Interchromosomal distribution of gamma ray-induced chromatid aberrations in Chinese hamster ovary cells[*]

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Abstract

Interchromosomal distributions of breakpoints from chromatid-type aberrations induced by gamma rays in Chinese hamster ovary (CHO) cells were analyzed. In most chromosomes the distribution was as expected from chromosome lengths for simple breaks or the respective relative corrected length in case of exchanges. There were deviations from expectation in a few chromosomes for chromatid breaks, interchanges, intra-arm intrachanges and inter-arm intrachanges. Especially interesting are the results concerning chromosomes 2 and 8, which were more often involved in exchanges than expected. An "exchange phenotype" for these chromosomes is proposed and possible explanations for the non-random distribution of chromosome breakpoints are presented.

INTRODUCTION

One of the ultimate lesions for the formation of chromosomal aberrations (CA) are DNA double-strand breaks (DSB) which are induced by agents such as ionizing radiation or endonucleases (Obe *et al.*, 1992, Pfeiffer *et al.*, 2000). Considerable evidence exists on the influence of chromatin structure or transcriptional activity in the processing of DNA lesions other than DSB (Mullenders *et al.*, 1990; Friedberg *et al.*, 1995; Proietti De Santis *et al.*, 2000). However, little information is available about the role of chromatin structure in the processing of DSB and the formation of CA (Pfeiffer *et al.*, 2000). Giemsa light bands and especially their subset, the T-bands, are regions rich in active chromatin, where cancer-associated exchange breakpoints, constitutional chromosome breakage sites and X-ray-induced breakpoints are clustered (Holmquist, 1992; Saccone *et al.*, 1992).

The Chinese hamster genome contains short and long interspersed repeated elements (SINEs and LINEs). SINEs mainly harbour the *Alu* family and LINEs the MIF-1 (or *Bam*HI) family. SINEs are clustered in G-light bands and LINEs in G-dark bands (Holmquist and Caston, 1986). Therefore, chromosomal breakpoints induced with the restriction endonucleases *Alu*I or *Bam*HI in Chinese hamster ovary (CHO) chromosomes should map to G-light and G-dark bands, respectively. However, the majority of breakpoints produced by both enzymes were located in G-light bands (Folle and Obe, 1995), irrespective of the cell cycle stage exposed (G1- or S-phase) (Folle and Obe, 1996). Moreover, breakpoint patterns produced by DNase I in S-phase CHO cells are nearly identical to those induced by *Alu*I or *Bam*HI (Folle *et al.*, 1997). When Chinese hamster embryonic (CHE) cells were X-irradiated in the G1-phase, breakpoints preferentially mapped to Giemsa-light bands (Slijepcevic and Natarajan, 1994a). However, it should be noted that breakpoint mapping for chromosome-type exchanges is subject to a pattern recognition artefact that biases pale-band location (Savage, 1977).

In 11 G-banded chromosomes of CHO cells the major breakpoint clusters produced by endonucleases (*Alu*I, *Bam*HI and DNase I) co-localize with the ones induced by gamma rays or neutrons though radiation-induced hot-spots were not so pronounced as the ones induced by endonucleases (Folle *et al.*, 1998, Martínez-López *et al.*, 1998).

Dominguez *et al.* (1996), Grigorova *et al.* (1998) and Xiao and Natarajan (1999) analyzed the yield of radiation-induced symmetrical and asymmetrical exchanges by fluorescence *in situ* hybridization (FISH) and showed a non-random involvement of individual chromosomes or their arms in the production of chromosomal aberrations.

There are a variety of factors that will influence breakpoint localization. For example, it is assumed that endonucleases have more access to chromatin areas in which the DNA is not tightly packed, namely, G-light bands (Folle *et al.*, 1997). The pattern of breakpoint distribution may therefore, in this case, coincide with the pattern of primary lesions.

