



# Redirecting Normal and Cancer Stem Cells to a Mammary Epithelial Cell Fate

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## Abstract

Tissue microenvironments, also known as stem cell niches, influence not only resident cells but also cells in surrounding tissues. Physical and biochemical intercellular signals originating from resident stem cells or non-stem cells participate in the homeostasis of the tissue regulating cell proliferation, differentiation, wound healing, tissue remodeling, and tumorigenesis. In recent publications it has been demonstrated that the normal mouse mammary microenvironment can provide development and differentiation guidance to not only resident mammary cells but also cells of non-mammary origin including tumor-derived cells. When placed in reforming mammary stem cell niches the non-mammary cells proliferate and differentiate along mammary epithelial cell lineages and contribute progeny to reforming mammary gland outgrowths. The tumor-derived cells that are redirected to assume mammary epithelial phenotypes lose their cancer-forming capacity and shift their gene expression profiles from a cancer profile towards a normal mammary epithelial expression profile. This review summarizes the recent discoveries regarding the ability of the normal mouse mammary microenvironment to dictate the cell fates of non-mammary cells introduced into mammary stem cell niches.

**Keywords** Breast · Mammary · Microenvironment · Niche · Redirection · Stem cells

## Abbreviations

BMSC	Bone marrow-derived stem cell
CMDS	Classic multidimensional scaling
CNS	Central nervous system
ECM	Extracellular matrix
EMT	Epithelial-mesenchymal transition
ER	Estrogen receptor
ESC	Embryonic stem cell
HER	Human epidermal growth factor receptor
K	keratin
LTR	Long terminal repeat
MEC	mammary epithelial cell
MMP	Matrix metalloproteinase
MMTV	Mouse mammary tumor virus
NSC	Neural stem cell
NT2	NTERA-2 cl
TEB	Terminal endbud
TDLU	Terminal ductal lobular unit

TGF	Transforming growth factor
TNBC	Triple negative breast cancer
TSC	Testicular-derived stem cell
WAP	Whey acidic protein promoter

## Introduction

In 1978 Shofield investigated the self-renewing ability of hematopoietic stem cells after transplantation in mice, hypothesizing that cell phenotype depends on the environment, specifically on cell-cell interactions, with neighboring non-HSC cells [1]. Later his theory was expanded and self-renewal abilities of stem cells was stated to be dependent not only on cell-cell interactions, but also dependent on diffusible factors, inflammation tissue components, extracellular matrix (ECM), physical parameters (shear stress, stiffness), and environmental signals such as hypoxia [2, 3]. Microenvironments that surround stem cells and direct cellular activity through short and long distance signaling are referred to as stem cell niches.

The normal mouse mammary gland provides an excellent animal model for the study of stem cells and normal growth and development as the majority of glandular expansion and differentiation occurs post-natally during puberty [4]. In the

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late 1950's it was determined by DeOme et al. that when mammary epithelial cells were transplanted into mammary fat pads of pre-pubescent female mice devoid of endogenous epithelium an entire functional mammary outgrowth could be recapitulated regardless of age or parity status of the transplanted cells [5]. Mammary tissue fragments and dissociated mammary epithelial cells were equally effective in generating mammary outgrowths [5–7]. The transplanted epithelial cells, in conjunction with the endogenous mammary stroma, were able to establish new mammary microenvironments, niches, that directed normal mammary development as the recipient mice progressed through puberty.

The normal mammary niche is comprised of numerous different cell types including epithelial cells, myoepithelial cells, nerve cells, endothelial cells, and stromal adipocytes and fibroblasts. Immune cells such as macrophages are also common in mammary microenvironments. Somatic mammary stem cells reside in niches and are influenced by the numerous biochemical and biophysical factors produced by the surrounding cells. Each of those cell types participate in heterologous cell-cell interactions that control stem cell differentiation. For example fibroblasts control epithelial morphogenesis [8, 9], while cytokines secreted by macrophages and eosinophils, such as stimulating factor -1 and eotaxin, influence terminal endbud TEB development. Moreover, in the absence of those cells, regenerative potential is severely compromised [10]. Adipocytes are involved in pubertal ductal tree development and regulate alveolar bud formation. Absence of fatty tissue will lead to restriction of branching morphogenesis [11, 12]. Those results demonstrate that each of the cell components of mammary gland is essential for proper development.

