

Mutagen sensitivity and p53 expression in colorectal cancer in China

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Abstract

Objective—This study was designed to investigate DNA damage and/or repair capability, non-random chromatid breakage, and p53 expression in patients with colorectal cancer.

Methods—The bleomycin sensitivity assay was used in a case-control study to compare the DNA damage repair system between colorectal cancer patients and controls. G-banding was used to search for non-random chromatid breaks. Immunocytochemistry was used to investigate p53 expression in tumour tissues and adjacent normal tissues.

Results—It was found that cases typically had a higher number of chromosome breaks than controls (0.84 v 0.69 breaks/cell, $p < 0.01$). After correction by sex and age, the difference was still significant ($F = 4.38$, $p < 0.05$). The correlation coefficient between mutagen sensitivity and age was 0.31 ($p < 0.05$) in controls and 0.18 ($p > 0.05$) in cases. The ratio of odds ratios among bleomycin resistant, sensitive, and hypersensitive classes was 1:2.31:3.85. Overexpression of p53 was detected in 25 of 47 tumour tissues independent of tumour stage. Cases who had a family history of cancer were susceptible to the p53 aberration ($p < 0.05$). Chromosomes 1p, 5q, and 14q were susceptible to breakage in patients with colorectal cancer.

Conclusion—Patients with colorectal cancer show increased bleomycin induced chromatid breaks and may have minor DNA repair deficiencies. p53 aberration is an early event in the development of colorectal cancer, but no definite correlation is found between p53 overexpression and mutagen sensitivity.

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developed an assay in which chromosomal breakage induced by in vitro exposure to bleomycin is used as an indirect measure of DNA repair capability.^{3,4} Hsu *et al* assume that bleomycin induced chromatid breakage may be related to defective DNA repair mechanisms and is not an all or none phenomenon in the general population.³ This assay is particularly useful for the study of the evolution and development of cancers in tissues exposed to environmental carcinogens such as lung, colon, head, and neck.

p53 is at the crossroads of a network of cellular pathways including cell cycle checkpoints, DNA repair, chromosomal segregation, and apoptosis. These pathways have evolved to maintain the stability of the genome during cellular stress from DNA damage.⁵ Abnormalities of p53 have frequently been detected in solid tumours.^{6,7} Moreover, the association between environmental carcinogens and p53 gene mutations or overexpression in head and neck cancer suggests that p53 may be a genetic target of environmental carcinogens.⁸

Both mutagen sensitivity and p53 may play a part in tumorigenesis in environmental exposed tissues such as lung, colon, head, and neck. They may have certain relationships in carcinogenesis. Furthermore, a significant correlation between p53 overexpression and mutagen sensitivity in patients with head and neck cancer with multiple malignancies and in lung cancer patients has been reported.^{9,10} Colorectal cancer is a classic malignant tumour in which genetic and environmental factors both play an important part.¹ Increased mutagen sensitivity has already been reported in patients with colorectal cancer, but the relationship between mutagen sensitivity and p53 expression has never been investigated.^{11,12} The purpose of this study was to examine bleomycin induced chromatid breaks in peripheral blood lymphocytes and p53 expression in colorectal cancer tissues.

Subjects and methods

PATIENTS/SAMPLES

We studied 52 patients with colorectal cancer from the First Affiliated Hospital of Zhejiang University who presented from April 1998 to December 1998. Colorectal cancer was defined as histologically confirmed and previously untreated adenocarcinoma of the colon or rectum. Fifty two blood samples were obtained before treatment; 47 paired tumour tissues and adjacent normal tissues were collected after excision. The control population was made up of health checkers and patients who had no cancer history or infectious diseases at that time.

