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Effect of Smoking on Chromosomes Compared with That of Radiation in the Residents of a High-Background Radiation Area in China

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Low dose radiation/Chromosome aberrations/Smoking/Human lymphocytes

Cytogenetic investigation of stable-type aberrations (translocations) was carried out with our improved methods on 28 elderly individuals in a high-background radiation area (HBRA) in China, and on 24 elderly individuals in a control area (CA). The level of radiation in HBRA is 3 to 5 times higher than in CA. The mean frequencies of translocations per 1,000 cells in HBRA and CA were 12.4 ± 5.3 and 10.0 ± 3.8, respectively. No significant difference was found in the frequencies between HBRA and CA ($P>0.05$, Mann-Whitney U test). When elderly individuals in HBRA and CA were classified into four subgroups of HBRA nonsmokers, HBRA smokers, CA nonsmokers, and CA smokers, a significant difference was found in the frequencies between CA smokers and CA nonsmokers ($P<0.05$, Mann-Whitney U test). Furthermore a tendency of difference (a near T-value of 0.05 level) was found in a comparison of HBRA smokers vs. CA nonsmokers. The present results indicate that the elevated level of natural radiation in HBRA plays a less significant part than smoking in bringing about the induction rate of stable-type aberrations (translocations) in those areas.

INTRODUCTION

Chromosome aberrations in circulating lymphocytes are considered the most reliable indicator of radiation exposure, especially at low doses. When evaluating the effects of chronic low-dose radiation on health, the confounding factor of smoking is always taken into consideration[1–3].

There is a high background radiation area in the south of China (HBRA) where the level of natural radiation is 3 to 5 times higher than in a control area (CA) because the soil and building materials contain nuclides of Th-232 and U-238 decay products. Epidemiological studies performed in this area detected no significant increase in either cancer morbidity or mortality[4], but a significant increase was found in the frequency of unstable chromosome aberrations (dicentrics and rings)[5]. Our previous studies of the stable aberrations (translocations) by the chromosome painting method showed no significant difference in the frequencies between these two areas[6,7].

In the present study, more age-matched individuals were studied in these areas, and statistical analyses focused on the effect of the smoking habits.

MATERIALS AND METHODS

Chromosome translocations in peripheral lymphocytes were investigated in 28 elderly individuals in HBRA and in 24 elderly individuals in CA. Populations in these two areas share almost the same culture and genetic background in the same province. The exposed radiation dose of each individual was estimated from the dose measured by an electric pocket dosimeter (Aloka PDM-101) for 24 hours in all subjects except TO01, YO01, and XO01. The doses of these 3 subjects were estimated from the dose rate measured by an NaI scintillation survey meter (Aloka TCS-166) at their homes. The methods for estimating individual doses were reported in detail by Morishima *et al*.[8] and Yuan *et al*,[9].

Blood samples were collected in their hamlets and brought by car to a cytogenetic laboratory established in Enping Municipal Hospital. They were processed within 7 hours after collection, basically under our improved method of chromosome preparation for low-dose study[10], except

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that the blood culture was performed in a water bath at 37°C without CO_2 gas. Colcemid was added for the entire 48 hours during the culture. The samples were cultured and fixed in 1996, 1998, and 2000, respectively. The fixed-cell suspensions were stored in a freezer until we prepared air-dry slides in a warm and humidified box[10,11].

Chromosome painting was performed according to the method by Yamada *et al.*,[12] with slight modifications. Chromosomes 1, 2, and 4 (representing 22.71% and 22.34% of the human genome in male and in female, respectively[13]) were painted by the use of Biotin-labeled whole chromosome painting probes (Cambio) specific for each chromosome. The fluorescence of the avidin-FITC signal was enhanced by another avidin-FITC staining following Biotin-Goat-Anti-Avidin treatment. Counterstaining was done with propidium iodide. Rearranged chromosomes were detected by the use of a 2B filter, and translocations were distinguished from dicentrics with a 2G filter in a NIKON fluorescence microscope. All abnormal or suspect abnormal cells were photographed, and their positions on the slide

