

LET Dependence of Response of Irradiated and Bystander Cells to Very Low Fluences of Charged Particles in 2D and 3D

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INTRODUCTION

In space, cells in an astronaut's body are not only traversed by ionizing particles and electrons from delta rays, but unirradiated cells could also experience bystander effects as a result of intercellular signaling from irradiated cells. We have shown previously that there is ~2-fold increase in the fraction of normal human fibroblasts that contain micronuclei (MN) in both the irradiated cell population and bystander cells sharing medium with the irradiated cells at doses as low as 0.47 mGy of 1 GeV/n Fe ions (~0.02 Fe ions/cell) or 70 μ Gy of 1 GeV protons (~2 protons/cell). From that lowest damaging dose to about 1 cGy of either ion, there is no further increase in the fraction of damaged cells in either cell population. At higher doses, the level of damage in irradiated cells increases, while the fraction of damaged bystander cells remains at the plateau level. In the current work we have extended these studies to investigate the importance of LET, particle track structure, and cell microenvironment or tissue architecture in the bystander effects.

METHODS

For 2D studies, responses were determined in AG01522 human skin fibroblasts using a transwell insert system in which irradiated and unirradiated bystander cells were co-cultured in shared medium, starting immediately after irradiation. Endpoints were formation of MN 72 h after irradiation, a surrogate for chromosome aberrations, and formation of nuclear foci of the DNA repair-related protein 53BP1, a surrogate for DNA DSBs, measured 5 h after irradiation. In the 3D studies, the fibroblasts were embedded in a collagen matrix with an epidermal layer containing h-TERT-immortalized human keratinocytes, which formed a skin-like, stratified epithelium. Bystander studies were conducted by splitting the constructs in half, irradiating one half, then placing the halves together in a transwell insert for up to 72 h and quantifying 53BP1 foci. Irradiation was performed using 1 GeV/n Fe ions (LET 151 keV/ μ m), 300 MeV/n Fe ions (LET 250 keV/ μ m) or 1 GeV protons (LET 0.24 keV/ μ m) at the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory (BNL).

RESULTS

In the 2D monolayers, at the higher LET from 300 MeV/n Fe ions compared to 1 GeV/n Fe ions, the lowest fluence at which DNA damage increases in AG01522 cells is shifted to lower fluences. The fraction of cells with MN or 53BP1 foci is increased in both irradiated and bystander populations at a fluence of 200 ions/cm², or 0.002 Fe ions/cell, a 10-fold lower fluence than the minimum at which damage is seen with 1 GeV/n Fe ions. The data suggest that the minimum charged particle fluence at which damage occurs in irradiated and bystander populations depends on LET, although the fraction of damaged cells, at doses less than 1 cGy, does not. Monte Carlo simulations of particle track structure for iron ions and protons are being conducted, and the results will be compared with the biological outcomes to study whether the apparent LET-dependent differences reflect the importance of energy imparted to a cell per particle traversal or whether delta-rays produced by the particles are important for stimulating cells to send a bystander response.

In the 3D system, we have recently shown that there is a significant reduction (4-6-fold) in the percentages of both irradiated and bystander fibroblasts positive for 53BP1 foci compared to the levels seen in 2D. In the 3D constructs, the minimum fluence of 1 GeV protons needed to elicit the bystander response is about 2×10^5 protons/cm² (~2 protons/cell or ~70 μ Gy), similar to the minimum effective proton fluence in cells in 2D. In contrast, when cells in 3D are exposed to 1 GeV/n Fe ions, a much higher minimum fluence of 4.1×10^5 Fe ions/cm² (~4.1 Fe ions/cell; ~10 cGy) is required to stimulate the bystander response. The data clearly indicate that cellular microenvironment or architecture influences radiation-induced intercellular signaling.

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