Analysis of high-LET radiation-induced HPRT mutations in mammalian cells

The main problem of radiation genetic investigations is the clarification of molecular mechanisms of mutagenic effects induced by different types of ionizing radiation in cells. It has been shown that during the irradiation of living cells, one of the main targets (and probably the most important one) is DNA. It has been established that under accelerated heavy ion irradiation, clustered DNA lesions are formed [1]. The repair of such lesions is much more complex than, for example, single strand breaks, which make up the main component of DNA damage yield caused by low-LET radiation. Damage to DNA induced by accelerated heavy ions and gamma rays (or X-rays) is therefore fundamentally different. The higher effectiveness of high-LET radiation in causing biologically relevant damage in comparison with ^{60}Co gamma radiation can be seen in Fig. 1 [2].

The action of ionizing radiation on the cell culture may be observed on several levels: survival of cells, number of mutants created, increase in the number of chromosomal aberrations, changes in phenotype, etc. The radiation damage to DNA can be severe; parts of the genetic material can be changed or even missing, causing chromosome aberrations and/or mutations. In either case, the information contained in DNA is altered, and this leads to an improperly working gene (as regards obtaining a correctly functioning protein). Moreover, the genome can become unstable, and the chromosome aberrations can be passed on to next generations. In human body, these irregularities in genome information can lead to various problems and can evolve into serious diseases such as cancer or Lesh-Nyhan disease in the case of HPRT gene.

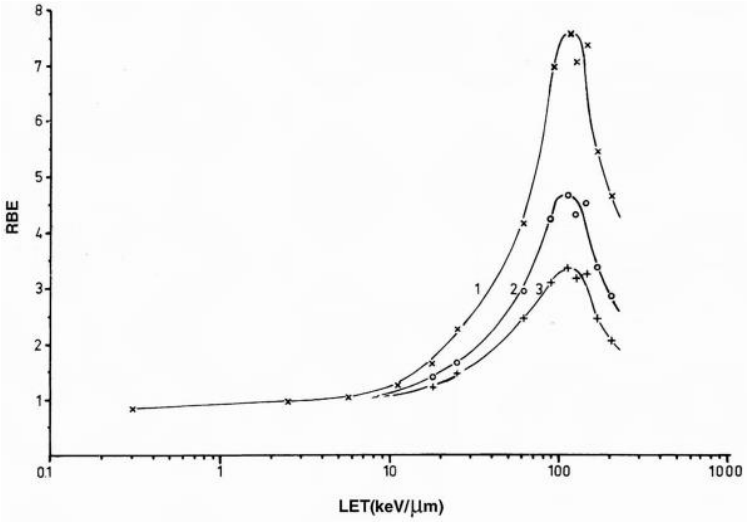

Fig. 1 - Dependence of Relative Biological Effectiveness (RBE) on the Linear Energy Transfer (LET) value of the tested radiation compared to gamma radiation of ^{60}Co. Curves 1, 2, and 3 correspond to cell survival levels of 0.8, 0.1, and 0.01, respectively. [2]

This project is a part of a systematic investigation of the biological effects of accelerated heavy charged ions on the mammalian cells (JINR Thematic Plan: Research on the Biological Action of Heavy Charged Particles; 04-9-1077-2009/2020). The Group of Radiation Cytogenetics is particularly interested in the changes in the genetic material of the cells after irradiation (induced mainly by accelerated heavy ions, Fig. 2). The main focus is aimed to the HPRT-mutant cells [3]. Survival, mutagenesis, chromosomal aberrations, structural changes in the HPRT gene, and other parameters induced by radiation are observed. This project should help to clarify the correlation between the number of radiation-induced mutants, number (and quality) of chromosome aberrations, and the number and type of structural mutations in the HPRT-mutant subclones [4].

