

ASSESSMENT OF INDIVIDUAL VARIATION IN DNA DOUBLE-STRAND BREAK REPAIR CAPACITY IN HUMAN DIPLOID FIBROBLASTS

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The induction of bi-stranded clustered DNA damage (BCD), which includes direct DNA double-strand breaks (DSBs), is a hallmark of ionizing radiation (IR) exposure. Incorrectly repaired DSBs can cause chromosomal rearrangements and an increased risk of genomic instability and cancer. Because there is polymorphic variation in DNA repair genes and much of this variation is predicted to have a functional impact, healthy people likely vary in their capacity to repair DSBs and other BCD. This project will quantify the capacity of a collection of human low-passage “apparently normal” primary fibroblast cell strains to recognize, signal, and repair DSBs and other BCD by measuring damage-responsive nuclear foci levels after exposure to 0, 5, 10, and 25 cGy of ^{137}Cs γ -rays. Radiosensitive cell strains from cancer-prone genetic disorders defective in DSB/BCD recognition, signaling, and repair will be used as controls to assess the degree of variation measured among the apparently normal cell strains.

The first aim of this project, which is currently underway, is to evaluate post-irradiation DNA damage foci kinetics in confluent G_0/G_1 -phase fibroblast cultures arrested by 0.2% serum starvation at 10, 30, 120, and 1440 minutes (24 hours) post-irradiation. γ -H2AX foci (H2AX phosphoserine-139) are detected by immunofluorescence and provide, to a first approximation, a direct measure of DSBs. To minimize ambiguity in scoring these foci, especially for smaller foci, we have defined conditions for co-staining for phospho-ATM (ATM phosphoserine-1981; pATM) and γ -H2AX. Our scoring criteria involve counting large, distinct γ -H2AX foci and smaller γ -H2AX foci that colocalize with pATM foci. We find that both γ -H2AX and pATM foci are readily detectable 10 minutes post-irradiation at 37°C. At 10 minutes and 120 minutes there is ~85% colocalization of the two foci types, with ~20% more γ -H2AX foci present than pATM foci. An example of these two types of foci and their colocalization is shown in **Figure 1**. To date, we have examined over 25 apparently normal primary fibroblast strains from the Coriell Institute of Medical Research (CIMR, Camden, NJ) and have observed significant variation in all parameters: background level of foci, initial post-irradiation yield of foci, kinetics of foci disappearance, and the residual level of foci present 24 hours post-irradiation. An example of the level of induced γ -H2AX foci and the kinetics of their disappearance after 5, 10, and 25 cGy for the apparently normal fibroblast strain AG01522 is shown in **Figure 2**.

These studies should advance development of predictors of risk of cancer from low-dose ionizing radiation exposures by quantifying inter-individual variation and the contributions of the various DNA damage-response and repair pathways. This work was performed under the auspices of the U.S. DOE by the University of California and the Lawrence Livermore National Laboratory under contract W-7405-Eng-48 and supported by the U.S. DOE Low Dose Radiation Research Program.

