Survival of Cells with Bleomycin-induced Chromosomal Lesions in the Cultured Lymphocytes of Lung Cancer Patients[1]

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Abstract

In a previous study of lung cancer, we showed that bleomycin, a radiomimetic agent, induced breaks preferentially on chromosomes 4 and 5. The molecular cytogenetic study reported here, using chromosome painting and G banding, was designed to assess whether the chromatid breaks induced by bleomycin could survive as chromosome-type aberrations after treated lymphocyte populations were allowed to recover in a drug-free medium for one or two cell generations and whether the survival rates of lesions on chromosomes 4 and 5 differed in cases with lung cancer and controls. The findings from 16 cases and 14 controls showed that in samples allowed to recover for 48 h, most aberrations were of the chromosome type. The proportion of chromosome 5 abnormalities surviving as chromosome-type aberrations was significantly higher in the cells of lung cancer cases (13.4%) than in controls (4.6%; $P < 0.0001$). However, no significant differences in survival of chromosome 4 abnormalities were detected between cases and controls. The proportions of chromosome 5q13-q22 abnormalities were 5.3% in the cases and 0.6% in the controls ($P < 0.0001$). 5q13-q22 regions encompassed 38.4% of all abnormalities on chromosome 5 in the cases but only 14.5% in the controls. Therefore, the survival rate of chromosome 5 lesions (especially those at 5q13-q22) in lymphocytes might be used as a biomarker to identify populations at high risk for lung cancer.

Introduction

There is substantial interindividual variation in susceptibility to carcinogenesis. One measure of such differential susceptibility is mutagen sensitivity, which can be measured by an *in vitro* assay of chromatid breaks induced in cultured lymphocytes by various mutagens. In our laboratory, mutagen sensitivity has been estimated by counting the chromatid breaks induced by the radiomimetic clastogen bleomycin. Increased sensitivity to clastogenesis has been shown to be a risk factor for upper aerodigestive tract and lung cancers and for secondary cancers of the head and neck (1–4). The distribution of these chromosomal lesions as a response to *in vitro* mutagen exposure was found to be nonrandom. Some chromosomes sustain more damage than others relative to their lengths, and some chromosomal loci have higher rates of breaks than others (5–7). A high frequency of breaks at these sites may indicate a genetic predisposition to mutagenic damage (5). These breakpoints could have significant effects on the risk for cancer development if they are at the sites of oncogenes, tumor suppressor genes, or growth factors and if the cells that contain the breaks continue to proliferate in the target tissue.

In our pilot case-control study (7), we challenged cultured lymphocytes from 78 cases with lung cancer and 75 controls with bleomycin for 5 h and counted the chromatid breaks on all chromosomes. We found that chromosomes 4 and 5 showed significantly higher rates of chromatid breaks in cases than in controls and that these chromosomes had higher rates of breakage than other chromosomes. The breakpoints occurred most frequently at 4p14, 4q27, 4q31, 5q21-q22, 5q31, and 5q33, where tumor suppressor genes (*MCC* and *APC* at 5q21 and *IRF1* at 5q31), oncogenes (*LCA* at 2q14), immunofactor genes (*IL1A*, *IL1B*, and *IL1RN* at 2q14; *IL2* at 4q25; and *IL3–5*, *IL9*, and *IL13* at 5q31), and growth factor genes (*EGF* at 4q25 and *FGF2* at 5q31) are located.

Survival of cells with chromatid lesions at specific loci is relevant to mutagenesis and carcinogenesis, because cells with severe chromosome damage usually perish, and a considerable number of induced lesions are probably repaired in the ensuing cell generations. Moreover, those that do survive as deletions or rearrangements may have no functional effect.

The molecular cytogenetic study reported here was designed to assess whether chromatid breaks induced by bleomycin survive as chromosome-type aberrations (deletions, ring chromosomes, dicentric chromosomes, inversions, and marker chromosomes) after the treated cells are allowed to recover in a drug-free medium for one or two generations. Because our previous study showed that bleomycin-induced chromatid breaks occurred preferentially on chromosomes 4 and 5, we examined only those chromosomes in the study presented here.

