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▣ 論 文 ▣

Analysis of radiation-induced micronuclei and aneuploidy involving chromosome 1 and 4 by FISH technique

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FISH 기법을 이용한 방사선에 의한 소핵과 이수성 분석

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Abstract - The cytokinesis-block micronucleus (CBMN) assay in combination with FISH technique using chromosome-specific centromeric probes for chromosome 1 and 4 was performed in mitogen stimulated human lymphocytes which were exposed to x-radiation to identify different sensitivity of chromosomes to the induction of micronuclei(MN) and aneuploidy by radiation. The frequencies of micronucleated cytokinesis-blocked(MNCB) cells and MN in binucleated lymphocytes(BN) increased with the increase in radiation dose. A significant induction of aneuploidy of chromosome 1 and 4 were found. The frequency of aneuploidy of chromosome 1 and 4 in the control were 9 per 2,000 BN cells and this increased to 47 and 71 following irradiation at a dose of 1 and 2 Gy, respectively. The induction of aneuploidy of chromosome 1 was higher than that of chromosome 4. The frequency of aneuploid BN cells with MN exhibiting positive centromere signal for either chromosome 1 and/or 4 increased in a dose dependent manner, and that for chromosome 1 is higher than that for chromosome 4. Among the total induced MN in irradiated lymphocytes, smaller proportion of MN exhibit centromeric signal of chromosome indicating that radiation-induced MN are mainly originated from chromosomal breakage rather than chromosomal non-disjunction. These results suggest that x-radiation can induce aneuploidy and supports the finding that chromosome vary in their sensitivity to aneuploidy induction by x-irradiation.
Key words : x-radiation, cytokinesis-block micronucleus assay, FISH, aneuploidy

요약 - 본 연구는 소핵분석과 염색체 1번 및 4번의 DNA probe를 이용한 FISH 기법을 병행하여 방사선에 의한 소핵과 이수성에 관여하는 각 염색체의 감수성을 평가하고자 하였다. 방사선 선량에 따라 소핵의 빈도는 증가하였으며 염색체 1번과 4번의 이수성도 대조군, 1 Gy 및 2 Gy 에서 각각 2000개의 BN세포 당 9개, 47개 및 71개로 유의하게 증가하였다. 염색체 1번의 이수성 빈도는 4번에 비해 높게 관찰되었다. 염색체 1번 및 4번을 포함하는 소핵도 방사선의 선량에 따라 증가하였으며, 소핵내 염색체 1번의 포함빈도가 4번보다 높게 관찰되었다. 또한 방사선에 의한 소핵 중 낮은 빈도의 염색체 signal를 포함하는 소핵이 관찰됨으로써 방사선에 의한 소핵은 대부분 절단에 의한 것임을 확인할 수 있었다. 따라서 본 연구 결과 방사선은 이수성을 유도하며 이에 염색체가 다르게 관여할 수 있음을 보여준다
중심어 : 방사선, 소핵분석, FISH, 이수성

Introduction

The use of the cytokinesis-block micronucleus (CBMN) assay in combination with the fluorescence *in situ* hybridization(FISH) technique with a centromeric probe for human peripheral

lymphocytes has been developed to characterize the origin of micronuclei(MN) occurring either spontaneously or following exposure to ionizing radiation and various chemical agents(1, 2). Radiation can induce chromosome breakages and spindle disruption that may be involved in the formation of MN. MN derived from acentric chromosome fragment can be distinguished from those from whole chromosome by FISH technique using a pancentromeric probe that will detect the centromere in the MN. It is known that radiation-induced MN originate primarily from acentric chromosome fragment and minor number of MN may arise from whole chromosome due to spindle disruption during a mitotic cycle(2). It is assumed that ionizing radiation-induced DNA lesions are distributed and repaired randomly along the whole genome.(3-6) However several studies reported that ionizing radiation induced break points nonrandomly among the chromosomes. Heterogeneity among the chromosomes for involvement in the formation of chromosome aberration induced by ionizing radiation has been reported(7-10).

