A Critical Examination of the Adaptive Response for Cytogenetic Damage in Human Cells, and Insights into the Adaptive Response Mechanism

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Goal: Task 1 of the Berkeley Lab SFA is designed to identify adaptive response (AR) mechanisms that may affect risk of developing radiation-induced cancer and to assess the linearity with dose of processes that influence mammary gland carcinogenesis. We use both in vitro and in vivo experimental systems in a parallelogram strategy. Our human cell culture model will elucidate the molecular mechanisms of the AR and investigate the relationships in target *vs.* non-target cells between a range of cancer-relevant endpoints potentially affected by adaptation such as chromosomal damage, cellular senescence, genomic instability, and epigenetic change. It considers effects both on the epithelial cells that are at risk of developing malignant features (target cells) and on cells of the stroma that do not themselves become malignant (non-target cells) but can impact radiation-induced carcinogenesis through changes in the tissue microenvironment. In parallel, mouse models provide crucial *in vivo* information while the *in vitro* studies of human and mouse cells enable assessment of the extent to which human response biology is mirrored by that in the mouse. The *in vitro* and *in vivo* components are being integrated through comparative characterization of gene expression profile and metabolomic changes in response to low dose ionizing radiation and adaptive regimens.

Background and Significance: The AR for ionizing radiation is a well-documented but highly variable protective phenomenon in which cells or individuals exposed to low-dose or low-dose-rate ionizing radiation are transiently protected against a variety of effects from subsequent higher exposures. Its occurrence 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 the AR into estimation of risk from low dose exposures.

Approach: Adaptation and low dose responses are being investigated in both human fibroblasts (major contributors to the mammary gland microenvironment; non-target cells for breast cancer) and human mammary epithelial cells (HMECs; breast cancer target cells) from individual women, with comparative studies in primary skin or lung fibroblasts and in an established HMEC line MCF10A. Our approach includes both testing of specific hypotheses concerning adaptive mechanisms and employing systems-based approaches to discover novel regulatory and mechanistic elements of the AR. We have used micronuclei formation as a cancer-relevant, highly quantifiable measure of cytogenetic damage to define conditions that reproducibly allow adaptation and to identify reproducibly adapting cells. These conditions are then applied to characterize possible dependence of the AR on cell-type and/or culture conditions, to compare AR outcomes for different cellular endpoints, and to investigate common underlying AR mechanisms. For systems-based studies, transcription profiling has been carried out in cultured human fibroblasts under high-dose challenge vs adaptive conditions as a function of time after the challenge dose. These results will be compared to ongoing complementary studies in mammary gland from cancer-sensitive and resistant strains of mice (Snijders et al). This comparison is an integral part of the “mouse-human parallelogram” strategy for assessing risk from low dose exposures to human beings. Key changes seen in the array analysis will be validated by qRT-PCR and then compared for non-adaptive conditions, as well as to transcriptional changes after low dose exposure only. Positive results will be fed back into mechanistic hypothesis-testing experiments. In parallel as a second systems-based approach, changes in response to low-dose exposure in the metabolomic profile of cultured

human fibroblasts known to exhibit the AR are being investigated for comparison to similar studies in genetically variable mice.

Results: Our human cell studies show that the AR appears to be dependent both on culture conditions and cell type. Analysis of >20 different cell strains (human and mouse fibroblasts and HMECs) to determine if a prior (4-hr) low-dose exposure (usually 10 cGy) provided adaptive protection from a subsequent challenge dose of 2 Gy using micronuclei (MN) formation as an assay yielded variable but predominantly negative results. We therefore hypothesized that culture or exposure conditions themselves may cause endogenous cellular stress, masking effects from the low priming doses. Oxidative stress was one obvious candidate, since cells in tissues *in vivo* are in a low-oxygen environment (~1-7%) compared to the 20% atmospheric oxygen concentration used for standard cell culture. Accordingly, we tested the effect of culturing cells in low-oxygen (3%) incubators and using sealed chambers during irradiation or sham exposures to minimize changes in oxygen concentration. A series of experiments with primary WI38 human fibroblasts convincingly established that significant AR protection against MN formation is achieved in cells grown under low oxygen, but NOT in standard oxygen conditions. Cell growth rate is increased in low oxygen vs atmospheric oxygen, and growth in atmospheric oxygen produces a DNA damage response (DDR) in unirradiated cells, as judged by western analysis for DDR-responsive proteins. Importantly, cells grown in 3% oxygen resolve the DDR induced by high dose X-ray (2 Gy) much more rapidly than those grown in 20% oxygen, as measured by western analysis. These results establish that standard culture conditions can induce the AR and mask low dose effects. The particular serum used in the cell culture medium is also critical for the AR, thus implicating microenvironmental effects.

Adaptive protection against chromosomal damage could, in principle, be due a prolonged G1/S checkpoint delay, thus allowing more time to repair lesions before they are encountered by break-inducing replication complexes, or to an increase in repair efficacy per se. Our studies of isogenic cells revealed that TP53, which can impact both checkpoint responses and repair, is essential for the adaptive response in primary fibroblasts, providing initial mechanistic insights.

The AR may also be cell-type specific. In contrast to our results with primary fibroblasts, we consistently failed to observe the AR for MN formation in the immortalized HMEC cell line MCF10A, whether grown in 20% or 3% oxygen. Our early results with paired primary HMECS and mammary-derived fibroblasts from the same individual under standard cell culture conditions also suggest the possibility that primary stromal fibroblasts exhibit the AR for cytogenetic damage but HMECs (target cells) do not. This result requires further testing, in particular to determine the effect of growth in 2D- vs tissue-mimetic 3D-conditions.

Time-dependent transcriptional profiling has been performed for human primary fibroblasts grown under adaptive conditions (3% oxygen). The results reveal distinct sets of genes whose expression is altered very early after a challenge dose in comparison to the adaptive regimen (low, then high dose), and different, also distinct, sets of genes with altered expression profiles at later times (8 hrs). These results will be compared to transcription profiles in prior studies of low dose and AR profiling in human blood-derived cells and in ongoing studies of mammary gland tissues from irradiated mice.

Conclusions / Future Directions: Our findings have both theoretical and practical implications. Conceptually, they suggest similarity between cellular responses to low doses of IR and to endogenous oxidative damage. In practical terms, now that we have identified conditions that reproducibly confer AR protection, we are well positioned to gain mechanistic information on the adaptive response in more complex 3D tissue models, the conditions under which it occurs, its genetic and microenvironmental determinants, and the cancer-relevant endpoints affected by it.

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