The initial lesions induced by low LET radiation are probably randomly distributed throughout the chromosomal complement (Savage, 1993). A deviation of observed breakpoint localization from a random pattern may therefore rather be a result of differential repair (Pantelias, 1994). In this paper we show that not only the intra- but also the interchromosomal distribution of observed breakpoints in chromatid-type aberrations do not appear to be random in CHO cells.

MATERIAL AND METHODS

CHO karyotype

A subclone of CHO cells (CHO9 from A.T. Natarajan, Leiden) derived from a CHO fibroblast culture established by Puck *et al.* (1958) was used. CHO9 contains a modal number of 21 chromosomes (Figure 1a), with 9 seemingly normal Chinese hamster chromosomes (1, 2, 5, 7, 9, 10, X, two chromosomes 8) and 12 rearranged Z-chromosomes (Z1 to Z10, Z12 and Z13) (Deaven and Petersen, 1973; Siciliano *et al.*, 1985). Table I shows the origin of Z-chromosomes. The long arm of the X chromosome is nearly entirely heterochromatic with a secondary constriction in the middle (Ray and Mohandas, 1976).

Cell culture and irradiation procedures

Cells were grown on plastic Petri dishes (95 mm) in McCoy's 5A medium supplemented with 10% foetal calf serum, 200 mM glutamine and antibiotics (100 U/ml penicillin and 125 µg/ml dihydrostreptomycin sulfate) at 37°C in a 5%-CO_2 incubator.

Exponentially growing cells (2×10^6) were seeded in 25-cm^2 culture flasks and monolayers were exposed to 2 Gy gamma rays using a THERATRON-780 (^{60}Co) gamma irradiator (Faculty of Medicine, Montevideo) at a dose rate of 1.07 Gy/min at room temperature.

Cells were sampled 8 h after irradiation, following a 2-h colcemid (0.08 µg/ml) treatment. Controls were processed in the same way but not irradiated. Mitotic cells were collected by shake-off, treated with 1% sodium citrate for 10 min at 37°C and fixed (2x) in methanol-acetic acid (3:1). Preparations were made following standard procedures. G-banding was carried out as reported previously (Folle and Obe, 1995).

Scoring of chromosome breakpoints

The frequencies of all types of chromosomal aberrations (with the exception of achromatic lesions, which were not considered) were analyzed in 100 Giemsa-stained cells from both control and irradiated cells. In the irradiated cells we found 46% damaged cells, mainly with chromatid-type aberrations (Figure 1b-c). About 5% of metaphases contained very heavy damage, making accurate breakpoint as-

signment impossible. These were omitted from our analyses. Only 5% of the cells contained chromosomal damage in the controls.

In the irradiated cells the distribution of 1405 observed aberration breakpoints amongst all the G-banded CHO chromosomes was analyzed for chromatid breaks, interchanges, intra-arm intrachanges and inter-arm intrachanges using an Axioplan microscope (Zeiss) with a 100X-Neofluar phase contrast objective.

Statistical analysis

Given the assumption that the initial radiation-induced breakpoints are randomly allocated to the arms, then the probability of simple open chromatid breaks (in so far as these are a direct manifestation of the initial breaks) will be directly proportional to the relative arm length, size, or DNA content.

For exchanges, which result from the interaction of (a minimum of) two breaks, the allocation of observed
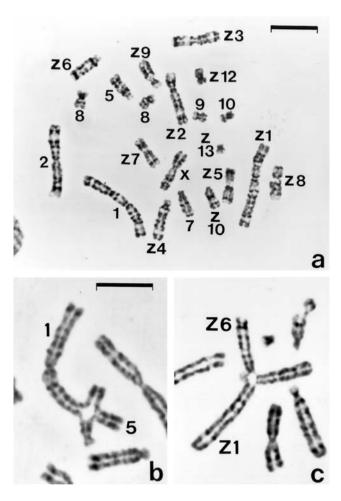

