Imperceptible Effect of Radiation Based on Stable Type Chromosome Aberrations Accumulated in the Lymphocytes of Residents in the High Background Radiation Area in China

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Natural radiation/Translocations/Chromosome painting/Environmental mutagen/Lymphocytes.

Cytogenetic investigation of stable type aberrations (translocations) was performed with our improved methods in 6 children and 15 elderly persons in a high background radiation area (HBRA) in China, and in 8 children and 11 elderly persons in a control area. The total numbers of cells analyzed in elderly persons were 68,297 in HBRA and 35,378 in controls and in children were 45,535 in HBRA and 56,198 in controls. On average 5138 cells per subject were analyzed. The variation in the frequencies of translocations per 1000 cells was small in children while it was large in elderly persons. No significant difference was found in the frequencies between HBRA and control ($P > 0.05$, Mann-Whitney U test). On the other hand, correlation between age and translocation frequencies was significant at the 1% level ($r_s = 0.658$ with 37DF, Spearman rank correlation test). The contribution of an elevated level of natural radiation in HBRA in China to the induction of stable type chromosome aberrations does not have a significant effect compared with the contribution of chemical mutagens and/or metabolic factors. The present study suggests that the probability of the risk of causing malignant and/or congenital diseases by the increased amount of radiation is imperceptible in HBRA where the level of natural radiation is 3 to 5 times higher than that in the control area.

INTRODUCTION

Chromosome aberrations in circulating lymphocytes are considered the most reliable indicator of radiation exposure, especially at low doses. The health effects of low dose (rate) radiation has been an important subject of study in radiation biology. The significance of radiation-induced stable chromosome aberrations (translocations) has come to be the focus of general interest[1–5].

There are several places in the world where the level of natural radiation is unusually high. In order to study the health effect of such high radiation levels at extremely low dose rates, we have been analyzing chromosomes in the lymphocytes of residents in a high background radiation area in the South of China (HBRA) using our improved cytogenetic method. The level of radiation in this area is 3 to 5 times higher than that in a control area 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[6], while a significant increase was found in the frequency of unstable chromosome aberrations (dicentrics and rings)[7]. Preliminary studies of the stable aberrations (translocations) by the chromosome painting method seemed to show no difference in the frequencies between these two areas[8,9].

In the present report we describe the result of further studies on translocations.

MATERIALS AND METHODS

Cytogenetic investigation was performed in 6 children and 15 elderly persons in HBRA and in 8 children and 11 elderly persons in the control area. Populations in these two areas have almost the same culture and genetic background in the same province. Except for one subject (HW14), they had no significant medical exposure other than occasional chest X-ray examination which contributes marginally to the cumulative dose of the individuals. Therefore we excluded HW14 in the statistical analyses. The radiation dose of each individual was estimated from the dose measured by an electric pocket dosimeter (Aloka

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PDM-101) for 24 h in all subjects other than SO01, SO03, TO01, TO03, YO01, YO03 and XO01. The doses of those 7 subjects were estimated from the dose rate measured by a NaI scintillation survey meter (Aloka TCS-166) in their homes. The methods for estimation of individual doses were reported in detail by Morishima *et al.*[10] and Yuan *et al.*[11]. The calculation of individual doses in our studies[8,9] is corrected in the present study and the values are about 15% less than previously reported in both areas.

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 following our improved method of chromosome preparation for low dose study[12]. The only difference was that the blood culture was performed in a water bath at 37°C without CO_2 gas in the present study. Colcemid was added for the entire 48 hours of culture. Seven samples (SO01, SO03, TO01, TO03, YO01, YO03 and XO01) were cultured and fixed in 1996, and the others were cultured and fixed in 1998. The fixed cell suspensions were stored in a freezer until we prepared air-dry slides in a warm and humidified box[12,13].