It is reported that chromosome DNA content is not the only factor to be considered for the induction of exchange-type aberration. The involvement of a particular chromosome in exchange-type aberration was reported. Among human chromosomes, higher frequency of chromosome aberration in chromosome 4 and lower frequency of translocation in chromosome 1 induced by x-irradiation was found than expected frequency calculated according to their DNA content.(9)

There has been contradictory results on the involvement of specific chromosomes in radiation induced aberrations analysed by FISH technique. Cigarran S. et al.(11) reported that there was a tendency that chromosome with higher DNA contents (1 to 12 including X) were less frequently involved in the formation of exchange type aberration and breaks than expected according to their DNA content whereas lower DNA content chromosome (13 to 22 including Y) were more frequently involved. (11, 12)

Among long chromosomes, significantly higher number of breaks were observed than expected for chromosome 1 and 2 (13). One of the possible factors contributing to this higher sensitivity of chromosome 4 is its low concentration of transcribing gene compared to chromosome 1(14,15).

In the present study, we analysed x-ray induced MN in combination with FISH technique using chromosome-specific centromeric probe to identify different involvement of specific chromosome in the formation of MN and aneuploidy thus to identify different sensitivity to chromosome aberration induction by radiation.

Materials and Methods

Irradiation and culture condition

Peripheral blood from a health male donor was obtained by venipuncture and collected into heparinized tube. The blood was irradiated with 1 and 2Gy x-ray(LINAC, Varian 6/100) at a dose rate of 2Gy/min.

Immediately following exposure, the blood was cultured in RPMI 1640(GIBCO) supplemented with 10% fetal bovine serum(FBS, GIBCO), 100 units/ml penicillin (GIBCO) and 1% phytohemagglutinin (PHA, GIBCO). The blood was cultured at 37℃ in an atmosphere of 95% air and 5% CO2.

Cytokinesis-Block Micronucleus(CBMN) assay

Human lymphocytes were cultured and Cytochalasin-B (4.0 g/ml, Sigma) was added after 44 hours from the start of the culture, then followed by another incubation for 28 hours. The cells were collected and treated with 0.075M KCl hypotonic solution for 3 minutes and fixed in the mixture of methanol : acetic acid (3:1).

FISH using centromeric probe

Fluorescent hybridization was performed using chromosome specific centromeric probe

(Vysis). Dual color cocktail probes that hybridized to the centromere of chromosome 1 and 4 were applied to the interphase spread from lymphocytes as described by manufacturer's instructions (Vysis).

Briefly, the slides were denaturated in 70% formamide, 2xSSC(ph 7.3) at 73℃ for 5min. The DNA probe was denaturated at 73℃ for 5min. Following an overnight hybridization at 42℃, slide were washed with 2x SSC(ph 7.3) at 73℃ for 2min and 0.4 X SSC/0.1% Nonidet P-40 at 37℃ for 1min. 4',6'-diamidino-2-phenylindole (DAPI) was used in the anti-fade solution of p-phenylenediamine dihydrochloride/glycerol (Vysis, Downers Grove, Illinois, USA) for counterstaining. The hybridized slides were viewed using a Nikon fluorescence microscope that was equipped with an epifluorescent illuminator. The triple bandpass filter for DAPI/FITC/Texas Red(Chroma Technology Corp., Bratleboro, Vermont, USA) was used for visualization of the markers. The stained slides were randomized and coded before examination. For each slide, 2000 BN cells were scored.

Statistical analysis

Statistical analyses were carried out using the SAS 8.1. The association of aberration yield with radiation dose was tested by Kendall rank correlation coefficient(τ). The Fisher's exact test was used to investigate difference of frequencies of aneuploidy and signal positive MN between chromosomes 1 and 4.

Table 1. Frequency of micronuclei in human lymphocyte induced by x-radiation.

Dose (Gy)	No. of BN[a] cells	No. of MNCB	No. of MNi			Total No. of MN
			+1	+2	+3	
control	2,000	3	2	1	0	4
1	2,000	47	43	4	0	51
2	2,000	189	158	29	2	222

a: BN=binucleated

Table 2. The frequencies of different types of aneuploid binucleated cells and micronuclei with positive signals of chromosome 1 in human lymphocyte induced by x-radiation.

Dose (Gy)	No. of BN[a] cells	Normal cells	Aneuploid BN cells					Total aneuploidy
			without MN		with signal positive MN			
		2+2	3+1	4+0	(2+1)+1	(1+1)+2	MN+	
control	2,000	1994	6	0	0	0	0	6
1	2,000	1970	25	0	3	2	5	30
2	2,000	1952	39	1	5	3	8	48

a: BN=binucleated

2+2: 2 signals in each daughter nuclei(normal).

3+1: 3 signals in one daughter nuclei and 1 signal in another daughter nuclei.