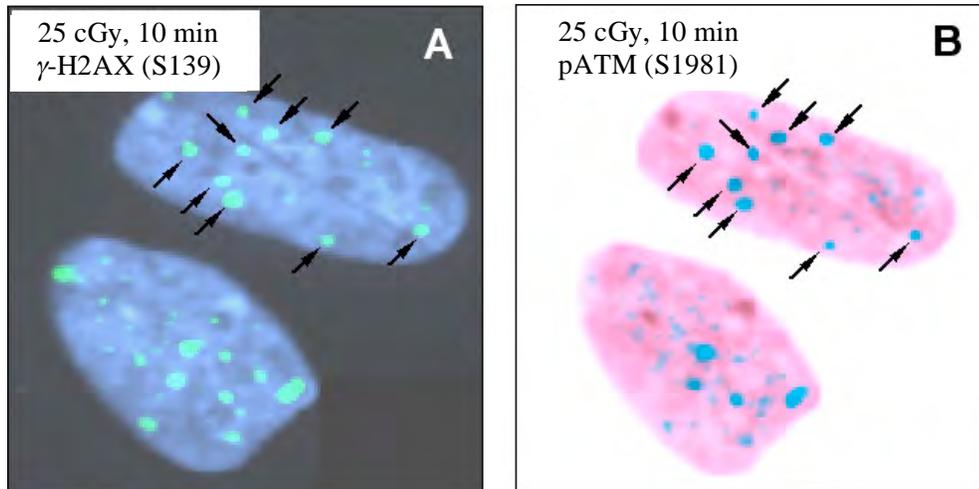


Figure 1. Panel A shows immunostaining for γ -H2AX (Upstate Cell Signaling Solutions) and Panel B shows immunostaining for pATM (Rockland Immunochemicals). The majority of γ -H2AX foci show colocalization with pATM foci, as indicated by the arrows; γ -H2AX foci are formed immediately after IR by the activation of the kinase activity of ATM (through phosphorylation of ATM serine-1981) and its phosphorylation of serine-139 of H2AX.

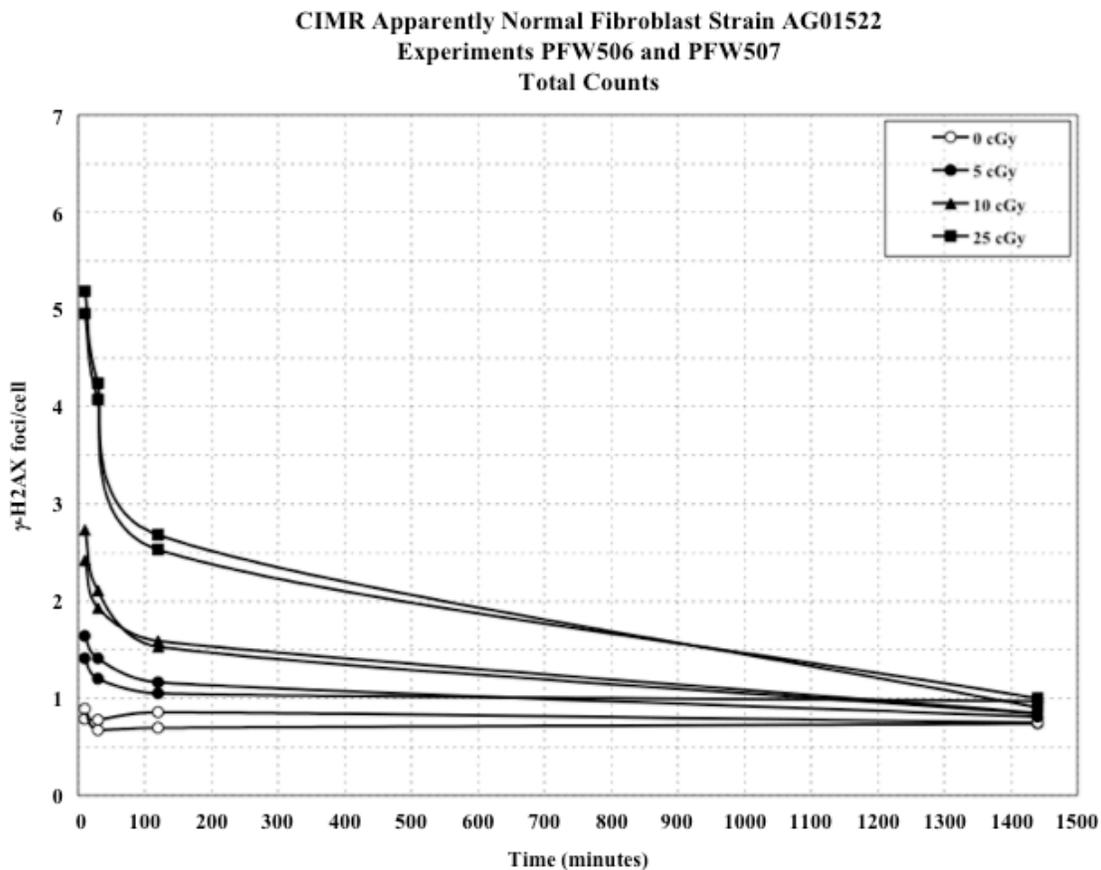


Figure 2. Kinetics of γ -H2AX foci formation and disappearance during 24 hours post-irradiation incubation at 37°C. Each pair of lines at a given dose represents data from two independent experiments. This apparently normal fibroblast strain AG01522 exhibits nearly complete DSB repair by 24 hours.