Optimizing dual detection of epithelial cell markers and EGFP for use in bystander studies

S. Gauny, S. Ravani, M.H. Barcellos-Hoff, M. Jasin[2] and A. Kronenberg
Lawrence Berkeley National Laboratory, Berkeley, CA 94720 and Memorial Sloan Kettering Cancer Center, New York, NY

The goal of this project is the assessment of bystander signaling in mouse mammary tissue. The project depends on the quantitative detection of un-irradiated bystander cells that undergo a homologous recombinational repair event leading to the expression of enhanced green fluorescent protein (EGFP) following their transplantation into either an un-irradiated cleared mammary fat pad or into a previously irradiated mammary fat pad. Mammary explants from transgenic mice that contain an expression cassette with two disabled EGFP genes will be transplanted in this protocol, and these tissue fragments include epithelial cells, stromal cells, and other cell types. Thus, the study requires quantitative detection of EGFP-positive cells in cells that are clearly identified as being epithelial in origin to monitor the fraction of transplanted epithelial cells that may undergo homologous recombinational repair.

Two approaches have been proposed to provide quantitative data - one in which flow cytometry will be done on fresh mammary epithelial cell preps derived from the transplanted tissues, and the second which involves *in situ* analysis using fluorescence microscopy. A major challenge to the work is the need to preserve the signal from EGFP, which fluoresceses "green" and to simulataneously and reproducibly detect mouse epithelial cells using an antibody-based approach that involves a primary antibody that detects validated epithelial cell markers along with a secondary antibody that will fluoresce in the "red" channel for flow cytometry. Optimization has focused on preserving EGFP flourescence using different fixatives, starting with cell lines that uniformly express EGFP, and then confirming the detection (and quantification) in mouse mammary epithelial cell preps from Tg(GFPU)5Nagy/J mice that carry an integrated, complete EGFP gene in the germline. Optimization has also been required to select an antibody that uniquely identifies mouse mammary epithelial cells that is compatible with the EGFP detection protocol and suitable for flow cytometry. Here we have worked with antibodies against cytokeratins as well as antibodies against mouse E-cadherin. A series of experiments indicates that coincident detection of E-cadherin and EGFP can be achieved by flow cytometry using formaldehyde fixation and an antibody against E-cadherin previously validated for use in histological applications in mouse mammary epithelial cells (Lochter, et al. *J. Cell Biol. 139*:1861-1872, 1997; Lochter et al. *J. Biol. Chem. 272*:5007-5015, 1997).