Adaptive Responses to Low Dose/Low Dose-Rate γ-Rays in Normal Human Fibroblasts: The Role of Oxidative Metabolism

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To investigate low dose/low dose-rate effects of low linear energy transfer ionizing radiation, we used γ-irradiated cells adapted to grow in three-dimensional architecture that mimics cell growth *in vivo*. We determined cellular, molecular and biochemical changes in these cells. Quiescent normal human fibroblasts were irradiated with *single* acute or chronic doses (1-10 cGy) of cesium-137 γ-rays. Whereas exposure to acute-10 cGy increased micronucleus formation (a surrogate form of DNA damage), protraction of the dose over 48 h reduced its frequency to levels similar or lower than background. The latter also up-regulated cellular content of the antioxidant glutathione. These changes correlated with modulation of phospho-TP53 (serine15), a stress-marker that was regulated by doses as low as 1 cGy. The DNA damage that occurred by exposure to acute- 10 cGy was protected against by two ways: 1) Up-regulation of cellular antioxidant enzyme activity by ectopic overexpression of Mn-superoxide dismutase, catalase or glutathione peroxidase, and 2) inhibition of superoxide anion generation by flavin-containing oxidases. These results support a critical role of oxidative metabolism in mediating low dose radiation effects.

The role of oxidative metabolism in low dose γ-ray effects was further supported by studies of mitochondrial protein import and membrane potential in irradiated cells. Dose response studies clearly showed that effects at low dose cannot be predicted from effects at high dose. When density-inhibited normal human fibroblasts were exposed to a toxic dose of 400 cGy (330 cGy/min), protein import into mitochondria isolated from these cells was decreased. In contrast, protein import into mitochondria isolated from low dose-irradiated (10 cGy, 0.2 cGy/h) cells was enhanced. Radiation-induced changes in mitochondrial membrane potential mirrored the changes in import at high and low dose exposures. These data suggest that mitochondria, which are active participants in oxidative metabolism, play a crucial role in low dose-induced adaptive responses.

Low dose/low dose-rate γ-ray-induced adaptive responses against chromosomal damage caused by a subsequent acute challenge dose of γ-rays were also observed when cells pre-exposed to 1, 5, or 10 cGy (0.2 cGy/h) were subsequently challenged with 100 cGy (80 cGy/min). The induced mitigating effects were transient and disappeared by 48 h. Oxidative metabolism, DNA repair and cell cycle checkpoints are involved in the induced responses. Importantly, the biological effects induced by reactive oxygen species generated by moderate γ-ray doses delivered at low dose rate, were similar to those generated from normal oxidative metabolism.

The modulation of oxidative metabolism in the progeny of control and low and high dose-irradiated cells will be discussed.

Supported by Research grant FG02-02ER63447from the US Department of Energy