Chromosome painting was performed according to the method by Yamada *et al.*[14] with slight modifications. Chromosome Nos. 1, 2 and 4 (representing 22.71% and 22.34% of the human genome in male and in female, respectively[15]) were painted using specific Biotin-labeled whole chromosome painting probes (Cambio). The fluorescence of the avidin-FITC signal was enhanced by another avidin-FITC staining following Biotin-Goat-Anti-Avidin treatment. Counter-staining was with propidium iodide. Rearranged chromosomes were detected using a 2B filter, and translocations were distinguished from dicentrics using a 2G filter in a NIKON fluorescence microscope (Fig. 1). All abnormal or suspect abnormal cells were photographed and their positions on the slide recorded by an automated stage system and/or by a special coordinate slide. Judgment of translocations was made directly in the microscope as well as in the photograph 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 the majority of apparently incomplete (one-way) exchanges are indeed complete (reciprocal) and probably originate from terminal exchange[16–18], we included one-way translocations in scored translocations. The frequencies of translocations per 1000 cells were scaled to genome-equivalent frequencies (F_G) by the formula reported by Lucas *et al.*[19] 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) or 35.6% (for female) of the translocations induced in the whole genome are detected.

The age ranges in elderly persons were 53.2–89.5 (61.6 ± 9.9) in HBRA and 55.3–70.5 (60.4 ± 4.6) in controls, and those in children were 10.8–13.5 (12.5 ± 0.9) in HBRA and 10.3–13.8 (12.3 ± 1.3) in controls.

The Mann-Whitney U test was used to compare the frequencies of the translocations in the two areas. A variance test of the homogeneity of the Poisson distribution was used to test homogeneity. The Spearman rank correlation test was used to perform a correlation analysis.

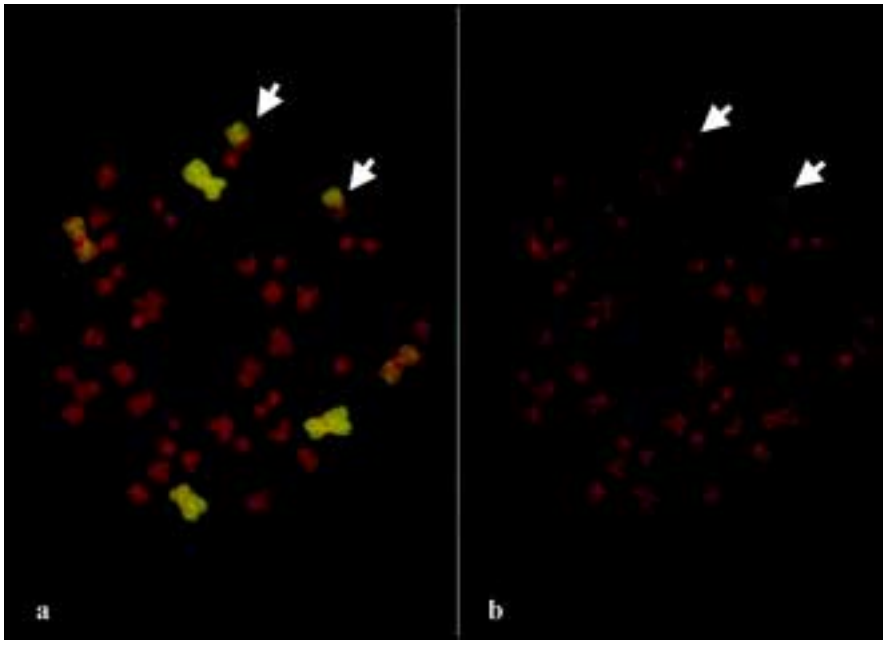

Fig. 1. A metaphase lymphocyte with a translocation detected in the present study. a: Rearrangement between chromosomes 4 and C (arrows) is shown by a chromosomal painting method with whole chromosome painting probes for Nos. 1, 2 and 4. b: A PI counter-stained preparation of the same metaphase showing centromere constrictions of both rearranged chromosomes (arrows).

Table 1. Chromosome translocations detected in 21 cases in HBRA.

