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Effect of high-level natural radiation on chromosomes of residents in southern China

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Abstract. To study the effect of low-dose (rate) radiation on human health, we analyzed chromosomes of peripheral lymphocytes of residents in a high background radiation area (HBRA) and compared the results with those obtained from residents in a control area (CA) in Guangdong Province, China. Unstable types of chromosome aberrations (dicentrics and rings) were studied in 22 members of eight families in HBRA and 17 members of five families in CA. Each family consists of three generations. On average 2,600 cells per subject were analyzed. 27 adults and six children in HBRA and 25 adults and eight children in CA were studied with respect to translocations. On average 4,741 cells per subject were examined. We found an increase of the frequency of dicentrics and rings in HBRA, where the natural radiation level is three to five times higher than in the control area. But the increase of translocations in HBRA was within the range of individual variation in the controls.

In the high background radiation area (HBRA) in Guangdong Province of China the level of natural radiation is three to five times higher than it is in other places. The high level of radiation is caused by natural radionuclides such as ^{238}U, ^{232}Th and ^{226}Ra in the soil and in the materials for building houses (Yuan et al., 1997). Most of the residents in the HBRA are farmers and have been living in this area for several generations. The area is rural and far from an air-polluted city.

Chromosome aberrations are extremely sensitive indicators of radiation exposure. Increase of dicentric (Dic) and centric ring (Rc) chromosomes accompanied by fragments can be detected at a dose of 20 mSv in acutely irradiated lymphocytes when chromosomes were analyzed immediately after exposure (Lloyd et al., 1992). Dic and Rc are specific indicators of the effect of radiation, while translocations (Tr) are an indicator of the total effect of all kinds of mutagenic agents such as chemicals and metabolic factors as well as radiation (Hayata et al., 2000). Dic and Rc are unstable aberration types and are gradually eliminated from the body at a rate of 50% loss per cell division. Fragments are also unstable aberration types and they are 100% eliminated at each cell division. On the other hand, Tr are stable, not lost by cell division, and accumulate in the body.

To determine the effect of low-dose (rate) radiation on human health, we analyzed the chromosomes of peripheral lymphocytes of the residents in the HBRA and compared the results with those obtained from the residents in a control area in Guangdong Province (Hayata, 2000; Hayata et al., 2002; Jiang et al., 2000; Zhang et al., 2003).

In the present paper we review our cytogenetic studies and discuss the effect of low dose (rate) radiation on human health.

Materials and methods

Subjects

HBRA and control area (CA) are nearby hamlets and the genetic and cultural backgrounds of the residents in both areas are very similar. For the determination of unstable chromosome aberrations (Dic and Rc), 22 members from eight families in HBRA and 17 members from five families in CA were studied. Each family consists of three generations. For stable chromosome aberrations (Tr), 27 adults and six children in HBRA and 25 adults and eight children in CA were studied.

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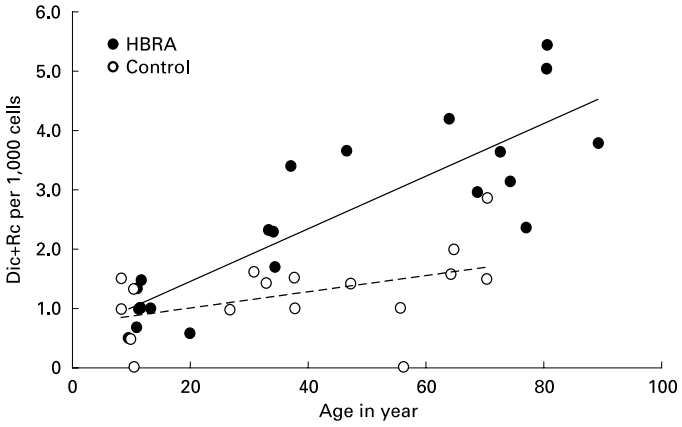

Fig. 1. Frequencies of dicentric and ring chromosomes (Dic + Rc) in relation to age of donors. Open circles: control individuals. Filled circles: HBRA individuals. Solid regression line: HBRA; broken regression line: controls.