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The development of cancer is thought to be associated with the accumulation of relevant genetic changes in the target tissue.¹ The risk of tumour development is increased by thousands-fold in individuals who are defective in their DNA damage and/or repair system. A deficient DNA repair system could increase the probability of mutations such as inactivation or deletion at cancer predisposing loci. Mutagen sensitivity can be used to evaluate DNA repair capability properly as individuals who have DNA damage and/or repair deficiency express increased chromatid breaks when exposed to environmental mutagens.² Hsu *et al* have

Table 1 Characteristics of patients and controls

	Colorectal cancer (n=52)	Control (n=127)
Age range	29–84	16–81
Mean (SD) age	57 (13)	45 (18)
Sex		
Male	31	70
Female	21	57
Mean (SD) spontaneous breaks/cell	0.020 (0.025)	0.023 (0.024)
Mean (SD) bleomycin induced breaks/cell	0.84 (0.32)	0.69 (0.25)
Site		
Right colon*	3	—
Left colon†	8	—
Rectum	41	—
Family cancer history		
+	11	—
-	41	—
p53 overexpression		
+	25	—
-	22	—

*Including ascending and transverse colon.

†Including descending and sigmoid colon.

DATA COLLECTION

The epidemiological data were collected by personal interview. A table was designed to include common variables (sex, age, height, body weight), lifestyle factors (smoking, alcohol), and presence of a family history of cancer.

MUTAGEN SENSITIVITY

A peripheral blood sample of 3 ml from each patient and control was collected in a heparinised tube, and lymphocytes were cultured by standard procedures.³ On the third day of incubation, cultures were treated with bleomycin (30 µg/ml) for five hours. During the last two hours, the cells were treated with colcemid (0.06 µg/ml) to accumulate mitoses before harvesting. Blank cultures were treated with colcemid only. The conventional cell harvest was followed, using 0.075M hypotonic potassium chloride and 3:1 methanol glacial acetic acid for fixation. Slides were coded for a blind study and stained with Giemsa without banding for directly counting chromatid breaks.

CHROMOSOMAL ANALYSIS

From stained preparations of each sample, 50 well spread metaphases were routinely examined and expressed as the average number of breaks/cell. In recording chromatid-type aberrations, we followed the recommendation of Hsu *et al.*³ To evaluate the location of the breaks, slides of five controls and 19 cases were aged in atmosphere for 3–7 days and digested by 0.25% trypsin to make G-banding. Fifteen to 40 metaphases per sample were analysed to record the location of breaks.

P53 EXPRESSION

Formalin fixed, paraffin embedded sections of the tumour tissues and adjacent normal tissues from 47 patients were made. Immunocytochemistry was performed using murine monoclonal antibody, DO-7, against human p53 protein (Dako, Copenhagen, Denmark). The staining pattern was assessed by Ms Huang and Dr Lai. Only nuclear staining was regarded as specific staining. Both negative and equivocal staining were regarded as p53 (-), ≥1% of positive cells were regarded as p53 (+).

STATISTICAL ANALYSIS

Specific chromosomal breaks were categorised by using the 75th centile value in the control group, selected a priori, as the arbitrary cut off point. In this study, a value of 0.85 breaks/cell was selected as the 75th cut off point to separate the bleomycin sensitive from the less sensitive class. And a value of 1.05 breaks/cell was taken as the arbitrary cut off point to separate the hypersensitive from the sensitive class. Differences between groups with respect to mutagen sensitivity and age were assessed by Student's *t* test. The influence of age and sex on mutagen sensitivity was corrected by the use of multiple factor analysis of variance. Intraclass correlation coefficient between age and mutagen sensitivity was calculated. Odds ratios and the χ^2 test were used to evaluate the distribution difference of mutagen sensitivity between groups.

Results

Characteristics of patients with colorectal cancer and the control group are shown in table 1. There was no significant difference in sex between cases and controls, but a significant difference was revealed in age between these two groups. The mean age for the cases was 57 years, compared with 45 years for the controls ($p < 0.01$). The correlation coefficient between chromatid breaks and age was 0.31 in controls ($p < 0.01$) and 0.18 in cases ($p > 0.05$). The overall spontaneous chromosome breaks/cell value was low (0–0.12) and the mean spontaneous breaks/cell value of the control group was not significantly different from that of the cancer group. There were significant differences in overall bleomycin induced chromatid breaks between cases and controls. The mean breaks/cell was 0.84 for cases and 0.69 for controls ($p < 0.01$). After adjustment by sex and age, the significant difference still existed ($F = 4.48$, $p = 0.036$). The population was divided into three groups using 0.85 and 1.05 breaks/cell as cut off points respectively. The ratio of bleomycin sensitive and hypersensitive classes was significantly higher in cases than in controls ($\chi^2_{trend} = 9.72$, $p < 0.01$). The ratio of odds ratios among bleomycin resistant, sensitive, and hypersensitive classes were 1:2.31:3.85 (table 2).

