CHROMATID INTERCHANGES AT INTRACHROMOSOMAL TELOMERIC DNA SEQUENCES

José Luís Fernández[1], Fernando Vázquez-Gundín[1], Aurora Bilbao[2], Jaime Gosálvez[3], Vicente Goyanes[4].

1.- Centro Oncológico de Galicia. Avda. de Montserrat s/n. 15009 La Coruña.Spain
2.- Departamento de Ciencias Morfológicas, Universidad del País Vasco. Spain
3.- Unidad de Genética, Departamento de Biología, Universidad Autónoma de Madrid. Spain
4.- Unidad de Genética, Hospital "Teresa Herrerra". La Coruña.Spain

ABSTRACT

Chinese hamster Don cells were exposed to X-rays, mitomycin C and teniposide (VM-26) to induce chromatid exchanges (quadriradials and triradials). After fluorescence in situ hybridization (FISH) of telomere sequences it was found that interstitial telomere-like DNA sequence arrays presented around five times more breakage-rearrangements than the genome overall. This high recombinogenic capacity was independent of the clastogen, suggesting that this susceptibility is not related to the initial mechanisms of DNA damage.

INTRODUCTION

Telomeres are differentiated structures that constitute the physical end of chromosomes. In vertebrate chromosomes, the functional telomeric DNA sequences are $(TTAGGG)_n$ repeats [1]. These repeats are organized with specific proteins constituting structures that prevent chromosome fusion and exonucleolytic degradation.

Besides classical $(TTAGGG)_n$ terminal sequences, a wide variety of vertebrates show interstitial $(TTAGGG)_n$ sequences in their chromosomes. These areas have been considered as sites of preferential chromosome breakage, fragility and recombination [2]. Chinese hamster Don cells present a remarkable centromeric block of interstitial telomeric-like DNA sequences in most of their chromosomes, which is easily delineated by FISH [3]. We have analysed the presence or absence of intrachromosomal telomeric blocks at breakage-rearrangements sites of chromatid-type interchanges induced by three clastogens of different mechanism of DNA damage induction.

MATERIALS AND METHODS

Chinese hamster Don cells were grown in monolayer, in RPMI medium. Twelve hours before harvesting, mitomycin C (0.5μg/ml) or Teniposide (VM-26) (0.05μg/ml) was added. Some cultures were exposed to X-rays, 55kVp, from a Philips RT-100 machine. The administered dose was 3Gy, 3h before harvesting. Colchicine (0.5μg/ml) was added for the last 2h of cultures.

After cytogenetic processing, chromosomes in slides were denatured and incubated with a telomeric probe biotin-labelled. Slides were washed and the bound probe detected by avidin-FITC (yellow-green fluorescence) with a round of signal amplification. Chromosomes were counterstained with propidium iodide (PI) (red fluorescence).

Chromatid interchanges such as quadriradials and triradials were randomly scored, since these lesions were easy to detect under the PI filter set the microscope. Furthermore, these exchanges maintain strict pairing of sister chromatids, so breakage-rearrangement sites can be accurately located. The FITC filter set allowed visualization of the position of telomere-like sequences in the rearrangement.

RESULTS AND DISCUSSION

Figure 1a shows the chromosomes of a Chinese hamster Don cell after FISH with a telomeric probe. It is clearly visualized the FITC signal at centromeric areas of most of the chromosomes, revealing the presence of intrachromosomal telomeric sequence arrays. Figure 1b and 1c present a quadriradial and a triradial respectively, where an interstitial telomere block is associated with a breakage-rearrangement site (arrows).

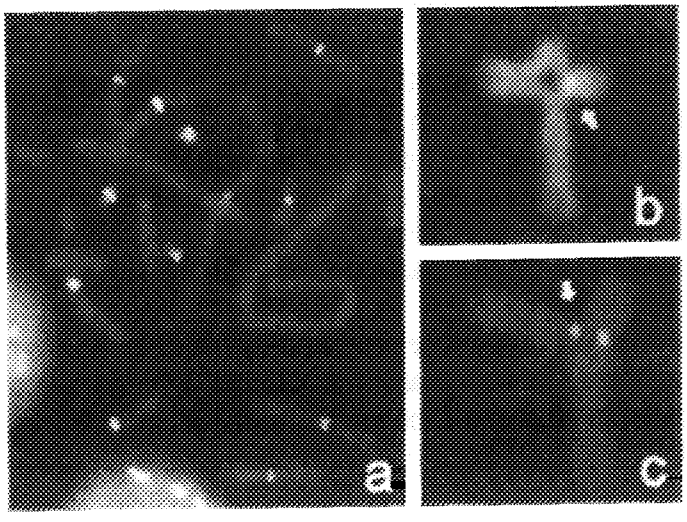

Figure 1. Chinese hamster Don chromosomes showing hybridization signals of telomeric DNA sequences at centromeric location of most of chromosomes. (a) Normal mitosis. Quadriradial (b) and triradial (c) with a block of intrachromosomal telomeric sequences involved in a breakage-rearrangement site (arrows).