Case	Sex	Age (year)	Cells scored	Translocation	FG/1000 cells ± SEM*	Dose/year** (mSv)	Total dose** (mSv)
YO01	M	89.5	2233	11	13.7 ± 4.1	2.92	261.3
XO01	M	74.5	2093	13	17.3 ± 4.8	3.08	229.5
HO01	M	59.0	4454	15	9.4 ± 2.4	2.39	141.1
HO02	M	53.3	5669	18	8.8 ± 2.1	2.78	148.2
HO03	M	58.1	8375	30	10.0 ± 1.8	3.18	184.5
HO04	M	53.2	4514	17	10.5 ± 2.5	2.76	146.6
HO05	M	66.2	2333	6	7.1 ± 2.9	2.62	173.7
HO06	M	55.5	1145	5	12.1 ± 5.4	2.97	165.0
HO07	M	61.7	5100	21	11.4 ± 2.5	3.15	194.1
HO12	M	58.5	10079	28	7.7 ± 1.5	2.65	154.9
HO13	M	55.4	4829	18	10.4 ± 2.4	2.96	164.3
HO14	M	54.6	1101	8	20.2 ± 7.1	2.53	137.9
HO15	M	54.0	1629	7	11.9 ± 4.5	2.54	137.4
HW08	F	61.5	9736	31	9.0 ± 1.6	2.82	173.2
HW14	F	69.2	5007	42	23.6 ± 3.6	1.91	132.3***
YO03	F	10.8	1015	1	2.8 ± 2.8	3.02	32.6
HC01	M	12.8	7543	11	4.1 ± 1.2	2.48	31.6
HC02	M	12.1	10443	16	4.3 ± 1.1	2.15	25.9
HC03	M	13.5	3826	3	2.2 ± 1.3	2.84	38.4
HC04	F	12.8	9469	18	5.3 ± 1.3	3.24	41.4
HC05	F	12.8	13239	19	4.0 ± 0.9	2.74	s35.1

FG: genome-equivalent frequency of translocation. *Standard error of the mean. **Air kerma. ***Medical irradiation is not included.

RESULTS

The accumulated doses (air kerma) in elderly persons ranged from 132.3 to 261.3 mSv (172.3 ± 36.0mSv) in HBRA and from 32.5 to 49.1 mSv (39.6 ± 4.3 mSv) in the control, respectively. Those in children were from 25.9 to 41.4 mSv (34.2 ± 5.4 mSv) in HBRA and from 5.6 to 11.1 mSv (8.9 ± 2.2 mSv) in the control. The difference of the average doses between the elderly persons in these two groups was about 130 mSv which is higher than the doses received in more than half (693,470 out of 1,136,216) of A-bomb survivors[20]. The total numbers of cells analyzed in elderly persons were 68,297 in HBRA and 35,378 in controls, and in children 45,535 in HBRA and 56,198 in controls. Age, sex, accumulated dose and the result of chromosome analysis for each subject are summarized in Tables 1 and 2. Frequencies of translocations in relation to dose and age are shown in Figures 2 and 3, respectively.

The mean frequencies of translocations in children were 3.8 ± 1.1 (from 2.2 to 5.3, $\chi^2 = 1.6$ with 5 DF) in HBRA and 3.2 ± 2.0 (from 0 to 5.8, $\chi^2 = 8.8$ with 7 DF) in controls and in elderly persons 11.4 ± 3.6 (from 7.1 to 23.6, $\chi^2 = 14.8$ with 13 DF) in HBRA and 12.0 ± 3.8 (from 4.3 to 17.8, $\chi^2 = 12.0$ with 10 DF) in controls, showing good homogeneity. The ratios of one-way, reciprocal and three-way translocations (involving three chromosomes) were 24%, 73% and 3%, respectively. No significant difference was found in the frequencies of translocations between HBRA and the control ($P > 0.05$, Mann-Whitney U test) in both children and elderly persons. On the other hand, correlation between age and translocation frequencies was significant at the 1% level ($r_s = 0.658$ with 37 DF, Spearman rank correlation test).

DISCUSSION

The frequency of translocations in the lymphocytes observed in the present study was about 3 to 6 times higher on average than those of dicentrics reported by Jiang *et al.*[7] from the same areas. Statistical analysis revealed no difference in the frequency of translocations between HBRA and control groups in both children and elderly persons. However, the frequencies are significantly different between elderly persons and children in the present study. A positive effect of age on the induction of translocations was demonstrated here as in results reported by others[21–24].

