Current Cytogenetic Methods for Detecting Exposure and Effects of Mutagens and Carcinogens

Adayapalam T. Natarajan,[1,2] Jan J.W.A. Boei,[1] Firouz Darroudi,[1,2] Paul C.M. Van Diemen,[1] Fernando Dulout,[3] M. Prakash Hande,[1] and Adriana T. Ramalho[4]

[1]MGC Department of Radiation Genetics and Chemical Mutagenesis, Leiden University, Leiden, The Netherlands; [2]J.A. Cohen Institute, Inter-University Research Institute for Radiopathology and Radiation Protection, Leiden, The Netherlands; [3]Centro de Investigaciones en Genetica Basica y Aplicada (CIGEBA), Facultad de Ciencias Veternerias, Universidad Nacional de la Plata, La Plata, Argentina; [4]CNEN-Insituto de Radioprotectcao e Dosimetria Commissao Nacional de Energia Nuclear, Rio de Janeiro, Brazil

Most mutagens and genotoxic carcinogens are efficient inducers of chromosomal alterations in exposed cells. Two important classes of aberrations, namely structural and numerical, are recognized and both types of aberrations are associated with congenital abnormalities and neoplasia in humans. These alterations can be easily detected and quantified in human peripheral blood lymphocytes. Conventional staining techniques can be used to detect these aberrations; this technique was used to estimate absorbed dose in the case of a radiation accident in Goiania, Brazil. A recently introduced fluorescent *in situ* hybridization technique (FISH) using DNA probes has increased the sensitivity and ease of detecting chromosome aberrations, especially stable chromosome aberrations. This technique allows, to some extent, the estimation of absorbed radiation dose from past exposures. Numerical aberrations can be directly estimated in metaphases by counting the number of FISH-painted chromosomes. Micronuclei are formed by lagging chromosome fragments or whole chromosomes during the anaphase stage of cell division. The nature of micronuclei as to whether they possess a centromere can be determined either by CREST staining (calcinosis, Raynoud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia) or FISH with centromere-specific DNA probes. In several carcinogen-exposed populations, such as heavy smokers or people exposed to arsenic, aneuploidy appears to be more common than structural aberrations. In victims of radiation accidents, aneuploidy (hyperploidy) has been found to be common in addition to structural aberrations. — Environ Health Perspect 104(Suppl 3):445–448 (1996)

Key words: chromosome aberrations, micronuclei, aneuploidy

Introduction

Exposure of human populations to mutagenic carcinogens can be monitored using different chemical and biological end points. The chromosomal aberration, a very sensitive biological end point, reflects the effect on the whole genome in contrast to point mutation, which reflects the effect on a small target (about 45 kb as in the case of *HPRT* gene). Human peripheral blood lymphocytes, because of their easy availability, have been traditionally used to monitor the effects of exposure to known or suspected mutagens. This methodology is very well established, and the criteria to be used in such studies are well documented (*1,2*). Two types of chromosome aberrations, namely structural and numerical ones, are recognized and both these types of aberrations are associated with human health, especially congenital malformations and cancer. A recent Nordic study has found a correlation between increased cancer risk and increased levels of chromosomal aberrations in lymphocytes (*3*). Frequencies of structural chromosomal aberrations (especially dicentrics) in human lymphocytes have been used as biological dosimeters for exposure to ionizing radiation (*4,5*). Recent introduction of the fluorescent *in situ* hybridization (FISH) technique using chromosome-specific DNA probes has increased the resolution of detecting and evaluating structural chromosomal aberrations, especially translocations in human (*6*) and rodent cells (*7,8*). In addition, the FISH technique allows us to study the nature of spontaneous or induced micronuclei in diverse cell types. In this paper, we summarize some of the approaches and results obtained in our studies using FISH.

