CHROMOSOME PAINTING IN BIOLOGICAL DOSIMETRY: SEMI-AUTOMATIC SYSTEM TO SCORE STABLE CHROMOSOME ABERRATIONS

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ABSTRACT

From the beginning of the description of the procedure of chromosome painting by fluorescence in situ hybridization (FISH), it was thought its possible application to score induced chromosomal aberrations in radiation exposition. With chromosome painting it is possible to detect changes between chromosomes that has been validated in radiation exposition. Translocation scoring by FISH, contrarily to the unstable dicentrics, mainly detect stable chromosome aberrations that do not disappear, it allows the capability of quantify delayed acute expositions or chronic cumulative expositions.
The large number of cells that have to be analyzed for high accuracy, specially when dealing with low radiation doses, makes it almost imperative to use an automatic analysis system.
After validate translocation scoring by FISH in our, we have evaluated the ability and sensitivity to detect chromosomal aberrations by chromosome using different paint probes used, showing that any combination of paint probes can be used to score induced chromosomal aberrations.
Our group has developed a FISH analysis that is currently being adapted for translocation scoring analysis. It includes systematic error correction and internal control probes. The performance tests carried out show that 9,000 cells can be analyzed in 10 hr. using a Sparc 4/370. Although with a faster computer, a higher throughput is expected, for large population screening or very low radiation doses, this performance still has to be improved.

RESUMEN

Desde la descripción de la técnica de chromosome painting mediante hibridación in situ fluorescente (FISH), se pensó en aplicarla a la detección de anomalías cromosómicas inducidas como la exposición a radiaciones ionizantes. La técnica de chromosome painting es capaz de detectar intercambios entre cromosomas y ha sido validada en exposición a radiaciones. Esta técnica detecta, principalmente, translocaciones que son anomalías estables - al contrario de los dicéntricos usados hasta ahora en dosimetría biológica-. La estabilidad de estas aberraciones cromosómicas permite cuantificar exposiciones agudas dilatadas en el tiempo o exposiciones crónicas acumuladas.
Debido al gran número de células que hay que analizar, sobre todo si se trata de exposiciones a bajas dosis, hace imperativo el uso de sistemas automáticos de análisis.
Después de haber validado la técnica de chromosome painting, nosotros hemos evaluado el uso de diferentes librerías de sondas estableciendo que es posible usar cualquier combinación de sondas siempre que se armonicen los datos.
Nuestro grupo que ha desarrollado un sistema automático para análisis de técnicas FISH, esta adaptándole para su dedicación al análisis de translocaciones cromosómicas mediante FISH. El sistema es capaz actualmente de analizar 9.000 células en 10 h usando una Sparc 4/370. Aunque con una computadora más rápida es posible aumentar la velocidad de análisis, se necesita mejorar el sistema pensando en la necesidad de analizar grandes poblaciones o exposiciones a muy bajas dosis.

INTRODUCTION

From the second half of 60's, biological dosimetry has been prove as a useful procedure and complementary to physical procedures. This biological dosimetry has been successfully carried out by cytogenetic methodology based in dicentric scoring. Biological dosimetry by dicentric analysis is highly effective at high doses, but insensitive at low doses where biological adverse effects exists.

From the beginning of the description of the procedure of chromosome painting by fluorescence in situ hybridization (FISH), it was thought its possible application to score induced chromosomal aberrations as in radiation exposition. The first approach was based in simultaneous hybridization of centromeric probes and telomeric probes for chromosome 1 (Lucas et al, 1989). More recently, with the uses of libraries of whole chromosome probes -paint probes- (Cremer et al, 1990, Collins et al 1991), it is possible to detect changes between chromosomes, that has been validated in radiation exposition (Tucker, 93). Chromosome painting is based upon different stain of specific chromosomes using labeled DNA probes for whole chromosomes. It allows to detect color changes into the chromosomes painted that means chromosome exchanges with other chromosomes not painted. Therefore, it is an easy technique detecting chromosome translocations by visualizing color changes instead of the time consuming G-banded analysis. Furthermore, in low doses of radiation where the rate of dicentrics is under detection, the use of FISH techniques scoring chromosome translocations could be due to the easiness in scoring metaphases.

But, translocation scoring by FISH mainly detect stable chromosome aberrations that do not disappear, contrarily to the unstable chromosome aberrations as dicentrics (Gray et al, 1992; Tucker et al, 1994) that quantify acute recent expositions. The circumstance that symmetric chromosome aberrations as translocations do not disappear allows the capability of quantify delayed acute expositions or chronic cumulative expositions.

Following this hypothesis, it should be possible to analyze risk populations, as workers, not only to establish the mutagenic history of individuals, but the suitability for a specific work under risk of exposition to ionizing radiation. Confirming the hypothesis of cumulative stable aberrations, Tucker et al (1994) showed that translocations detected by chromosome painting in a control population increase with age.

After validate translocation scoring by FISH in our laboratory (Garcia-Sagredo et al, 1994) we have evaluated whether the ability and sensitivity to detect chromosomal aberrations by chromosome painting is independent or not to the specific paint probes used, showing (Garcia-Sagredo et al, 1996) that any combination of paint probes can be used to score induced chromosomal aberrations because, after data correction according to the paint probes used, the amounts of translocations are dose dependent and quite homogeneous independently of chromosomes painted.

