SINFONIA short course "Measuring radiation-induced DNA damage, DNA repair and cell death: radiation biology in lab practice"

Activity report for PIANOFORTE travel grant

The course took place in Stockholm at the Wojcik laboratory within the Stockholm University from the 16th to the 21st of October 2022. During the course a state of the art in terms of cellular effects of ionizing radiation and methods adopted to investigate molecular and cellular effects were provided, including hands on work on the most utilized cytogenetics and immunogenetics techniques.

Following theoretical lectures on basic introduction to biological effects of radiation, factors involved in cellular radiosensitivity, explanation of molecular biology techniques used in radiobiology and individual radiosensitivity, the practical part of the course consisted of three main methods for the assessment of chromosomal aberrations, DNA repair through detection of ɣ-H2AX foci and clonogenic cell survival assay. Basic knowledge on cell culture were provided prior proceeding with the molecular techniques. For the assessment of chromosomal aberrations two different methods were conducted. The first method consisted of Giemsa staining of a representative cell sample treated with Colcemid for 30 minutes, in order to induce a higher number of cells in metaphase for chromosomal aberrations scoring. The scoring process was performed on preexisting samples of human blood cells irradiated *in vitro* to 4 different doses of neutrons, cultured for 50h and treated with Colcemid after 24h until harvesting. An average of 30 metaphases per slide were scored under an optical light microscope (100x magnification in oil) and the different types of chromosomal aberrations annotated in order to calculate frequency of aberrations per dose of exposure. Fluorescence in situ hybridization (FISH) was the second method demonstrated to assess chromosome aberration, in particular this method allows the identification of reciprocal translocations, a type of stable aberration that cannot be detected by Giemsa staining. For this purpose, two specific probes complementary for chromosomes 1 (red fluorescence signal) and 2 (green fluorescence signal) were adopted, in addition to counterstaining with DAPI to mark the rest of the chromosomes. Analysis of the prepared slides was performed using a fluorescence light microscope equipped with a digital camera (Metasystem software) at 100x magnification in oil.

DNA repair dynamics were assessed through the induction of ɣ-H2AX foci on irradiated and non-irradiated cells, by using immunohistochemistry technique on adherent cells grown on 22x22 mm coverslips. The protocol was performed in 3 cm Ø Petri dishes and consisted of fixation in 70% EtOH, permeabilization with 0.2% Triton X and immunostaining with primary and secondary specific antibody. DAPI counterstaining was performed to visualize the genetic material inside cell nuclei, prior mounting in Vectashield mounting media and sealing with nail polish on glass slides. After fluorescence light microscopy analysis at 100x magnification in oil, the number of foci was assessed

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on digital images by using ImageJ software with an inhouse macro plug-in specifically developed for this assay.

The clonogenic cell survival assay was demonstrated by using preexisting 6-well plates where cells had been cultured, irradiated and stained with Giemsa. Two sets of samples were used, one consisted of wild-type MRI cells and one of RP1-KD radio-sensitized cells, both exposed to increasing doses of gamma radiation. After counting cell colonies in triplicates, Plating efficiency and Surviving fraction were calculated based on standard formulas which take into account the relative number of cells seeded and the plating efficiency under control conditions.

Results were plotted for each of the described methods and presented on the last day of the course.

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