Optimization of the chromatid paint FISH assay for the detection of radiation-induced inversions *

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Inversions are intra-chromosomal events originating from two miss-rejoined breaks, where the resulting chromosome segment is reversed and re-inserted into the chromosome. Since insertions are stable aberrations, i.e. are transmissible to further cell generations, they are an ideal tool for retrospective biodosimetry. Moreover, based on the differences in the track structure of low and high LET radiation it has been proposed that insertions may represent a biomarker of high LET exposure [1].

So far, the available data base is small, mainly attributable to the fact that the detection rate of inversions by routine cytogenetic techniques is low. Recently, a new molecular cytogenetic assay named chromatid paint FISH has been developed enabling the detection of small insertions [2] through the use of fluorescence-labeled directional probes specific to unique sequences along each chromatid. Inversions are microscopically visible as a double-color switch of the hybridization signal from one chromatid to the other as depicted in Figure 1.

For chromatid painting, cells are cultured in medium supplemented with the nucleotide analogues BrdU and BrdC until the first mitosis, and chromosome spreads are prepared. The newly synthesized (BrdU/BrdC substituted) strands are degraded by UV exposure and exonuclease III treatment. Slides are hybridized with Cy3-labeled directional probes resulting in single chromatid painting. Chromosomes are counterstained with DAPI and analyzed under a fluorescence microscope. A Cy3-labeled chromatid appears red, while the other (counter-stained) chromatid appears blue (see figure 1).

We performed chromatid paint FISH assay in peripheral blood lymphocytes, a frequently used cell system to study radiation effects and to estimate risks associated with an exposure. Chromatid painting probe for chromosome 3 was obtained from Kromatid Inc. (Fort Collins, Colorado 80521, USA). Lymphocytes were cultured for 48h in medium supplemented with 5µM BrdU and 1 µM BrdC. Then, chromosome spreads were prepared and the chromatid FISH assay was performed following the manufacturer instructions. Z-stack image acquisition and processing was done with the ISIS software (MetaSystems GmbH, D-68804 Altlussheim, Germany). In parallel, a control slide provided by the manufacturer (i.e. fibroblasts cultured in medium with BrdU and BrdC) was hybridized. Comparison of the samples showed a strong FISH signal intensity along fibroblasts' chromosomes, but weak signal intensity along lymphocytes' chromosomes. Since signal quality will largely depend on the completeness of the denaturation process of the target DNA, we optimized the denaturation temperature for lymphocytes. Samples were denatured for 3 minutes in a temperature range from 68 to 73°C. Optimal signal intensity was achieved by incubation at 70°C. The volume of the probe necessary for hybridization was adjusted to 10µl and spreads were mounted with an 18x18mm cover glass. Notably, hybridization of fresh frozen slides resulted in better FISH signals than air-dried aged slides.

In a first experiment, the background frequency of inversions in chromosome 3 of lymphocytes was determined. Inversions are characterized by a double color switch between sister chromatids, while a single color switch is considered as a false inversion and excluded from the analysis. In total, 86 chromosomes were scored and 2 inversions were observed, i.e. yielding a frequency of 0.02 ± 0.02 in agreement with data reported by Ray et al. [2]. Currently, the frequencies of inversions in lymphocytes after X-ray and after alpha-particles exposure are analysed.

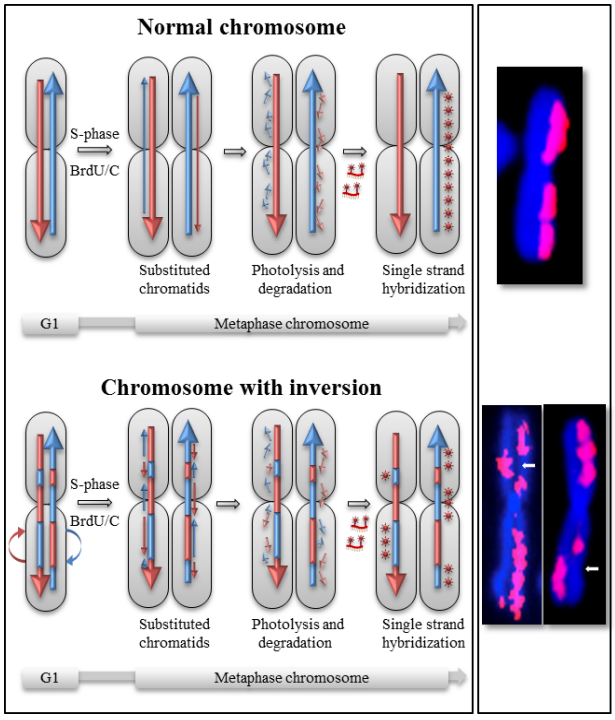

Figure 1: Chromatid paint FISH assay (adapted from Ray et al. [2])

References

[1] M. Hada et al., *Radiat. Res.* (2007) 168: 98-105.
[2] F. A. Ray et al., *Radiat Environ Biophys* (2014) 53:255-263.

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