Application of Translocation, γ-H2AX, and Sam68 as a biological indicators for the assessment of radiation exposure in nuclear power plant workers

Kwang Hee Yang*, Hyung Sun Park, Seon Young Nam
Korea Hydro Nuclear Power Co,. RHRI, 308, Uicheon-ro, Do-bong Gu, Seoul, Korea,132-703
**Corrseponding author: ykhee@khnp.co.kr*

1. Introduction

For many years, ionizing radiation has been known to damage cells, and well-documented effect is alterations in the structure of chromosomes. Radiation workers are exposed to ionizing radiation from various sources. Ionizing radiation produces several types of DNA lesion, including DNA base alterations, DNA–DNA cross-links, and single- and double-strand breaks. As a protocol for biological dosimetry recommended by IAEA (2001) [1], the analysis of solid stained dicentric chromosomes has been used since the mid 1960s. The intervening years have seen great improvements bringing the technique to a point where dicentric analysis has become a routine component of the radiological protection programs of many Member States.

However, a recognized drawback of the dicentric and cytokinesis-block micronucleus (CBMN) assays is that the damage is unstable. For retrospective biological dosimetry, FISH (fluorescence in situ hybridization) method is possible because stable aberrations such as a reciprocal translocation will pass successfully through mitosis and into the daughter cells [2,3].

The purpose of this paper is to investigate how FISH can be best used for retrospective biological dosimetry in the radiation workers, and to test how new biological indicators can be used for it in cell lines of γ – irradiation *in vitro*.

2. Analysis of Methods

2.1 Characterization of the Examined Groups of Radiation Workers

Cytogenetic examination was performed during 2009~2012 in groups of nuclear workers in Wol-sung nuclear power plant, who had been exposed to the chronic impacts of various ionizing radiations for 5–20 years during their occupational activities, namely, β-radiation of tritium and its oxide and external γ-radiation with the dominant contribution of γ-radiation to the total dose. The mean age of the nuclear workers by the time of the examination was 45 ±13 years. A group of people unexposed by radiation was used as a negative control.

2.2 Cytogenetic Analysis of Human Peripheral Blood

The peripheral blood lymphocytes were cultivated and chromosomes were prepared by standard manipulation protocol. To analyze the stable chromosome aberrations, The metaphase chromosomes were prepared using fluorescence staining based on molecular hybridization of a DNA probe with the metaphase chromosomes fixed on a glass slide (in situ), followed by fluorescence microscopy examination for detecting hybridization (the FISH method). In this work, we used MetaSystems GmbH kit containing the specific DNA probes for human chromosomes 1, 2, 4 labeled with direct fluoro chromosome probes (Fig. 1).

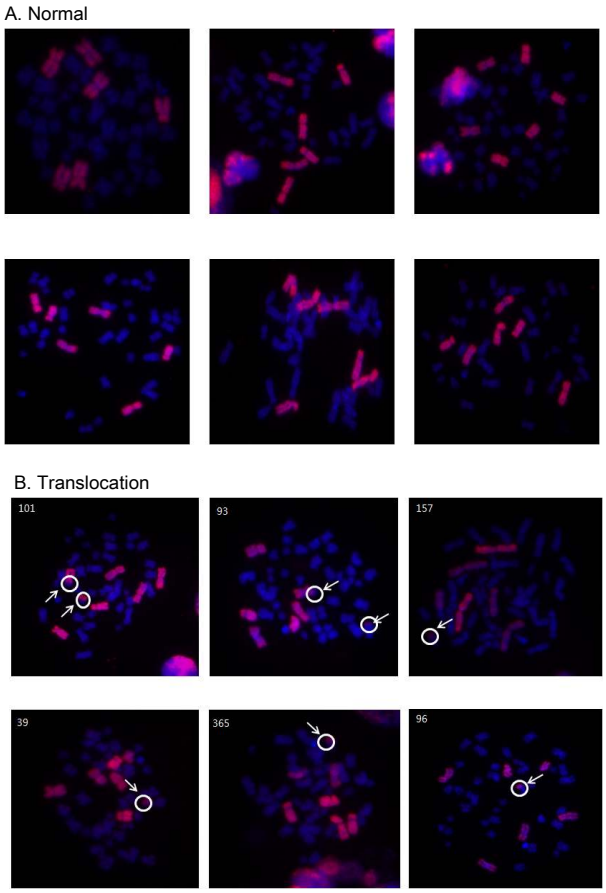

Fig. 1. Translocation assessment using FISH method.

2.3 Application of new biological indicators for the identification of radiation exposure in vitro.

IM-9 B lymphoblast cells and Jurkat lymphoma cells were cultured in RPMI 1640 medium. Cells were uniformly irradiated at room temperature with various doses of γ-irradiation using a ^{137}Cs γ-source (dose rate, 5.41 Gy/ min; IBL 437 C type H, CIS Biointernational,

France). Cells simultaneously exposed to sham irradiation were used as negative control. Western blot analysis was performed to measure the protein levels of γH2AX, H2AX, Sam68, and Actin.