Around 40% of the chromatid interchanges induced by each of the three clastogens showed a breakage-rearrangement site related to a block of telomere-like DNA sequence arrays (Table I)[4]. Using image analysis software it was found that these arrays constitute the 8.13 $\pm$ 0.62% of the genome. Therefore, the frequency of interchanges at interstitial telomeric sites was around five times that expected if assuming induction at random (Table I). This was a similar result to that referred by Alvarez et al. (1993)[5] in Chinese hamster cells exposed to gamma-rays, analysing chromosome damage. Moreover, Balajee et al. (1994) [6] also reported that around 22%-39% of chromosome aberrations induced by several restriction endonucleases involved internal telomeric repeat sequences.

Table I: Frequencies of chromatid exchanges induced by clastogens in Chinese hamster Don chromosomes.

Agent	Dose	Scored triradials and quadriradials			
		Total	Expected in telomeric-like DNA sequences (%)	Observed in telomeric-like DNA sequences (%)	Obs/Exp
X-rays	3Gy	63	8.13	42.86	5.27
Mitomycin C	0.5μg/ml	59	8.13	44.07	5.42
Teniposide (VM-26)	0.05μg/ml	148	8.13	37.84	4.65

These data support that telomere-like sequence arrays could promote breakage and recombination in themselves or the neighbouring chromatin once DNA damage has been locally induced. This increased susceptibility may be related to a specific organization of chromatin in this areas. In fact, evidence exists of a differential structuration of telomeric sequences either on terminal or on interstitial localizations [7]. Furthermore, telomerase activity could also play an important role in sensitivity to radiation-induced chromosomal breakage [8]. This sensitivity has possibly practical consequences, since an interstitial telomere array is present at a murine chromosome 2 fragile site, which could be involved in leukaemogenesis [9].

The mechanism of DNA damage produced by the three agents employed in our study was different among them. Ionizing radiation induces a broad spectrum of DNA lesions, including strand breaks, in clusters of interaction of the incident tracks. Teniposide (VM-26) is an inhibitor of DNA topoisomerase II. Finally, mitomycin C produces monoadducts and crosslinks in DNA. Since the sensitivity of interstitial telomere like sequences was similar, independently of the agent employed (Table I), our results suggest that this sensitivity is not related to the initial mechanisms of DNA damage. Furthermore, analysis of aberrations at this specific sequence areas can not reveal the feature of a characteristic aetiological agent.

Acknowledgements: This work was supported by the Consejo de Seguridad Nuclear (Spain).

REFERENCES

[1] Moyzis, R.K., Jones, M.D., Meyne, J., Ratliff, R.L., Wu, J.R., A highly conserved repetitive DNA sequence, $(TTAGGG)_n$ present at the telomeres of human chromosomes, Proc Natl Acad Sci USA 85 (1988) 6622-6626.

[2] Hastie, N.D., Allshire. R.C., Human telomeres: fusion and interstitial sites. Trends Genet., 5 (1989) 326-331.

[3] Meyne, M., Baker, R.J., Hobart, H.H., et al.., Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes. Chromosoma 99 (1990) 3-10

[4] Fernández, J.L., Gosálvez, J., Goyanes, V., High frequency of mutagen-induced chromatid exchanges at interstitial telomere-like DNA sequence blocks of Chinese hamster cells. Chrom Res 3 (1995) 281-284.

[5] Alvarez, L., Evans, J.W., Wilks, R., Lucas, J.N., Brown, J.M., Giaccia, A.J., Chromosomal radiosensitivity at intrachromosomal telomeric sites. Genes Chrom Cancer. 8 (1993) 8-14
[6] Balajee, A.S., Oh, H.J., Natarajan, A.T., Analysis of restriction enzyme-induced chromosome aberrations in the interstitial telomeric sequences of CHO and CHE cells by FISH. Mutat Res 307 (1994) 307-313.
[7] Balajee, A.S., Domínguez, I., Bohr, V.A., Natarajan, A.T., Immunofluorescent analysis of the organization of telomeric DNA sequences and their involvement in chromosomal aberrations in hamster cells. Mutat Res 372 (1996) 163-172.
[8] Slijepcevic, P., Xiao, Y., Domínguez, I., Natarajan, A.T., Spontaneous and radiation induced chromosomal breakage at interstitial telomeric sites.Chromosoma 104 (1996) 596-604.
[9] Bouffler, S., Silver, A., Coates, J., Papworth, D., Cox, R., Murine myeloid leukaemogenesis: the relationship between interstitial telomere-like sequences and chromosome 2 fragile sites, Genes Chrom Cancer 6 (1993) 98-106.