Figure 1 - a) G-banded CHO9 metaphase with the single chromosomes indicated. **b)** Chromatid interchange between chromosomes 1 and 5. **c)** Chromatid interchange between chromosomes Z1 and Z6. Bars indicate: **a)** 10 µm; **b,c)** 5 µm.

breakpoints will be directly proportional to the product of the arms involved. Using a probability matrix containing the products of all possible arm interactions, the selective summation of exchange components results in a new set of arm lengths, termed relative corrected lengths or RCLs (Savage and Papworth, 1982; Savage, 1991). Under the assumption of randomness both for the initial lesions and for the exchanges formed by these lesions, we expect the allocation of observed exchange breakpoints to be directly proportional to RCLs. These expectations are shown in Table II.

The overall difference between observed and expected frequencies of chromosomal breakpoints was tested by using χ^2-analysis. In order to decide whether single chromosomes were more or less frequently involved in the formation of breaks or exchanges, a partitioned χ^2-test was used (Savage and Papworth, 1982).

RESULTS AND DISCUSSION

Most of the previous investigations on the distribution of observed chromosome breakpoints amongst Chinese hamster chromosomes were carried out in G0/G1-phase of the cell cycle (Slijepcevic and Natarajan, 1994a,b; Dominguez et al., 1996; Grigorova et al., 1998; Xiao and Natarajan, 1999). Detection of chromosome aberrations for such analyses is limited to interchanges and inter-arm intrachanges. Very few intra-arm aberrations can be mapped, either using G-banding or FISH. In contrast, chromatid-type aberrations allow one to identify, and locate the breakpoints of, a much larger range of events, providing an enhanced analysis.

Radiation-induced breakpoints from 122 chromatid breaks, 483 interchanges (chromatid/chromatid and chromatid/isochromatid), 710 intra-arm intrachanges (iso-chromatid breaks, interstitial and duplication deletions) and 80 inter-arm intrachanges (chromatid rings and inversions) were investigated and found to be nonrandomly distributed among the 21 chromosomes of CHO9 cells (Table III).

Some chromosomes showed more (1, Z1, Z3) or less (8) chromatid breaks than expected. No breakpoints were observed in chromosomes 9, 10 and Z13. Breakpoint frequencies in chromatid interchanges were higher (8, Z3) or lower (2) than expected according to RCLs. In relation to chromatid intra-arm intrachanges we observed more breakpoints than expected in chromosomes 10, Z5 and Z9 and less in chromosomes Z8 and Z10. Chromatid inter-arm intrachanges showed a highly significant frequency of breakpoints in chromosome 2 (Table III).

Chromosome 8 is an interesting case. It has significantly fewer breakpoints corresponding to chromatid breaks but more corresponding to interchanges. In irradiated primary Chinese hamster fibroblasts it was found that chromosome 8 is overrepresented in chromosome-type interchanges (Slijepcevic and Natarajan, 1994a,b; Dominguez et al., 1996; Grigorova et al., 1998; Xiao and Natarajan,

Table I - Description of rearranged CHO9 chromosomes (Z). In addition to Z-chromosomes there are the following seemingly normal Chinese hamster chromosomes: 1, 2, 5, 7, 8, 9, 10, X. With the exception of two chromosomes 8, all the others including the Z-chromosomes are present in one copy.

Z-chromosomes	Origin of Z-chromosomes [main Chinese hamster chromosome in the respective Z-chromosome]
Z1	t (1p;5q) [1]
Z2	del (2q) [2]
Z3	t (3p;4p) [3]
Z4	inv (3) [3]
Z5	del (4) [4]
Z6	t (1p;5q) [5]
Z7	t (3p;4p) [4]
Z8	t (6p, [a]) [6]
Z9	t (6p, [a]) [6]
Z10	Unknown origin
Z12	Unknown origin
Z13	Unknown origin

[a]Second partner of translocation is unknown.

Table II - Relative length and relative corrected lengths (RCLs) for interchanges, intra-arm intrachanges and inter-arm intrachanges of CHO chromosomes.

