Low dose radiation combines with the Src oncoprotein to transform pre-malignant human breast cells

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Goal: Determine whether low dose radiation exerts persistent epigenetic effects that promote malignancy.

Background and Significance: Some persistent carcinogenic effects of radiation may not be traceable to specific DNA sequence alterations and may not be linearly related to dose. Through the biochemical initiation of positive feedback loops, ionization-induced events may have heritable non-linear effects on cellular behavior. Inflammatory responses involving the transcription factor NFκB may be subject to such effects. Increased NFκB activity has been strongly linked to carcinogenesis in a number of published *in vitro* and *in vivo* studies (reviewed in [1]). Since radiation has been shown to affect NFκB activity [2], perturbation of NFκB activity by low dose radiation may affect the threshold, and therefore the frequency, of oncogenic transformation. Such nonlinear threshold-dependent epigenetic changes involved in malignant transformation may affect radiation risk assessment.

Approach and Results: To examine persistent carcinogenic effects of low dose radiation, we have employed a pre-malignant human mammary epithelial cell line, MCF10A, which is susceptible to oncogenic transformation by individual events. In initial experiments, MCF10A cells grown under adherent conditions were irradiated with 0, 10 cGy (low dose), or 200 cGy (high dose) of X-rays, then incubated for 7 days prior to harvest for transcriptional profiling. Unsupervised clustering of gene expression profiles of triplicate samples revealed subsets of gene transcripts exhibiting consistent changes in abundance 7 days after radiation exposure. We analyzed low dose transcriptome data using classical differential gene expression and sparse association methods. Very little overlap was noted between gene expression changes after low or high dose exposures. For example, gene expression changes obviously related to changes in growth (e.g., c-myc, EGR1, c-fos, p21) were observed after high, but not low dose radiation. Bioinformatics analyses of low dose expression profiles indicated: (i) subnetwork enrichment of NFκB and IL6 as common regulators, and (ii) pathway enrichment of Stat1 and Stat3 transcription factors.

Notably, MCF10A cells transiently exposed to sufficiently high levels of an inducible Src oncoprotein exhibit stable epigenetic transformation mediated by a positive feedback loop involving NFκB and IL6 [3]. Once activated, the transformed state is self-sustaining in the absence of Src. Additional evidence indicates that the NFκB/IL6 feedback loop is likely to play a role in maintaining the malignant growth of a variety of different human cancers. Using MCF10A cells expressing sub-optimal levels of Src, we are testing the hypothesis that low dose radiation-induced increases in NFκB activity affect the threshold of Src-induced transformation. Our preliminary results confirm that 10 cGy of X-rays are sufficient to increase the frequency of Src-induced transformation, as measured by anchorage-independent growth in soft agar.

Conclusion: Low dose radiation exerts an epigenetic effect that promotes oncogenic transformation of susceptible cells.

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