Flow-cytometry applications to characterize the radiation response of colorectal adenocarcinoma cells

L. Lonati[1], I. Guardamagna[1], M. Savio[2], L.A. Stivala[2], A. Ottolenghi[1], G. Baiocco[1]

1 Lab. of Radiation Biophysics and Radiobiology, Department of Physics, University of Pavia, Pavia, Italy
2 Immunology and General Pathology Unit, Department of Molecular Medicine, University of Pavia, Pavia, Italy

Introduction

According to the Global Cancer Observatory (GCO), colorectal cancer (CRC) is the third most common in terms of incidence and the second leading cause of cancer death. It is usually treated with surgery, with chemotherapy and radiotherapy as adjuvant/neoadjuvant therapies. Despite advances in treatment modalities however, patients' survival remains poor. This requires further research efforts to better understand the in vivo response to treatment, and well-characterized in vitro cell systems represent important pre-clinical tools to study the main mechanisms underlying cell response to therapeutic agents, in particular ionizing radiation (IR). Based on these premises, we present an experimental characterization of the in vitro response to X-rays of Caco-2 cells, a cell line derived from human colorectal adenocarcinoma. We measured a variety of radiobiological endpoints with different techniques, including flow-cytometry applications.

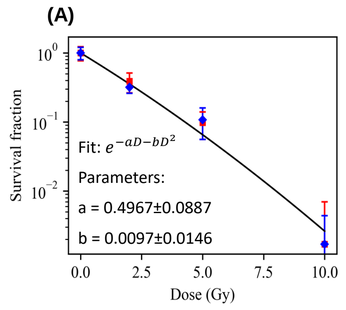
Materials and methods

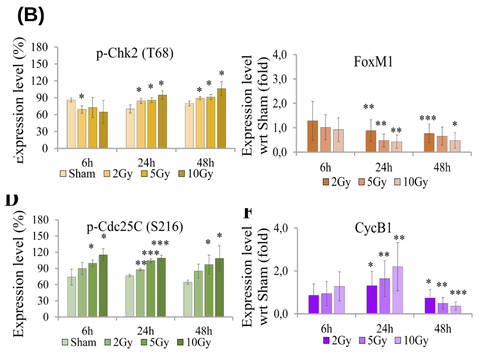
Caco-2 cells were exposed to X-rays from 2 Gy up to 10 Gy from a LINAC (linear accelerator) used in radiotherapy. Clonogenic survival was assessed at 2 weeks after irradiation. We further focused on measuring several endpoints in the early timepoints after exposure up to 48 hours: cell-cycle perturbations and cell death were investigated via flow cytometry, respectively using a triple staining with PI (Propidium Iodide), EdU (5-ethynyl-2´-deoxyuridine) and anti-pH3, and with FITC-conjugated Annexin-V/PI staining.

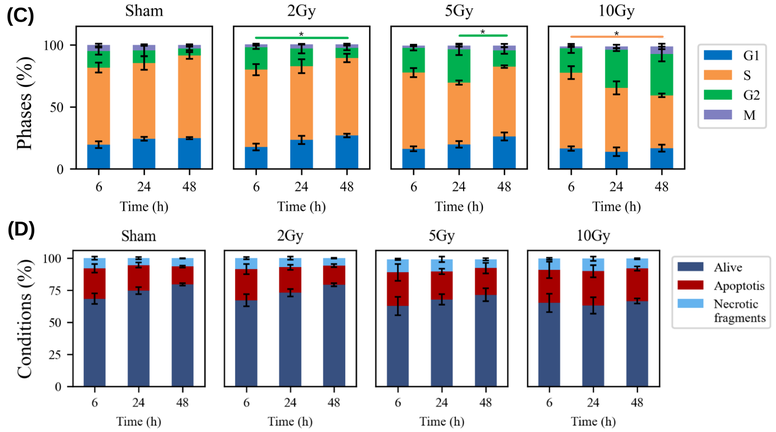
We also scored micronuclei and atypical mitosis with immunofluorescence microscopy. To complement and better interpret flow-cytometry data on cell cycle from a molecular point of view, we quantified via Western Blot techniques several proteins involved in the regulation of the G2/M checkpoint, among which FoxM1, Chk2, Cdc25C, CyclinB1, and in the DNA response by γH2AX signal. Finally, activation of matrix metalloproteases (MMPs) was measured via gelatin zymography.

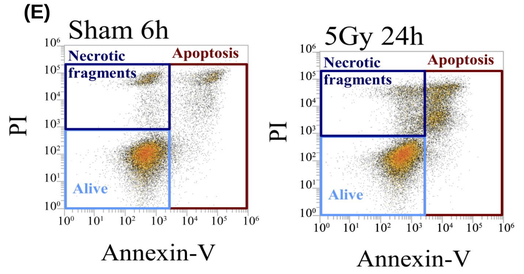
Results

We found that: Caco-2 clonogenic potential is conserved up to 5 Gy **(A)**; radiation mainly induces an arrest in the G2-phase, peaked at 24 hours **(C)**, confirmed by the associated molecular markers from the integrated analysis of flow cytometry and Western blotting **(B)**; the number of apoptotic cells increases as a function of the dose **(D, E)** and the dependency of the death pathways on dose and time correlates with the inhibition of MMPs (not shown here, see Ref.); genomic instability markers, as the number of atypical mitosis and micronuclei, are also dose-dependent (not shown here, see Ref.).









(A) Clonogenic survival assay
(B) Western blot quantification of G2/M checkpoint regulatory proteins
(C) % of cell in each phase as function of time and dose
(D) % of cells alive or dead as function of time and dose
(E) Representative biparametric plot of AnnV-PI staining

Conclusions

Integrating flow-cytometry applications and other experimental techniques, we achieved a more detailed characterization of Caco-2 early response to radiation, with further correlation to their long-term replicative potential. This integrated analysis offers the chance to gain knowledge on mechanisms underlying radiation effects on such model, that can be exploited in perspective to identify therapeutic targets for colorectal cancer treatment.

Reference I. Guardamagna, L. Lonati, et al. «An Integrated Analysis of the Response of Colorectal Adenocarcinoma Caco-2 Cells to X-Ray Exposure». *Frontiers in Oncology* 11 (2021):2029. https://doi.org/10.3389/fonc.2021.688919.

Email leonardo.lonati01@universitadipavia.it