Table 1. Chromosome translocations detected in 28 elderly individuals in HBRA

Case	Sex	Age (years)	Smoking habits	Cells scored	Translocation	FG/1000Cells ± SEM/1000*	Dose/year** (mSv)	TotalDose** (mSv)
HW01	F	64.3	n	2,411	7	8.2 ± 3.1	2.38	153.4
HW03	F	56.7	n	1,598	6	10.6 ± 4.3	3.29	186.5
HW04	F	58.1	n	2,436	8	9.2 ± 3.3	2.75	159.7
HW05	F	67.8	n	1,195	2	4.7 ± 3.3	2.67	181.2
HW06	F	70.4	n	2,552	13	14.3 ± 4.0	2.79	196.4
HW07	F	55.4	n	8,731	23	7.4 ± 1.5	2.76	153.1
HW08	F	61.5	n	9,736	31	9.0 ± 1.6	2.82	173.2
HW09	F	64.7	n	3,290	17	14.5 ± 3.5	2.60	168.4
HW10	F	69.4	n	2,670	10	10.5 ± 3.3	2.07	143.6
HW11	F	69.3	n	2,595	27	29.3 ± 5.6	2.74	189.5
HW12	F	65.1	n	4,047	13	9.0 ± 2.5	2.84	184.6
HW13	F	68.2	n	4,737	19	11.3 ± 2.6	2.39	163.0
HW14	F	69.2	n	5,007	42	23.6 ± 3.6	1.91	132.3
HW15	F	65.3	n	6,573	33	14.1 ± 2.5	2.89	188.9
YO01	M	89.5	n	2,233	11	13.7 ± 4.1	2.92	261.3
HO08	M	66.3	n	5,678	38	18.6 ± 3.0	2.50	166.0
HO13	M	55.4	n	4,829	18	10.4 ± 2.4	2.96	164.3
XO01	M	74.5	y	2,093	13	17.3 ± 4.8	3.08	229.5
HO01	M	59.0	y	4,454	15	9.4 ± 2.4	2.39	141.1
HO02	M	53.3	y	5,669	18	8.8 ± 2.1	2.78	148.2
HO03	M	58.1	y	8,375	30	10.0 ± 1.8	3.18	184.5
HO04	M	53.2	y	4,514	17	10.5 ± 2.5	2.76	146.6
HO05	M	66.2	y	2,333	6	7.1 ± 2.9	2.62	173.7
HO06	M	55.5	y	7,144	41	15.9 ± 2.5	2.97	165.0
HO07	M	61.7	y	5,100	21	11.4 ± 2.5	3.15	194.1
HO12	M	58.5	y	10,079	28	7.7 ± 1.5	2.65	154.9
HO14	M	54.6	y	1,357	9	18.4 ± 6.1	2.53	137.9
HO15	M	54.0	y***	1,629	7	11.9 ± 4.5	2.54	137.4

FG: genome-equivalent frequency of translocation. *Standard error of the mean.
**Air Kerma ***Former smoker

were recorded by an automated stage system and/or by a special coordinate slide. The judgment of translocations was made directly under the microscope as well as in the photographs by at least 2 observers. Scored translocations included one-way and reciprocal translocations between 2 chromosomes and three-way translocations involving 3 chromosomes. A three-way translocation involving 3 chromosomes was counted as 2 translocations. Since most incomplete (one-way) exchanges are most likely originated from reciprocal exchange[14–16], we included one-way translocations in scored translocations. The frequencies of translocations per 1,000 cells were scaled to genome-equivalent frequencies (F_G) by the formula reported by Lucas *et al.*[17] as follows: $F_G=F_p/2.05f_p(1-f_p)$, where F_p is the frequency of translocations detected by painting and f_p is the fraction of the genome painted. When chromosomes 1, 2, and 4 are painted, 36.0% (for male) and 35.6% (for female) of the translocations induced in the whole genome are detected.

The age ranges in elderly individuals were 53.2–89.5 (63.0 ± 8.0) in HBRA and 53.0–70.8 (63.1 ± 5.5) in CA.

The Mann-Whitney U test was used to compare the frequencies of the translocations between two groups. A variance test of the homogeneity of the Poisson distribution was used to test homogeneity.

