P8 - A novel EdU-based protocol for the investigation of cell cycle kinetics of irradiated human lymphocytes

Molecular and cellular effects

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Introduction: In radiation biology, gaining insights into the effects of irradiation on the proliferation and kinetics of cells is imperative. A powerful tool to accurately quantify cell cycle kinetics is the use of nucleoside analogues, such as 5-ethynyl-2'-deoxyuridine (EdU). EdU is a thymidine analogue that can be incorporated into DNA during replication. Via bivariate analysis of cellular DNA content and EdU incorporation, a distinction between cell cycle phases can be made. By performing a time-lapse analysis of EdU pulse-labeled cells, kinetics of the cell cycle can be investigated. Lymphocytes are widely used in radiation research. However, knowledge of the effects of radiation on their cell cycle kinetics is still limited. In this study, we optimized an EdU-labeling protocol on whole blood cultures to study the cell cycle progression of irradiated human lymphocytes.
Methods: Whole blood was cultured and cell division was stimulated by the addition of phytohaemagglutinin (PHA). After 4 days of culture, the cells were pulse-labeled with EdU and irradiated *in vitro* with 1, 2 and 4 Gy of 220 kV X-rays. Time-lapse analysis was performed from 0 up to 25 hours of incubation, with one-hour intervals. After counterstaining with DAPI to measure DNA content, the cells were analyzed by flow cytometry.
Results: G2-arrest after irradiation of the lymphocytes could be detected with our EdU-based cell cycle analysis protocol. The length of the G2 arrest depends on the irradiation dose.
Conclusion: We propose a novel protocol of EdU-based cell cycle analysis to determine the proliferation kinetics of human lymphocytes in whole blood cultures.