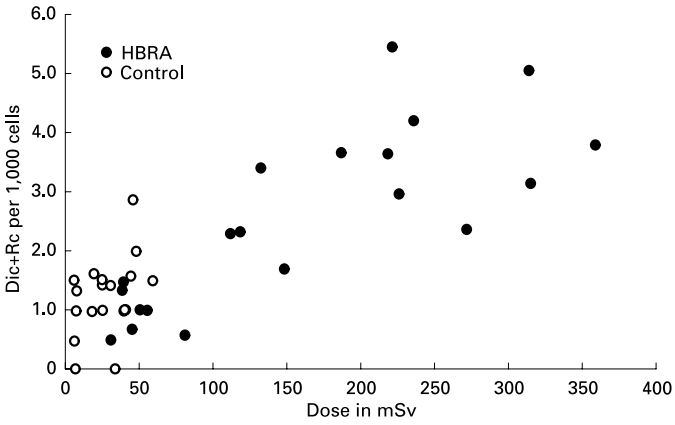

Fig. 2. Frequencies of dicentric and ring chromosomes (Dic + Rc) in relation to accumulated dose. Open circles: control, filled circles: HBRA.

Measuring individual dose

Prior to the cytogenetic study, each individual's exposed dose was measured with electronic pocket dosimeters (Aloka PDM-101) for 24 h and/or thermoluminescence dosimeters (TLD, National UD-200S) for 2 m (Morishima et al., 1997). Average dose rates per year were 3.11 mSv in HBRA and 0.71 mSv in CA. Accumulated doses were calculated by multiplying the measured dose rate with the age of each individual at the time of blood sampling.

Cytogenetic preparation

About 3 ml of blood was taken from each subject in HBRA and CA and brought to the Enping Municipal Hospital in CA where we established a laboratory for chromosome preparation. Lymphocyte cultures were set up within 7 h after taking the blood. Colcemid was added to the cultures from the beginning for 48 h. Cultured cells (T cells) were processed for chromosome preparation according to the high-yield chromosome preparation method (Hayata et al., 1992). Fixed cell samples were distributed between two laboratories, one in Beijing, China and the other in Chiba, Japan. Air-dried slides were made at both laboratories and were stained with Giemsa solution for the study of Dic and Rc or with FISH using whole chromosome painting probes for chromosomes 1, 2, and 4 for the analysis of Tr (for details see Zhang et al., 2003).

Observation

Observations were performed at both Chinese and Japanese laboratories using microscopes equipped with an automated stage. All the results were reexamined by at least two examiners, one from each laboratory. The average life span (period of observation) of T cells in the body under normal conditions is 22 weeks for CD45RO and 3.5 years for CD45RA cells (IAEA, 2001). In the present subjects, a considerable number of T cells should be in second or later cell division cycles after exposure to radiation and should not have any fragments. Therefore, Dic with or without fragments are pooled in the present results.

Tr included both one-way and reciprocal Tr between two chromosomes, and three-way Tr involving three chromosomes. A three-way Tr involving three chromosomes was counted as two Tr. The frequencies of translocations per 1000 cells were scaled to genome-equivalent frequencies (F_G) by the formula reported by Lucas et al. (1992) as follows: $F_G = F_p/2.05\ f_p(1 - f_p)$, where F_p is the frequency of Tr detected by painting and f_p is the fraction of the genome painted.

Statistical analysis

The frequencies of chromosome aberrations in the two areas were compared by using the Mann-Whitney U test. A variance test of the homogeneity of the Poisson distribution was used to test for homogeneity and the Spearman rank correlation test to perform a correlation analysis.

Results and discussion

Regarding unstable aberrations (Dic + Rc), 101,395 cells in total and 2,600 cells average per subject were analyzed. Dic without fragments were found in about equal ratios among total Dic both in HBRA and in CA. The results are summarized in Figs. 1 and 2. As shown in Fig. 1, the frequencies of Dic + Rc seem to increase with age in both groups. However, there is a clear difference in the degree of increase between HBRA and control, with the former being much steeper than the latter (4.44 per 100 years for HBRA and 1.37 per 100 years for control). The contribution ratios due to the regression were much higher in HBRA than in CA ($r^2 = 0.7544$ for HBRA and $r^2 = 0.2111$ for control). The frequencies of Dic + Rc per age group among the subjects excluding children are significantly different ($P < 0.01$) between HBRA and CA. The frequencies of Dic + Rc in relation to accumulated doses are shown in Fig. 2, which indicates dose response of the frequencies of Dic + Rc. No factor except radiation was found to explain the difference of the frequencies of Dic + Rc between HBRA and CA. Therefore, the age-related increase of the frequencies of Dic + Rc was considered to be attributable to the high level of background radiation. The dose rate in HBRA is less than 1 cGy per year. This dose rate means that less than one track of radiation passes a cell in more than two months time, which would mean that most of the chromosome aberrations observed are caused by one track of radiation. Therefore, there seems to be no threshold dose for the induction of chromosome aberration. Activation of DNA repair enzymes to reduce subsequent lesions (adaptive response) seems not to be effective at such low dose rates as seen in HBRA.