4+0; 4 signals in one daughter nuclei only.

(2+1)+1: 2 signals in one daughter nuclei and 1 signal in another daughter nuclei plus a micronuclei with positive signal of chromosome 1.

(1+1)+2: 1 signal in each daughter nuclei plus micronucleus with 2 positive signals of chromosome 1.

MN+: Micronucleus with positive signal of chromosome 1.

Results

As shown in table 1 the frequencies of micronucleated cytokinesis-blocked(MNCB) cells and MN in binucleated(BN) lymphocytes increased with radiation dose.

The frequency of MN in the control were 4 per 2,000 BN cells and this increased to 51 and 222 following irradiation at a dose of 1 and 2Gy, respectively. The induction of aneuploidy by radiation was observed as shown in tables 2, 3 and figure 1. A significant induction of aneuploidy of chromosome 1 and 4 were found. The induction of aneuploidy of chromosome 1 was higher than that of chromosome 4(figure 1). The frequency of aneuploidy BN cells with MN exhibiting positive centromere signal for chromosome 1 was also higher than that of chrpmosome 4 indicating chromosome 1 is suggested to be more involved in the formatioin of MN and aneuplidy than chromosome 4.

Among the total induced MN in irradiated lymphocytes, smaller proportion of MN exhibit centromeric signal of chromosome indicating that radiation-induced MN are mainly originated from chromosomal breakage rather than chromosomal non-disjunction (table4).

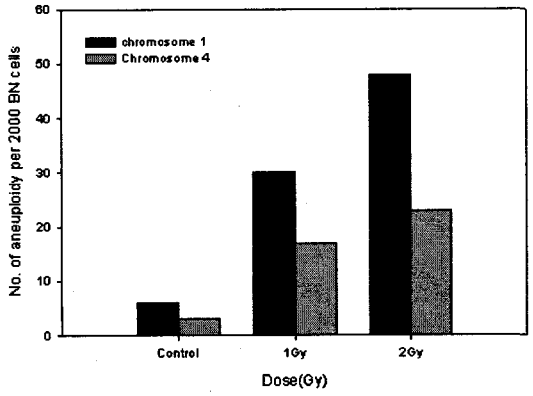

Fig. 1. Frequency of aneuploid BN cells with positive fluorescent signal of chromosome 1 and 4 in human lymphocytes induced by x-radiation.

Discussion

A non-random distribution of aberrant chromosome induced by ionizing radiation were reported elsewhere. The different involvement of different chromosome in the formation of chromosome aberration was suggested and the same may be true for the chromosome or chromosome fragment that form MN.(7-10).

Table 3. The frequencies of different types of aneuploid binucleated cells and micronuclei with positive signals of chromosome 4 in human lymphocyte induced by x-radiation.

Dose (Gy)	No. of BN[a] cells	Normal cells	Aneuploid BN cells					Total aneuploidy
			without signal in MN		with signal in MN			
		2+2	3+1	4+0	(2+1)+1	(1+1)+2	MN+	
control	2,000	1997	3	0	0	0	0	3
1	2,000	1983	16	1	0	0	0	17
2	2,000	1977	22	0	1	0	1	23

a: BN=binucleated

2+2: 2 signals in each daughter nuclei(normal).

3+1: 3 signals in one daughter nuclei and 1 signal in another daughter nuclei.

4+0; 4 signals in one daughter nuclei only.

(2+1)+1: 2 signals in one daughter nuclei and 1 signal in another daughter nuclei plus a micronuclei with positive signal of chromosome 4.

(1+1)+2: 1 signal in each daughter nuclei plus micronucleus with 2 positive signals of chromosome 4.

MN+: Micronucleus with positive signal of chromosome 4.

Table 4. The frequency of micronuclei with positive fluorescent signal of chromosome 1 and 4 in human lymphocytes induced by x-radiation.

Dose (Gy)	No. of BN[a] cells	No. of MN	No. of MN with chromosome 1	No. of MN with chromosome 4	No. of MN with chromosome 1 and 4
control	2,000	4	0	0	0
1	2,000	51	7	0	7
2	2,000	222	11	1	12

a: BN=binucleated

b: Kendall's τ was calculated on cell bases.

A combination of micronucleus and a FISH technique using chromosome-specific centromeric probes allow to measure the structural chromosome aberration that did not form MN from chromosomal non-disjunction and aneuploidy resulting from chromosome loss and non-disjunction in interphase cells(1,2). An induction of non-disjunction by low doses of x-ray was demonstrated elsewhere(16,17).