Materials and Methods

Lymphocyte Culture. Peripheral blood samples were collected in heparinized tubes from 16 previously untreated, histologically confirmed cases with lung cancer and 14 healthy controls. The subjects were identified from an ongoing molecular epidemiological study of lung cancer. The test mutagen used was bleomycin (Nippon Kayaku Co., Ltd., Tokyo, Japan),

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Table 1 Chromosomal aberrations present after various recovery periods

Recovery period	Mean no. of cells (%) with aberrations[a]		
	G_2-S	G_1-G_0	Both
None	25.8 (51.6)	0	0
24 h	7.1 (14.2)[b]	2.3 (4.6)	0.6 (1.4)
48 h	3.4 (6.8)	4.0 (8.8)[c]	0.3 (1.0)

[a] Fifty cells were examined from each of 10 subjects including 5 cases and 5 controls.
[b] $P < 0.05$ compared with no-recovery and 48-h recovery groups.
[c] $P < 0.05$ compared with 24-h recovery group.

which induces 1-bp deletions, exon deletions, and gene deletions, as well as single- and double-stranded DNA breaks. Bleomycin was selected as the clastogenic agent because: (*a*) its mechanism of action has been well elucidated (8); (*b*) sensitivity to bleomycin is a risk factor for lung cancer (3); and (*c*) bleomycin is a radiomimetic agent, and lung cancer risk is increased in uranium miners who are exposed to radon daughters, in cigarette smokers (cigarettes contain radioactive constituents, such as radon and its decay products, as well as lead-210, bismuth-210, and polonium-210 (9), and in patients treated with radiation (10).

Lymphocyte cultures were established as follows: 1 ml whole blood was added to 9 ml RPMI 1640 (Gibco Laboratories, Inc., Grand Island, NY) supplemented with 20% fetal bovine serum, 2 mM L-glutamine, 50 units/ml penicillin, 100 μg/ml streptomycin (Sigma Chemical Co., St. Louis, MO), and 1.3% phytohemagglutinin (Wellcome Research Laboratories, Research Triangle Park, NC). The cultures were incubated at 37°C for 2 or 3 days.

Recovery Experiments. On day 2 of incubation, the cultures were treated with bleomycin (0.03 units/ml) for 5 h and washed twice with drug-free RPMI medium. The cultures were then reincubated for another 24 or 48 h in drug-free growth medium before harvesting.

Cytogenetic Preparations and Banding. Chromosome preparations were made after routine blocking with Colcemid (0.04 μg/ml for 50 min; Sigma), pretreating with a hypotonic solution (0.06 M KCl for 12 min), and fixing in acetic acid:methanol (1:3). Slides were prepared by the standard air-drying procedure. To count chromatid breaks and obvious chromosomal abnormalities such as rings, dicentric chromosomes, and fragments, the slides were stained with Giemsa without banding. For G banding, the slides were allowed to age for 7–9 days before being trypsinized. The slides were coded before analysis. A minimum of 50 metaphases from each sample was examined for chromatid or chromosomal anomalies with both a light microscope and Genetiscan (PSI, Houston, TX).

FISH.[3] For FISH experiments, the slides were stored at −20°C until use. Probes for FISH were prepared from whole DNA from human-rodent cell hybrids GM10115 and GM10114, which contain only human chromosomes 4 and 5, respectively. The methods for preparation of human-specific FISH probes from hybrid cells by inter-Alu PCR, biotin or digoxigenin labeling, competitive hybridization blocking of repeat sequences, FISH, avidin-fluorescein or rhodamine-fluorescein detection, fluorescence microscopy, and photography were described by Liu *et al* (11). An average of 100 metaphases from each sample was examined for chromosome 4 and 5 abnormalities.

[3] The abbreviation used is: FISH, fluorescence *in situ* hybridization.