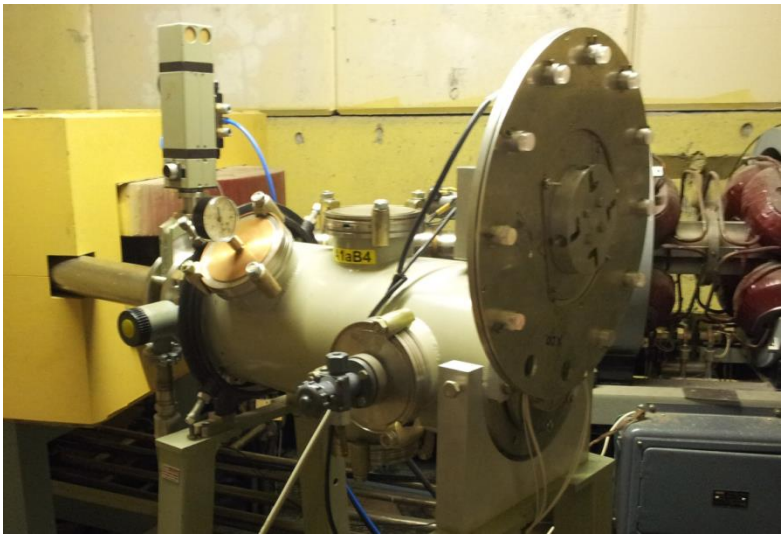

Fig. 2 - Revolving disc holding the irradiation chambers at the Genome-M setup of the U400M accelerator used for accelerated ion irradiation of biological samples.

References

1. Goodhead. D. T. Mechanisms for the biological effectiveness of high-LET radiations. *Journal of Radiation Research*, 1999, vol. 40 (Suppl.), p. 1 – 13.
2. Barendsen, G. W. Responses of cultured cells, tumours, and normal tissue to radiations of different linear energy transfer. *Current topics in radiation research quarterly*, 1968, vol. 4, p. 293 - 356.
3. Little, J. B.; Nagasawa, H.; Pfenning, T.; Vetrovs; H. Radiation-Induced Genomic Instability: Delayed Mutagenic and Cytogenetic Effects of X Rays and Alpha Particles. *Radiation Research*, 1997, vol. 148, p. 299 – 307.
4. Rossiter, B. J.; Fuscoe, J. C.; Muzny, M. D.; Fox, M.; Caskey, C. T. The Chinese hamster HPRT gene: restriction map, sequence analysis and multiplex PCR deletion screen. *Genomics*, 1991, vol. 9, p. 247 – 256.

Student tasks and activities:

- Acquaintance with the basic procedures of handling the cell cultures and the preparation of metaphase samples for chromosome aberration analysis
- Preparation of DNA samples for analysis based on polymerase chain reaction (PCR)
- HPRT gene structure analysis in the selected HPRT mutant-subclones induced by accelerated heavy ions
- Discussion of the obtained results and preparation of the final presentation

During this short course, participants will be given basic lecture on radiobiology focused mainly on: general cytological principles, radiation damage to DNA, differences between low-LET and high-LET radiation, induction of mutations, and genomic instability. Students will get familiar with the basics of the work in the biological laboratory. They will learn the proper handling of a mammalian cell culture (Chinese hamster cells, line V79; Fig. 3), which involves: maintaining the cell culture, recultivation, counting the concentration of cells in the suspension, distinguishing the dead cells from the living ones (trypan blue assay or colony formation assay), sample preparing for the irradiation, etc. The participants will be also shown how to prepare the metaphase samples for chromosome aberration analysis and the fundamentals of their evaluation will be explained.

This project is focused primarily on the analysis of the partial and/or total deletions (caused by heavy-ion irradiation) of exons in the HPRT gene using the PCR method (Fig. 4). After completion, the students should be able to isolate DNA from mammalian cells, measure the DNA concentration, and mainly prepare the samples for a successful PCR run. After the reaction, students should be capable to evaluate the obtained data and verify the PCR products using the gel electrophoresis.

Fig. 3 – Work with the cell culture under sterile conditions in the laminar flow hood.

Requirements

Basic knowledge of biology and the interaction of ionizing radiation with matter is welcomed.

Number of students

The maximum number of participants is four. After the introductory lessons and general explanation, they will be divided into 2 groups. Every group will be using different methods at the same time. For example: one group is running PCR, and other one is doing the gel electrophoresis of the previously amplified samples.

Fig. 4 – Laboratory room, with PCR box and PCR amplifier, where the DNA samples are isolated, measured and prepared for further analysis.

Recommended literature

1. Hall, E. J; Giaccia, A. Radiobiology for the radiologist. 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2006.
2. Ritter, S.; Durante, M. Heavy-ion induced chromosomal aberrations: A review. *Mutation Research*, 2010, vol. 701, p. 38 – 46.
3. Sorensen, B. S.; Overgaard, J.; Bassler, N. In vitro RBE-LET dependence for multiple particle types. *Acta Oncologica*, 2011, vol. 50, p. 757 – 762.

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