

# Detection and study of the formation and repair of DNA double-strand breaks after irradiation

## Introduction

In everyday life the DNA in our cells is exposed to a number of different influences which can damage it. In DNA of an ordinary mammalian cell can be generated endogenously (spontaneously) more than  $10^4 - 10^5$  lesions per cell, per day. If these lesions were to persist, they would result in a significant genetic damage which could induce chromosomal aberrations or even carcinogenesis. Fortunately, during the evolution, cells invented very elaborate system of DNA damage repair thanks to which the vast majority of such spontaneously generated damage can be effectively and quickly repaired. DNA damage can be caused also exogenously – e.g. by chemical agents or by ionizing radiation (IR). IR is possible to divide depending on the physical parameter: linear energy transfer (LET). The LET describes average energy loss of the particle per unit of distance in the traversed material. High-LET radiation for example alpha or heavy charged particles cause dense ionizations along its track. On the other hand, low-LET radiation as  $\gamma$ -rays or X-rays, produce ionizations sparsely along their tracks and almost homogenously within a cell. Therefore the DNA damage strongly depends on the ionization density of used radiation. The number of simple damage to DNA (base damage, single strand break of DNA, double strand break of DNA (DSB) etc.) increases with increasing ionization density. Consequently, the quantity of simple damage is directly proportional to the probability of formation of clustered DNA lesions along the path of the passing particles. Moreover, DNA damage caused by dense IR is more difficult and sometimes not possible to repair [1].

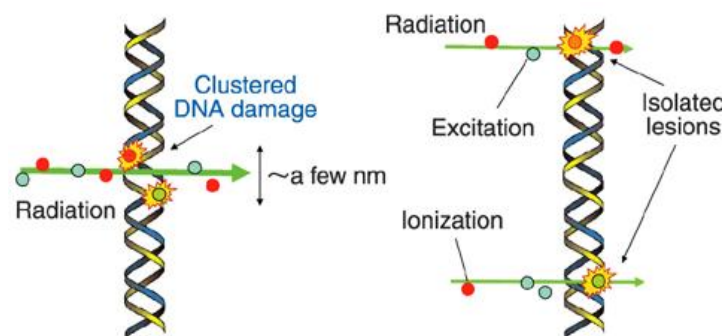


Figure 1. Differences between the character of DNA damage caused by high-LET (left picture) and low-LET radiation (right picture) [1].

From the simple damage, DNA DSBs represent one of the most serious lesions of DNA that can occur in the genome. Their proper repair is crucial for the avoidance of chromosomal translocations and cancer. In the higher eukaryotic cells they evoke a coordinated multi-step response including damage recognition, signal transduction and repair. One of the first responses ongoing immediately after DNA DSBs formation is the phosphorylation of the histone H2AX variant at Serine 139. A typical DSB results in phosphorylation of several Mbp (mega base pairs) around the break, involving  $10^2$  to  $10^3$  phosphorylated H2AX ( $\gamma$ H2AX) proteins [2]. At the microscopic level, this huge accumulation of  $\gamma$ H2AX leads to the formation of  $\gamma$ H2AX foci which can be detected by immunofluorescent labeling. In fact, several proteins involved in DNA repair and DNA damage signaling have been shown to create discrete foci in response to IR. These foci are called ionizing radiation-induced foci (IRIF) or DNA repair foci [3].

In our group, we are focusing on the comparison of the extent of the DNA damage and kinetics of reparation of DNA DSBs and cluster damage, when radiation with different LET are used. We use the immunofluorescence detection of  $\gamma$ H2AX for the visualization of DNA DSBs. However, the  $\gamma$ H2AX can be also generated in response to replication stress – in order to be sure we measure only the DSBs, we detect yet another marker of DBSs - damage sensor p53-binding protein 1 (53BP1). As the number of  $\gamma$ H2AX/53BP1 closely corresponds to the number of DSBs, visualization of these two proteins in colocalization gives us a sensitive *in vitro* assay for the detection of DNA DSBs [4].

### **References and recommended literature**

1. [http://www.rerf.jp/radefx/basicno\\_e/radcell.htm](http://www.rerf.jp/radefx/basicno_e/radcell.htm)
2. Kuo LJ, Yang LX. Gamma-H2AX - a novel biomarker for DNA double-strand breaks. *In Vivo*. 2008;22(3):305-9.
3. Kinner A, Wenqi W, Staudt C, Iliakis G.  $\gamma$ -H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. *Nucleic Acids Res*. Oct 2008; 36(17): 5678–5694.
4. Ježková L, Falk M, Falková I, Davidková M, Bačíková A, Štefančíková L, Vachelová J, Michaelidesová A, Lukášová E, Boreyko A, Krasavin E, Kozubek S. Function of chromatin structure and dynamics in DNA damage, repair and misrepair:  $\gamma$ -rays and protons in action. *Appl Radiat Isot*. 2014;83 Pt B:128-36.