RESULTS

The accumulated doses (air kerma) in elderly individuals ranged from 132.3 to 261.3 mSv (170.7 ± 28.4 mSv) in HBRA and from 36.1 to 73.6 mSv (45.6 ± 9.3 mSv) in CA,

Table 2. Chromosome translocations detected in 24 elderly individuals in CA

Case	Sex	Age (years)	Smoking habits	Cells scored	Translocation	FG/1000 Cells ± /1000*	Dose/year** (mSv)	TotalDose** (mSv)
CO16	M	59.1	n	5,749	22	10.6 ± 2.3	0.66	39.1
CW01	F	61.2	n	2,306	9	11.0 ± 3.7	0.62	38.2
CW04	F	67.0	n	3,519	7	5.6 ± 2.1	1.10	73.6
CW05	F	70.8	n	4,666	18	10.8 ± 2.6	0.75	52.9
CW06	F	68.1	n	2,409	6	7.0 ± 2.9	0.68	46.0
CW07	F	53.0	n	2,860	9	8.8 ± 2.9	0.69	36.7
CW09	F	70.3	n	4,138	9	6.1 ± 2.0	0.71	49.7
CW10	F	61.5	n	2,657	4	4.2 ± 2.1	0.65	40.2
CW11	F	55.5	n	5,932	10	4.7 ± 1.5	0.85	47.2
CW12	F	63.1	n	6,157	14	6.4 ± 1.7	0.70	44.0
CW13	F	65.8	n	1,777	7	11.1 ± 4.2	0.68	44.7
CW14	F	67.3	n	2,924	14	13.5 ± 3.6	0.96	64.8
CW16	F	70.3	n	9,633	43	12.6 ± 1.9	0.77	53.9
CW17	F	65.2	n	5,950	13	6.1 ± 1.7	0.66	43.1
CW18	F	69.3	n	3,464	6	4.9 ± 2.0	0.82	56.6
CO12	M	63.3	n	3,369	14	11.5 ± 3.1	0.64	40.7
TO01	M	70.5	y	1,424	5	9.8 ± 4.4	0.53	37.4
CO01	M	61.2	y***	3,023	19	17.5 ± 4.0	0.80	49.1
CO02	M	57.5	y	2,208	9	11.3 ± 3.8	0.65	37.2
CO03	M	57.1	y	3,275	21	17.8 ± 3.9	0.67	38.4
CO04	M	65.3	y	1,186	6	14.1 ± 5.7	0.63	41.0
CO11	M	55.6	y	3,345	17	14.1 ± 3.4	0.65	36.1
CO14	M	61.1	y	6,282	21	9.3 ± 2.0	0.68	41.3
CO13	M	55.3	unknown	4,864	20	11.4 ± 2.6	0.78	43.3

FG: genome-equivalent frequency of translocation. *Standard error of the mean.
**Air Kerma ***Former smoker

Table 3. Translocation frequency and homogeneity test in each subgroup

Subgroups	No.of subjects	Agerange (years)	Average age	Cells scored	Range of FG	FG/1000 cells ± SE	χ^2	DF
HBRA								
smokers	11	53.2–74.5	59.0	52,747	7.1–18.4	11.67 ± 3.86	13.0	10
Nonsmokers	15	55.4–89.5	65.2	62,716	4.7–18.6	11.03 ± 3.49	15.6	14
CA								
smokers	7	55.6–70.5	61.2	20,743	9.3–17.8	13.41 ± 3.45	5.3	6
Nonsmokers	16	53.0–70.8	64.4	67,510	4.2–13.5	8.43 ± 3.12	17.2	15

DF: Degrees of freedom.
FG: genome-equivalent frequency of translocation.
SE: Standard error.
Note: In HBRA nonsmokers, 2 outliers were excluded. The smoking habits of CO13 was unknown, and this subject was excluded from the statistical analysis.

Table 4. Results of Mann-Whitney U test between any two subgroups

Combination of two subgroups	T-value	0.05 Level of T-value	Significant difference
HBRA smokers vs. HBRA nonsmokers	133	106	no
HBRA smokers vs. CA smokers	56	44	no
HBRA smokers vs. CA nonsmokers	115.5	114	no
CA smokers vs. CA nonsmokers	45	54	yes
HBRA nonsmokers vs. CA nonsmokers	180	169	no
HBRA nonsmokers vs. CA smokers	60.5	52	no

respectively. The difference of accumulated doses between HBRA elderly individuals and CA elderly individuals was about 130 mSv. The total numbers of cells analyzed in elderly individuals were 123,065 in HBRA and 93,117 in CA. Age, sex, smoking habits, scored cells, the result of chromosome analysis and the accumulated dose for each subject are summarized in Tables 1 and 2.

