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Analysis of cell-cycle regulation following exposure of lung-derived cells to γ-rays

D. Trani [a,b], C. Lucchetti [a,c], M. Cassone [a], L. D'Agostino [a,c], M. Caputi [b], A. Giordano [a,c,*]

[a] Sbarro Institute for Cancer Research and Molecular Medicine, College of Science and Technology, Temple University, Philadelphia, PA, United States
[b] Dipartimento di Scienze Cardio-Toraciche e Respiratorie, Facoltà di Medicina e Chirurgia, Seconda Università degli Studi di Napoli, c/o Ospedale V. Monaldi, via L. Bianchi 80128, Napoli, Italy
[c] Dipartimento di Patologia Umana ed Oncologia, Università di Siena, Siena, Italy

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Abstract

Acute exposure of mammalian cells to ionizing radiation results in a delay of cell-cycle progression and/or augmentation of apoptosis. Following ionizing radiation-induced DNA damage, cell-cycle arrest in the G_1- or G_2-phase of the cell-cycle prevents or delays DNA replication or mitosis, providing time for the DNA repair machinery to exert its function. Deregulation or failing of cell-cycle checkpoints and/or DNA repair mechanisms may lead normal cells bearing chromosome mutations to acquire neoplastic autonomy, which in turn can trigger the onset of cancer. Existing studies have focused on the impact of p53 status on the radiation response of lung cancer (LC) cell lines in terms of both cell-cycle regulation and apoptosis, while no comparative studies have been performed on the radiation response of lung derived normal and cancerous epithelial cells. To investigate the radiation response in normal and cancerous phenotypes, along with the role and impact of p53 status, and possible correlations with pRb/p105 or other proteins involved in carcinogenesis and cell-cycle regulation, we selected two lung-derived epithelial cell lines, one normal (NL20, p53 wild-type) and one non-small cell lung cancer (NSCLC), H358 (known to be p53-deficient). We compared the levels of γ-induced cell proliferation ability, cell-cycle arrest, apoptotic index, and expression levels of cell-cycle regulating and regulated proteins. The different cell sensitivity, apoptotic response and protein expression profiles resulting from our study for NL20 and H358 cells suggest that still unknown mechanisms involving p53, pRb/p105 and their target molecules might play a pivotal role in determining cell sensitivity and resistance upon exposure to ionizing radiation.

Keywords: p53; Lung cancer; Cell-cycle; Ionizing radiation; Cosmic rays

1. Introduction

Since the last century, the humankind had to face an increased exposure to natural and man made sources of radiation. Ionizing radiation (IR) represents both a carcinogenic agent for healthy tissues and a therapeutic tool against solid tumors. The effects of prolonged exposure of normal tissues to low doses of high energy radiation are still not known at the molecular and clinical level. Understanding their molecular effects will aid not only in developing more tailored therapeutic strategies, but also in assessing the oncogenic potential of galactic cosmic rays and in implementing radio-protective measures, essential prerequisite for the long-time permanence of men in space (Cucinotta and Durante, 2006).

Cells exposed to ionizing radiation show delays in the progression through the cell-cycle: DNA damage induced by radiation exposure triggers the complex network of cell-cycle checkpoints and surveillance mechanisms, developed by organisms in order to ensure genetic integrity.

* Corresponding author. Present address: Temple University, Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, Bio Life Sciences Building, Suite 333, 1900 North 12th Street, Philadelphia, PA 19122-6099, United States.
E-mail address: giordano@temple.edu (A. Giordano).

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Following radiation-induced DNA damage, cell-cycle arrest in G_1 or G_2 prevents or delays DNA replication or mitosis, providing time for DNA repair machinery to exert its function (Iliakis et al., 2003). Deregulation or failing of cell-cycle checkpoints and/or DNA repair mechanisms may lead normal cells bearing chromosome mutations to acquire neoplastic autonomy, which in turn can trigger the onset of cancer (Weinert and Lydall, 1993; Jeggo and Lobrich, 2006). Most organisms have developed programmed cell death processes, referred to as apoptosis, in order to eliminate severely damaged cells and to minimize the risk of disorders and malignancy (Evan and Littlewood, 1998). Defects in the apoptotic and DNA repair pathways are nowadays believed to contribute to both carcinogenesis and resistance to radio- and chemotherapy of many human malignancies (Thompson, 1995).

