

Persistent Phenotypic Responses of Human Mammary Epithelial Cells Induced by Ionizing Radiation

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Ionizing radiation (IR) is a known human breast carcinogen. Although the mutagenic capacity of IR is widely acknowledged as the basis for its action as a carcinogen, we and others have shown that IR can also induce growth factors and extracellular matrix remodeling. We have shown that irradiating human mammary epithelial cells (HMEC) cultured with transforming growth factor β 1 (TGF β) can generate a persistent phenotype in daughter cells characterized by spindle cell morphology, increased mesenchymal markers, decreased epithelial markers and increased cellular motility and invasion, which are hallmarks of epithelial to mesenchymal transition (EMT). Neither radiation nor TGF β alone elicited EMT, although IR increased chronic TGF β signaling and activity. Gene expression profiling revealed that double-treated cells exhibit a specific 10-gene signature associated with Erk/MAPK signaling. We hypothesized that IR-induced MAPK activation primes nonmalignant HMEC to undergo TGF β -mediated EMT. Consistent with this, Erk phosphorylation was transiently induced by irradiation and persisted in irradiated cells treated with TGF β , and inhibition of Erk activation, blocked the EMT phenotype. Preliminary studies suggest that eqi-toxic doses of sparsely and densely ionizing radiation resulted in comparable EMT when cells were cultivated in the presence of TGF β . Furthermore radiation dose response studies show that this effect has a very low threshold in that a single exposure of 3-200 cGy radiation elicits the 'same' phenotypic switch, which is consistent with non-targeted effects. Together, these data show that the interactions between radiation-induced signaling pathways elicit heritable phenotypes that could contribute to radiation carcinogenesis.

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Introduction

An emerging concept in cancer biology is that carcinogens can compromise tissue integrity by eliciting altered phenotypes and tissue composition (reviewed in ref. 1). Intercellular and extracellular signals are critical to the suppression of neoplastic growth, whereas disruption of cell-cell and cell-matrix interactions is implicated, if not required, for neoplastic progression. Indeed, reversion of the malignant phenotype by modulating extracellular signals suggests that cancer cells are susceptible to signals from the microenvironment.²

It has been shown that ionizing radiation (IR) activates multiple signaling pathways depending on the cell type, radiation dose, and cell status (reviewed in ref. 3). We have previously shown that transforming growth factor β 1 (TGF β) is activated following IR, and that it, in turn, mediates cellular and tissue radiation responses.^{4,5} Although TGF β is considered to be a potent tumor suppressor during the ini-

tial stage of tumorigenesis, numerous reports show that TGF β can switch to tumor promoter during neoplastic progression (reviewed in ref. 6). Some epithelial tumors, particularly those that overexpress TGF β , exhibit mesenchymal characteristics and are more aggressive.⁷ Several lines of evidence have led researchers to link this morphologic shift during carcinogenesis to the physiologic process of epithelial to mesenchymal transition (EMT). We have postulated that IR alters cell phenotypes, which in turn contribute, directly or indirectly, to carcinogenesis.⁸ Thus we examined the persistent radiation-induced phenotypes in non-malignant HMEC that affect pathways that precede neoplasia.

Materials and Methods

Human mammary epithelial cells (HMEC) were cultured in se-

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rum-free medium as previously described for HMT-3522 S1 (S1; passages 55–60), MCF10A (ATCC, Manassas, VA), 184 HMEC (184v; passage 7–10) and HMT-3522-S2 (S2).⁹ Recombinant human TGF β (400 pg/ml) was added at the time of plating or of irradiation. HMEC were irradiated 4–5h (S1, MCF10A and S2) or 10–14h (184v HMEC) post plating. Three dimensional (3D) cultures were done as previously described.¹⁰ Radiation treatments were performed with either 160 kV X-ray or ⁶⁰Co γ -radiation with a total dose of 2 Gy. Some cells were irradiated with a 5600 curie source of ¹³⁷Cs for γ -radiation over a dose range of 0–2Gy or with 1 GeV/amu ⁵⁶Fe ions from the NASA Space Radiation Laboratory of the Brookhaven National Laboratory. Microarray analysis, migration and invasion assays were done as described previously.⁹