The mean frequencies of translocations in HBRA elderly individuals were 12.4 ± 5.3 (from 4.7 to 29.3, χ^2=61.16 with 27DF) and show a poor homogeneity. Two individuals (HW11 and HW14) in HBRA had unusually high stable aberration frequencies. HW11 had fluoroscopy in the past, but we could not find a suspect confounding factor for HW14. Since those two cases were clearly outliers, we excluded them from the statistical analyses. Then the mean frequency of translocations becomes 11.3 ± 3.6 (from 4.7 to 18.6, χ^2=28.67 with 25DF), showing a fairly good homogeneity. The mean frequency of translocations in CA elderly individuals was 10.0 ± 3.8 (from 4.2 to 17.8, χ^2=33.21 with 23DF), which also shows a fairly good homogeneity. Whether two outliers (HW11 and HW14) were excluded or not, a somewhat higher frequency of translocations was observed in HBRA elderly individuals compared with CA elderly individuals. The increase, however, was not significant (P>0.05, Mann-Whitney U test). The elderly individuals in HBRA and CA were classified into four subgroups of HBRA nonsmokers, HBRA smokers, CA nonsmokers and CA smokers. The smoking habits of CO13 was unknown, and this subject was excluded from the statistical analysis. The results of the test of homogeneity for these four subgroups are shown in Table 3. A good homogeneity is shown in all subgroups. Mann-Whitney U tests between all two subgroups are shown in Table 4. A significant difference was found in the frequencies between CA smokers and CA nonsmokers (P<0.05, Mann-Whitney U test). The tendency of difference (a near T-value of 0.05 level) was found in a comparison of HBRA smokers vs. CA nonsmokers. Smoking habits have an effect on the translocation yield. However, no difference was found in a comparison of HBRA smoker vs. HBRA nonsmokers. There is a possibility that the elevated radiation in HBRA suppressed chromosome aberrations caused by smoking.

DISCUSSION

Jiang *et al.*[5] found a significant increase in the frequency of unstable chromosome aberrations (dicentrics and rings) in HBRA in comparison with CA. The unstable aberrations and

the stable aberrations are induced by radiation with about equal frequency[18]. However, the frequency of stable chromosome aberrations (translocations) was much higher than that of dicentrics and rings in both areas, and the significant difference was not detected between those areas[6,7]. Therefore the present result is consistent with epidemiological studies performed in HBRA where no significant increase in either cancer morbidity or mortality was found[4]. In our present study where we studied more subjects than before, a statistical analysis still revealed no difference in the frequency of translocations between HBRA elderly individuals and CA elderly individuals. Furthermore, the present result indicates that the contribution of an elevated level of natural radiation in HBRA to the induction rate of stable type aberrations (translocations) is smaller than that of smoking in those areas. It indicates that the effects of confounding factors are much larger than those of radiation in those areas.

Most studies on the stable aberrations relating to the low-dose radiation report similar conclusive results as follows: Salomaa *et al*.[19] could not observe an increase in the mean translocation yields among residents living in the area contaminated by radionuclides from the Chernobyl fallout seven years after the reactor accident in comparison with the control cohort. Stephan *et al*.[20] analyzed translocations in the lymphocytes of individuals living near the Semipalatinsk nuclear test site. The mean frequency of translocations in these subjects, who had been irradiated during childhood, did not differ from a control value. Littlefield *et al*.[3] observed no increase in translocation yields among 118 Estonian cleanup workers compared with 50 control individuals. Snigiryova *et al*.[21] could find no correlation between individual translocation frequencies and documented doses in Chernobyl cleanup workers. Akleyev *et al*.[22] found no elevated translocation frequency in 34 subjects from the Techa River population with a reconstructed mean cumulative RBM dose of 1.52 Gy (0.28-2.66 Gy). Lindholm *et al*.[23] detected no increase of the translocations in a Finnish population that had been chronically exposed to high concentrations of domestic radon. On the other hand, Bauchinger *et al*.[24] detected an increase in the frequency of translocations in the Techa River population. Tucker *et al*.[1] reported a significant correlation between translocation frequency and documented cumulative dose in 58 workers at the Sellafield nuclear facility. The exposure ranged from 173 to 1,108 mSv, and the average working period was over 30 years. Also, nonsmokers showed a stronger dose response than smokers. Most of the studies[1,2,3,25] observed increased translocation frequencies as a result of smoking. Pressl *et al*.[26] found a marginal impact of smoking habits on the translocation yield.

In conclusion, it seems that the low-dose radiation at least up to 3–5 times higher than the normal level has no considerable effect on human health, if it is compared with the effects of all kinds of mutagenic factors in normal living circumstances.

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