Normal somatic cells exhibit a tight regulation of the cell-cycle, involving the interaction of many different proteins. One of the pivotal molecules in the checkpoint pathways is the tumor suppressor p53, which coordinates DNA repair with cell-cycle progression and apoptosis: therefore it has been considered the guardian of genome, responsible for the maintenance of genomic stability (Vogiatzi et al., 2006). The retinoblastoma (Rb) gene, either, has been proved to play a key role in cell-cycle control, also in response to radiation-induced DNA damage (Weinberg, 1995; Genovese et al., 2006). Rb and p53 contribute in determining cell sensitivity and resistance upon exposure to ionizing radiation.

p53 is mutated in ~50% of Non Small Cells Lung Carcinomas (NSCLCs) and ~70% of Small Cell Lung Carcinomas (SCLCs) (Viktorsson et al., 2005). Numerous studies have focused on the impact of p53 status on the radiation response of lung cancer (LC) cell lines, in terms of both cell-cycle regulation and apoptosis, while no comparative study has been published about the radiation response of lung derived normal and cancerous epithelial cells. Our belief is that investigating the response to IR of lung derived normal epithelial cells might lead to the understanding of molecular mechanisms underling LC onset event and/or generating resistant phenotypes. This study was designed to *in vitro* investigate the radiation response (proliferation ability, cell-cycle regulation, and apoptosis) in normal and cancerous phenotypes, along with the role and impact of p53 status, and the possible correlations with Rb or other proteins known to be involved in carcinogenesis and cell-cycle regulation. The two selected lung-derived cell lines were the normal epithelial NL20 cell line (p53 wild-type) and the NSCLC cell line H358, known to be p53 deficient. We compared the levels of γ-induced cell proliferation ability, cell-cycle arrest, apoptotic index, and expression levels of cell-cycle regulating proteins. All the obtained data were analyzed and correlated leading us to hypothesize that p53- and pRb-dependent mechanisms might be responsible for the normal radiosensitive and the malignant radioresistant phenotypes.

2. Materials and methods

2.1. Cell lines and radiation treatment

The non-tumorigenic human bronchial epithelial NL20 cell line and the Non Small Cell Lung Cancer (NSCLC) cell line H358 were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). NL20 cells were cultured in Ham's F12 medium supplemented with sodium bicarbonate (1.5 g/L), glucose (2.7 g/L), L-glutamine (2.0 mM), non-essential amino acids (0.1 mM), insulin (0.005 mg/ml), epidermal growth factor (10 ng/ml), transferrin (0.001 mg/ml), hydrocortisone (500 ng/ml) and 4% fetal bovine serum. H358 cells were grown in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum. Both cell lines were grown in 5% CO_2/95% air at 37 °C and subcultured according to ATCC recommendations. Before radiation treatments, the cells were counted using a Z2™ Coulter Counter (Beckman Coulter, Inc., Fullerton, CA, USA) and seeded at a density of $2.0 \times 10^3/cm^2$ in 500 cm^2 polystyrene dishes (Nalgene Nunc International, Rochester, NY, USA). Four days later, asynchronously proliferating cultures (~1.0×10^5 cells/cm^2, ~70–75% of confluence) were exposed to 4 or 10 Gy of γ-radiation from a ^{137}Cs source at 100 cGy/min. After the irradiation all the samples were incubated again at 37 °C for 4, 12, 24, 48, 72 or 96 h. Control samples were maintained in the same culturing conditions of the correspondent treated samples.

2.2. Cell proliferation analysis

For each time point, cells were collected, counted and cell concentration (number of cells/mL) was estimated. The cell concentration was then reported as function of post-irradiation time (hours) and dose (0, 4 and 10 Gy). The obtained curves describe the modulation of cell proliferation ability in absence or presence of radiation-induced DNA damage.

2.3. Flow cytometry analysis

Experiments were performed on asynchronously proliferating cultures. Cells were plated in 500 cm^2 dishes at a cell density of $2.0 \times 10^3/cm^2$ and exposed to radiation when the confluence had reached ~70–75%. At 4, 12, 24, 48, 72 or 96 h after the irradiation, treated and control samples were harvested, washed in phosphate-buffered saline (PBS) and fixed by cold 70% ethanol. The cells were then re-suspended in PBS containing 10 μg/mL of propidium iodide (PI) and 250 μg/mL of RNase, and incubated for 30 min at 37 °C. Flow cytometry analyses for cell-cycle distribution and apoptosis were performed in triplicate on a Coulter Elite Apparatus (Beckman Coulter, Inc., Fullerton, CA, USA).

2.4. Western blotting

Total proteins were extracted from control and irradiated samples from both cell lines at all time points, and