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Comparison of Radiosensitivity of Human Chromosomes 1, 2 and 4 from one Healthy Donor

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ABSTRACT

In general, it was assumed that the chromosome aberration induced by ionizing radiation is proportional to the chromosome size. From this viewpoint, the higher chromosome size, the more resistant to radiation. However, different opinions, in which chromosomes are particularly sensitive or resistant to radiation, are also still followed until now. Here in this research, we compared the chromosome sensitivity between chromosomes number 1, 2, and 4 using the FISH (fluorescence *in situ* hybridization) technique. From this research, we expect that the information obtained could show clearly whether a longer chromosome is more frequently involved in translocations and also more resistant to radiation than a shorter one. The type of chromosome aberration considered was limited only to translocation and we used one sample donor in order to avoid donor variability. The whole blood from a healthy female was irradiated with γ-rays with doses of 1, 3 and 5 Gy, respectively. Isolated lymphocytes from the whole blood were then cultured for 48 hours. After the culture process was completed, preparations of harvest and metaphase chromosomes were carried out. Chromosomes 1, 2, and 4 were stained with different fluorochromes. The translocation of each chromosome at each dose point was subsequently evaluated from 50 images obtained from an automated metaphase finder and capturing system. An additional analysis was performed to identify which chromosome arm was more frequently involved in translocation. Further analyses were also conducted with the aim of determining which chromosome band had a higher frequency of radiation-induced breakage. The experimental results showed that chromosome number 4 was more frequently involved in translocations compared to chromosomes 1 and 2 at 5 Gy. In contrast, at doses of 1 and 3 Gy translocations involving chromosomes number 1 and 2 were more numerous compared to the ones involving chromosome 4. However, if the number of translocation was accumulated for all the doses applied, the chromosome number 4 was the chromosome most frequently involved in translocations. Breakpoint analysis revealed that in chromosome 1, chromosome 2, and chromosome 4, the highest chromosome bands as break position were in band q32, p13, and q21, respectively. It can be concluded that chromosome 4 is more sensitive to radiation in all doses point, despite having less DNA content than chromosomes 1 and 2. Thus, it was showed that our research cannot support the general assumption about chromosome aberration induced by radiation being proportional to DNA content.

INTRODUCTION

The chromosomal radiosensitivity is representative of the individual radiosensitivity [1]. It can be measured using several cytogenetic techniques, and one of the most useful method for assessing it is *fluorescence in situ hybridization* (FISH). FISH has been shown to be a useful method for assessment of chromosomal radiosensitivity [2]. A differential susceptibility of chromosomes for aberration induction will be discernible through a

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systematic and extensive analysis. In general the chromosome aberration induced by radiation will be proportional to deoxyribonucleic acid (DNA) content that is proportional to the chromosome size [3].

Pandita *et al*. [4], using *premature chromosome condensation-fluorescence in situ hybridization* (PCC-FISH) in human lymphocytes at G_0 stage, has shown that there is a direct relationship between chromosome size and aberration frequency. Luomahaara *et al*. [5] examined the distribution of breakpoints that were induced by radiation chromosomes 1, 2, and 4. In their study, they obtained their samples from donors from radiation accident victims in Estonia in 1994 and from *in vitro* irradiated lymphocytes. They showed the localization of the breaks in the chromosome and examined the correlation of the break points with DNA proportion content. They also showed that the yield of chromosome exchanges was equal to DNA content for both the accidental radiation exposure victims, *in vivo*, and irradiated lymphocytes, *in vitro*. However, Wojcik and Streffer [6] showed that, in general, chromosome 1 was more frequently involved in translocations compared to chromosome 2, even though this result was not always reproducible.

The translocation is a type of chromosomal aberration in which a large segment of one chromosome breaks off and attaches to a different chromosome. Chromosome translocations are now considered as a valuable biomarker of radiation exposure and cancer risk [7,8]. Several studies showed that chromosome translocations in peripheral blood lymphocytes can be considered as the most reliable biomarker for measurement of low-dose radiation effects and for retrospective biodosimetry [9-12]. Another study also revealed that the analysis results of translocations using FISH after *in vitro* irradiation correlated with clinical response to radiation in prostate cancer patients. Based on that result, the authors suggested that the cytogenetic assay should be considered as a potential predictor of radiosensitivity [13]. Even now, there are techniques that can be used as radiosensitivity predictors, such as the γ-H2AX assay. Djuzenova *et al*. showed that the γ-H2AX assay shows the potential for use in screening the individual radiosensitivities of breast cancer patients [14].