Dual analyses by the conventional Giemsa staining method and by chromosome painting of the same metaphase demonstrated that the induction rate of dicentrics and translocations in the peripheral lymphocytes after in vitro exposure to radiation is about 1:1[25,26]. A dicentric chromosome accompanied by a fragment found in 48 h-cultured lymphocytes is a highly specific indicator of radiation exposure. The dicentric chromosome is an unstable aberration that is destined to disappear by cell division and therefore it is not accumulated in the body. On the other

Table 2. Chromosome translocations detected in 19 cases in the control.

Case	Sex	Age (year)	Cells scored	Translocation	FG/1000 cells ±SEM*	Dose/year** (mSv)	Total dose** (mSv)
TO01	M	70.6	1424	5	9.8 ± 4.4	0.53	37.4
SO01	M	58.7	653	1	4.3 ± 4.3	0.56	32.5
CO01	M	61.2	3023	19	17.5 ± 4.0	0.80	49.1
CO02	M	57.5	2208	9	11.3 ± 3.8	0.65	37.2
CO03	M	57.1	3275	21	17.8 ± 3.9	0.67	38.4
CO04	M	65.3	1186	6	14.1 ± 5.7	0.63	41.0
CO11	M	55.6	3345	17	14.1 ± 3.4	0.65	36.1
CO12	M	63.3	3369	14	11.5 ± 3.1	0.64	40.7
CO13	M	55.3	4864	20	11.4 ± 2.6	0.78	43.3
CO14	M	61.1	6282	21	9.3 ± 2.0	0.68	41.3
CO16	M	59.1	5749	22	10.6 ± 2.3	0.66	39.1
TO03	M	10.4	1742	3	4.8 ± 2.8	0.54	5.6
SO03	M	10.3	1056	1	2.6 ± 2.6	0.56	5.8
CC01	F	13.8	8185	17	5.8 ± 1.4	0.81	11.1
CC02	F	12.3	6886	13	5.3 ± 1.5	0.69	8.5
CC03	M	12.9	6564	8	3.4 ± 1.2	0.71	9.2
CC04	M	12.9	3004	0	0.0 ± 0.0	0.82	10.7
CC05	M	12.8	21710	19	2.4 ± 0.6	0.78	10.1
CC11	M	13.0	7051	4	1.6 ± 0.8	0.81	10.5

FG: genome-equivalent frequency of translcation. *Standard error of the mean. **Air kerma.

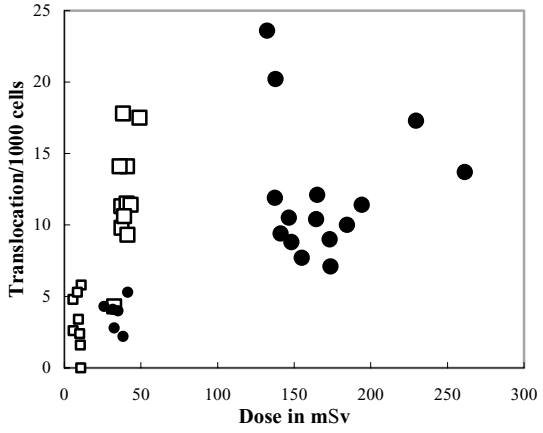

Fig. 2. Frequency of translocations in relation to dose. Smaller circles, HBRA children; larger circles, HBRA elderly persons; smaller squares, control children; larger squares, control elderly persons.

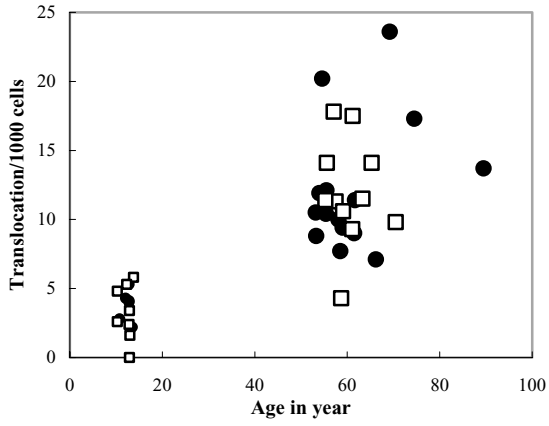

Fig. 3. Frequency of translocations in relation to age. Smaller circles, HBRA children; larger circles, HBRA elderly persons; smaller squares, control children; larger squares, control elderly persons.