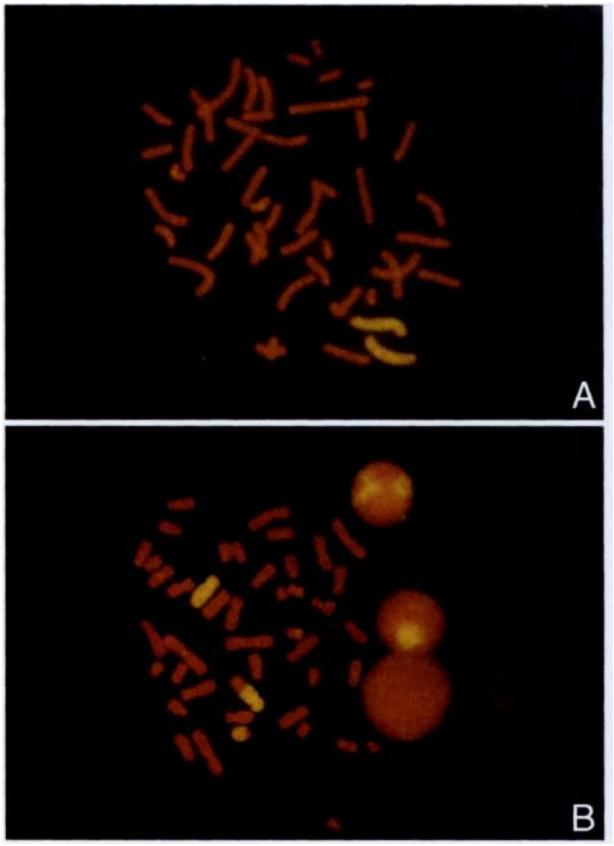

Fig. 1. *A*, FISH preparation using biotin-labeled chromosome 5 probe (yellow). This metaphase is from a control individual. The cell has two normal copies of chromosome 5. *B*, FISH preparation using biotin-labeled chromosome 5 probe (yellow). This metaphase is from peripheral blood lymphocytes of a lung cancer case 48 h after treatment. The cell has one intact chromosome 5. The other chromosome 5 has a translocation involving 5q with a breakpoint at or near 5q21.

Statistical Analysis. The percentages of abnormalities on chromosomes 4 and 5 and at specific chromosomal subregions were calculated for each sample. Student's *t* test and the Mann-Whitney test (applied to variables not distributed normally) were used to compare the frequency of abnormalities between cases and controls.

Results

Chromatid Breaks and Obvious Chromosome Abnormalities. To score breaks and chromosome-type abnormalities, we classified chromosomal aberrations into three types: G_2-S, G_1-G_0, and both. G_2-S breaks are exclusively chromatid-type breaks, whereas G_1-G_0 breaks are chromosome-type breaks.

Table 1 summarizes the chromosomal aberrations detected in cultured lymphocytes immediately after a bleomycin challenge and in cell populations after 24 and 48 h of recovery. In the cell populations treated with bleomycin for a short duration (5 h), the overwhelming majority of aberrations were the chromatid type. In the recovering cell populations, both chromatid and chromosome types of the aberrations were observed, but the frequency of chromatid aberrations in the 24-h recovery samples was significantly lower than that of samples without

Table 2 Chromosome 5 abnormalities in lymphocytes of lung cancer cases and controls after 48 h of recovery