Chromosomal Aberrations

Ionizing Radiation

Ionizing radiation induces chromosomal aberrations very effectively in all stages of the cell cycle. When cells are irradiated prior to replication (G_0, G_1), chromosome-type aberrations are induced; following irradiation of cells in the postsynthetic period (G_2), chromatid-type aberrations are induced. Circulating lymphocytes are in the G_0 stage of the cell cycle; on exposure to radiation (as in the case of accidents), chromosome-type aberrations are induced immediately following exposure (*9*). The frequencies of induced aberrations are dose related; therefore, they can be used as biological dosimeters in the case of radiation accidents. Conventionally, the frequencies of dicentrics have been used as biological dosimeters. The large amount of data available from *in vitro* studies using radiations of different qualities, different dose rates, etc., could be used for calibration and thus for dose estimation. However, for the use of dicentric yields for dose estimation, the blood samples should be taken as soon as possible because

This paper was presented at the 2nd International Conference on Environmental Mutagens in Human Populations held 20–25 August 1995 in Prague, Czech Republic. Manuscript received 22 November 1995; manuscript accepted 28 November 1995.

This work was funded in part by grants from the European Union's Radiation Protection Programme and European Union Environment Programme to ATN.

Address correspondence to A.T. Natarajan, Department of Radiation Genetics and Chemical Mutagenesis, Leiden University, Wassenarseweg 72, 2333 Al Leiden, The Netherlands. Telephone: 31-71-527-6164. Fax: 31-71-522-1615. E-mail: natarajan@rullf2.medfac.leidenuniv.nl

Abbreviations used: FISH; fluorescent *in situ* hybridization; CREST, calcinosis, Raynoud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia; LET, linear energy transfer.

dicentrics are unstable and the lymphocytes carrying this aberration are eliminated with time (with an average lifetime of 150 to 220 days depending on the dose received) (*10*).

Unlike dicentrics, balanced translocations are stable and perpetuate during cell proliferation. With conventional techniques such as G banding, gross translocations can be recognized; but this method is very laborious to be employed for large-scale studies. Recently, human chromosome-specific DNA libraries have become available; by employing these probes and the FISH technique (commonly called the chromosome painting technique), it is possible to easily identify and quantify translocations. It is generally believed that dicentrics and reciprocal translocations are induced with equal frequencies following irradiation of cells in G_0 or G_1 phase. Since the resolution of the painting technique is much higher than the chromosome banding techniques, one can, in addition to reciprocal translocations, identify terminal (incomplete) and interstitial translocations. If we take into account all translocations induced, the frequencies of translocations are higher by a factor of about 1.5 to 2 in comparison to dicentrics (*11,12*). This increase is more evident at doses higher than 2 Gy of low linear energy transfer (LET) radiation. Since it is possible to score both dicentrics and translocations in the same cell, the efficiency of detecting exchange aberrations (both translocations and dicentrics) is improved, thus enabling a higher confidence in estimating absorbed radiation dose in biological dosimetry.

Estimation of radiation dose from past exposures using the frequencies of dicentrics is possible if the frequencies are high, i.e., the initial dose has been high. There are two methods available for such estimation: one method uses the observed frequencies and the average life span of lymphocytes (generally taken as 3 years, which is a conservative upper estimate) in computing the original frequencies and thus the dose (*4*); and the other method, the QdR method, takes into account the frequencies and distribution of aberrations among the aberrant cells (*13*).

Because translocations are stable, one can use their frequencies to retrospectively estimate past radiation exposures. A good correlation between the frequencies of translocations observed in the lymphocytes of the atom bomb victims of Hiroshima and Nagasaki and the *in vitro*-induced frequencies for estimated TD 86 doses has been reported (*14*); however, in this case it was not possible to know the initial yield of dicentrics.