Until now, there has been no agreement on to which extent the chromosomes are particularly sensitive or resistant to radiation. However, several studies supported the assumption that the higher the chromosome size, the more resistant the chromosome is to radiation. It seems that donor variability is probably a contributing factor to the disagreement, as most studies were performed using lymphocytes of a homogeneous donor [15,16]. The works of Wojcik and Streffer [6] and of Sommer *et al*. [16] seem to support this argument. Their works show that the types of aberration studied affected the radiation sensitivity of chromosomes; for example, human chromosome number 1 was more susceptible to translocations than that of chromosome 2, while Wojcik and Streffer [6] showed that chromosome number 2 was more prone to deletions than that of chromosome 1.

The aim of this research was to compare the sensitivities of chromosomes number 1, 2 and 4 using FISH technique. The aberration type analyzed was limited only to translocation, and only one sample donor was used in this research to avoid donor variability. In this work, we assumed that the higher the chromosome size is, the more prone the chromosome is to be involved in the translocation and also the more radiation-resistant it is. Additional analyses were also conducted to identify the chromosome arms that more involved in the translocation and to detect the higher frequency bands as breakpoint position induced by radiation.

EXPERIMENTAL METHODS

Subjects and irradiation

Eighty milliliters of blood was collected by venipuncture from one 41-year-old healthy female donor without a history of ionizing radiation exposure beyond routine diagnostic exposures. The whole blood was then irradiated with γ-rays from ^{137}Cs in doses of 1, 3, and 5 Gy, respectively, with a dose rate of 0.649 Gy/min at the Institute for Environmental Sciences in the Rokkasho village, Aomori prefecture, Japan.

Blood culture

Lymphocytes to be cultured were isolated from whole blood using Vacutainer CPT Tube (BD Biosciences USA). Cultures were set up in Roswell Park Memorial Institute 1640 (RPMI 1640) culture medium supplemented with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and L-glutamine, 20% fetal bovine serum (FBS), kanamycin, colcemid 0.05 µg/mL, and phytohaemagglutinin (PHA). The culture was maintained in a 5%-humidified CO_2 incubator at 37°C for 48 hours. Cells were then treated with hypotonic shock (0.075 M KCl) for 15 min at 37°C and fixed in acetic acid and methanol

(1:3). Subsequently, 20-25 µL of cell suspension was dropped onto clean slides for metaphase chromosome preparation and allo wed to air-dry. Then, slides were kept overnight at -80°C.

Fluorescence *in situ* hybridization (FISH)

The slides that were kept overnight in the previous procedure were hardened for 1 hour at 65°C. Commercial chromosome DNA probes (MetaSystems, Altlussheim, Germany) were used to directly label chromosome 1 (Texas Red), 2 (fluorescein isothiocyanate/FITC), and 4 (FITC/Texas Red) following the manufacturer's recommended protocol. The chromosome DNA probe cocktail was prepared as a premix containing equal amounts of the probe for each chromosome. Seven microliters of the probe mixture were applied on the slide depending on the size of the hybridization area. The area was then covered with 15 mm × 40 mm coverslips and sealed with rubber cement. Slides and probes were denatured simultaneously at 70°C for two min. The slides were incubated at 37°C overnight in a humidified atmosphere. The rubber cement was then removed from the slides and they were treated for the post-hybridization step washed with 0.4× saline sodium citrate (SSC) buffer at 73°C for two min. Subsequently, the slides were treated in 0.5× SSC / 0.05% of Tween 20 at room temperature for 30 s. Finally, they were rinsed twice briefly in distilled water to prevent salt crystal formation. Air-dried slides were then embedded and counterstained with 15 µL of 4', 6-diamidino-2-phenylindole (DAPI) and mounted with cover glass; nail polish was used to prevent the DAPI from leaving the area in cover slip.

Fluorescence image capture and analysis

The automatic metaphase finder was performed with a Zeiss Axioplan 2 Imaging epifluorescent microscope connected to a Cool Cube (MetaSystems, Altlussheim, Germany) and the Metafer 4 imaging system (MetaSystems, Altlussheim, Germany). Fluorescent images of metaphase spreads were captured and analyzed with the ISIS software from MetaSystems and ImageJ 1.49. The translocation in each chromosome was evaluated separately, and at each dose used, 50 metaphases were scored and the yields of the translocations involving chromosomes 1, 2, and 4 were recorded.