Subjects	No. of cells examined	No. (%) of cells with chromosome 5 aberrations					
		5q	5q13-q22	5p	Monosomy 5	Other	Total[a]
Cases							
1	50	5 (10.0)	4 (8.0)	2 (4.0)	2 (4.0)	1 (2.0)	10 (20.0)
2	99	5 (5.1)	2 (2.0)	0 (0.0)	2 (2.0)	1 (1.0)	8 (8.1)
3	119	8 (6.7)	3 (3.0)	2 (1.7)	1 (0.8)	4 (3.4)	15 (12.6)
4	144	11 (7.6)	9 (6.0)	0 (0.0)	2 (1.4)	4 (2.8)	17 (11.8)
5	24	2 (8.3)	1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.3)
6	35	5 (14.3)	4 (11.0)	0 (0.0)	1 (2.9)	1 (2.9)	7 (20.0)
7	150	15 (10.0)	13 (9.0)	3 (2.0)	1 (0.7)	2 (1.3)	21 (14.0)
8	51	3 (5.9)	2 (4.0)	0 (0.0)	3 (5.9)	1 (2.0)	7 (13.7)
9	77	8 (10.4)	6 (8.0)	0 (0.0)	1 (1.3)	0 (0.0)	9 (11.7)
10	91	12 (13.2)	3 (3.0)	0 (0.0)	2 (2.2)	2 (2.2)	13 (14.3)[b]
11	204	11 (5.4)	4 (2.0)	1 (0.5)	0 (0.0)	4 (2.0)	16 (7.8)
12	66	7 (10.6)	4 (6.0)	1 (1.5)	3 (4.5)	1 (1.5)	12 (18.2)
13	210	26 (12.4)	13 (6.0)	0 (0.0)	0 (0.0)	0 (0.0)	26 (12.4)
14	45	5 (11.1)	2 (4.0)	0 (0.0)	3 (6.7)	0 (0.0)	8 (17.8)
15	139	11 (7.9)	5 (4.0)	2 (1.4)	1 (0.7)	1 (0.7)	14 (10.1)[b]
16	139	8 (5.8)	6 (4.0)	3 (2.2)	5 (3.6)	3 (2.2)	18 (12.9)[b]
Mean	102.7	8.9 (9.0)[c]	5.1 (5.3)[c]	0.9 (0.9)	1.7 (2.4)[c]	1.6 (1.5)	12.7 (13.4)[c]
Controls							
1	263	2 (0.8)	1 (0.0)	1 (0.4)	1 (0.4)	4 (0.2)	8 (3.0)
2	211	3 (1.4)	1 (1.0)	0 (0.0)	1 (0.5)	1 (0.5)	5 (2.4)
3	282	4 (1.4)	0 (0.0)	2 (0.7)	0 (0.0)	1 (0.4)	7 (2.5)
4	65	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)	1 (1.5)
5	230	2 (0.9)	2 (1.0)	0 (0.0)	0 (0.0)	4 (1.7)	6 (2.6)
6	50	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.0)	2 (4.0)
7	398	5 (1.3)	3 (1.0)	1 (0.3)	8 (2.0)	3 (0.8)	17 (4.3)
8	130	5 (3.8)	1 (1.0)	3 (2.3)	0 (0.0)	1 (0.8)	9 (6.9)
9	78	3 (3.8)	1 (1.0)	1 (1.3)	0 (0.0)	0 (0.0)	4 (5.1)
10	59	3 (5.1)	2 (3.0)	1 (1.7)	1 (1.7)	0 (0.0)	5 (8.5)
11	74	1 (1.4)	1 (1.0)	0 (0.0)	2 (2.7)	0 (0.0)	3 (4.1)
12	83	2 (2.4)	0 (0.0)	0 (0.0)	2 (2.4)	0 (0.0)	4 (4.8)
13	87	5 (5.7)	0 (0.0)	0 (0.0)	2 (2.3)	0 (0.0)	7 (8.0)
14	51	2 (3.9)	0 (0.0)	0 (0.0)	1 (2.0)	0 (0.0)	3 (5.9)
Mean	147.2	2.7 (2.4)	0.9 (0.6)	0.6 (0.4)	1.3 (1.0)	1.1 (1.1)	5.8 (4.6)

[a] Values represent the sum of aberrations on 5q, 5p, monosomy 5, and other.
[b] Two types of aberrations were observed simultaneously in the same cells.
[c] $P < 0.0001$ compared with controls.

recovery but was significantly higher than that of 48-h recovery.

Chromosome 4 and 5 Abnormalities Detected by FISH. Fig. 1 shows typical results of FISH analyses. Table 2 lists the numbers and types of chromosome 5 aberrations in lymphocytes of lung cancer cases and controls after 48 h of recovery. Chromosome 5 aberrations were categorized as 5q aberrations (including 5q13-q22 aberrations), 5p aberrations, monosomy 5, other aberrations of 5q or 5p, and the total number of chromosome 5 aberrations. The average number of metaphases for each sample examined was 103 for the cases and 147 for the controls. The percentages of cells with any chromosome 5 aberration (13.4%), with 5q aberrations (9.0), with 5q13-q22 aberrations (5.3%), and with monosomy 5 (2.4%) in cases were significantly higher than those of controls (4.6, 2.4, 0.6, and 1.0%, respectively; $P < 0.0001$).

Table 3 lists the numbers and types of chromosome 4 aberrations in the lymphocytes of lung cancer cases and controls after 48 h of recovery. The average number of metaphases of each sample examined was 72 for the cases and 104 for the controls. The percentage of chromosome 4 abnormalities was similar in cases and controls.