Overexpression of p53 was detected in 25 of 47 colorectal cancer tissue samples (53.2%) compared with 46.8% that were negative; none of the paired normal tissues stained positively. p53 overexpression was detected in both early and late stage tumours: 11 of 18 Dukes A tumours (61.1%), seven of 13 Dukes B tumours (53.8%), and seven of 16 Dukes C and D tumours (43.8%) had detectable p53 protein. The mean value of breaks/cell was

Table 2 Distribution of mutagen sensitivity in cases and controls

Bleomycin	Colorectal cancer (%)	Control (%)	Odds ratio
Resistant	28 (53.8)	97 (76.4)	1
Sensitive	14 (26.9)	21 (16.5)	2.31
Hypersensitive	10 (19.2)	9 (7.1)	3.85

$\chi^2_{trend} = 9.72$, $p < 0.01$.

Table 3 p53 expression and mutagen sensitivity

p53 overexpression	No	Mean (SD) breaks/cell
Positive	25	0.76 (0.28)
Negative	22	0.93 (0.36)

Student's *t* test: $p > 0.05$.

Table 4 Comparison of mutagen sensitivity between left and right colon cancer

Site	No	Mean (SD) breaks/cell
Right*	3	0.78 (0.26)
Left†	8	1.03 (0.14)

Student's *t* test: $p > 0.05$.

*Including ascending and transverse colon.

†Including descending and sigmoid colon.

Table 5 p53 expression and family history of cancer

p53 overexpression	No	Family cancer history	
		Positive	Negative
Positive	25	9	16
Negative	22	2	20

$\chi^2 = 4.73$, $p < 0.05$.

higher in p53 protein negative classes than that in p53 protein overexpression classes, but no significant difference was found (table 3).

In 52 cases, eight were left sided colon cancer and three were right sided colon cancer. Mutagen sensitivity of left colon cancer was higher than that of right colon cancer, though no significant difference was found (table 4).

Eleven of 52 cases had one or two first degree relatives affected by cancer, mostly of the digestive tract, but no patients with hereditary non-polyposis colon cancer were included according to the obtained material. These patients were younger than those who had no relative affected by cancer: the mean age of the cases who had a family history of cancer was 50 years and those without was 59 years ($p < 0.05$). In patients who had a family history of cancer, the p53 protein positive rate was significantly raised compared with those without cancer in their family history (table 5).

The results of G-banding showed that in cases and controls the larger chromosomes (1–8) were more susceptible to bleomycin induced breaks than the smaller ones (14–22). Altogether 83.3% of the total breaks were on large chromosomes in controls and 78.9% in cases ($p > 0.05$). Susceptibility to break was found in 1q, 2q, 3p, 4q in controls and in 1p, 5q, 14q in those with colorectal cancer, but no significant difference was found between controls and cases.

Discussion

Previous studies have shown that patients with colorectal cancer have a greater sensitivity to bleomycin induced chromosomal breaks.^{11 12} The results of our current study are consistent with Hsu *et al*'s hypothesis and other scholars' reports, suggesting that patients with cancer in their aerodigestive tracts exhibit increased chromosome fragility when their lymphocytes are exposed in vitro to bleomycin.^{3 13} The ratio of odds ratios of bleomycin resistant, sensitive, and hypersensitive classes was 1:2.31:3.85,

which suggests that individuals with higher chromatid breaks have a higher risk of colorectal cancer. Hsu *et al* suggest that age has no effect on mutagen sensitivity.¹⁴ But our results show that there is a weak correlation between age and mutagen sensitivity in controls, though the correlation coefficient is small. Cloos *et al* also found that age had a large influence on mutagen sensitivity when they examined the bleomycin induced chromatid breaks in the offspring or siblings of patients with head and neck cancers, but no explanation was given.¹⁵ It is possible that individuals have decreased DNA repair capability with aging and the shortening of telomeres.