hand, radiation-induced translocations seen in 48 h-cultured lymphocytes can not be distinguished from those induced by mutagens such as chemicals or metabolic factors[27]. The translocation is a stable type aberration that accumulates in the body and therefore may increase the probability of the risk of causing malignant and/or congenital diseases. The present result indicates that the contribution of an elevated level of natural radiation in HBRA in China to the induction rate of stable type aberrations (translocations) does not have a significant effect compared with the contribution from all other sources such as chemical mutagens and/or metabolic factors. This may explain why the epidemiological study could not detect an increase in cancer in the residents in this area despite the elevated natural radiation causing an increase in unstable type aberrations (dicentrics and rings).

Stephan *et al.*[28] 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. Snigiryova *et al.*[29] could not find a correlation between

individual translocation frequencies and documented doses in Chernobyl clean-up workers. Akleyev *et al.*[30] did not find an 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.*[31] could not detect an increase of the translocations in a Finnish population who had been chronically exposed to high concentrations of domestic radon. On the other hand, Bauchinger *et al.*[2] detected an increase in the frequency of translocations in the Techa River population. Tucker *et al.*[32] reported a significant increase in translocations and a dose dependency in radiation workers at the Sellafield nuclear facility. The threshold dose above which the frequency of translocations in exposed people become significantly higher than that of controls is to be revealed in future studies.

Factors related to lifestyle and living circumstances influence the yield of translocations. Ramsey *et al.*[33] showed that cigarette smoking greatly increased the frequencies of stable type chromosome aberrations (translocations and insertions). Smoking is a much-favored habit in the South of China and all the elderly males in our study are smokers.

Further study on nonsmokers in those two areas is under way.