Table 4 summarizes the mean proportions of cells with various abnormalities in cases and controls. Chromosomal region 5q13-q22 seemed to be a target for mutagenic damage in the lung cancer cases. Forty eight h after treatment, this region exhibited 38.4% of all abnormalities on chromosome 5 in the lung cancer cases but only 14.5% in the controls ($P < 0.0001$). Therefore, the survival rates of chromosome damage were not random with respect to the break sites. Chromosome 7 painting analysis showed no difference between cases and controls (data not shown), similar to our findings in a previous study (7).

Abnormalities Detected by G Banding. G banding was used to further confirm the location of break points on chromosome 5 in lymphocytes after 48 h of recovery in five lung cancer cases. The most frequent breakpoints were located around 5q21-q22 and 5q31. Fig. 2 shows the typical results of G banding analyses.

Discussion

In vitro chromosomal analyses have been widely used to study individual sensitivity to genotoxicity and cancer risk. Using a molecular epidemiological approach, we and other investigators have found that lymphocyte analysis of individuals can be used to identify those at a higher risk of developing cancer (1–4, 12). In a comprehensive study of the clastogenic effects of 16 mutagens, Yunis *et al.* (5) found that these mutagens

Table 3 Chromosome 4 abnormalities in lymphocytes of lung cancer cases and controls after 48 h of recovery

Subjects	No. of cells examined	No. (%) of cells with chromosome 4 aberrations				
		4q	4p	Monosomy 4	Other	Total
Cases						
1	48	3 (6.3)	1 (2.1)	2 (4.2)	0 (0.0)	6 (12.5)
2	46	2 (4.3)	1 (2.2)	0 (0.0)	0 (0.0)	3 (6.5)
3	42	3 (7.1)	0 (0.0)	1 (2.4)	0 (0.0)	4 (9.5)
4	91	6 (6.6)	1 (1.1)	2 (2.2)	0 (0.0)	9 (9.9)
5	109	3 (2.8)	0 (0.0)	4 (3.7)	0 (0.0)	7 (6.4)
6	28	3 (10.7)	1 (3.6)	1 (3.6)	0 (0.0)	5 (17.9)
7	60	5 (8.3)	1 (1.7)	0 (0.0)	1 (1.7)	7 (11.7)
8	43	2 (4.7)	1 (2.3)	1 (2.3)	0 (0.0)	3 (7.0)[a]
9	184	14 (7.6)	0 (0.0)	6 (3.3)	4 (2.2)	24 (13.0)
Mean	72.3	4.6 (6.6)	0.7 (1.4)	1.9 (2.3)	0.6 (0.4)	7.6 (10.5)
Controls						
1	103	1 (1.0)	1 (1.0)	7 (6.8)	4 (3.9)	13 (12.6)
2	121	2 (1.7)	0 (0.0)	3 (2.5)	4 (3.3)	9 (7.4)
3	56	7 (12.5)	0 (0.0)	1 (1.8)	0 (0.0)	8 (14.3)
4	124	2 (1.6)	2 (1.6)	0 (0.0)	0 (0.0)	4 (3.2)
5	49	6 (12.2)	0 (0.0)	3 (6.1)	0 (0.0)	9 (18.4)
6	121	5 (4.1)	1 (0.8)	2 (1.7)	0 (0.0)	8 (6.6)
7	61	4 (6.6)	0 (0.0)	0 (0.0)	0 (0.0)	4 (6.6)
8	42	8 (19.0)	1 (2.4)	1 (2.4)	1 (2.4)	10 (23.8)[a]
9	43	6 (14.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (14.0)
10	315	11 (3.5)	0 (0.0)	2 (0.6)	1 (0.3)	14 (4.4)
Mean	103.5	5.2 (7.8)	0.5 (0.6)	1.9 (2.3)	1.0 (0.9)	8.5 (11.1)

[a] Both 4q and 4p aberrations were observed in the same cells.

