Non-targeted effects of low dose ionizing radiation act via TGFβ to promote mammary carcinogenesis. M.H. Barcellos-Hoff, New York University School of Medicine, NY, NY

The carcinogenic action of ionizing radiation is usually ascribed to targeted DNA damage and resulting mutations, but non-targeted radiation effects can alter phenotype and microenvironment. Recent studies show that signaling and cellular crosstalk lie at the heart of carcinogenesis. We initiated experiments to identify whether low dose radiation affects stromal-epithelial interactions that contribute to carcinogenesis.

Transforming growth factor β1 (TGFβ) activation is a non-targeted radiation effect that mediates microenvironment composition. TGFβ is activated in mouse mammary gland following whole body exposure to doses of as low as 0.1 Gy and persists in the stroma for at least a week where it mediates stromal extracellular matrix remodeling and cell fate decisions following DNA damage (1). To comprehensively examine the TGFβ dependence of low dose radiation effects, the mammary glands of *Tgfb1* wildtype and heterozygote littermates irradiated with 0.1 Gy were analyzed at 1 week and 1 month. Gene expression profiling using Affymetrix of total tissue mRNA extracts showed that 234 genes were similarly regulated in both irradiated wildtype and *Tgfb1* heterozygote mice. Notably, 736 genes regulated by radiation in the wildtype mice were not affected by radiation in *Tgfb1* heterozygote littermates. The top ranked KEGG pathways significantly regulated by radiation in a TGFβ dependent manner were ErbB signaling, insulin signaling, circadian rhythm, glycolysis/gluconeogenesis, and VEGF signaling.

To determine whether persistent changes evident by expression profiles contribute to radiation carcinogenesis, we used a radiation chimera model. Pubertal mammary glands of BALB/c mice were cleared of endogenous epithelium at 3 weeks. These mice were irradiated at 10-12 weeks with total body exposure to 0.1 or 1 Gy and transplanted three days later with syngeneic *Trp53* null mammary fragments. Tumor development was monitored by palpation for 12 months. Palpable tumors were evident beginning at 6 months after transplantation in both sham and irradiated hosts. By 300 days, all of the transplants to hosts that had been irradiated with either 0.1 or 1 Gy had developed tumors, compared to 54% of transplants in unirradiated hosts. At the termination of the experiment 1 year after transplantation, 73% of the transplants had developed tumors in sham-irradiated mice compared to 100% of irradiated hosts ($p=0.05$). Tumors also grew faster in hosts irradiated with 1 Gy.

The specific epithelial actions of TGFβ suggest that it functions as a tumor suppressor in early cancer (3), yet its effects on carcinogenesis in the context of irradiated tissue are unknown. To investigate whether host TGFβ status influences to tumor promotion via host irradiation, the above described protocol was repeated by transplanting *Trp53* null epithelium to syngeneic *Tgfβ1* heterozygote mice. Neither frequency, latency nor growth rate of tumors was affected by *Tgfβ1* host irradiation. These data indicate that TGFβ mediates tumor promotion of *Trp53* null in irradiated hosts.

Moreover, gene expression profiling of tumors generated in irradiated versus non-irradiated mice showed that a core gene set among tumors discriminated host status, even though host irradiation had occurred months before. Notably some of these genes were also regulated at 1 month post irradiation. Thus non-targeted radiation effects influence the timing, biology and type of carcinomas that develop from unirradiated Trp53 null mammary epithelium.

References

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