippold W, Meyer zum Büschenfelde K-H, zur Hausen H. An amplification unit in human melanoma cells showing partial homology with sequences of human papillomavirus type 9 and nuclear antigen 1 of the Epstein-Barr virus. *Cancer Res* 1987; **47**: 4485-92.
 - 33 Cohen JB, Walter MV, Levinson AD. A repetitive sequence element 3' of the human C-Ha-ras1 gene has enhancer activity. *J Cell Physiol* 1987; Suppl 5: 75-81.
 - 34 Spandidos DA, Holmes L. Transcriptional enhancer activity in the variable tandem repeat DNA sequence downstream of the human Ha-ras1 gene. *FEBS Lett* 1987; **218**: 41-6.
 - 35 Bedford MT, van Helden PD. Allele specific methylation of the H-ras gene. *J Cancer Res Clin Oncol* 1990; **116** (suppl): 16.
 - 36 Chandler LA, Ghazi H, Jones PA, Boukamp P, Fusenig NE. Allele-specific methylation of the human c-Ha-ras-1 gene. *Cell* 1987; **50**: 711-7.
 - 37 Kurzrock R, Gallick GE, Gutterman JU. Differential expression of p21 ras gene products among histological subtypes of fresh primary human lung tumors. *Cancer Res* 1986; **46**: 1530-4.
 - 38 Gallick GE, Kurzrock R, Kloetzer WS, Arlinghaus RB, Gutterman JU. Expression of p21 ras in fresh primary and metastatic human colorectal tumors. *Proc Natl Acad Sci USA* 1985; **82**: 1795-9.
 - 39 Jansson DS, Radosevich JA, Carney WP, Rosen ST, Schlom J, Staren ED, *et al*. An immunohistochemical analysis of ras oncogene expression in epithelial neoplasms of the colon. *Cancer* 1990; **65**: 1329-37.