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Review

FLUORESCENCE *IN SITU* HYBRIDISATION IN DETECTING CHROMOSOME ABERRATIONS CAUSED BY OCCUPATIONAL EXPOSURE TO IONISING RADIATION*

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Received in July 2005
Accepted in February 2006

For more than two decades, chromosome aberration analysis has been used in biomonitoring of occupational and environmental exposure to ionising radiation. Chromosome aberration analysis is a method used to detect unstable aberrations in the lymphocytes of irradiated personnel. In turn, stable chromosome aberrations that arise some time after exposure are detected using the multicolour fluorescence *in situ* hybridisation. This is a technique which dyes each pair of chromosomes with different colour. Due to the dynamics of unstable aberration formation, chromosome aberration analysis is more suitable for genome damage assessment of recent exposures. On the other hand, fluorescence *in situ* hybridisation gives information on chromosome instability caused by long-time occupational exposure to ionising radiation. Considering the high cost of fluorescence *in situ* hybridisation and the uncertainty of the result, it should be used in biodosimetry only when it is absolutely necessary.

KEY WORDS: *biodosimetry, CA, FISH, genome damage, occupational exposure*

In 1986, the International Atomic Energy Agency (IAEA) published its annual report describing chromosome aberration (CA) analysis as the method of choice in the evaluation of biological effects of ionising radiation (1, 2). In the years that followed, the method was shown to be efficient in biodosimetry and cytogenetic biomonitoring of populations occupationally exposed to ionising radiation (3, 4). The method was later recommended by the World Health Organization (WHO) as a mandatory technique in ionising radiation risk assessment (5). It is well known that chromosome aberrations occur as the result of action of ionising radiation on the DNA molecule either directly or through the interaction between DNA and free radicals produced by the ionization of the surrounding molecules. One of the basic types of chromosome aberration is the double-strand break (6). Later, under certain conditions during DNA repair these breaks can transform into dicentric or ring chromosomes accompanied by acentric fragments. These types of chromosome aberrations involve larger DNA rearrangements and pose a higher risk for genome stability and integrity (6). Although dicentric and ring chromosomes are considered to be unstable aberrations, a clear correspondence with the development of malignant diseases was found (2, 7, 8). Unstable chromosome aberrations arise shortly after the exposure of cells to low and high linear energy transfer (LET) radiation. They can also arise from dose accumulation in long-term exposure to low doses of radiation (2, 3). Misrepair of unstable aberrations leads to stable chromosome aberrations known as translocations (3, 9). The number of stable chromosome aberrations rises within one

*Preliminary report presented at the 6th Symposium of the Croatian Radiation Protection Association with international participation, Stubičke Toplice, Croatia, 18-20 April 2005

year from exposure (2, 3). Since they do not lead to morphological abnormalities in chromosome structure, translocations could be detected only by specific cytogenetic techniques for visualization of distinct chromosome regions.

FLUORESCENCE *IN SITU* HYBRIDISATION

Since the beginning of the 1990s, fluorescence *in situ* hybridisation (FISH) has been used as a cytogenetic tool for the detection of genome damage involved in cancer development and development of hereditary diseases and malformations. This genome damage includes deletions, amplifications and translocations of specific genes within the genome (10). The technique is based on the hybridisation of a specific short DNA molecule (probe) with the complementary sequence in the genome. The probe is labelled with a fluorescent dye giving a clear signal on its binding location within the chromosome. Using a probe for specific genome sequence, it is possible to obtain the information on its copy number and location on the chromosome. Simultaneous hybridisation of different probes labelled with different fluorescent dyes makes it possible to locate several different sequences within the genome at the same time. This can provide information on sequence amplification, deletion or translocation, as well as on their location within the genome (11).

In the interaction between ionising radiation and cell genome it is impossible to predict which part of the chromosome will be damaged and which sequence will be translocated. Thus to monitor the effect of radiation on the formation of stable chromosome aberrations it is necessary to dye all the chromosomes with different dyes at the same time. This version of the FISH technique is known as multicolour FISH or mFISH (10).

Colour labelling of whole chromosome probes used in mFISH makes it possible to view each of the 22 chromosome pairs and sex chromosomes in different colour. This is achieved by using probes for different chromosomes labelled with three or more fluorescent dyes in different combinations and ratios (12). The translocation is viewed as a shift in colour, that is, an insertion of a colour specific for another chromosome whose sequence has been translocated (13).