Table 4 Percentage of cells with chromosome abnormalities in lymphocytes of lung cancer cases and controls after 48 h of recovery

Chromosome or region	Cases		Controls		*P*
	n	$\bar{P} \pm Sp$ (%)[a]	*n*	$\bar{P} \pm Sp$ (%)	
4	9	10.8 ± 3.8	10	11.1 ± 6.7	NS[b]
5	16	13.9 ± 4.6	14	4.6 ± 2.2	<0.001
5q	16	9.0 ± 2.8	14	2.4 ± 2.0	<0.001
5q13-q22[c]	16	5.3 ± 2.6	14	0.6 ± 0.8	<0.001

[a] $\bar{P}$ mean percentage of cells with chromosome abnormalities; Sp, standard error.
[b] NS, not significant.
[c] 5q13-q22 contained 38.4% of all chromosome 5 abnormalities in cases and 14.5% controls ($P < 0.001$).

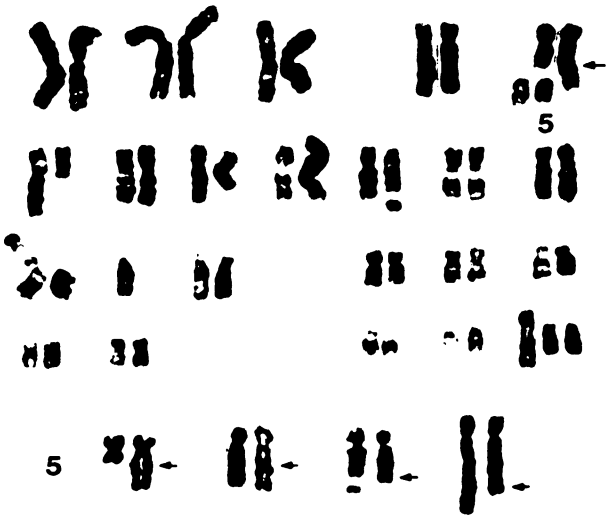

Fig. 2. Karyotype of lymphocyte of a lung cancer case 48 h after treatment. A portion of one chromosome 5 has been deleted. The breakpoint is around 5q21-q22. Several marker chromosomes involving chromosome 5 are visible at the *bottom* of the field (*arrow*).

acted on the cells in a wide variety of ways at the molecular level but produced breaks at similar sites in normal individuals. Dave *et al.* (6) studied the cellular responses of patients with melanoma and with head-and-neck carcinoma and noted that the sites of breakage in response to bleomycin were distinctly different in the two groups. In melanoma patients, breaks were more prevalent on chromosomes 1, 6, and 9, whereas in the head-and-neck cancer patients, there were more breaks on chromosomes 3 and 7. In our previous case-control study, the frequencies of bleomycin-induced chromatid breaks on chromosomes 2, 4, and 5 were higher in lung cancer patients than in controls. Moreover, the breaks clustered at several chromosomal regions, notably 5q21-q22, 5q31, and 4q25-q27 (7).

Deletion of 3p14-p21 is present in virtually all cases of small cell lung carcinoma (13). Our data on induced chromosome damage did not fit this pattern. However, biopsy specimens taken at one phase of cancer development may not reflect the entire sequence of genetic alterations. The predilection of induced breaks on 5q is of special interest because of the parallel chromosomal aberrations reported in lung tumors. Using the comparative genomic hybridization procedure to map DNA gains and losses in primary small cell carcinomas, Ried *et al.* (14) found not only 3p losses but also frequent 5q losses. Deletion of 5q has been frequently found in recent cytogenetic and molecular investigations of lung tumors (15–17).

Chromosome aberrations involving 5q have also been found in other neoplasms. Theodossiou *et al.* (18) reported a 5q deletion in acute lymphoblastic leukemia, and Benitez *et al.* (19) followed four patients with myelodysplastic syndromes from preleukemic to leukemic phases. In one patient, 5q− was the only aberration in the preleukemic stage, whereas in the

other three, 5q− occurred during disease development. These authors believe that deletion of 5q is an early event in tumorigenesis. Of more interest in relating 5q aberrations to carcinomas is the report of Hukku and Rhim (20), who immortalized human keratinocytes by treatment with 4-nitroquinoline-1-oxide or infection with adenovirus 12 and Simian virus 40. These investigators found that beginning at passage 10, a clone with a marker chromosome containing part of 5q appeared. These investigators also regarded the 5q aberration as a critical early event in tumorigenesis.