In the case of stable aberrations (Tr) 312,887 cells in total and 4,741 cells per subject on average were analyzed. The results are summarized in Figs. 3 and 4. In both areas, frequencies of Tr are much higher than those of Dic + Rc and they are lower in children than in adults. Individual variation is small in children but large in the adults. There are two outlyers with more than 20 Tr per 1000 cells, one had medical exposure by

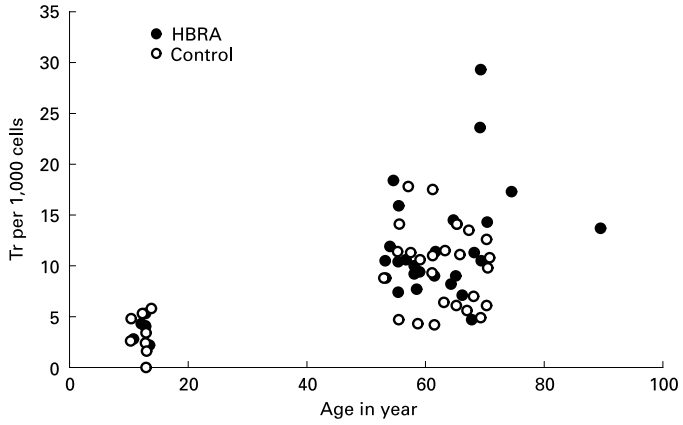

Fig. 3. Frequencies of translocations (Tr) in relation to age. Open circles: control, filled circles: HBRA.

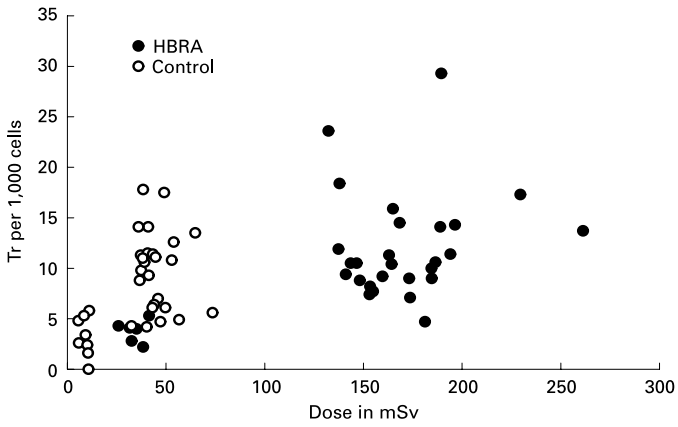

Fig. 4. Frequencies of translocations (Tr) in relation to the accumulated dose. Open circles: control, filled circles: HBRA.

fluoroscopy, for the other no reason was found. When those two cases are excluded, there is no significant difference of the frequencies of Tr between HBRA and CA.

Irrespective of the increased frequencies of Dic + Rc in HBRA, the frequencies were within the range of variation in the controls.

The ratio of Dic vs. Rc induced by radiation is about 1:0.1–0.2 (our unpublished data). Dic and Tr are derived from the same types of lesions in two chromosomes caused by radiation. Asymmetric rearrangements lead to a Dic accompanied by fragments and symmetric rearrangements result in Tr. Some types of asymmetric rearrangements are preferentially reduced in their production and therefore, the ratio of Dic vs. Tr is about 0.8–0.9:1 (Zhang and Hayata, 2003). The ratio of Dic + Rc vs. Tr induced by radiation in lymphocytes is about 1:1.

As mentioned above, Dic + Rc and Tr are induced by radiation with comparable frequencies. But, radiation-induced Tr can not be distinguished from Tr induced by other agents such as chemicals or metabolic factors. Tr are stable aberrations that accumulate in the cells and therefore may increase the risk of malignant and congenital diseases. The present results in HBRA indicate that the contribution of an elevated level of natural radiation to the induction rate of Tr does not have a significant effect compared to the contribution of other mutagenic factors such as chemicals and/or metabolic factors. This may explain why epidemiological studies do not show any increase in malignant or congenital diseases in residents in HBRA, despite the fact that the elevated natural radiation causes an increase of unstable type aberrations (Dic + Rc).

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