A link between stem cells and the EMT phenotype induced by IR and TGFβ

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Epithelial-Mesenchymal Transition (EMT) occurs as key developmental program but is also often activated during cancer progression. 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[1]. Approximately 18% of breast cancers exhibit evidence of EMT. We have shown that the progeny of irradiated human mammary epithelial cells (HMEC) undergo EMT when exposed to transforming growth factor β1 (TGFβ). Additionally, recent publication from Weinberg and colleagues showed that induction of EMT through engineered expression of Snail or Twist or exposure to TGFβ in immortalized human mammary epithelial cells results in the acquisition of stem-cell markers[2].

In the present study, we evaluated the dose dependence of IR-induced EMT in non-malignant MCF10A mammary epithelial cells and measured changes in the expression of pluripotentiality biomarkers during the transition process. We observed that the IR+TGFβ-induction of EMT in the MCF10A cells cultured in monolayer, measured as the reduction of E-cadherin expression, were acutely sensitive to low doses (<10 cGy) but showed no greater response with increasing dose; essentially radiation exposure acted as a switch that primed MCF10A cells to undergo TGFβ mediated EMT. Then, we performed the experiments in the cells cultured in the presence of low concentrations of exogenous Extra Cellular Matrix (ECM), a situation in which those cells still grow in monolayer without generating 3D structures. Surprisingly, under those conditions, we found no reduction of E-cadherin expression in the samples treated with TGFβ and different doses of IR. There are two types of mammary epithelial cells: luminal and basal, marked by cyotkeratin 18 (CK18) and cytokeratin 14 (CK14) respectively, which are expressed in distinct subpopulations of MCF10A. We analyzed the expression of both markers in the cells cultured with low concentrations of ECM. First we observed that all those cells were positive for CK14 and negative for CK18. Then we evaluated the expression of the basal marker p63 in the same cells. Again, we found that all of the cells cultured with low concentrations of ECM were positive for p63. Then we analyzed the expression of both markers in cells cultured in monolayer without ECM. We observed an increase in the number of IR+TGFβ treated cells that express both markers when compared with the control culture. Interestingly, when we measure the radiation dose-response in the co-expression of CK14 and CK18, we found that the lowest dose was sufficient, similar to that found for E-cadherin. Taken together, these data suggest an important role of the acquisition of pluripotentiality during IR+TGFβ induced EMT and support the idea that phenotypic responses to radiation can contribute to neoplastic progression.

1.- Andarawewa, K., *et al.* Cancer Res 2007; 67: 8662-8670
2.- Mani, S. A., *et al.* Cell 2008; 133 (4): 704-715