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BIOLOGY CONTRIBUTION

LACK OF RADIATION DOSE OR QUALITY DEPENDENCE OF EPITHELIAL-TO-MESENCHYMAL TRANSITION (EMT) MEDIATED BY TRANSFORMING GROWTH FACTOR β

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Purpose: Epithelial-to-mesenchymal transition (EMT) is a phenotype that alters cell morphology, disrupts morphogenesis, and increases motility. Our prior studies have shown that the progeny of human mammary epithelial cells (HMECs) irradiated with 2 Gy undergoes transforming growth factor β (TGF-β)–mediated EMT. In this study we determined whether radiation dose or quality affected TGF-β–mediated EMT.

Methods and Materials: HMECs were cultured on tissue culture plastic or in Matrigel (BD Biosciences, San Jose, CA) and exposed to low or high linear energy transfer (LET) and TGF-β (400 pg/mL). Image analysis was used to measure membrane-associated E-cadherin, a marker of functional epithelia, or fibronectin, a product of mesenchymal cells, as a function of radiation dose and quality.

Results: E-cadherin was reduced in TGF-β–treated cells irradiated with low-LET radiation doses between 0.03 and 2 Gy compared with untreated, unirradiated cells or TGF-β treatment alone. The radiation quality dependence of TGF-β–mediated EMT was determined by use of 1 GeV/amu (gigaelectron volt / atomic mass unit) ^{56}Fe ion particles at the National Aeronautics and Space Administration's Space Radiation Laboratory. On the basis of the relative biological effectiveness of 2 for ^{56}Fe ion particles' clonogenic survival, TGF-β–treated HMECs were irradiated with equitoxic 1-Gy ^{56}Fe ion or 2-Gy ^{137}Cs radiation in monolayer. Furthermore, TGF-β–treated HMECs irradiated with either high- or low-LET radiation exhibited similar loss of E-cadherin and gain of fibronectin and resulted in similar large, poorly organized colonies when embedded in Matrigel. Moreover, the progeny of HMECs exposed to different fluences of ^{56}Fe ion underwent TGF-β–mediated EMT even when only one-third of the cells were directly traversed by the particle.

Conclusions: Thus TGF-β–mediated EMT, like other non-targeted radiation effects, is neither radiation dose nor quality dependent at the doses examined. © 2011 Elsevier Inc.

TGF-β, Ionizing radiation, Mammary epithelial cell, EMT.

INTRODUCTION

The assembly of cells into sheets is a fundamental property of epithelia. The further assembly into tissue-specific structures requires E-cadherin that links cells via a homophilic extracellular domain and is anchored intracellularly to the cytoskeleton via dynamic interactions with the catenins (1). Notably, E-cadherin acts as a central modulator by controlling the molecular factors that govern the stability of cell phenotype; its loss during malignant progression is frequently attributed to a process called epithelial-to-mesenchymal transition (EMT) (2). EMT is characterized by a shift from epithelial to bipolar cell morphology, increased expression of mesenchymal markers, decreased expression of epithelial markers, and increased cell motility. The normal EMT program is used in organ formation during development, in wound healing responses to tissue injury, and subversively by cancer cells to support motility and invasion (3).

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Conflict of interest: none.

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Transforming growth factor β (TGF-β) is frequently involved in EMT in malignant cells (2) and induces a heritable phenotypic transition in irradiated nonmalignant human mammary epithelial cells (HMECs) that fulfills the criteria of EMT (4, 5). TGF-β is ubiquitous as an extracellular latent complex in tissues and is abundantly produced in tumors. Radiation induces TGF-β activation (6, 7). TGF-β mediates epithelial fate decisions by regulating proliferation and apoptosis [reviewed previously (8)] and has been widely implicated in radiation responses. Terzaghi-Howe (9) showed that TGF-β produced by normal epithelial cells inhibited the growth and phenotype of radiation-transformed cells. Bauer and colleagues (10) described three distinct roles for TGF-β during neoplastic transformation [reviewed previously (11)]: helping to maintain the transformed state of mesenchymal cells, enabling non-transformed neighbors to recognize transformed cells, and triggering an apoptosis-inducing signal. The latter two processes are enhanced after very low radiation doses.