3. Results and Some Considerations

For the scoring of translocations by FISH analysis, two blood cell cultures were set up, followed by FISH analysis. 1,000 metaphases per donor were scored for the presence of aberrations. We found 0 translocations in 2,000 metaphases of group A, 14 translocations in 7,000 metaphases of group B, 16 translocations in 9,000 metaphases of group C, and 15 translocations in 2,000 metaphases of group D of control groups (Table 1). In worker groups, we found 4 translocations in 3,000 metaphases of group A, 27 translocations in 27,000 metaphases of group B, 38 translocations in 16,000 metaphases of group C and 52 translocations in 15,000 metaphases of group D (Table 2). However, we couldn't find the significant difference of translocation frequency in between control and worker groups taking into account the 95% confidence limits.

Table 1. The frequency of translocations in control group.

Group	Age	No. of translocation	yield (translocation /total cell)	Total cell
A (2 donors)	20 ~ 29	0	0.00±0.00	2,000
B (16 donors)	30 ~ 39	14	0.88±1.45	7,000
C (9 donors)	40 ~ 49	16	1.78±1.86	9,000
D (2 donors)	50<	2	1.00±1.41	2,000

Table 2. The frequency of translocations in worker group.

Group	Age	No. of translocation	yield (translocation /total cell)	Total cell
A (3 donors)	20 ~ 29	4	1.33±1.15	3,000
B (27 donors)	30 ~ 39	54	2.00±2.15	27,000
C (16 donors)	40 ~ 49	38	2.38±2.22	16,000
D (15 donors)	50<	52	3.47±3.16	15,000

To identify new biological indicators for radiation exposure, we examined radiation-induced cellular responses. γ-H2AX has been known as one of DNA repair factors in radiation damaging cells. Thus, we examined whether γ-H2AX is applicable as radiation damage marker. We found that H2AX was transiently phosphorylated by irradiation at 0.1 Gy and the induced level of H2AX phosphorylation was highest at 1h post irradiation. Moreover, at 2 Gy, H2AX was stably phosphorylated and it was increased in a time dependent manner. However, the level of H2AX protein was not changed in both 0.1 Gy and 2 Gy.

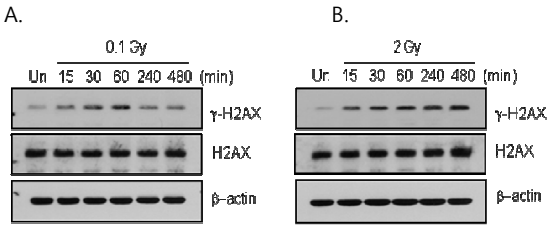

Fig. 2. Application of γH2AX and H2AX as a biological indicator in IM-9 cell line.

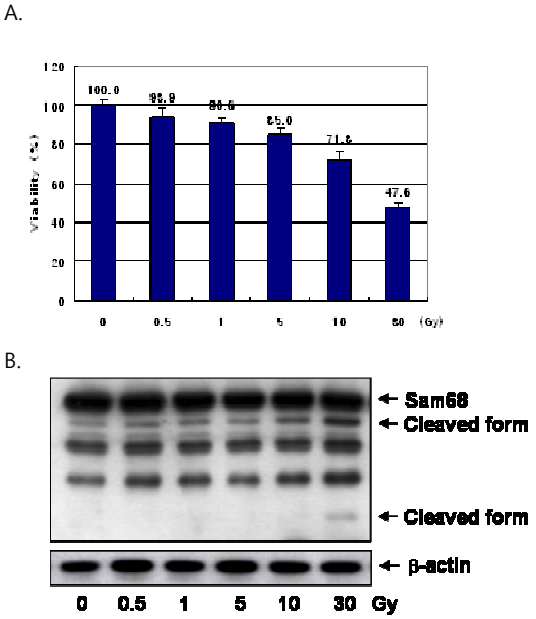

Fig. 3. Application of Sam68 as a biological indicator in Jurkat cell line.

In previous study, we identified Sam68 as a protein interacted with caspases. Thus, we examined whether Sam68 is applicable as a new biological indicators. Our result showed that γ-irradiation induced cell death of Jurkat cells in a dose-dependent manner (Fig. 3A). Interestingly, we found that the cleaved form of Sam68 was increased at 5 to 30 Gy (Fig. 3B).

4. Conclusions

This study showed that confirmation of the initial dose estimated by dicentric analysis is provided by the subsequent FISH analysis for translocation frequency and provides further evidence for the valid use of FISH as a retrospective biological dosimeter. The IAEA manual on cytogenetic dosimetry recommends a half-time value of 3 y to correct for the decrease of dicentrics in case of delayed sampling based on the patient data of Buckton [1,2]. Support for this comes from the cytogenetic follow up of an individual exposed to tritium, which also indicated a decline in dicentrics with a half-time of ~3 y. Naturally, the RBE of tritium, as well as other kinds of ionizing radiation, depends on the dose, exposure conditions, and studied parameters. The information about the RBE of tritium that is most important from an applied standpoint is that associated

with the range of low doses [4]. In our study, the dose dependence of tritium RBE was not identified because of very low dose Tritium (< 1mSv). However, The strong smooth relationship between translocation yield and age is shown in Table 2. The translocation yields reported here are only slightly lower than already published [5, 6, 7]. The implication is that the increase of yield with age could be due to environmental factors, to a natural aging process or both.

In addition, we confirmed that γ-H2AX and Sam68 associated with DNA damage and apoptosis, can be new biological indicators for radiation exposure.

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