Patients with colorectal cancers from different sites have different mutagen sensitivities; this has been shown by Fireman *et al*.¹¹ In our study, left sided colon cancer patients show higher mutagen sensitivity than those with right sided cancer (table 4). These data support the hypothesis that environmental carcinogens might play a greater part in the aetiology of left sided rather than right sided colon cancer, where genetic factors might be more important. But the result is limited by the small sample of patients with colon cancer, because cancer of the rectum makes up nearly three quarters of the cases of colorectal cancer in China and it is not easy to collect samples from those with colon cancer.

p53 overexpression is commonly raised in the tissues from patients with colorectal cancer that have been studied, and the incidence of 53% (25/47) p53 positive tumour specimens analysed is consistent with the reported studies in colorectal cancer and other solid tumours.^{6 7} p53 overexpression is present in both early and late stage tumours, which is consistent with the report that p53 protein accumulation happens in the adenoma-carcinoma sequence in colorectal carcinogenesis.¹⁰ To our surprise, we find that individuals who have p53 positive tumour tissues have lower mutagen sensitivity than those who have p53 negative tumour tissues, though the difference is not significant. Studies on environmental related cancers report that p53 overexpression is more common in bleomycin sensitive individuals than in bleomycin resistant ones.^{9 10} Mutagen sensitivity and p53 aberration may have a major impact on carcinogenesis of the colon and rectum. The mutation of p53 in colorectal cancers is always acquired and inherited mutation leading to the Li Fraumeni syndrome happens rarely. In the peripheral blood of patients with colorectal cancers, p53 is always the wild type. Though it is reported that p53 may be the target gene of smoking related carcinogens in head and neck cancers. In aerodigestive cancer, other genes such as APC (adenomatous polyposis coli) or β -catenin may be the target genes of heterocyclic compounds, while p53 may be inactivated by other mechanisms.⁸ Furthermore, p53 mainly functions in G1-S and induces cell cycle arrest or apoptosis in response to DNA damage, while bleomycin induced chromatid breaks mainly reflect the DNA repair capability in G2. Hence, we assume that the function of p53 in DNA repair system cannot be reflected

by measuring mutagen sensitivity in peripheral lymphocytes. Moreover, the p53 protein overexpression is far more common in patients with colorectal cancer who have a familial cancer history than those without. We can infer from this that aberrant p53 expression may play a more important part in carcinogenesis in members of families with cancer.

The development of certain cancers is related to constitutional chromosome breakage. Wu *et al* find that chromosomes 4 and 5 are preferentially involved in lung cancer.¹⁷ But Ankathil *et al* find that patients with colorectal cancer, their unaffected family members, and controls don't show any constitutional chromosomal abnormalities.¹² In our studies, 1p, 5q, and 14q are more susceptible to breakage. The rate of 1p deletion in sporadic colorectal cancer is 50% by conventional banding technique; 45% of colorectal cancers and 38% of adenomas have the deletion of 1p by fluorescence in situ hybridisation.^{18 19} So it is thought that the deletion of 1p is an early event in colorectal carcinogenesis. Abnormalities in chromosome 5 are often detected in solid tumours. APC and MCC (mutated in colorectal cancer) are both situated on 5q and are the most frequently mutated genes in colorectal cancers. Alterations in 14q are rarely reported in tumours. A recent study reported that loss of heterozygosity of 14q happened more frequently in early onset colorectal cancers than in those of late onset.²⁰ 14q harbours a colorectal cancer related tumour suppressor gene candidate, SNC73, which has been cloned on 14q32.²¹ The aberration of 14q may lead to the inactivation of SNC73.

From the results shown above, we infer that sensitivity to bleomycin induced chromatid breaks is a risk factor for colorectal cancer, and the breaks induced by bleomycin appear to be non-random. p53 overexpression happens in the adenoma-carcinoma sequence, but no definite correlation is found between p53 and mutagen sensitivity.

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