Chromosome arm identification

The identification of which chromosome arm (p or q arm) that was getting involved in translocation was carried out manually for chromosomes number 2 and 4. Since the chromosomes 2 and 4 were submetacentric, the shortest arm was identified as the p arm. For example, in Fig. 1 it can be seen clearly that the centromere in chromosome 4 was in the dashed line and the shorter arm is defined as the chromosome's p arm. Therefore, the longer arm was taken as the q arm. For chromosome 1 the arm identification was performed by measuring the length of each arm to define the centromeric ratio. The arm that gives a centromeric ratio in the 0.510-0.520 range, or closer to it, is defined as the q arm based on Morton [17]. For instance, in Fig. 1 the length of the upper arm (red arrow) is 27.203 pixels and the length of the bottom arm (orange arrow) is 35.355 pixels. The total length of the upper and bottom arms is 62.558 pixels. If the upper arm is assumed as the q arm then the centromeric ratio would be 0.43, while if the bottom arm is considered as the q arm it will give a centromeric ratio of 0.56. Since the bottom arm gives the closer centromeric ratio to 0.510 then it was defined as the q arm. The centromeric ratio is defined by equation (1) as follows.

$$CR = \frac{q}{(p+q)} \quad (1)$$

q : Length of q arm
p : Length of p arm
CR: Centromeric ratio

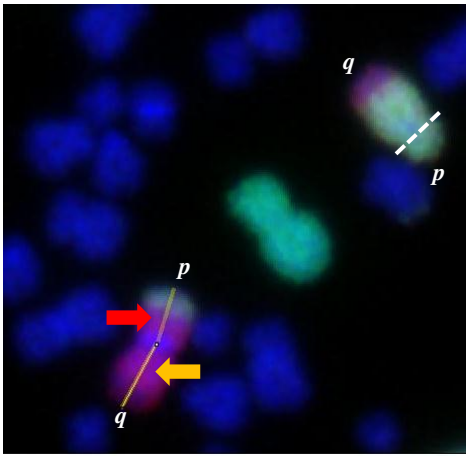

Fig. 1. Chromosomes 1 and 4 arm identification using centromeric ratio (CR) and manual process.

Breakpoint analysis

The breakpoint analysis was carried out by measuring the loss of chromosome area from the

original chromosome one through observation of the short arm. The area loss percentage was converted to chromosome length, and then it was plotted to chromosome image obtained from International System for Human Cytogenetic Nomenclature (ISCN) 2009 from the Atlas of Genetics and Cytogenetics in Oncology and Haematology website (http://atlasgeneticsoncology.org/index.html) [18]. A detailed process of this method is available in additional file of this paper. The method used in this paper was a modified form of Schilling *et al*. [19].

RESULTS AND DISCUSSION

Many studies have previously been conducted for many years up to present to identify the chromosomes that are particularly sensitive to ionizing radiation. Here in this research, it was clearly found that for the highest dose used (5 Gy), chromosome number 4 underwent more translocations than did chromosomes 1 and 2, while at 1 and 3 Gy, the number of translocations of chromosomes number 1 and 2 was higher than that of chromosome 4, as shown in Fig. 2. However, for the total number of translocation for all doses, chromosome number 4 showed the most, as shown in Fig. 3. The results of total translocation in chromosomes 1, 2 and 4 from 50 metaphases at doses of 1, 3, and 5 Gy were summarized on Table 1, Fig. 2, and Fig. 3.

Table 1. Translocation number at doses of 1, 3, and 5 Gy

Radiation Doses (Gy)	Chromosomes Number	Number of Translocations	Chromosomes Arm Number Involved in Translocation
1	1	20	11 in *p* arm and 9 in *q* arm
	2	20	10 in *p* arm and 10 in *q* arm
	4	11	11 in *q* arm
3	1	26	14 in *p* arm and 12 in *q* arm
	2	12	4 in *p* arm and 8 in *q* arm
	4	24	3 in *p* arm and 11 in *q* arm
5	1	19	7 in *p* arm and 12 in *q* arm
	2	15	5 in *p* arm and 10 in *q* arm
	4	24	6 in *p* arm and 18 in *q* arm

Our experimental results showed that the *q* chromosome arms underwent more translocation that did the *p* arms. In total, for all doses, *q* chromosome arms underwent 101 translocations, while *p* arms underwent 60. Moreover, at 1 Gy, there was no translocation for the *p* arm of chromosome 4. At 3 and 5 Gy, the *q* arms of chromosome 4 underwent three times as many translocations as did the *p* arms (Table 1). Possibly, a factor that causes *q* arms to get translocated more frequently is the larger size of the *q* arm compared to the *p* arm. As can be seen in Table 1 and Fig. 2, for all doses, the *q* arms of chromosome 2 and 4 underwent more translocations than the *p* arms. The *p* arms of chromosome 1 also underwent fewer translocations than the *q* arms for all doses, even though the difference was smaller. It is possible that the difference is smaller because chromosome 1 is metacentric; it has equal-sized *p* and *q* arms.

