Course Title: Cytogenetic methods in radiation biology and human biodosimetry

Content:

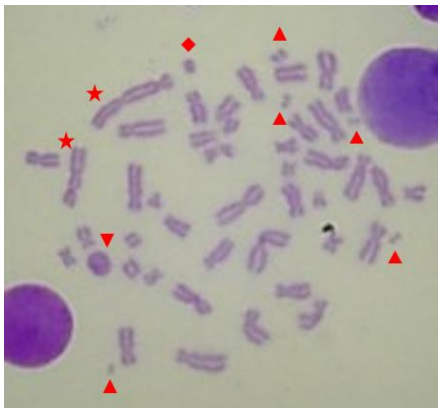
An extended course on cytogenetic methods and their application in radiation biology and human biodosimetry offered over three weeks, focusing on leveraging chromosome preparation techniques in the fields of radiobiology and radiooncology. The course is designed to suit master students and junior research staff. A total of 4 lecture sessions will be offered: 3 in the first two days, with each session lasting 2 hours and 1 at the end of course. First 3 lectures represent introduction to cytogenetics and overview of modern methods of chromosome visualisation. The last one concerns statistical methods of cellular radiobiology. The rest of time is spent on intensive hands-on training sessions in the cell and microscopy labs.

Scope of the course:

The main goal of radiation biology is to visualize the damage produced by radiation in living cells. Radiation cytogenetics is detecting lesions in chromosomes, which become visible during cell division due to enormous extent of DNA condensation: a mitotic chromosomes is 10 thousand –fold shorter than DNA extended length. The reason for such incredible package - precise segregation of genetic material to daughter cells during division. Ionizing radiation produces in DNA many types of lesions the vast majority of which are successfully removed by cell repair systems. Chromosome aberrations are originated from the most severe radiation-induced DNA lesions – DNA double-strand breaks (DSB), non-repaired or misrepaired, and are considered as a marker of radiation exposure.

There are several main techniques to visualize chromosome damage which will be learned during our course, particularly, metaphase method, anaphase assay and premature chromosome condensation technique. The

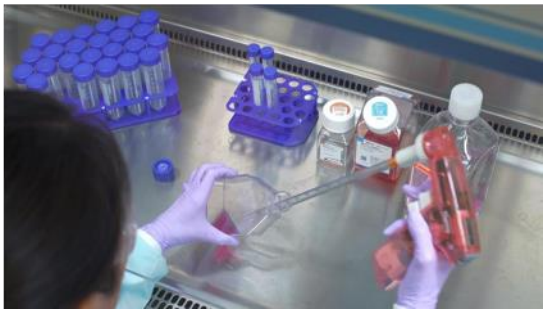
most powerful and widely used is metaphase method which will recieve the main attention (Fig. 1).



There are several most important applications of this cytogenetic method. 1) For more than seven decades there is the only valuable method of human biodosimetry: we estimate the dose to which an individual has been exposed occupationally, accidentally or therapeutically, by comparison of CA frequency in lymphocytes of individual with calibration curve obtained on the blood of healthy donors irradiated in vitro. 2) Metaphase method allows estimating individual radiosensitivity of patients: aberration yield induced in lymphocytes by *in vitro* irradiation may predict severe side effects after tumor radiotherapy. 3) CA are an important biomarker of cancer risk: epidemiological investigations revealed that increased level of spontaneous CA in healthy individuals associated with cancer risk (cancer incidence and mortality) *(Hagmar et al. 2004).*

Course Outline:

Module 1: Cell culture and Metaphase method



Every day hands-on training sessions will take place in the Cell Labs (R. 311 and 301) of LRB. The practical part includes the study of aseptic techniques of cell culturing by all the participants, long- and short-term cultures of normal (peripheral blood lymphocytes) and tumor (breast carcinoma line Cal 51) human cells, cell culture irradiation (X-rays SARRP, LRB).

Hands-on training sessions will be mainly focused on metaphase method of chromosome aberration analysis: as a first step, cell culture preparation, fixation, slide preparation, staining and mounting and as a second step, microscopic analysis of different aberration types. The main goal is the estimation of the chromosome aberrations frequency induced by different doses of radiation. The result is the generation of dose-response curves, fit parameters and statistical methods of their analysis and evaluation of biological efficiency of radiation.
Additionally, we apply Fluorescence plus Giemsa technique of chromosome staining (FPG) which allows to distinguish between first- second- and third postirradiation mitoses and evaluate the radiation-induced cell cycle delay.

Module 2: mFISH

Next module focuses on the most advanced method of modern cytogenetics, mFISH, which allows the simultaneous detection of all human chromosomes and analysis of all rearrangements between them. Practical part includes the hybridization procedure, capture and processing of the images, karyotyping of normal and malignant human cells, recording the CA, summarizing the results.

Module 3: PCC

Premature chromosome condensation (PCC) technique allows visualizing the chromatin damage in interphase cells. The first step of practical part includes cell culture samples preparation, fixation, slide preparation and staining. The second step includes the microscopic analysis of initial chromatin damage, repair kinetics of chromatin breaks and reconstruction of cell cycle composition.

Recommended Literature:

CYTOGENETIC DOSIMETRY: Applications in preparedness for and response to radiation emergencies. IAEA, 2011

This project is designed for simultaneous work of no more than 6 participants.

Supervisor:
Elena Nasonova, PhD, Head of group, LRB JINR, expert in radiation cytogenetics. Research interests are biological effectiveness of densely ionizing radiation, production and repair kinetics of chromatin lesions, long-term consequences of tumor radiotherapy.

Project and Assessment: On the final day, participants will have the opportunity to work on a project, developed during the hands-on sessions.

By completing this course, participants will be equipped with a most powerful and advanced cytogenetic techniques which they can use in their radiobiological and radiooncological research.

13-31января 2025 года в iThemba LABS (Кейптаун, ЮАР) прошла очередная летняя студенческая школа (*SAINTS Summer School).* Со стороны Объединенного института доклады представили директор УНЦ Дмитрий Каманин, ведущий н.с. ЛНФ Ваэль Бадави, руководитель группы исследования комплексных хромосомных аберраций ЛРБ Елена Насонова и другие. Впервые наряду с физическими практиками был организован практический курс по радиобиологии, который в течение двух дней провела Елена Насонова. Руководимая ею группа заняла первое место по итогам конкурса финальных презентаций и с 16 июня по 4 июля этого года в полном составе приедет в Дубну на 1 этап Международной студенческой практики для студентов ЮАР для продолжения обучения в ЛРБ.