Abstract #4315

Column-free FACS-compatible Immunomagnetic Enrichment of EpCAM Positive and CD49f Positive Human Mammary Epithelial Progenitor Cells

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Abstract

The process by which mammary epithelial cells differentiate from a putative stem cell compartment within normal human mammary tissue is not well understood. We have previously shown that three types of human mammary epithelial progenitor cells can be isolated from normal human mammary tissue. These are luminal-restricted, myoepithelial-restricted and bipotent progenitors, with the latter being able to generate colonies of demonstrable single cell origin *in vitro* that contain both luminal and myoepithelial cells. Using FACS technology, we have shown that the luminal-restricted and bipotent progenitors are characterized by co-expression of epithelial cell adhesion molecule (EpCAM) and CD49f and loss of expression of either of these two markers is correlated with decreased clonogenic potential. We now describe a simple and rapid method for obtaining enriched populations of the luminal-restricted and bipotent progenitor populations using an immunomagnetic cell separation procedure, EasySep™. This procedure utilizes a unique tetrameric antibody complex (TAC) to cross-link targeted cells to magnetic nanoparticles for column-free magnetic selection. Immunomagnetic separation of EpCAM$^+$ cultured human mammary epithelial cells using a TAC incorporating the anti-EpCAM antibody clone VU-1D9 resulted in purities of 93±1% and recoveries of 47±5% (mean ± SEM, n=7). Immunomagnetic separation of CD49f-expressing human mammary cells was performed by pre-labelling the cultured cells with R-phycoerythrin (PE)-conjugated anti-CD49f (clone GoH2), followed by incubation with a TAC cross-linking PE to the magnetic nanoparticles, and subsequent magnetic separation. Purities of 97±2% and recoveries of 32±4% (n=5) of CD49f-expressing cells were obtained. The magnetic nanoparticles are so small that they do not impede subsequent FACS analysis and further sorting of enriched subpopulations. The EasySep™ strategy is flexible enough to isolate cells on the basis of their expression (or not) of a variety of cell surface markers, and will likely be a useful tool in the future isolation of phenotypically distinct, rare subpopulations of progenitors.

Materials and Methods

Preparation of Cell Suspension. Normal human mammary tissue obtained from reduction mammoplasty surgeries was minced and enzymatically digested overnight in collagenase and hyaluronidase to yield a suspension of epithelial organoids. These organoids were collected and further digested with trypsin, dispase and deoxyribonuclease 1 (DNAse), filtered to generate a single cell suspension, resuspended in Hank's + 2% fetal bovine serum (FBS) and 0.1 mg/mL DNAse, and incubated with a blocking antibody for 15 minutes on ice.

EasySep™ Cell Labeling - EpCAM$^+$ or MUC1$^+$ Cells. Cells were incubated for 30 minutes on ice with a TAC (Tetrameric Antibody Complex) targeting EpCAM or MUC1, and then for a further 15 minutes with magnetic nanoparticles.

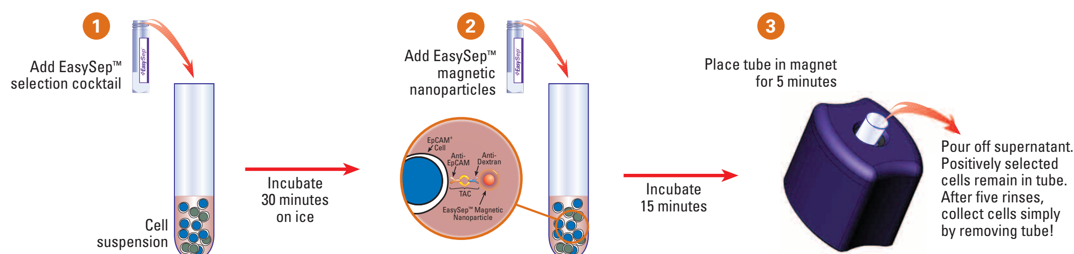
EasySep™ Cell Labeling - CD49f$^+$ Cells. Cells were incubated for 10 minutes with a PE-conjugated anti-CD49f antibody, then for 30 minutes with an anti-PE TAC. Finally, cells were incubated for 15 minutes with magnetic nanoparticles.

EasySep™ Cell Separation. The labeled cell suspension was placed in the EasySep™ magnet for 5 minutes, and the cells that were not magnetically labeled were poured off. The labeled cells were resuspended and the separation repeated a total of 6 times.

Evaluation of EasySep™ Cell Separation by Flow Cytometry. The purity of EpCAM$^+$ cells was assessed with FITC-labeled anti-epithelial cell 5E11 antibody. The purity of MUC1$^+$ cells was assessed with FITC-labeled anti-dextran (which would recognize the dextran on the magnetic nanoparticles). CD49f$^+$ cells were already labeled with PE from the cell separation procedure.

HBEC Colony Assay. Purity of luminal-restricted progenitors before and after MUC1-based separations was assessed by seeding 1.5 - 3 x 10^4 pre- or post-enrichment cells into 3 x 35 mm tissue culture plates in EpiCult-B™ medium supplemented with 5% FBS and in the presence of 6 x 10^3 cells/cm^2 irradiated NIH 3T3 feeder layers. Twenty-four hours later, the media was changed to EpiCult-B™ alone and the cultures maintained for a further 5 - 6 days. Cultures were fixed and stained with Wright's Giemsa to visualize colonies, and the number of myoepithelial cell-containing and pure luminal cell colonies quantified under a dissecting microscope.