In this study, the induction of aneuploidy of chromosome 1 by radiation was higher than that of chromosome 4, suggesting that chromosome 1 is more involved in the process of aneuploidy induction. Chromosomes 1 and 4 were widely studied due to their relative higher content of break points. Although some authors have reported that the frequencies of radiation-induced chromosome aberration was proportional to the DNA content of the target chromosome (3,4). many studies showed that the involvement of individual chromosome in the radiation induced chromosomal aberration was not relative to their DNA content(6-10). Different sensitivity of chromosome to aberration induction byionizing radiation has been reported. Possible explanations for this phenomenon were proposed as follows. Due to the fact that heterochromatin regions repaired the lesions much slower than the enchromatin region, chromosome that are rich in heterochromatin are expected to be more sensitive to aberration induction(6). Another possible explanation is the richness of interstitial telomere repeat in the chromosome, because increased possibility for interaction between induced breaks has been reported in the interstitial telomere sequence due to the fact that this sequence tend to aggregate in the interphase forming chromocenters(14, 18). The differential involvement of different chromosome in the formation of chromosomal aberration is still controversial.

There have been contradictory results on the sensitivity of chromosomes 1 and 4 to ionizing radiation. Boei et al.(9) reported higher radiation sensitivity of chromosome 4 compared to chromosome 1. They reported similar frequency of dicentrics for chromosome 1 and 4, but the frequency of reciprocal translocation involving chromosome 4 was overrepresented.

Since no direct comparison of the relative sensitivity of chromosomes 1 and 4 to aneuploidy and MN induction by ionizing radiation was reported until now, it is of interest to compare our results with that analysed thorough bleomycin exposure.

Bleomycin is radiomimetic agent which can efficiently induce double strand break in DNA like ionizing radiation although there are differences between bleomycin and radiation in the way they break DNA. A recent study by Adema et al.(19) reported that bleomycin seems to be a good alternation to radiation for G2 chromatid radiosensitivity test.

Whereas Puerto s. et. al(20) reported that chromosomel and 4 were equally sensitive to the clastogenic effect of bleomycin, Ellard et al.(21) reported that chromosome 1 was overrepresented in bleomycin-induced chromosomal aberration which were calculated according to its DNA content.

In summary chromosome 1 is more involved than chromosome 4 in the process of aneuploidy and MN induction by radiation and combined application of CBMN assay with FISH technique can be used to obtain information on the origin of aneuploidy and MN.