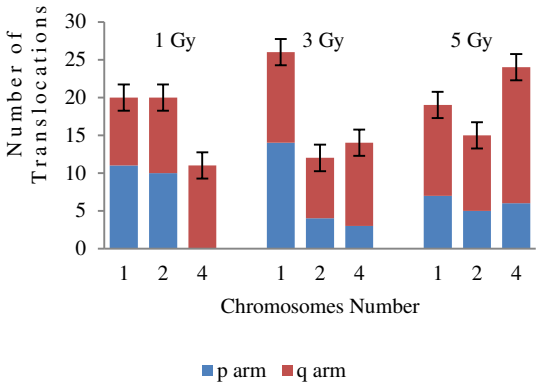

Fig. 2. Number of translocations in chromosomes 1, 2, and 4 at doses of 1, 3, and 5 Gy.

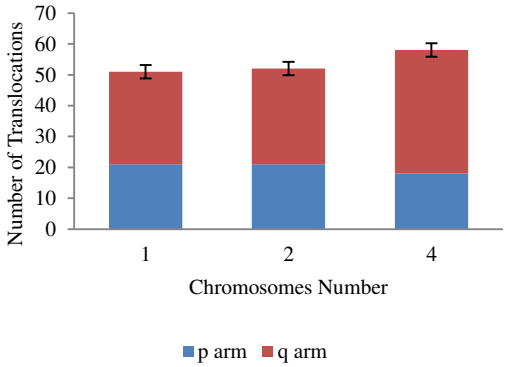

Fig. 3. Total number of translocations in chromosomes 1, 2, and 4 for all applied doses.

In our study, it has been shown that the most frequent break position of chromosome 1 was in band *q32*. This experimental result is in good agreement with the results of Barrios *et al*. that examined the lymphocytes from cancer patients after radiotherapy [20]. Barrios *et al*. argued that the band *q32* is a hot point for clastogenic effects (causing chromosomal breakage) of ionizing radiations. For chromosome 2, the break position was depicted in band *p13*, while for chromosome 4 was in band *q21*. This research only considered the break positions that are located in light bands, as only those bands contain the active genes.

