Low Dose Responses in Human Cells and Molecular Mechanisms of Adaptation

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The radiation Adaptive Response (adaptation, or AR) is a well documented, although evidently highly variable, protective phenomenon in which exposures to low-dose or low-dose-rate ionizing radiation result in reduced deleterious effects of subsequent higher exposures. Protection has been reported against a variety of biologically important endpoints, but its variability as a function of cell and tissue type and its genetic control are not well understood. The adaptive response is predicted to result in a non-linear dose response for cancer risk in the low dose range. However, the molecular mechanism(s) remain unknown, and such information is essential for incorporating adaptive responses into estimation of risk from low dose exposures. The goals of our studies are to characterize molecular functions that may differ between low dose and high dose responses, to elucidate AR mechanisms – proposed to include low-dose inducible DNA repair and DNA damage signaling pathways – and to investigate the relationships between a range of endpoints affected by adaptation, including chromosomal damage, radiation-induced cellular senescence, genomic instability, and epigenetic changes. We use human mammary epithelial cells (HMECs) and fibroblasts, which are the target and non-target cells for mammary gland carcinogenesis, as well as mouse mammary fibroblasts from both radiation-sensitive and resistant strains. These in vitro studies are essential to enable assessment of the extent to which human radiation response biology is mirrored by that in the mouse, which is employed as a crucial model for in vivo effects of low-dose radiation.

Our working hypothesis is that ability to induce efficient repair of oxidative DNA damage is critical for cell and tissue responses to low doses of ionizing radiation, as well as being a key determinant of the effects of endogenous DNA damage on the organism. The p53 protein plays central roles in early responses to DNA damage and in suppression of damage-induced tumorigenesis. It has been implicated in both the AR and low-dose hypersensitivity, but its role is unclear. We are testing the hypotheses that DNA damage signaling and/or DNA repair processes critical for removing oxidative DNA damage and maintaining genomic integrity are non-linear with dose, that their induction is required for the adaptive response, and that p53 plays a role in this induction.

In order to investigate the mechanism of the AR, it is necessary to identify cell types and cell culture conditions that reproducibly adapt. An extensive search for mammalian cell strains consistently exhibiting adaptive protection against formation of micronuclei (MN; a highly quantitative surrogate marker for the types of chromosome aberrations that can result in cancer) was carried out among a variety of human fibroblasts and HMECs. However, using a standardized adaptive regimen of a 0.1 Gy priming dose followed by 4 hr of incubation prior to a challenge dose of 2 Gy, adaptation was only occasionally observed in certain fibroblast strains, while the majority of experiments yielded negative results; i.e., no reduction in MN frequency was seen as a result of prior low dose exposure. In light of our hypothesis that increased efficiency of responding to oxidative damage underlies the AR, we wondered whether standard culture conditions might almost maximally induce these responses, so that the additional contribution of a low priming dose would be negligible. Tissues in vivo experience much lower oxygen concentrations than the atmospheric oxygen used in standard cell culture. We therefore compared results with cells cultured in 3-5% oxygen (using specialized tri-gas incubators in which nitrogen is used to reduce the oxygen concentration) to cells grown in standard incubators at 20% oxygen. Remarkably, we find that significant AR protection against micronuclei formation can be reproducibly observed in primary human fibroblasts grown under low oxygen, but NOT in cells grown in standard, atmospheric oxygen culture conditions. Suggesting that the cells grown in standard conditions are under oxidative stress prior to any exposure to ionizing radiation, we found that markers of the DNA Damage Response (DDR), including p53, p21, and γH2AX protein levels, are elevated in

cells grown in 20% oxygen compared to the same cells grown in low oxygen culture. Consistent with this idea, the growth rate of cells grown under low oxygen is substantially faster. These findings suggest that standard culture conditions alone can induce the AR, at least in fibroblasts, thus masking the effect of low doses of radiation. This observation has important implications that are both practical and theoretical.

Having now identified conditions in which AR protection can be reproducibly observed, we are engaged in a systematic investigation to gain mechanistic understanding of the AR. Protection against MN formation after a 2 Gy challenge dose by a prior 10 cGy exposure has been observed for primary human fibroblasts from two different individuals when grown in low oxygen, but not in an established HMEC line, MCF10A. These studies will be extended to compare primary mammary-derived fibroblasts and HMECs from the same individual, both grown under low oxygen. To investigate possible p53-dependence of the AR, we have compared normal fibroblasts to the same cells expressing a p53 Genetic Suppressor Element that prevents p53 activation by DNA damage. The preliminary results suggest that AR in human fibroblasts is entirely dependent on p53 function. These experiments are being extended to determine the effect of loss of other candidate AR proteins on the ability to mount the adaptive response for protection against MN formation after a challenge dose of radiation. In addition, the AR for MN formation is being compared to adaptive protection against cellular senescence as indicated by reduction in the radiation-induced Senescence-Associated Secretory Phenotype (SASP) as described by our collaborator J. Campisi. We are currently implementing a systems-biology approach in which gene expression profiles in control and low-dose, high-dose, or adaptive regimen-exposed cells will be correlated with functional outcomes including radiation-induced foci (RIF) formation, protein markers of the DDR, and MN formation. Our studies will determine (a) the extent to which different AR endpoints coincide with each other, with molecular mechanisms that are predicted from gene expression signatures, and with susceptibility to radiation-induced breast cancer and lymphoma, and (b) the extent to which the AR might alter breast cancer risk through mechanisms that act in cancer target cells vs their microenvironment.

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