RADIATION INDUCED CHROMOSOME ABERRATIONS AND INTERPHASE DNA GEOMETRY

N. Nasazzi, D. Otero* and M. Di Giorgio

Ente Nacional Regulador Nuclear
* Comisión Nacional de Energía Atómica
ARGENTINA

SUMMARY

Ionizing radiation induces DNA double strand breaks (DSBs) and their interaction and illegitimate recombination produces chromosome aberrations. Stable chromosome aberrations comprise inter-chromosomal events (translocations) and intra-chromosomal events (inversions).

Assuming DSBs induction and interaction is completely random and neglecting proximity effects, the expected ratio of translocations to inversions is F=86, based on chromosome arm lengths.

We analyzed the number of translocations and inversions using G-banding, in 16 lymphocyte cultures from blood samples acutely irradiated with γ rays (dose range: 0.5Gy - 3Gy). Our results give F=13.5, significantly smaller than F=86. Literature data show similar small F values but strongly spread.

The excess of inversions could be explained by a "proximity effect", it means that more proximate DSBs have an extra probability of interaction. Therefore, it is possible to postulate a special chromosome arrangement during irradiation and the subsequent interval.

We propose a model where individual chromosomes show spherical confinement with some degree of overlapping and DSBs induction proportional to cross section. We assume a DSBs interaction probability function with cut-off length = 1μm. According to our results the confinement volume is $\approx$ 6.4% of the nuclear volume. Nevertheless, we propose that large spread in F data could be due to temporal variation in overlapping and spatial chromosome confinement.

INTRODUCTION

Ionizing radiation damages DNA and produces chromosome aberrations, generally recognized in metaphase using conventional staining, G banding or fluorescence in situ hibridization (Fish).(1-3)

The primary mechanism for the production of exchange-type chromosomal aberrations after irradiation of resting lymphocytes is the pairwise interaction of two double-strand breaks (DSBs) an their illegitimate recombination.(4)

The simplest aberrations to score in metaphase spreads are dicentrics, which are interchromosomal events, and centric rings, which are intrachromosomal events. These are unstable aberrations and they do not survive after cell division. The stable counterparts are reciprocal translocations and pericentric inversions, respectively, and they are used particularly in retrospective dosimetry due to the fact that they remain long after overexposure to ionizing radiation. The correspondence between unstable and stable aberration production has been generally confirmed.(5)

The observed ratio of interchromosomal interactions (dicentrics or reciprocal translocations) to intrachromosomal events (centric rings or pericentric inversions) indicates that it is smaller than predicted assuming complete randomness, it is there are more centric rings or pericentric inversions than expected.

The observed excess of intrachromosomal events may be due to a proximity effect (6): two DSBs induced in a short distance have an extra probability of pairwise interaction and recombination. Furthermore, there is evidence of chromosome localization during interphase (7).

Assuming that the excess of intrachromosomal events is due to the above mentioned facts, we propose a lymphocyte DNA spatial arrangement during interphase, where individual chromosomes are confined to spherical domains which interact among them under several constrains and we estimated, according to our experimental data, the size of the chromosome domain.

MATERIAL AND METHODS

The number of radiation induced translocations and pericentric inversions were obtained from 16 lymphocyte cultures of both sex donors, which are part of our calibration curve data set for low LET radiation using stable chromosome aberrations. Blood samples were acutely irradiated with γ rays from a Co^{60} source (Picker C4M60) with doses ranging from 0.5Gy to 3Gy (mean dose-rate: 0.5Gy/min). Heparinized whole blood from each donor was irradiated and cultured using micromethod for 48 h in 10 ml of RPMI 1640 medium containing 20% fetal calf serum and PHA (0.15 mg/ml). Colchicine was added to the cultures after 47 h of incubation at 37°C and air dried metaphase spreads were prepared.

Translocations and inversions were identified by G banding, according to Seabright technique, modified.(8) All metaphases were photographed and analyzed using enlarged prints. We observed 325 reciprocal translocations and 24 pericentric inversions in 1040 banded metaphases. The ratio of interchromosomal to intrachromosomal events is F = 13.5

MODEL

Let F represent the ratio of translocations to pericentric inversions. Assuming that DSBs are produced at random on a chromosome arm, proportional to its length in base pairs following a Poisson distribution and assuming, in addition, that pairwise DSBs interactions and recombinations are completely random and neglecting proximity effects, it is possible to estimate a theoretical value of F. (9-11)

$$F = \frac{\sum_{i=1}^{46}(L_i + S_i)(T - S_i - L_i)}{2\sum_{i=1}^{46} L_i S_i} = 86$$

where: L_i = length in megabases of *i*th chromosome long arm.

S_i = length in megabases of *i*th chromosome short arm.