Fluorescence *in situ* hybridisation has revealed that beside acentric fragments, the formation of dicentric and ring chromosomes involves translocations at the position where there was a misrepair. There are various types of translocations. The most common are reciprocal translocations occurring when two different sequences switch their positions within the genome. One-way translocations are those where only one sequence changes position within the genome. Terminal translocations occur there where a sequence from the end of the chromosome changes position, and interstitial translocations involve sequences from inside the chromosome arm (13, 14). There are also complex mechanisms of translocation involving more than three double-strand breaks and an interchange of chromosome sequences originating from more than two chromosomes (14). The use of FISH in biomonitoring has shown that the frequency of translocations in individuals occupationally exposed to low doses of radiation over a period of few years is seven times as high as the frequency of dicentric chromosomes (14). The frequency of one-way translocations is 10 % to 40 %, of which 76 % are interstitial and 24 % terminal (2). Sixty-five percent of all translocations involve the formation of acentric fragments (15). Fluorescence *in situ* hybridisation has shown that DNA strand breaks caused by ionising radiation are not randomly distributed within the genome. Most occur in the euchromatin region. However, the repair efficiency of those breaks is higher than in the heterochromatin region. Due to its characteristic base pair sequence in the telomeric region, chromosome 8 is more susceptible to ionising radiation than other chromosomes (16). Since FISH gives an insight into the mechanism of the formation of stable chromosome aberrations, it was possible to determine the correlation between radiation dose and translocation frequency. For all these achievements, FISH has been increasingly used in low-dose radiation risk assessment (17, 18).

CONCLUSION

Unlike CA analysis, FISH gives an insight into the mechanism of translocation. As the number of translocations decreases with time, dose assessment based on effects should include a correction of data using the Quadratic Discriminant Rule (QDR) method. The same correction is used in the dose assessment based on chromosome aberrations. Thus both CA analysis and FISH display the same limitations

when used in retrospective dosimetry. An additional disadvantage of FISH is its ineffectiveness in dose assessment at the individual level when exposure is below 20 cGy or above 1.5 Gy (2). Considering the high cost and uncertain outcome of the results, the use of FISH in biodosimetry remains limited to exceptional situations when all other cytogenetic methods fail (2). This is why the WHO recommends that CA analysis should be used for routine biomonitoring of populations occupationally exposed to ionising radiation (2, 4).

Acknowledgement

This study was supported in part by the Croatian Ministry of Science, Education and Sports (Grant No. 0022020).

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Sažetak

FLUORESCENCIJSKA HIBRIDIZACIJA *IN SITU* (FISH) U DETEKCIJI KROMOSOMSKIH OŠTEĆENJA NASTALIH KAO POSLJEDICA PROFESIONALNE IZLOŽENOSTI IONIZIRAJUĆEM ZRAČENJU

Posljednjih dvadesetak godina analiza kromosomskih aberacija rabi se kao glavna citogenetička tehnika u biodozimetriji osoba profesionalno izloženih zračenju. Metoda omogućava utvrđivanje prisutnosti nestabilnih tipova oštećenja u limfocitima ozračenih osoba. Međutim, njome nije moguće istodobno utvrditi i prisutnost stabilnih tipova oštećenja kao što su translokacije. U tu svrhu potrebno se koristiti višebojnom fluorescencijskom hibridizacijom *in situ* (mFISH) kojom je moguće svaki par kromosoma obojiti drugom bojom. Zbog same dinamike nastanka nestabilnih kromosomskih oštećenja analiza kromosomskih aberacija, kao citogenetička tehnika, prikladnija je za procjenu genomskih oštećenja u kratkom vremenu nakon ozračivanja, dok bi FISH mogao naći primjenu u detekciji stabilnih oštećenja genoma koja nastaju kao posljedica dugotrajne profesionalne izloženosti zračenju. Međutim, zbog visokih troškova same tehnike i zahtjevnosti njezine izvedbe, FISH kao tehnika u biodozimetriji rabila bi se samo u slučajevima kada podatke o stupnju oštećenja genoma nije moguće dobiti primjenom ni jedne druge citogenetičke tehnike.

KLJUČNE RIJEČI: *biodozimetrija, citogenetika, genomska oštećenja, kromosomske aberacije*

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