A good cohort for such a study is the victims of the radiation accident in Goiania, Brazil. We have been following these victims since the accident. Initial individual dose estimates were made immediately after the accident using the frequencies and distribution of dicentrics among the cells (*5*). The frequencies of translocations in this cohort have been studied from 1992 onward (*15,16*). The frequencies of translocations were found to be lower than the originally observed frequencies of dicentrics (Figure 1), indicating that we have to use a correction factor if we want to use the frequencies of translocations for estimating past exposures retrospectively. This factor is dose dependent and appears to be higher (~3) at high doses (> 3 Gy). This clearly indicates that the frequencies of lymphocytes carrying translocations decline with time *in vivo*. This is not unexpected because *a*) there is a constant turnover of the lymphocyte population in the body; *b*) immediately following irradiation, there is strong depletion of lymphocytes *in vivo* as seen by moderate to severe leukopenia; *c*) apoptosis occurs, which could eliminate highly damaged cells; *d*) most of the translocation-bearing lymphocytes observed several years after the radiation accident most probably derived from irradiated differentiating stem cells from the bone marrow, a heterogenous population with radiosensitivity that may be different from circulating lymphocytes; and *e*) cells carrying multiple aberrations and complex exchanges will be eliminated during cell division.

To determine whether the frequencies of translocations in lymphocytes decline with time, we used the mouse as a model. For studying translocations we used DNA libraries specific for mouse chromosomes 1, 11, and 13, which were generated in our laboratory (*7,17*). Mice were whole-body irradiated with X rays (2 Gy) and sacrificed at different times, namely 0, 1, 3, 7, 14, 28, 56, and 112 days; and the frequencies of translocations and dicentrics were determined in the splenocytes. Frequencies of dicentrics and translocations were equal in number immediately following irradiation. The frequencies of splenocytes carrying dicentrics declined very fast in the beginning (0–14 days) and slower at later times (Figure 2). The average lifetime of splenocytes was calculated to be around 70 days. The frequencies of translocations were constant up to 7 days and declined linearly with time, reaching a 50% reduction at day 112 (*18*). These results clearly demonstrate that the frequencies of radiation-induced translocations do not remain constant *in vivo* over time, thus supporting our results from the radiation accident in Goiania.

Exposure to Chemical Mutagens

Although there are many biomonitoring studies of populations exposed to chemical mutagens using conventional methods, there are only a very few studies using FISH on human populations exposed to chemical mutagens.

Recently a study on the frequencies of dicentrics and translocations in the lymphocytes of 12 smokers and 30 nonsmokers was carried out in our laboratory (*19*). The frequencies of dicentrics were determined by scoring slides stained with Giemsa solution, whereas translocations were detected by FISH using DNA libraries specific to nine different human chromosomes in different cocktails (representing about 50% of

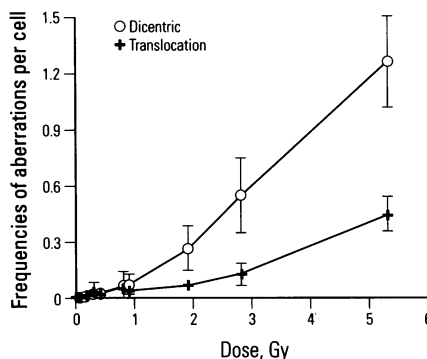

Figure 1. A comparative study of dicentrics (1987) and translocations (1993).

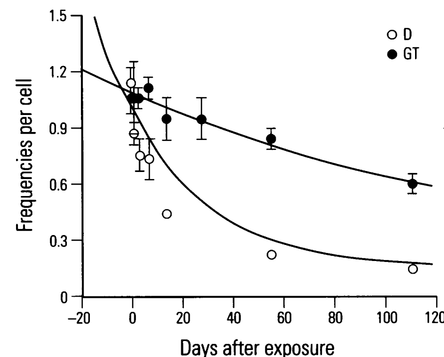

Figure 2. Frequencies of dicentrics (D) and translocations (GT) at different days following irradiation (2 Gy, X rays) of mice.

the genome). No significant difference was observed between smokers or nonsmokers in the frequencies of stable and unstable aberrations. It is easy to identify hyperploid cells involving any of the painted chromosomes in such a study because this shows up as an extra painted whole chromosome. A significantly higher frequency ($p < 0.05$) of hyperploidy was evident in smokers compared to the controls. When a cocktail for chromosomes 1, 3, and X was used, the X chromosome was involved in about 50% of cases of hyperploidy.