Chromosome	Relative length	RCL interchange	RCL intra-arm intrachange	RCL inter-arm intrachange
1	0.113	0.107	0.182	0.215
2	0.092	0.089	0.119	0.145
5	0.038	0.039	0.029	0.014
7	0.034	0.035	0.023	0.011
8	0.046	0.047	0.030	0.036
9	0.019	0.020	0.005	0.006
10	0.017	0.018	0.004	0.005
x	0.059	0.059	0.050	0.057
z1	0.107	0.102	0.165	0.189
z2	0.071	0.071	0.080	0.076
z3	0.062	0.062	0.054	0.066
z4	0.057	0.058	0.069	0.027
z5	0.048	0.048	0.032	0.038
z6	0.044	0.045	0.040	0.018
z7	0.044	0.044	0.027	0.032
z8	0.045	0.046	0.030	0.032
z9	0.040	0.041	0.027	0.023
z10	0.031	0.032	0.024	0.002
z12	0.021	0.022	0.006	0.008
z13	0.012	0.013	0.003	0.002
Total	1.000	1.000	1.000	1.000

1999). Breaks in chromosome 8 seem to be preferentially transformed to interchanges in primary Chinese hamster cells as well as in CHO cells derived a long time ago from the primary cells. Chromosome 8 has been reported to be enriched in interstitial telomeric sequences which could be involved in the formation of radiation-induced chromosomal aberrations (Balajee et al., 1994). This fact could explain the over-representation of interchanges in chromosome 8.

Table III - Interchromosomal distribution of radiation-induced breakpoints involved in chromatid breaks, interchanges and intrachanges in CHO cells.

Chromosome	Chromatid breaks	Chi-square	Interchanges	Chi-square	Intra-arm intrachanges	Chi-square	Inter-arm intrachanges	Chi-square
1	20	*2.76(+)	40	2.70	124	0.22	16	0.08
2	10	0.14	31	*3.44 (-)	89	0.27	28	***23.26 (+)
5	5	0.02	24	1.30	20	0.03	0	1.15
7	4	0.00	21	1.02	9	3.44	0	0.84
8	2	*2.32 (-)	41	***14.86 (+)	17	0.84	2	0.26
9	0	*2.34 (-)	12	0.54	6	1.44	0	0.50
10	0	*2.10 (-)	15	*4.57 (+)	8	**8.44 (+)	0	0.40
x	8	0.09	33	0.66	44	2.00	2	1.46
z1	19	*2.71(+)	37	3.08	123	0.33	18	0.54
z2	6	0.84	25	2.47	48	1.38	8	0.61
z3	16	**9.28(+)	40	*3.23 (+)	49	2.79	2	2.03
z4	4	1.27	26	0.13	42	1.05	2	0.01
z5	4	0.56	22	0.08	34	*5.60 (+)	0	*3.02 (-)
z6	8	1.25	16	1.56	22	1.53	0	1.41
z7	2	2.06	19	0.28	19	0.00	0	*2.53 (-)
z8	5	0.05	28	1.50	10	*6.22 (-)	2	0.13
z9	6	0.24	20	0.00	30	*6.22 (+)	0	1.80
z10	1	1.99	15	0.00	8	*4.98 (-)	0	0.16
z12	2	0.12	12	0.18	8	3.11	0	0.60
z13	0	1.49	6	0.01	0	1.78	0	0.16
Total	122	*31.64	483	**41.60	710	***51.67	80	**40.96

*P < 0.05; **P < 0.005; ***P < 0.0005; (+) more breakpoints than expected; (-) less breakpoints than expected.

By the same token, chromosome 10 also shows an intense hybridization signal for interstitial telomeric repeats and presents a significant increase of interchanges and intra-arm intrachanges (Table III).

