γ-Radiation Induces Distinct DNA Damage Responses in a Human Lung Epithelial Cell Three-Dimensional Culture Model

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DNA damage and consequent mutations initiate the multi-step carcinogenic process. Most mechanistic studies of the DNA damage response *in vitro* have been performed in monolayer (2D) cultures. Previous work using 53BP1 fused to yellow fluorescent protein (YFP-53BP1) as a surrogate marker, demonstrated that the number of DSBs formed was linear with dose from 5 mGy to 1 Gy. The DSBs induced by very low radiation doses (5 mGy) were repaired with efficiency similar to repair of DSBs induced at higher doses. Although these systems provide useful and powerful information, 2D systems do not recapitulate the three-dimensional (3D) structural organization or functional differentiation of the cells *in vivo*. We have developed a novel human lung 3D model and have assessed cellular responses to DNA damage induced by low-linear energy transfer (LET) ionizing radiation (IR). We provide evidence that the number of double-strand breaks (DSBs) formed in the 3D lung model, as measured by the number of EGFP-53BP1 foci formed, was linear with γ-radiation dose from 10 to 100 cGy. Though the number of DSBs induced after low-LET IR was comparable between 2D and 3D structures, DSBs were repaired with slower kinetics in 3D structures as compared to 2D. Interestingly, in contrast to cells in 2D, cells in 3D structures were less sensitive to IR and displayed limited types of gross-chromosome aberrations. Thus, cells in 2D and 3D culture responded to radiation differently. This data will have important implications for evaluating risks due to radiation exposure.

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