Automation of the Analysis

The large number of cells that have to be analyzed for high accuracy (specially when dealing with low radiation doses), makes it almost imperative to use a semi- or fully automatic analysis system. However the complexity and variability of the cases that can be found has made that only in the last few years some automatic or more often semi-automatic system have been proposed.
A system for FISH translocation scoring needs the following basic steps:

- Metaphase finder
- Translocation scoring
- Manual review for increased accuracy.

Metaphase finder: For brightfield several commercial tools are available, but fluorescence metaphase finder is a more difficult problem (due to the low light intensity and bright and non-uniform background) and just recently some metaphase finder have appeared [Piper et al. 1994, Vrolijk et al. 1994]. They comprise two steps:

- ROI determination in low resolution images and background correction
- actual metaphase detection in full resolution images: segmentation and classification.

The method can also provide a quality figure for each of the metaphases located so that subsequent analysis can be restricted to the highest quality metaphases available.

FISH translocation scoring: the number (or size) of painted chromosomes in the located metaphases is determined to classify each one as normal or abnormal, without having to obtained the full karyotype (always difficult and subject to errors). In [Fantes et al 1995] a system is proposed that uses as criteria to classify the metaphases an increase in the number of painted objects or a large asymmetry in the area distribution of the expected number of painted objects.

Manual review: present analysis systems still have an undesirable proportion of cells that are classified as abnormal when they actually should be rejected (they have paint artifacts, non-specific hybridization or they have been incorrectly segmented). The analysis results are then greatly improved if the dubious cases are presented to a trained operator that visually classifies them.

Our group has developed a FISH analysis system [Santos et al 1997a, Malpica et al 1996] that is currently being adapted for translocation scoring analysis [Santos et al 1997b]. It includes systematic error correction and internal control probes. The performance tests carried out show that 9,000 cells can be analyzed in 10 hr. using a Sparc 4/370. Although with a faster computer, a higher throughput is expected, for large population screening or very low radiation doses, this performance still has to be improved.

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References

Cremer T, Popp S, Emmerich P, Lichter P, Cremer C: Rapid metaphase and interphase detection of radiation-induced chromosome aberrations in human lymphocytes by chromosomal suppression in situ hybridization. Cytometry 11:110-118, 1990.

Fantes J A, Green DK, Hill W, Stark MH, Gordon JM, Piper J. A because it is an easy technique and it can detect chromosome translocations by visualizing color changes instead of the time consuming G-banded analysis application of automation to the detection of radiation damage using FISH technology. Int J Radiat Biol 68:263-276, 1995.

García Sagredo JM, Resino M, Cabello P. Utilizacion de técnicas de citogenética molecular (Chromosome painting) para la detección de mutaciones cromosómicas: Posible aplicación a radiaciones electromagnéticas. In: Avances en Bioelectromagnetismo (Bardasano JL, ed). Universidad Alcalá de Henares, Inst. de Bioelectromagnetismo Alonso de Santa Cruz, pp.153-165, 1994.

García-Sagredo JM, I Vallcorba, A López-Yarto, MC Sanchez-Hombre, M Resino, Ferro MT. Chromosome painting in biological dosimetry: Assessment of the ability to score stable chromosome aberrations using different pairs of paint probes. Env. Health Perspect. 104:475-477, 1996.

Gray JW, Lucas J, Pinkel D, Awa A. Structural chromosome analysis by whole chromosome painting for assessment of radiation-induced genetic damage. J Radiat Res. 33:80-86, 1992.

Knehr S, Zitzelsberger H, Braselmann H, Bauchinger M. Analysis for DNA-proportional distribution of radiation-induced chromosome aberrations in various triple combinations of human chromosomes using fluorescence in situ hybridization. Int J Radiat Biol 65:683-690, 1994.

Lucas JN, Tenjin T, Straume T, Pinkel D, Moore H, Litt M, Gray JW: Rapid human chromosome aberration analysis using fluorescence in situ hybridization. Int.J.Radiat.Biol. 56:35-44, 1989.

Malpica N, Ortiz C, Santos A, Vaquero JJ, del Pozo F, Vallcorba I, García-Sagredo JM. Automation of FISH spot counting. A feasibility and usefulness study. IV Plenary Workshop of the European Concerted Action "Automation of Molecular Cytogenetic Analysis". Leiden. 1996

Piper J, Poggensee M, Hill W, Jensen R, Ji L, Poole I, Stark M, Sudar D. Automatic Fluorescence Metaphase Finder Speeds Translocation Scoring in FISH Painted Chromosomes. Cytometry 16:7-16, 1994.

Santos A, Ortiz de Solórzano C, Malpica N, Vaquero JJ, del Pozo F. Automatic system for FISH cytogenetic analysis. Cytogenet Cell Genet. 77:36, 1997a.

Santos A, García-Sagredo JM. Automation in environmental mutagenic analysis. Cytogenet Cell Genet. 77:30, 1997b.

Tucker JD, Lee DA, Ramsey MJ, Brinner J, Olsen L, Moore DH. On the frequency of chromosome exchanges in a control population measured by chromosome painting. Mutat Res 313:193-202, 1994.

Tucker JD, Ramsey MJ, Lee DA, Minkler JL. 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.

Vrolijk J, Sloos WCR, Darroudi F, Natarajan AT, Tanke HJ. A system for fluorescence metaphase finding and scoring of chromosomal translocations visualized by in situ hybridization". Int J Radiat Biol 66:287-295, 1994.