Another example of interest is chromosome 2. It exhibits fewer interchanges than expected but shows a significantly high number of inter-arm intrachanges. Dominguez *et al.* (1996) studying translocations and dicentrics induced by X-rays in Chinese hamster splenocytes and fibroblasts by means of chromosome painting also observed a low frequency of interchanges in chromosome 2 in both cell types according to RCLs. However, Grigorova *et al.* (1998) detected, with FISH, a random frequency of exchanges produced by neutrons and X-rays in chromosome 2 of Chinese hamster splenocytes, based on DNA content of the chromosomes.

Chromosome Z3 is involved more frequently than expected in chromatid breaks and interchanges. As shown with antibodies against acetylated histone H4, Z3 exhibits a high level of acetylation (Martínez-López *et al.*, 2000) which may be considered a cytogenetic marker for gene expression (Jeppesen, 1997). Recently, we have shown a correlation between chromosome regions enriched in hyperacetylated H4 with breakpoint clusters induced in S-phase CHO cells by the endonucleases *Alu*I, *Bam*HI and DNase I as well as by neutrons and gamma rays (Martínez-López *et al.*, 2000). Several authors have stressed the importance of chromatin activity for the localization of chromosome breakpoints (Savage, 1993; Cremer *et al.*, 1995; Folle *et al.*, 1998).

We propose that chromosomes may have different phenotypes with respect to their characteristics to form different types of radiation-induced chromosomal aberrations. In some chromosomes the distribution of breakpoints may deviate from randomness in a manner characterizing the chromosomes in which this occurs. It seems as if chromosomes 2 and 8 are preferentially participating in exchange-type aberrations, they have an "exchange phenotype". Similarly, chromosome Z3 may have both an "exchange" and a "break" phenotype.

Not only the primary lesions as such but also the reaction of chromosomes to them is important. Random induction of primary lesions does not necessarily lead to random formation of aberrations. There is a whole range of production steps, followed by a complex program of chromosome compaction in preparation for division that intervenes between initiation and metaphase observation. Furthermore, the initial DSB and subsequent interaction are at the molecular level, many orders of magnitude below resolution at light microscope level, and possibilities for modification or disguise are endless.

It is therefore very important to recognize the fact that the aberrations we observe are the processed forms. They may not reflect the initial distribution of the DNA damage. In any case, we are only able to score a residue of the initial lesions, namely those that enter into aberrations which can be seen and recognized (Savage and Harvey, 1994; Savage, 1996; Harvey and Savage, 1997). A whole spectrum of chromosomal lesions from unvisible to visible ones can be expected to occur and we do not know

whether the formation of all of these follows the same rules or whether they are differentially influenced by intra- and/or extranuclear factors.

There is increasing evidence that the intra-nuclear architecture, the arrangement and proximity of arm domains may have a profound influence upon the probabilities of break interaction. Thus, the topology of chromosome territories and the active chromatin loops protruding into the inter-domain channels can determine both the frequency, type and the position of aberrations in relation to the arms (Savage, 1993; Folle *et al.*, 1998).

Departures from random expectation for individual chromosomes, such as those reported here, alert us to the operation of these complicating factors, and remind us that we still have much yet to learn about the formation of chromosomal aberrations.

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RESUMO

As distribuições intercromossômicas de pontos de quebra vindos de aberrações tipo cromátide induzidas por raios gama em células de ovário de hamster chinês foram analisadas. Na maioria dos cromossomos a distribuição foi a esperada de comprimentos de cromossomos para quebras simples ou, em caso de trocas, o respectivo comprimento corrigido. Em uns poucos cromossomos, houve desvios a partir do esperado para quebras de cromátides, trocas, trocas dentro do braço e trocas entre braços. Especialmente interessantes foram os resultados relativos aos cromossomos 2 e 8, que se envolveram nas trocas mais freqüentemente do que o esperado. Um "fenótipo de troca" para esses cromossomos é proposto e possíveis explicações para a distribuição não-aleatória de pontos de quebra cromossômicas são apresentadas.

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