Analysis of cell cycle regulated and regulating proteins following exposure of lung derived cells to sub-lethal doses of γ-rays

Trani D.(1,2), Claudio P. P.(1), Cassone M.(1), Lucchetti C.(1,3), D'Agostino L.(1,3), Caputi M.(2), Giordano A.(1,3).

(1) Sbarro Institute for Cancer Research and Molecular Medicine, College of Science and Technology, Temple University, Philadelphia, Pennsylvania.

(2) Dipartimento di Scienze Cardio-Toraciche e Respiratorie, Facolta di Medicina e Chirurgia, Seconda Università degli Studi di Napoli, c/o V. Monaldi Hospital, via L. Bianchi 80128, Naples, Italy

(3) Department of Human Pathology and Oncology, University of Siena, Siena, Italy

dtrani@temple.edu

Introduction. Since the last century mankind had to face an increased exposure to man made and natural sources of radiation. Radiation represents a therapeutic instrument for radiosensitive cancers as well as a cytotoxic agent for normal human tissues. The effects of prolonged exposure to low doses of high energy radiation are still not well-known at the molecular and clinical level. Understanding their molecular effects will aid in developing more tailored therapeutic strategies as well as implementing radio-protective measures, essential prerequisite for the long-time permanence of men in space.

Objective of the study. The general aim of this study was to evaluate the susceptibility and the response of lung epithelial cells to DNA damage induced by ionizing radiations. We decided to study a panel of epithelial bronchial cell lines because of their fast-growth rate and their prominent exposure to both environmental and medical radiations. The specific objective of our study was to qualitatively and semi-quantitatively assess the involvement and behaviour of selected genes in DNA damage, DNA-repair mechanisms and apoptosis which follow radiation exposure, with the aim to determine the involvement of the most promising targets for the early detection of radiation-mediated lung damage before chronic disease develops.

Methods. Four epithelial cell lines (one normal and three neoplastic) were selected in order to detect and compare survival, cell cycle and protein expression differences related to their different genetic asset and/or baseline expression patterns. The non-tumorigenic human bronchial epithelial cell line NL20 and the non small cell lung cancer (NSCLC) cell lines A549, H358 and H23 were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and grown at 37 ^{o}C,

5% CO_2,in Ham's F12K medium supplemented with 500 ng/ml hydrocortisone and 4% fetal bovine serum (NL20), 10% fetal bovine serum (A549), in RPMI-1640 supplemented with 10 % fetal calf bovine serum (H358), or in DMEM supplemented with 10% FBS (H23). Subconfluent cultures (4.0 x 10^5 cells/cm^2, $\sim$ 70 % of confluence) were exposed to 4 Gy of γ-rays from a ^{137}Cs source (measured dose rate 100 cGy/min). Control samples were maintained in the same culturing conditions of the correspondent treated samples. All the samples were then incubated at 37 ^{o}C for 4, 12, 24 or 48 hours and, for each time point, both attached and floating cells were collected and used to perform FACS analysis, Western Blot, semi-quantitative PCR and cell viability assay. Appropriate loading and reaction controls were utilized.

Results. A markedly different response to radiation (4 Gy of γ-rays from a ^{137}Cs source) was observed over time between NL20 lung epithelial cell line and the three neoplastic cell lines. NL20 cells shifted to a high-apoptotic rate ($>$20%) in less than 12 hours. This event was paralleled by cellular growth arrest in the G0/G1 phase of the cell cycle, measured by FACS analysis, and by reduction of cell viability. Analysis of p53 and RB/p105 by Western Blotting demonstrated a positive correlation between their expression levels and the percentage of apoptotic cells.

The cell lines A549 and H358 showed no apoptotic shift at all in response to 4 Gy of γ-rays, with an apoptotic fraction never exceeding the $<$ 4% baseline at all time points. H358 (p53 null type) cells continued to replicate in linear-growth phase. H23 cells (p53 mutated) showed dramatic reduction of G0/G1 cellular fraction and an increase of the G2/M cell fraction in less than 12 hours from 4 Gy of γ-rays. In A549 cells cell line (p53 wild-type), we observed a transient arrest of cellular growth and a paradox decrease of apoptotic cell fraction, which averaged only 1% (30% of baseline) at all time points after a 4-hour lag phase. Interestingly, all three cancer cell lines showed a sharp peak of the G2/mitotic cells from 12 to 24 hours. The G2/M fraction doubled with respect to the control in the A549 and H358 cell lines. Additionally, we found some genes involved in DNA repair (RAD51, XRCC4, and XRCC5) modulated at the transcriptional level after 4 Gy of γ-irradiation.

Conclusions. Normal and lung cancer cells responded differently to ionizing radiation exposure by modulating key cell cycle regulatory genes in a very distinct manner. A more extensive study of the involvement of a broad panel of genes' expression can contribute to the prediction and understanding of cellular response to radiation-mediated damage and thus of individual radio-sensitivity. For this purpose, we are implementing a large scale genomic and expression survey on a wider set of cell lines, involving the use of high-throughput DNA- and protein-microarrays and of RealTime-PCR to gather an accurate quantitative assessment of individual pre- and post-radiation exposure sensitivity.