Study of the influence of ionizing radiation on cell cycle progression and apoptosis induction in human fibroblasts

Introduction

It is widely known that cell exposure to ionizing radiation leads to cellular damage primarily through a spectrum of lesions in DNA. While a cell progresses through the cell cycle, it determines whether it has to complete cell division, arrest growth to repair cellular damage, or undergo apoptosis if the damage is too severe to be repaired or if the cell is unable to repair DNA. The cell decides which of these options is suitable at the checkpoints that occur at the G1/S phase boundary, in the S phase, and during the G2/M phase. These checkpoints ensure that critical events in a particular phase of the cell cycle are completed before a new phase is initiated, thereby preventing the formation of genetically abnormal cells. When DNA damage is detected in the cell, growth arrest can be induced. Upon damage repair, cell cycle progression resumes. An alternative to repairing damaged cells is simply to eliminate them by apoptosis (programmed cell death) [1]. It is a highly regulated process by which an organism eliminates unwanted cells without eliciting an inflammatory response. Programmed cell death is involved in many physiological processes such as tissue homeostasis, embryonic development, and the immune response. As it is essential to identify and eliminate the cells proliferating inappropriately, apoptosis and proliferation are tightly coupled. The process of apoptosis was discovered in the second half of the 20th century; however, this field of research is quite timely nowadays. In spite of the growth of experimental data, the regularities and mechanisms of apoptosis after exposure to accelerated heavy charged particles are still unclear and represent a current direction for further research. This theme is really important in worldwide perspective with use of radiotherapy and planned missions to Mars, it is essential to know how space radiation influences human cells and take into account the astronauts' health problems, which may appear. Therefore, the research of apoptosis induced by ionizing radiation of different linear energy transfer (LET) is of great interest for future study.

The aim of the project is to find out how ionizing radiation influences apoptosis induction and cell cycle progression in human fibroblasts by the flow cytometric method.

Besides cleavage and degradation of specific cellular proteins, compaction and fragmentation of nuclear chromatin, etc., one of the characteristic apoptotic events is an externalization of phosphatidylserine (PS) to the cell surface. Annexin V is a calcium-dependent phospholipid-binding protein with a high affinity for PS. In the beginning of the apoptotic pathway, molecules of PS are translocated to the outer surface of the cell membrane, where Annexin V can readily bind them. In this way, it is possible to detect apoptotic cells and also live, early apoptotic, and dead cells using The Muse™ Annexin V & Dead Cell Assay (Millipore) and the Muse™ Cell Analyser (Millipore).

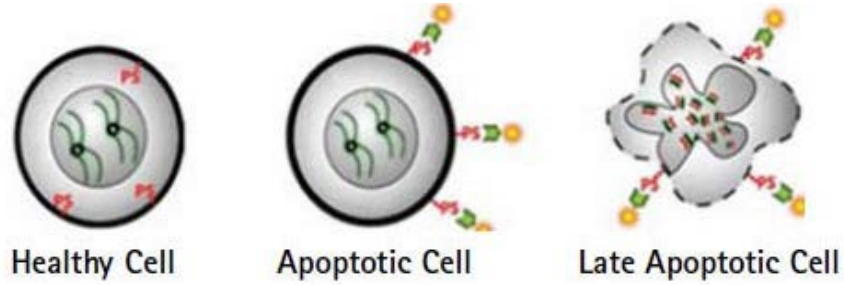

Fig.1. Localization of phosphatidylserine (PS) in healthy, apoptotic, and late apoptotic cells.