REFERENCES

1. Awa, A. A., Nakano, M., Ohtaki, K., Kodama, Y., Lucas, J. and Gray, J. (1992) Factors that determine the in vivo dose-response relationship for stable chromosome aberrations in A-bomb survivors. J. Radiat. Res. (Tokyo) **33 Suppl.**: 206–214.
2. Bauchinger, M., Salassidis, K., Braselmann, H., Vozilova, A., Pressl, S., Stephan, G., Snigiryova, G., Kozheurov, V. P. and Akleye A. (1998) Fish-based analysis of stable translocations in a Techa River population. Int. J. Radiat. Biol. **73**: 605–612.
3. Kanda, R., Suzuki, M., Minamihisamatsu, M., Furukawa, A., Odaka, T. and Hayata, I. (1998) Non-fluorescent chromosome painting using the peroxidase/diaminobenzidine (DAB) reaction. Int. J. Radiat. Biol. **73**: 529–533.
4. Lucas, J. N., Deng, W., Oram, S. W., Hill, F. S., Durante, M., George, K., Wu, H., Owens, C. L. and Yang, T. (1999) Theoretical and experimental tests of a chromosome fingerprint for densely ionizing radiation based of F ratios calculated from stable and unstable chromosome aberrations. Radiat. Res. **151**: 85–91.
5. Heieh, W. H., Lucas, J. N., Hwang, J. J., Chan, C. C. and Chang, W. P. (2001) Biodosimetry using chromosomal translocations measured by FISH in a population chronically exposed to low dose-rate 60Co γ-irradiation. Int. J. Radiat. Biol. **77**: 797–804.
6. Tao, Z., Zha, Y., Akiba, S., Sun, Q., Zou, J., Li, J., Liu, Y., Kato, H., Sugahara, T. and Wei, L. (2000) Cancer mortality in the high background radiation areas of Yangjiang, China during the period between 1979 and 1995. J. Radiat. Res. **41 Suppl.**: 31–41.
7. Jiang, T., Wang, C.-Y., Chen, D.Q., Yuan, Y.-L., Wei, L.-X., Hayata, I. Morishima, H., Nakai, S. and Sugahara, T. (1997) Preliminary report on quantitative study of chromosome aberrations following life time exposure to high background radiation in China. In: High levels of Natural Radiation 1996. Radiation Dose and Health Effects, Eds. Wei L, Sugahara T and Tao Z, pp. 301–306, Elsevier, Amsterdam.
8. Hayata, I., Wang, C., Zhang, W., Chen, D., Minamihisamatsu, M., Morishima, H., Yuan, Y., Wei, L. and Sugahara, T. (2000) Chromosome translocation in the residents of the high background radiation areas in southern China. J. Radiat. Res. **41 Suppl.**: 69–74.
9. Hayata, I., Wang, C., Zhang, W., Chen, D., Minamihisamatsu, M., Morishima, H., Yuan, Y., Wei, L. and Sugahara, T. (2002) Chromosome translocation in residents of high background radiation area in China. In High Level of Natural Radiation and Radon Areas: Radiation Dose and Health Effects, Eds. W. Burkart, M. Sohrabi and A. Bayer, pp. 199–205, Elsevier, Amsterdom.
10. Morishima, H., Koga, T., Tatsumi, K., Nakai, S., Sugahara, T., Yuan, Y., Sun, Q. and Wei, L. (1997) Study of the indirect method of personal dose assessment for the inhabitants in HBRA of China. In: High levels of Natural Radiation 1996. Radiation Dose and Health Effects, Eds. Wei L, Sugahara T and Tao Z, pp. 235–240, Elsevier, Amsterdam.
11. Yuan, Y., Shen, H., Sun, Q. and Wei, L. (1999) Estimation of individual doses from external exposures and dose-group classification of cohort members in high background radiation area in Yangjiang, China (in Chinese). Chinese J. Radiol. Med. Protect. **19**: 99–103.
12. Hayata, I., Tabuti, H., Furukawa, A., Okabe, N., Yamamoto, M. and Sato, K. (1992) Robot system for preparing lymphocyte chromosome. J. Radiat. Res. **33 Suppl.**: 231–241.
13. Hayata, I. (1997) Advanced cytogenetical techniques necessary for the study of low dose exposures. In: High levels of Natural Radiation 1996. Radiation Dose and Health Effects, Eds. Wei L, Sugahara T and Tao Z, pp. 293–300, Elsevier, Amsterdam.
14. Yamada, K., Kasama, M. and Takai, S. (1996) Technical improvement and development for automated detection. In: Report on Trial Researches of Nuclear Power by National Organizations, vol. **35**, Eds. Ministry of Health and Welfare, pp. 118-1–118-4 (in Japanese).
15. Morton, N. E. (1991) Parameters of the human genome. Proc. Natl. Acad. Sci. USA **88**: 7474–7476.
16. Wu, H., George, K. and Yang, T. C. (1998) Estimate of true incomplete exchanges using fluorescence in hybridization with telomere probes. Int. J. Radiat. Biol. **73**: 521–527.
17. Boei, J. J. W. A., Vermeulen S., Fomina, J. and Natarajan, A. T. (1998) Detection of incomplete exchanges and interstitial fragments in X-irradiated human lymphocytes using a telomeric PNA probe. Int. J. Radiat. Biol. **73**: 599–603.
18. Fomina, J., Darroudi, F., Boei, J. J. W. A. and Natarajan, A. T. (2000) Discrimination between complete and incomplete chromosome exchanges in X-irradiated human lymphocytes using FISH with pan-centromeric and chromosome specific DNA probes in combination with telomeric PNA probe. Int. J. Radiat. Biol. **76**: 807–813.
19. Lucas, J. N., Awa, A., Straume, T., Poggensee, M., Kodama, Y., Nakano, M., Ohtaki, K., Weier, H. U., Pinkel, D., Gray, J. and Littlefield, G. (1992) Rapid translocation frequency analysis in humans decades after exposure to ionizing radiation. Int.