Results

The progeny of irradiated nonmalignant HMEC cultured with TGF β (double-treated: IR + TGF β) exhibit compromised morphogenesis, polarity, and growth control when cultured in Matrigel.¹⁰ To examine the possibility that EMT underlies the response to radiation and TGF β , we compared the epithelial characteristics of HMEC in traditional monolayer culture. Epithelial markers, E-cadherin, β -catenin, and ZO-1 were reduced in double-treated cells, while mesenchymal markers, N-cadherin, fibronectin, and vimentin were dramatically increased. Double-treated cells also underwent disrupted morphogenesis when propagated in Matrigel in the absence of additional TGF β . The functional consequence of the response of irradiated HMEC to TGF β was analyzed by the migratory and invasive properties of double-treated HMEC. TGF β induced an increase in motility and invasiveness in irradiated HMEC. Although IR induced TGF β activity in cultured HMEC as it did in the mouse mammary gland, it was insufficient to disrupt HMEC acinar morphogenesis as shown in our previous study.^{4,10} Thus, chronic TGF β activation by irradiated HMEC is insufficient to drive the EMT phenotype. These data indicate that irradiated cells undergo TGF β mediated EMT.⁹

To identify the genetic programs underlying EMT, we compared transcript expression levels in sham, IR-, TGF β -, and double-treated HMEC. We identified 10 genes that constituted the double treatment signature. An extensive bibliography search showed that 5 of the 10 EMT signature genes are directly or indirectly associated with the Erk/MAPK pathway, suggesting a specific functional role of Erk/MAPK activation in inducing EMT upon double treatment. Consequently, we examined the possible involvement of the Erk/MAPK signaling cascade in the mediation of TGF β -induced EMT in irradiated HMEC and found persistent activation of Erk/MAPK in double-treated HMEC. Consistent with this, Erk phosphorylation was transiently induced by irradiation and persisted in irradiated cells treated with TGF β , and inhibition of Erk activation, blocked the EMT phenotype. These findings identify a previously un-described pathway in which interactions between radiation-induced signaling pathways elicit heritable phenotypes that could contribute to neoplastic progression.

Preliminary data comparing eqi-toxic doses of 1Gy of 1 GeV/amu ⁵⁶Fe vs 2Gy of ¹³⁷Cs γ -radiation showed comparable EMT in monolayer and disrupted 3D morphogenesis when cells were cultivated in the presence of TGF β . However, radiation dose response studies show that this effect has a very low threshold in that a single exposure of 3–200 cGy γ -radiation elicits the 'same' phenotypic switch.

Discussion/Conclusions

We have shown that the progeny of irradiated HMEC are dramatically sensitized to undergo TGF β -induced EMT. IR and TGF β cooperated to induce a phenotypic transition that occurred in the progeny of cells irradiated once and persisted even in the absence of TGF β . This resulted in increased motility, enhanced invasion, and disrupted epithelial morphogenesis and was accompanied by a distinct pattern of gene expression. Neither chronic TGF β signaling induced by irradiation nor supplementation with low TGF β concentrations was sufficient to induce the EMT phenotype in any of the three HMEC that were examined in our studies. EMT occurred only in irradiated HMEC in the presence of TGF β . Expression profiling distinguished between genes regulated by TGF β without concomitant EMT in non-malignant HMEC and genes that are differentially expressed under conditions resulting in EMT. These differentially expressed genes suggested the involvement of Erk/MAPK signaling, which was further supported by experimental studies showing that this is required for establishing and maintaining EMT and is essential for the functional response, i.e., enhanced migration. We propose a model in which IR-induced Erk/MAPK signaling is sustained by TGF β is necessary for EMT. Notably, EMT occurred in cells exposed to sparsely or densely ionizing radiation at very low doses, as does Erk/MAPK signaling.¹¹ These data are consistent with a non-targeted radiation effect.

Our current and earlier studies, have shown that irradiated nonmalignant HMEC undergo EMT only if they are exposed to additional TGF β , as might be derived from the stroma in intact tissues.¹⁰ If moderate radiation doses can prime pre-neoplastic cells to undergo EMT in vivo, it could accelerate cancer progression. An interesting implication from our study is that normal epithelia may undergo EMT in response to irradiation and TGF β , which could contribute to fibrosis following radiotherapy. If so, this would lend further credence to the potential application of TGF β inhibitors in radiotherapy.^{12,13} However, additional studies from our laboratory indicate that TGF β is instrumental in mounting a DNA damage response and in inducing apoptosis of genomically unstable cells.^{5,14,15} The complexity of radiation effects mediated by TGF β will require further study to determine whether it plays a proximal role in suppressing or promoting radiogenic carcinogenesis.

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