A B

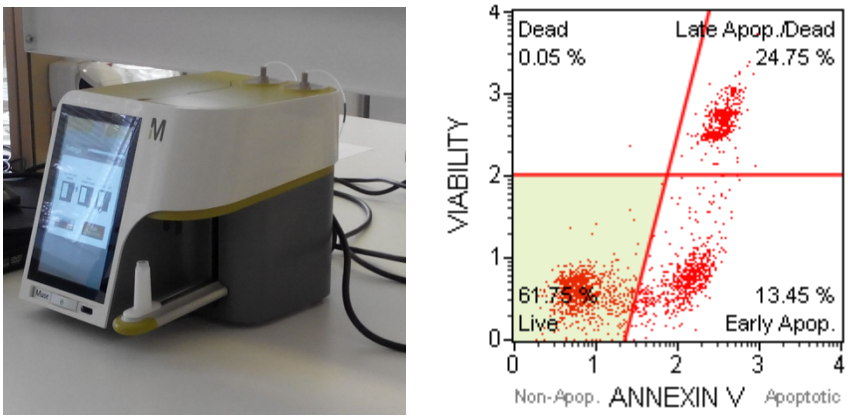

Fig.2. The Muse™ Cell Analyzer (A) and a sample apoptosis measurement plot (B).

For measurements of the percentage of cells in the G0/G1, S, and G2/M phases of the cell cycle on the Muse™ Cell Analyzer, the Muse™ Cell Cycle Assay reagent is used, which includes the nuclear DNA intercalating stain propidium iodide (PI) and RNAse A. PI discriminates cells at different stages of the cell cycle, based on differential DNA content in the presence of RNAse to increase the specificity of DNA staining.

A B

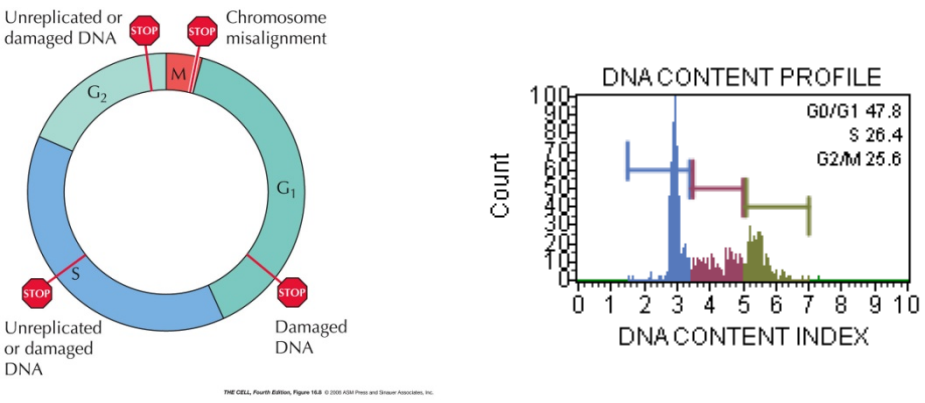

Fig.3. A cell cycle scheme (A) and a sample DNA content index histogram (B) of the cell population in each phase of the cycle.

Student activity:

1. Acquaintance with the basic principles of human fibroblast cell line cultivation and operating essential equipment.
2. Cell synchronization.
3. Preparation of samples for irradiation.
4. Irradiation of cells with ^{60}Co γ-rays (the Rokus-M facility) or accelerated protons (the therapeutic beam of the Phasotron).
5. Measurements of apoptotic induction and the percentage of cells in different phases of the cell cycle with the Muse^{TM} Cell Analyzer in various time intervals after irradiation.
6. Analysis of the obtained data.
7. Preparing a report.

Number of participants:

This project is meant for 1—2 students.

Experience: At least one of the courses: General Radiobiology, Biophysics, Biochemistry.

Recommended literature:

1. King K.L., Cidlowski J.A.. Cell cycle regulation and apoptosis. Annu. Rev. Physiol. 1998;60: 601-617.

2. Vermeulen K, Van Bockstaele DR, Berneman ZN. The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Prolif. 2003;36:131–149.

3. Sung T. et al. Boron neutron capture therapy induced cell cycle arrest and cell apoptosis of glioma stem/progenitor cells in vitro. Rad. Oncology. 2013; 8:195.

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