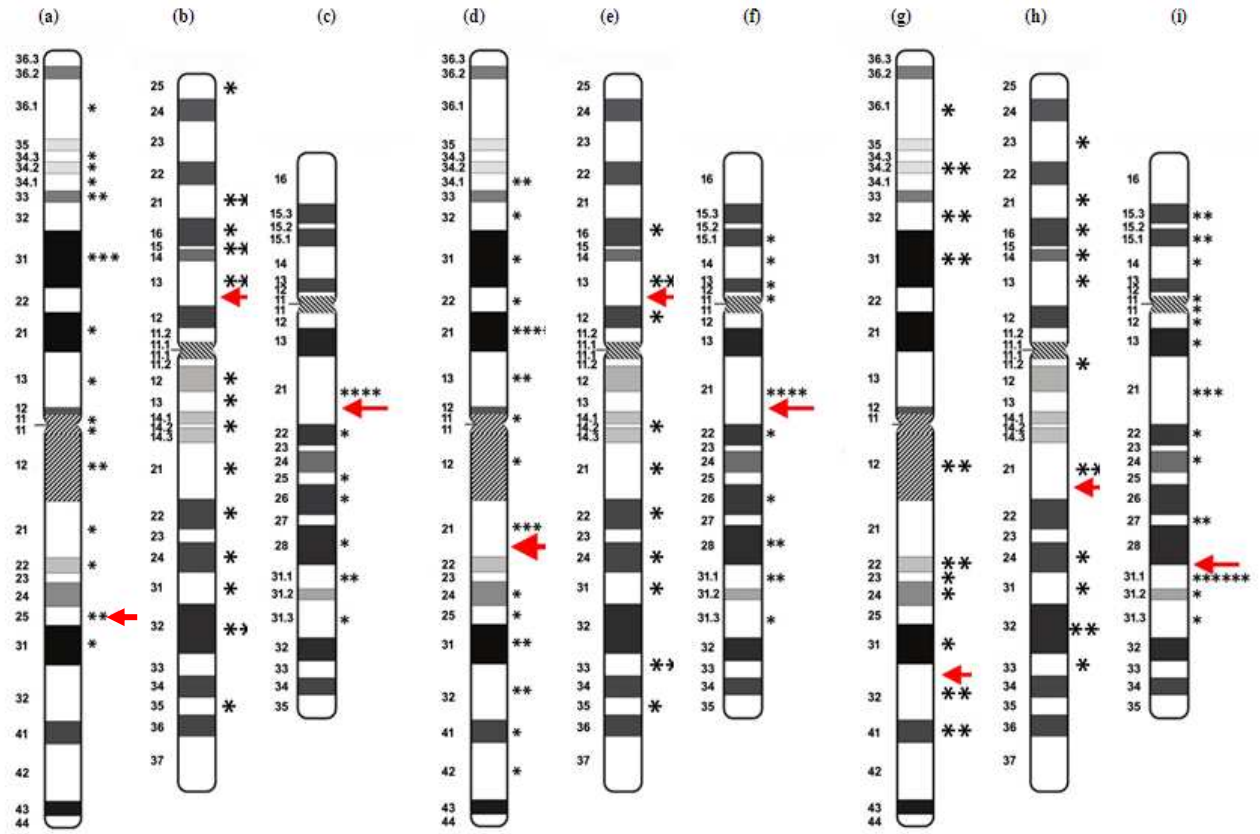

Fig. 4. Breakpoint positions in chromosome 1 at doses of (a) 1 Gy; (d) 3 Gy; and (g) 5 Gy; chromosome 2 at doses of (b) 1 Gy; (e) 3 Gy; and (h) 5 Gy; and chromosome 4 at doses of (c) 1 Gy; (f) 3 Gy; and (i) 5 Gy.

Our experimental results also showed that the ionizing radiation induced breakpoints in the chromosomes were not random. Figure 4 of our experimental data shows that the *q21* breakpoint on chromosome 4 seems to have a high breakage frequency for all doses. Breakages were more frequent in light bands compared to dark bands in all chromosome number at all doses. Possible explanation for these finding is that the light bands contain more active genes than do the dark bands. Our findings also support other several studies that found that breakages induced by radiation were more frequent in light bands that are considered as gene-rich regions [21,22].

From previous studies it was known that chromosomal radiosensitivity based on the translocation in lymphocytes can be proposed as a predictive assay for detection of radiosensitive individuals [23]. Several studies used chromosome translocations to identify the cancer patients with higher radiosensitivity. A study by Huber *et al*. showed that translocations can be used as a test to identify breast tumour patients with potentially elevated radiosensitivities [23].

In biodosimetry using painting of several chromosomes, there was an assumption that the frequencies of chromosomal aberrations were proportional to their size, which is important because it will extrapolate to the whole genome [24]. In contrast, our experimental research found that the frequencies of chromosome aberrations were not proportional to their size. Based on these findings, for a biodosimetry purpose, it is possible that biodosimetry using painting of all chromosomes (multicolor FISH) is better than with painting only several chromosomes. Multicolor FISH is also considered as the best method for assessment of a chromosome's structural damage because it allows unstable and stable aberrations to be detected [25].

Other methods such as telomeric and pancentromeric probes combined with chromosome paint probes can also be use in order to accurately discriminate between translocations and dicentrics [26]. This technique can also overcome the problem of the high cost of the probes for several laboratories [27]. A study by M'kacher . showed that using the telomere and centromere (TC) staining probes it can provides the most precise cytogenetic biological dosimetry currently available. Another advantage of TC staining method is that it does not require a high level of expertise to identify the chromosome aberrations induced by radiation exposure [28].

CONCLUSION

Based on the experimental results, it can be concluded that chromosome 4 was more sensitive to radiation in all doses point despite having less DNA content than chromosome 1 and 2. The general assumption about radiation-induced chromosomal aberration being proportional to DNA content cannot be supported by our experimental results. For a biodosimetry purpose it is possible that multicolor FISH will give a better result than painting only several chromosomes. A comparison of the radiation dose estimates using multicolor FISH and three-color FISH should be conducted in the next experiment to validate or invalidate this assumption.

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