Disrupted epithelial morphogenesis and aberrant lineage commitment following radiation and TGFβ

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In the present study, we evaluated cell lineage plasticity and its phenotypic effects in the non-malignant human mammary epithelial cell line MCF10A in response to ionizing radiation and TGFβ. We have recently shown that the molecular profiles of mammary tumors and normal mammary gland from irradiated mice are distinct from those arising in unirradiated mice by virtue of enriched stem cell and progenitor markers. Consistent with this, we found that radiation (10-100 cGy) increases the mammary repopulation capacity in a functional and marker assays (Nguyen et al. Cancer Cell 2011). Some studies have shown that epithelial-mesenchymal transition (EMT) is associated with greater mammary epithelial cell stem cell renewal (Mani et al, 2009). Our previous studies have shown that the progeny of irradiated mammary gland epithelial cells undergo a phenotypic change that strongly resembles EMT when exposed to transforming growth factor β1 (TGFβ) (Andarawewa et al. Cancer Res 2007).

EMT is characterized by loss of epithelial cell polarity, loss of cell-cell contacts, and acquisition of mesenchymal markers and phenotypic traits that include increased cell motility. We observed that IR+TGFβ exposure in the MCF10A cells acted as a sensitive switch (even at doses below 10cGy) to induce persistent heritable phenotype characterized by the lost of epithelial junction markers, gain of mesenchymal markers, and dysplastic acinar morphogenesis in Matrigel, but still retains expression of tissue-specific cytokeratins. MCF10A contain three types of mammary epithelial subpopulations (luminal, marked by cyotkeratin 18 (CK18), basal, marked by cytokeratin 14 (CK14) and bipotent, marked with both) and possess the capacity to recapitulate ductal morphogenesis composed of a CK18/CK14 bi-layer when co-cultured with human fibroblasts in a collagen:Matrigel matrix. If established co-cultures are treated with IR+TGFβ, ductal morphogenesis is not affected compared to controls In contrast, the progeny of IR+TGFβ treated MCF10A cells exhibit aberrant ductal morphogenesis lacking epithelial bi-layer organization when re-cultured in this model.

Aberrant morphogenesis has been linked to disruption of lineage commitment in BRCA1 mutations (Lim et al. Nat Med. 2009). We observed that progeny that express both markers (i.e. bipotent) are significantly increased following IR+TGFβ when compared with single treatments or control cultures. The dose response for this mirrored that of EMT, i.e. switch-like. Bipotent cells are thought to be progenitors, suggesting that lineage commitment is perturbed by either radiation, TGFβ or the execution of the EMT program. Similar to our studies in irradiated mouse mammary gland, we found significantly elevated active β-catenin and Notch1, which are critical signals in stem cell regulations. Taken together, these data suggest that the acquisition of pluripotentiality collaborates with EMT to disrupt mammary gland epithelial morphogenesis.

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