To determine which chromosomal lesions survive in the absence of the mutagen, we allowed the cells to recover in a drug-free medium. Because we did not know exactly how fast the drug-injured cells would recover and resume mitosis, we harvested the cells at two times, 24 and 48 h after removing the clastogen. Our data showed that the 24-h recovery samples contained predominantly metaphases with chromatid breaks, indicating that most of the cells still lingered in the original stages of the cell cycle. The 48-h recovery samples, however, had a higher percentage of metaphases with chromosome-type aberrations (Table 1). When cells are reincubated, after being exposed to a mutagen, in a drug-free medium, several explanations for chromatid damage may exist: (*a*) severely damaged cells may perish, and less damaged cells may have some of the genetic lesions repaired; (*b*) many chromatid aberrations, following the advance of the cell cycle, are converted into chromosome-type aberrations, such as reciprocal translocations or dicentric formations, that represent a form of repair (or misrepair); (*c*) a subpopulation of lymphocytes with little or no genetic damage may grow selectively faster than others; and (*d*) a combination of the above may occur. However, without a study of cell cycle kinetics, no definite conclusions can be made at this time.

Therefore, we used 48-h recovery samples for more detailed FISH experiments. Our data demonstrated that the breaks on chromosome 5, especially in 5q13-q22, did indeed survive better than the breaks at other loci in lung cancer cases. Whether this differential in break survival rates is due to differential repair rates or differential survival rates remains a question for future investigations.

We also found that the 5q lesions were mostly simple deletions or translocations with other chromosomes. Moreover, there was a significant difference ($P < 0.0001$) in the proportions of 5q aberrations that survived in lung cancer cases and controls. Using chromosome-painting techniques, we found that in one primary lung tumor tissue culture, 95% of the cells examined had four copies of chromosome 5, one of which had a 5q deletion. The breakpoint was 5q21-q22, the same breakpoint we found in the lymphocytes. It is, therefore, very likely that lesions in the regions 5q13, 5q21-q22, and 5q31 are not only the hot spots of intrinsic sensitivity but are also among the early genetic changes in lung cancers.

The finding of nonrandom mutagen-induced breaks and nonrandom survival of chromosome lesions in lung cancer patients raises the question of whether chromosome 5 changes are related to responses to mutagens such as cigarette smoke, radiation, and other occupational carcinogens implicated in lung carcinogenesis. This hypothesis has been supported by several reports that have suggested that chromosome 4 abnormalities in hematological cancers may result from exposure to environmental carcinogens (21). Occupational exposures have been implicated in cancer patients with monosomy 5 (22). Chromosome 5 abnormalities are an early event in chemical carcinogen-induced tumorigenesis (20). Many acute myeloid leukemia patients with del5/del(5q) have had substantial occupational exposures to putative environmental carcinogens, suggesting that abnormalities of chromosome 5 may be markers of mutagen exposure-related leukemia (23).

We believe that our experimental method of correlating mutagen-induced chromatid breaks with chromosome breaks after cellular recovery adds more useful information than measuring chromatids alone. Nevertheless, experimental data obtained from cell cultures must be correlated with findings from tumor cells *in vivo* or at least from short-term cultures before genetic changes related to the culturing process occur. We plan to conduct similar cytogenetic analyses of paired lymphocytes and tumor tissue.

Because a chromosomal band can contain dozens of genes, breakage at one band does not necessarily indicate that a critical gene is truncated, mutated, or severely damaged. Chromosome painting and G banding cannot precisely define breakpoints. However, chromosome painting has one distinct advantage: it can easily reveal whether a break actually results in a terminal deletion or translocation. If a crucial gene is located distal to the break, and the distal portion of the chromosome is lost, one can safely conclude that this gene is lost. On the other hand, if the breakpoint is in or near the gene in question, other procedures must be used to pinpoint the break locations. We plan to use specific 5q DNA cosmid or yeast artificial chromosome clones to help identify the breakpoints in both chromatid and chromosome aberrations. The results of these studies will determine whether further detailed molecular genetic and immunocytochemical experiments should be conducted. If the investigations proposed above prove to yield useful information, parallel studies on other types of cancer can be initiated using this approach.

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