Arsenic in drinking water is a great problem in several parts of the world (e.g., Argentina, Bolivia, Chile, India, Bangladesh, China, Taiwan, Thailand, Mongolia, Japan, etc.). Arsenic poisoning can lead to skin lesions, hyperkeratosis, skin cancer, liver diseases, etc. The mode of action of arsenic *in vivo* is not well understood. *In vitro* studies have indicated that arsenic can interfere with DNA replication, DNA repair, and cell division. Earlier studies have shown that arsenic can inhibit ligation of DNA strand breaks and, if present during DNA synthesis, it can induce chromosomal aberrations, sister chromatid exchanges, and malsegregation of chromosomes. We have studied a native population from San Antonio Cobres, Salta Province in Argentina exposed to arsenic in drinking water at concentrations as high as 150 to 200 µg/l along with an appropriate control population. There was no increase in the frequencies of sister chromatid exchanges in the exposed population compared to the control. Surprisingly, there was no increase in the frequencies of translocations, but tetraploid and hyperploid cells were found (*20*). This population was found to have a unique arsenic metabolism in that they can detoxify arsenic very efficiently and quickly (*21*).

Interphase Cytology

By applying FISH and specific probes for individual chromosomes, it is possible to detect aneuploidy events directly in interphase nuclei by counting the number of domains in each nucleus. In this methodology it is not necessary to culture the lymphocytes until mitosis; they can be transformed with phytohemagglutinin for a period of 6 hr, and the events reflect those that occur in the bone marrow. Employing this technique, it has been found that the frequencies of lymphocytes carrying an extra chromosome 1 (as detected by a probe specific for the centromeric repetitive DNA-puc 1.77) in the radiation victims of Goiania were increased up to 1.2% (*15*).

Malsegregation of Chromosomes

Micronuclei are formed from the lagging fragments or whole chromosomes during cell division; such a division is needed for the expression of the micronucleus. To make quantitative comparisons, it is important to know the frequencies of cells (with and without micronuclei) that have undergone one division following exposure. This has become possible by blocking cytokinesis by cytochalasin B, which leads to binucleated cells (*22*). Micronuclei formed by whole chromosomes can be detected by CREST antibodies that specifically stain kinetochore proteins or by FISH using a DNA probe specific to the centromeric regions, such as alphoid probes in human or major and minor satellite probes for mouse.

Although the presence of a micronucleus with centromeric signals suggests a possible loss of a chromosome, this does not reflect a true nondisjunction. A nondisjunction should lead to one of the daughter nuclei having additions of one or more chromosomes, with concomitent loss of chromosomes in the other daughter nucleus. To study induction of malsegregation of chromosomes, an *in vivo* model using transgenic mice has been standardized (*17,23*). By a complex breeding procedure, we have combined three different transgenic mice to generate a mouse strain that carries three pairs of marker chromosomes in the female and five marker chromosomes in the male. These marker chromosomes carry inserts of either λ sequences (in chromosomes 2 and X) and c-*myc* sequences (in chromosome 8). By employing the FISH technique using λ and c-*myc* probes, one can determine the number of these marker chromosomes even in interphase nuclei. In binucleated cells generated by cytochalasin B treatment, one can easily study the segregation of these marker chromosomes. Using this system, it could be demonstrated that ionizing radiation can effectively induce chromosomal nondisjunction (*23*). This type of technique can be used in human lymphocytes as well to monitor the effects of *in vivo* exposure to carcinogenic agents. Recently, using tandem labeling with two different fluorochromes of the 1-cen-1q12 region of the interphase nucleus of lymphocytes in a human population exposed to pesticides, an increase in hyperploidy and clastogenicity was detected (*24*). This technique, if adapted for more chromosomes, will have a great potential for large-scale monitoring of human populations for possible exposure to mutagenic and carcinogenic agents.

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