Radiation elicits cell behaviors that are strongly associated with epithelial cancer, including deregulation of differentiation, genomic instability, phenotypic transitions like EMT, and aberrant multicellular organization (4, 5, 12). Many of these radiation effects are classified as a nontargeted effect (NTE) because the response is delayed or is exhibited by the progeny of irradiated cells. With the exception of genomic instability, there are few studies that compare NTE as a function of radiation quality. Unlike the classic biophysical dose response relationship for mutation and DNA damage, which are both proportional to dose without a threshold, the dose responses of NTE frequently exhibit a relatively abrupt transition or threshold followed by saturation or a plateau, that is, radiation dependence suggests that the response is more like a switch rather than a dial. In this study we asked whether the ability of irradiated HMECs to maintain their epithelial phenotype when cultured with TGF-β was radiation dose or quality dependent. Our studies show that both high–linear energy transfer (LET) radiation and low-LET radiation prime HMECs to undergo TGF-β–mediated EMT. Consistent with other NTEs, TGF-β–mediated EMT is neither radiation dose nor quality dependent for the dose/fluence range examined herein.

METHODS AND MATERIALS

Cell culture

The MCF10A and 184v finite-lifespan HMECs (13) were previously shown to behave similarly in response to radiation and TGF-β (5). The 184v HMECs are finite-lifespan post-selected HMECs from 1 individual that retain most normal HMEC biology but have overcome a stress-associated senescence barrier (stasis) via silencing of the cyclin-dependent kinase inhibitor $p16^{INK4a}$. HMECs were cultured in the presence or absence of recombinant human TGF-β (400 pg/mL; R&D Systems, Minneapolis, MN) in serum-free medium added at the time of plating and added every other day thereafter, with medium change as previously described (5). Because radiation kills cells and TGF-β impedes HMEC proliferation, in some experiments the number of cells plated under each condition were increased 20% so that all cultures reached similar degrees of confluence at experiment termination. HMECs were irradiated and cultured as monolayer or in reconstituted basement membrane (Matrigel; BD Biosciences, San Jose, CA) as previously described (14).

Radiation exposure

Dose response was assessed with a Varian 2300 linear accelerator (Varian Medical Systems, Palo Alto, CA) with 6-MV x-rays at a rate of 4 Gy/min for doses from 0.4 to 2 Gy and 0.03 Gy/min for lower doses. X-radiation exposures for survival assays used a 160-kV X-ray source at a dose rate of 1.4 Gy/min. γ-Radiation exposures were performed with a 5,600-Ci ^{137}Cs source at a dose rate of 0.03 to 2 Gy/min. High-LET 1-GeV/amu (gigaelectron volt / atomic mass unit) ^{56}Fe ions were delivered at a dose rate of 0.2 to 1 Gy/min at the National Aeronautics and Space Administration's Space Radiation Laboratory of Brookhaven National Laboratory (BNL). The 1-GeV/amu iron (Fe) particles have an LET of about 148 keV/μm, which leads to a mean dose of 0.24 Gy per one particle traversal of a cell measuring 100 μm^2 in cross section. A fluence of 4.22 particles per 100–μm^2 cellular cross section leads to a mean dose of 1 Gy in which only 1.5% of cells will "miss" a particle traversal assuming a Poisson process. At a mean dose of 0.1 Gy, the mean number of hits per target area is 0.42; thus 65% of cells will be missed.

Clonogenic survival assay

We irradiated 184v HMECs as subconfluent monolayers. Two hours after irradiation, the cells were trypsinized and seeded on 100-mm plates at three different densities in triplicate for the dose response, which was repeated 2 to 3 times. Colonies containing more than 50 cells were counted at 7 to 8 days. Colony-forming efficiency was determined and normalized to that of the nonirradiated control. The mean plating efficiency of controls was 14% $\pm$ 2% (SE) (n = 6).

Immunofluorescence and image acquisition

Cells grown on LabTek 8-well chamber slides (Nalge Nunc International, Rochester, NY) or cryosections of colonies embedded in Matrigel (20 μm) were fixed and stained by use of antibodies against E-cadherin and β-catenin (BD Biosciences), β-actin, and fibronectin (Sigma, St Louis, MO) as previously described (5, 15). Cells were imaged by use of a 40$\times$ objective with a 0.95–numerical aperture Zeiss Plan-Apochromat objective on a Zeiss Axiovert (Zeiss, Wetzlar, Germany) equipped with epifluorescence. Total E-cadherin intensity for a field or acini perimeter was quantified by use of in-house scripts under the Metamorph imaging platform (Molecular Devices, Sunnyvale, CA) in which a background correction was applied and the mean intensity of the signal above this threshold was computed. The acinar perimeter was manually thresholded using DAPI signal. The significance of differences between double-treated groups compared with TGF-β alone was calculated by use of the KS test.

Statistical analysis

Survival curves were fitted with the least square fit tool of MATLAB (The MathWorks, Natick, MA) by use of the following linear–quadratic model: $S(D) = \exp^{-(\alpha D+\beta D^2)}$, where α and β are the linear and quadratic terms, respectively, of the percent survival S at a dose D. Relative biological effectiveness (RBE) was computed as the ratio between X-ray and Fe survival at 10% survival, based on