Figure 1. EasySep™ Procedure to Enrich EpCAM$^+$ Cells



Results

Table 1. Enrichment of EpCAM+ Cells From Freshly Dissociated and Cultured Human Breast Specimens Using EasySep™

Experiment	Start Purity (%)	End Purity (%)	Log Depletion of EpCAM⁻ Cells	# EpCAM+ Cells Start	# EpCAM+ Cells End	% Recovery EpCAM+ Cells
1	31.8	93.9	1.9	1.69×10^{6}	6.57×10^{5}	38.9
2	26.8	92.1	1.8	1.32×10^{6}	6.35×10^{5}	48.2
3	21.1	93.6	1.9	2.18×10^{6}	1.40×10^{6}	64.2
4	24.5	87.2	1.5	1.65×10^{6}	1.05×10^{6}	63.6
5	28.2	93.8	1.9	1.38×10^{6}	6.47×10^{5}	46.9
6	49.5	94.6	1.7	1.76×10^{6}	5.96×10^{5}	33.8
7	59.9	98.3	2.1	6.08×10^{6}	2.09×10^{6}	34.8
Mean ± SEM	34.5 ± 5.5	93.3 ± 1.3	1.8 ± 0.1			47.2 ± 4.8

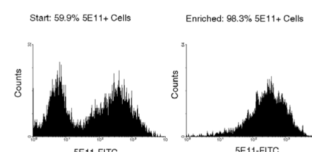


Figure 2. EpCAM+ cells were enriched from a 3 day-old primary culture of normal human mammary tissue using EasySep™. Purity of the enriched cells was determined by flow cytometry using the 5E11 antibody, which recognizes an epithelial cell surface antigen with a distribution identical to that of EpCAM.

Table 2. Enrichment of MUC1+ Cells From Freshly Dissociated and Cultured Human Breast Specimens Using EasySep™

Experiment	Start Purity (%)	End Purity (%)	Log Depletion of MUC1⁻ Cells	# MUC1+ Cells Start	# MUC1+ Cells End	% Recovery MUC1+ Cells
1	32.2	98.7	2.7	1.80×10^{6}	5.78×10^{5}	32.1
2	30.8	98.4	2.6	1.54×10^{6}	5.45×10^{5}	35.4
3	20.3	97.6	2.5	9.60×10^{5}	4.65×10^{5}	48.5
4	16.6	96.7	2.4	12.59×10^{6}	7.25×10^{5}	5.8
5	15.4	98.2	2.8	2.64×10^{6}	1.32×10^{6}	49.9
6	44.3	99.0	2.3	5.76×10^{6}	3.19×10^{6}	56.2
Mean ± SEM	26.6 ± 4.6	98.1 ± 0.3	2.6			38.0 ± 7.4

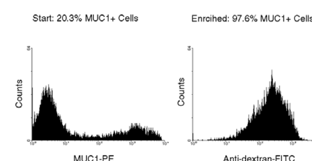


Figure 3. MUC1+ cells were enriched from a 3 day-old primary culture of normal human mammary tissue using EasySep™. Purity of MUC1+ cells was assessed with FITC-labeled anti-dextran (which would recognize the dextran on the magnetic nanoparticles).

Table 3. Enrichment of MUC1+ Luminal-Restricted Progenitors (LRP) Cells From Freshly Dissociated and Cultured Human Breast Specimens Using EasySep™

Experiment	Start Purity-LRP(%)	End Purity-LRP(%)	Log Depletion of non-LRP	# LRP Start	# LRP End	% Recovery LRP
1	44.5	98.8	2.12	1.49×10^{5}	8.28×10^{4}	55.6
2	27.5	90.2	1.43	1.07×10^{5}	9.57×10^{4}	89.7
3	48.9	92.6	2.28	9.30×10^{4}	7.58×10^{4}	76.3
Mean ± SEM	40.3 ± 6.5	93.9 ± 2.6	1.94 ± 0.26			73.9 ± 9.9

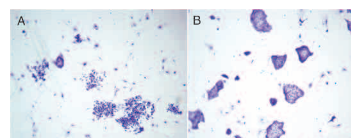


Figure 4. Human mammary epithelial cell colonies before (A) and after (B) positive selection of MUC1-expressing luminal-restricted progenitor cells.

Table 4. Enrichment of CD49f+ Cells From Freshly Dissociated and Cultured Human Breast Specimens Using EasySep™

Experiment	Start Purity (%)	End Purity (%)	Log Depletion of CD49f⁻ Cells	# CD49f+ Cells Start	# CD49f+ Cells End	% Recovery CD49+ Cells
1	53.9	98.1	2.1	10.23×10^{6}	3.87×10^{6}	37.9
2	9.7	90.8	2.6	2.66×10^{5}	0.68×10^{5}	25.6
3	66.8	98.7	1.9	5.21×10^{5}	2.37×10^{6}	45.5
4	64.7	96.3	1.7	15.65×10^{6}	4.52×10^{6}	28.9
5	60.2	99.5	2.8	11.32×10^{6}	2.43×10^{6}	21.5
Mean	51.1 ± 10.6	96.7 ± 1.6	2.2 ± 0.2			31.9 ± 4.4

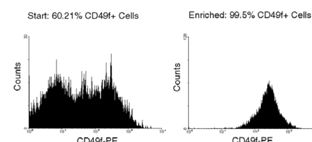


Figure 5. CD49f+ cells were enriched from freshly dissociated normal human mammary tissue using EasySep™. Purity of the enriched cells was determined by flow cytometry.

Conclusions

- Human mammary epithelial cells expressing either EpCAM, MUC1 or CD49f can be postively selected to 93-98% purity using EasySep™ immunomagnetic cell separation.
- Both freshly dissociated as well as previously cultured human mammary epithelial cells are suitable for EasySep™ cell separation.
- Immunomagnetic separation of human mammary epithelial cells using EasySep™ does not impede further FACS analysis or the proliferation of the postively selected cells in culture.

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