## Practical work

Goal of this project is to characterize kinetics of DNA DSBs repair in normal human fibroblasts after the irradiation. The main tasks for the students within the practice period will be:

- introduction into the work in laboratory, familiarization with the methods, with the plan of the experiment, and with the work with a cell culture of fibroblasts;

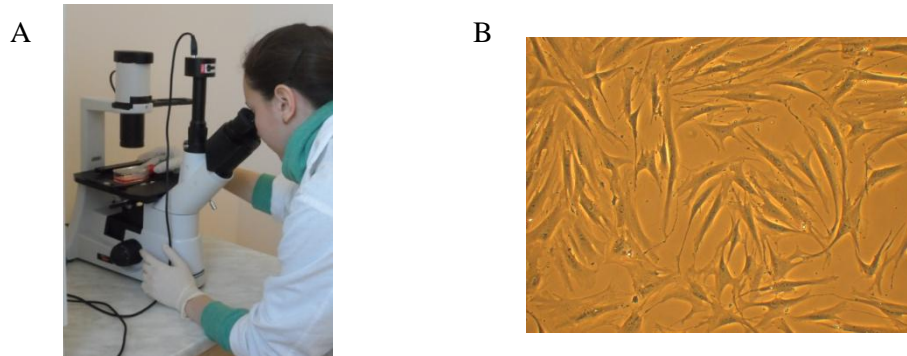


Figure 2. (A) Inverted microscope Biomed 3I F for the the cell culture monitoring.

(B) Cell culture of fibroblasts, main cell type in skin.

- preparation of cells for irradiation; irradiation with  $\gamma$ -rays (Rocus-M,  $\gamma$ -rays  $^{60}\text{Co}$ ) or with accelerated protons (Phasotron);
- processing of cells after irradiation - IRIF assay (fixation of cell structure in different times post irradiation, permeabilization of cell membrane, cell immunostaining with primary antibodies and secondary antibodies conjugated with fluorescent dyes, staining of cell nucleus with DAPI, preparation of microscopic samples);

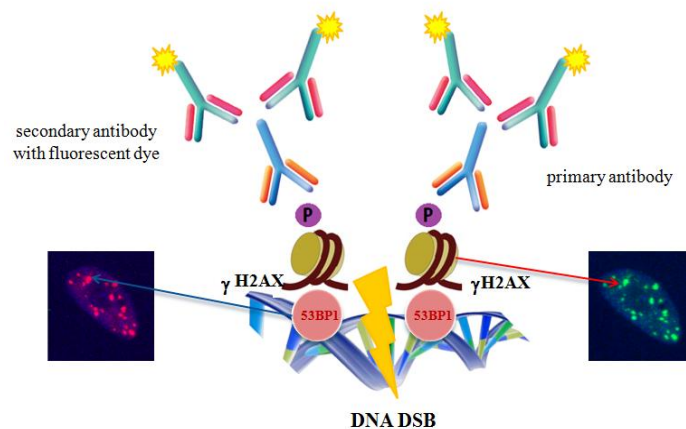


Figure 2. Scheme of the detection of  $\gamma\text{H2AX}/53\text{BP1}$  using IRIF.

- acquisition of images on a fluorescent microscope Axio Imager. M2;
- analysis of acquired images, evaluation of obtained data;

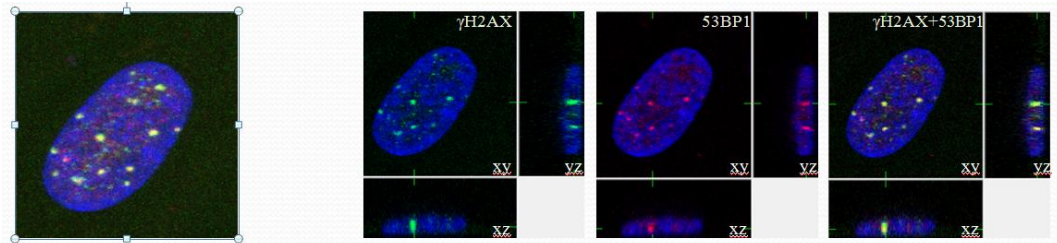


Figure 3. Analysis of acquired 3D images in software Acquarium.

- discussion of results, presentation of obtained results.

### **Number of participants**

Four participants can work on the project at the same time.

### **Requirements**

Basic knowledge of the cell biology or biochemistry is welcomed.

### **Project supervisor**

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