T_i = length in megabases of average diploid set weighted for both sexes.

representing the numerator the average number of reciprocal translocations and the denominator the average number of pericentric inversions.

The convention for translocation counting assumes complete reciprocity. It is important to point out that when it is said that the theoretical F value neglects proximity effects, it means that the individual chromosomes are considered freely distributed in the nuclear volume filling fractions of this volume just according to their lengths.

The obtained value F = 86 is significantly higher than our experimental result F = 13.5 an those obtained by other authors considering unstable and stable chromosome aberrations and using radiation of different linear energy transference (12).

A plausible explanation for the observed small F values is that DSBs produced on the two arms of the same chromosome are in fact and on average more proximal. It implies additional chromosome confinement in domains during interphase, when DSBs are induced and recombined. The proposed spherical domain volumes are smaller than the nuclear volume. We accept several experimental an theoretical considerations (13) which suggest that the probability g(r) that two induced DSBs separated by a distance *r* will interact and produce an exchange event is the cutoff form:

g = constant if $r \leq h$ and g = 0 if $r > h$

Here h is a maximum interaction distance and its value is ≈ 1μm (14). It is interesting to consider that even if two DSBs are produced at a distance more than h apart and are possible to move to get in contact, they may be restituted before interacting and, anyway, it is possible to consider $g \approx 0$ for $r \geq h$.

Then, assuming chromosome spherical domains, two DSBs produced in both arms of the same chromosome have an extra chance of interaction than DSBs produced in different chromosomes because they will more probably lie within 1μm each other.

Now, we propose to analyze DSBs interactions assuming a spherical domain maximum packing model with 12 nearest neighbors in contact, corrected by nuclear surface effect. Moreover, we postulate that the DSBs production is proportional to the geometric cross section , defined as (chromosome length)$^{2/3}$

Based on the above described assumptions the theoretical relationship between reciprocal translocations and pericentric inversions results:

$$F = \frac{\sum_{i=1}^{46} (L_i + S_i)^{2/3} \alpha^{2/3} \beta \delta}{2 \sum_{i=1}^{46} (L_i + S_i)^{4/3} \frac{L_i S_i}{(L_i + S_i)^2}}$$

where: α = chromosome average length in megabases (140.3)

β = average number of nearest neighbor corrected by surface effect (12 * 0.58)

δ = correction factor due to maximum interaction distance (1μm)

Assuming a cell nucleus diameter d = 8μm and a maximum interaction distance = 1μm we calculated that spherical chromosome domains in contact have approximately an average diameter = 2μm. Under these conditions results F = 10.7.

If we consider higher domain diameter, they get some degree of overlapping and consequently the probability of translocations and F values increase. The total overlapping condition corresponds to a domain diameter equal to the nuclear diameter = 8μm and results F = 86.

According to our experimental results, F = 13.5 indicates that domains overlap giving a domain volume = 6.4% of the nuclear volume, with a diameter $\approx$ 3.2μm.

Literature data show strongly spread F values, even considering experiments using the same radiation quality. The present model allows to represent such dispersion just varying the domain diameter but keeping fixed the maximum interaction distance (δ). We proposed that in vivo chromosome domain dimension could vary temporarily during interphase, offering different scenarios to ionizing radiation.

REFERENCES

1- Bender, MA; Awa, A; Brooks, A.L. et al. Mutat. Res. 196, 103-159 (1988).
2- Savage, J.R.K. Br. J. Radiol. 62, 507-520 (1989).
3- Lucas, J.N.; Tenjin,T.; Straume,T. et al Int. J. Radiat. Biol. 56, 35-44 56, 201 (1989).
4- Savage, J. R. K. Prog. Clin. Biol. Res. 340 B, 385-396 (1990).
5- Lucas, J.N. and Sachs, R. K. Proc. Natl. Acad. Sci. USA 90, 1484-1487 (1993).
6- Sax, K. Genetics 25, 41-68 (1940).
7- Manuelidis, L. Science 250, 1533-1540 (1990).
8- Seabright, M. Lancet 2, 971-972 (1971).
9- Cornfort, M. N. Radiat. Res. 121, 21-27 (1990).
10- Morton, N. E. Proc. Natl. Acad. Sci. USA 88, 7474-7476 (1991).
11- Hlatky, L. R.; Sachs, R. K.; Hahnfeldt P. Radiat. Res. 129, 304-308 (1992).
12- Brenner, D. J. and Sachs, R. K. Radiat. Res. 140, 134-142 (1994).
13- Brenner, D. J. Radiat. Res. 124, S29-S37 (1990).
14- Sasaki, M. S.; Kobayashi, K.; Hieda, T. et al Int. J. Radiat. Biol. 56, 975-988 (1989).