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References

1. H.W. Chung, S.J. Kang and S.Y. Kim, "A combination of the micronucleus assay and a FISH technique for evaluation of the genotoxicity of 1.2.4-benzotrial", Mutat. Res., 516, 49-56(2002)
2. A. Kryscio, W.U. Ulrich Müller, A. Owjcik, N. Kotschy, S. Grobelny and C.Streffer, "A cytogenetic analysis of the long-term effect of uranium mining on peripheral lymphocytes using the micronucleus- centromere assay", Int. J. Radia. Biol. 77(11), 1087-1093(2001)
3. J.N. Lucas, A. Awa, T. Straume, M. Poggensee, Y. Kodama, M. Nakano, K. Ohtaki, H.-U. Weier, D. Pinkel, J. Gray and G. Littlefield, "Rapid translocation frequency analysis in humans decades after exposure to ionizing radiation", Int. J. Radia. Biol., 62(1), 53-63(1992)
4. J.D. Tucker, M.J. Ramsey, D.A. Lee and J.L. Hinkler, "Validation of chromosome painting as a biodosimeter in human peripheral lymphocytes following acute exposure to ionizing radiation in vitro", Int. J. Radiat. Biol., 64, 27-37(1993)
5. J.F. Barqunero, S. Knehr, H. Braselmann, M. Figel and M. Bauchinger, "DNA-proportional distribution of radiation-induced chromosome aberrations analysed by fluorescence in situ hybridization painting of all chromosomes of a human female karyotype", Int. J. Radiat. Biol., 74(3), 315-323(1998)
6. K.L. Johnson, D.J. Brenner, J. Nath, J.D. Tucker and C.R. Greard, "Review radiation-induced breakpoint misrejoining in human chromosome: random or non-random?", Int. J. Radiat. Biol., 75(2), 131-141(1999)
7. P. Finnon, D.C. Lloyd and A.A. Edwards, "Fluorescence in situ hybridization detection of chromosomal aberrations in human lymphocytes: applicability to biological dosimetry", Int. J. Radiat. Biol., 68(4), 429-435(1995)
8. A. Wojcik and C. Streffer, "Comparison of radiation-induced aberration frequencies in chromosomes 1 and 2 of two human donors", Int. J. Radiat. Biol., 74(5), 573-581 (1998)
9. J.J.W.A. Boei, S. Vermeulen and A.T. Natarajan, "Differential involvement of chromosomes 1 and 4 in the formation of chromosomal aberrations in human lymphocytes after x-irradiation", Int. J. Radiat. Biol., 72(2), 139-145(1997)
10. S. Knehr, H. Zitzelsberger, H. Braselmann, U. Nahrstedt and M. Bauchinger, "Chromosome analysis by fluorescence *in situ* hybridization: further indications for a non DNA-proporional involvement of single chromosomes in radiation-induced structural aberration", Int. J. Radiat. Biol., 70(4), 385-392(1996)
11. S. Cigarran, L. Barrios, J.F. Barquinero, M.R. Caballin, M. Ribas and J. Egozcue, "Relationship between the DNA content of human chromosomes and their involvement in radiation-induced structure aberrations, analysed by painting, Int. J. Radiat. Biol., 74(4), 449-455(1998)
12. S. Knehr, H. Zitzelsberger, H. Braselmann, U. Nahrstedt and M. Bauchinger, Chromosome analysis by fluorescence in situ hybridization: further indications for a non-DNA-proportional involvement of single chromosome in radiation-induced structural aberrations, Int. J. Radiat. Biol., 70(4), 385-392(1996)
13. T.K. Pandita, V. Gregoire, K. Dhingra and W.N. Hittelman, "Effect of chromosome size on aberration levels caused by gamma

radiation as detected by fluorescence in situ hybridization", Cytogenet. cell genet., 67, 94-101(1994)
14. A.T. Natarajan, A.S. Balajee, J.J. Boei, F. Darroudi, I. Dominguez, H.P. Hande, M. Meifers, P. Slijepcevic, S.V. Vermeulen and Y. Xiao, "Mechanisms of induction of chromosomal aberrations and their detection by fluorescence in situ hybridization", Muatat. Res., 372, 247-258(1996)
15. J.M. Craig and W.A. Bickmore, "The distribution of CpG islands in mammalian chromosomes" Nature Genet., 7, 376-381 (1994)
16. A.T. Natarajan, S.E. Vlaslom, A. Manca, P.H.M. Lohman, J.A. Gossen, J. Vijg, F. Beerman, E. Hummler and I. Hansmann, "Transgenic mouse- an in vivo system for detection of aneugeus In Mutation and the Environment Edited by : M.L. Mendelshon and R-J. Albertini(New York, Wileg-Liss) 295-299(1990)
17. J.J. Boei and A.T. Natarajan, "Detection of chromosome malsegregation to the daughter nuclei in cytokinesis-blocked transgenetic mouse splenocytes", Chromosome Res., 3(1), 45-53(1995)
18. A.T. Natarajan, A.S. Balajee, J.J. Boei, S. Chatterjee, F. Darroudi, M. Grigorova, M. Noditi, H.J. Oh, P. Slijepcevic and S. Vermeulen, "Recent development in the assessment of chromosomal damage", Int. J. Radiat. Biol., 66(5), 615-623(1994)
19. A.D. Adema, J. Cloos, R.H. Verheijen, B.J. Braakhuis and P.E. Bryant, "Comparison of bleomycin and radiation in the G2 assay of chromatid breaks". Int. J. Radiat. Biol., 79(8), 655-661(2003)
20. S. Puerto, J. Surralles, M.J. Ramirez, E. Carbonell, A. Creus and R. Marcos, "Analysis of bleomysin- and cytosine arabinoside-induced chromosome aberration involving chromosomes 1and 4 by painting FISH", Mutat. Res., 439, 3-11(1999)
21. S. Ellard, E.M. Parry and J.M. Parry, "Use of multicolor chromosome painting to identify chromosome rearrangements in human lymphocytes exposed to bleomycin: a comparison with conventional cytogenetic analyses of Giemsa-stained chromosome", Envrion. Mol. Mutagen, 26(1), 44-54(1995)