THE EFFICIENCY OF HOMOLOGOUS RECOMBINATION AND NON-HOMOLOGOUS END JOINING SYSTEMS IN REPAIRING DOUBLE-STRAND BREAKS DURING CELL CYCLE PROGRESSION

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This study investigated the efficiency of Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR) repair systems in rejoining DNA double-strand breaks (DSB) induced in CCD-34Lu cells by different γ-ray doses. The kinetics of DNA repair was assessed by analyzing the fluorescence decrease of γ-H2AX foci measured by SOID (Sum Of Integrated Density) parameter and counting foci number in the time-interval 0.5-24 hours after irradiation. Comparison of the two methods showed that the SOID parameter was useful in determining the amount and the persistence of DNA damage signal after exposure to high or low doses of ionizing radiation. The efficiency of DSB rejoining during the cell cycle was assessed by distinguishing G1, S, and G2 phase cells on the basis of nuclear fluorescence of the CENP-F protein. Six hours after irradiation, γ-H2AX foci resolution was higher in G2 compared to G1 cells in which both NHEJ and HR can cooperate. The rejoining of γ-H2AX foci in G2 phase cells was, moreover, decreased by RI-1, the chemical inhibitor of HR, demonstrating that homologous recombination is at work early after irradiation. The relevance of HR in DSB repair was assessed in DNA-PK-deficient M059J cells and in CCD-34Lu treated with the DNA-PKcs inhibitor, NU7026. In both conditions, the kinetics of γ-H2AX demonstrated that DSBs repair was markedly affected when NHEJ was absent or impaired, even in G2 phase cells in which HR should be at work. The recruitment of RAD51 at DSB sites was, moreover, delayed in M059J and in NU7026 treated-CCD-34Lu, with respect to DNA-PKcs proficient cells and continued for 24 hours despite the decrease in DNA repair. The impairment of NHEJ affected the efficiency of the HR system and significantly decreased cell survival after ionizing radiation, confirming that DSB rejoining is strictly dependent on the integrity of the NHEJ repair system.