J. Radiat. Biol. **62**: 53–63.
20. Katayama, H., Matsuura, M., Endo, S., Hoshi, M., Ohtaki, M. and Hayakawa, N. (2002) Reassessment of the cancer mortality risk among Hiroshima atomic-bomb survivors using a new dosimetry system, ABS2000D, compared with ABS93D. J. Radiat. Res. **43**: 53–63.
21. Tucker, J. D., Lee, D. A., Ramsey, M. J., Briner, J. F., Olsen, L. A. and Moor II, D. H. (1994) On the frequency of chromosome exchanges in a control population measured by chromosome painting. Mutat. Res. **313**: 193–202.
22. Ramsey, M. J., Moor II, D. H., Briner, J. F., Lee, D. A., Olsen, L. A., Senft, J. R. and Tucker, J. D. (1995) The effect of age and lifestyle factors on the accumulation of cytogenetic damage as measured by chromosome painting. Mutat. Res. **338**: 95–106.
23. Lucus, J. N., Deng, W., Moore, D., Hill, F., Wade, M., Lewis, A., Sailes, F., Kramer, C., Hsieh, A. and Galvan, N. (1999) Background ionizing radiation plays a minor role in the production of chromosome translocations in a control population. Int. J. Radiat. Biol. **75**: 819–827.
24. Sorokine-Durm, I., Whitehouse, C. and Edwards, A. (2000) The variability of radiation yields amongst control populations. In: Fluorescence In Situ Hybridization (FISH) Biological Dosimetry. Radiation Protection Dosimetry, vol. **88**, Ed. Edwards AA, pp. 93–99, Nuclear Technology Publishing, Kent.
25. Nakano, M., Nakashima, E., Pawei, D. J., Kodama, Y. and Awa, A. (1993) Frequency of reciprocal translocations and dicentrics induced in human blood lymphocytes by X-irradiation as determined by fluorescence in situ hybridization. Int. J. Radiat. Biol. **64**: 565–569.
26. Kanda, R. and Hayata, I. (1996) Comparison of the yields of translocations and dicentrics measured using conventional Giemsa staining and chromosome painting. Int. J. Radiat. Biol. **69**: 701–705.
27. Hayata, I. (2000) Insignificant risk at low dose (rate) radiation predicted by cytogenetic studies. In: Proceedings of the 10th International Congress of the International Radiation Protection Association, T-17-3, P2a-90, Hiroshima (in CD).
28. Stephan, G., Pressl, S., Koshpessova, G. and Gusev, B. I. (2001) Analysis of FISH-painted chromosomes in individuals living near the Semipalatinsk nuclear test site. Radiat. Res. **155**: 796–800.
29. Snigiryova, G., Braselmann, H., Salassidis, K., Shevchenko, V. and Bauchinger, M. (1997) Retrospective biodosimetry of Chernobyl clean-up workers using chromosome painting and conventional chromosome analysis. Int. J. Radiat. Biol. **71**: 119–127.
30. Akleyev, A. V., Kossenko, M. M., Silkina, L. A., Degteva, M. O., Yachmenyov, V. A., Awa, A., Akiyama, M., Veremeyeva, G. A., Vozilova, A. V. and Kyozumi, S. (1995) Health effects of radiation incidents in Southern Urals. Stem Cells, **13 Suppl. 1**: 58–68**.**
31. Lindholm, C., Makelainen, I., Paile, W., Koivistoinen, A. and Salomaa, S. (1999) Domestic radon exposure and the frequency of stable or unstable chromosomal aberrations in lymphocytes. Int. J. Radiat. Biol. **75**: 921–928.
32. Tucker, J. D., Tawn, E. J., Holdsworth, D., Morris, S., Langlois, R., Ramsey, M. J., Kato, P., Boice, Jr. J. D., Tarone, R. E. and Jesen, R. H. (1997) Biological dosimetry of radiation workers at the Sellafield Nuclear Facility. Radiat. Res. **148**: 216–226.
33. Ramsey, M. J., Moor II, D. H., Briner, J. F., Lee, D. A., Olson, I. A., Senft, J. R. and Tucker J. D. (1995) The effect of age and lifestyle factors on the accumulation of cytogenetic damage as measured by chromosome painting. Mutat. Res. **338**: 95–106.

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