Secreted factors mediate indirect communication between stem cells and resident niche cells. Without the presence of hormones, such as estrogen and progesterone, normal mammary gland development is perturbed. Estrogen regulates ductal development during puberty, while progesterone controls branching of the ducts. Prolactin and the *erbB4* receptor are involved in alveolar development and milk-production [13]. *Wnt4* and *Wnt3A* cytokines regulate progesterone production, thus, mammary gland branching [14, 15]. While the TGF- $\beta$  receptor family supports tissue homeostasis by limiting terminal endbud (TEB) cell proliferation [16]. Furthermore, TGF- $\beta$ 1, TGF- $\beta$ 2 and TGF- $\beta$ 3 expression elevated during pregnancy and lactation causing robust increase in cell differentiation rates [17, 18].

Alteration of ECM composition is another way through which mammary gland niches direct stem cell actions [19, 20]. Integrins are involved in signaling pathways between ECM, mammary stroma, and surrounding cells. Removal of  $\beta$ 1 integrin expression in *Itg $\beta$ 1<sup>fx/fx</sup>*, *CreER $\delta$*  mice prevented mammary outgrowth development in 86% of transplantation cases [21].

All previously described mechanisms indicate that mammary microenvironments direct gland development and proliferation of epithelial cells through cell-cell interactions with non-multipotent cells, hormones, growth factors, and is dependent on ECM protein composition and physical properties.

Niches can be involved not only in normal development but are also known to drive tumor progression and metastasis [22]. Extracellular components, such as metalloproteases, and cell-cycle related genes are extensively upregulated in tumor-associated stroma. In the malignant epithelium, expression of genes participating in the immune response are drastically elevated and similar results have been shown in the neighboring normal tissue. Furthermore, decreased expression of cytoplasmic ribosomal proteins and increased expression of mitochondrial ribosomal proteins are also present in the surrounding microenvironment. These results suggest that cancer cell differentiation is not the only parameter involved in disease progression. Several matrix metalloproteases (MMP2, MMP11 and MMP14) exhibit elevated expression levels right before the switch from pre-invasive to invasive growth, indicating that mammary gland niches are not just influenced by tumor progression, but in some cases send signals allowing tumor cell colonization [23].

## Niche Vs Stem Cells

A hot topic in the field of stem cell biology in recent years has been the question “which component is more important, the stem cells or the microenvironment?” In order to demonstrate the deterministic nature of the normal mammary microenvironment over resident stem cells, G.H. Smith and colleagues introduced non-mammary stem cells into reforming mammary niches using the mouse mammary transplantation model pioneered by DeOme described above. Somatic stem cells were isolated from transgenic mouse seminiferous tubules and used as the source of stem cells for the new normal mammary niches [24]. The seminiferous-derived cells were mixed with normal mammary epithelial cells (MECs) and transplanted into recipient mouse mammary fat pads. These cells were derived from male *WAP-Cre/Rosa26R* mice. Expression of whey acidic protein (WAP) promoter is restricted to the mammary gland. Once activated, *WAP-Cre* resulted in constitutive expression of *lacZ* in the testicular cells and all their progeny allowing easy identification of the male cells. The testicular stem cells were able to survive and enter the new mammary niches, proliferate, and divide asymmetrically to provide progeny that helped regenerate a functional mammary gland. Male transgenic cells were found throughout the recapitulated mammary outgrowths as luminal epithelial cells and myoepithelial cells.

**Table 1** Results when normal non-mammary stem cells are transplanted into cleared mammary fat pads

Cells transplanted	#positive takes/ #implants	# <i>lacZ</i> <sup>+</sup> takes/ # positive takes	#tumors formed	# <i>lacZ</i> <sup>+</sup> tumors
TSCs alone	10/10	0/10	0	0
TSCs w/MECs (1:1)	20/38	12/20	0	0
NSCs alone	0/10	0/0	0	0
NSCs w/MECs (1:1)	22/30	22/22	0	0
ESCs alone	0/13	0/0	13/13	13/13
ESCs w/MECs (1:5)	5/13	5/5	3/13	3/3
ESCs w/MECs (1:50)	8/13	8/8	1/13	1/1
BMSCs alone	0/6	0/0	0	0
BMSCs w/MECs (1:1)	26/40	19/26	0	0

NSCs neural stem cells, TSC testicular-derived stem cells, BMSC bone marrow-derived stem cells, ESC embryonic stem cells

Additionally, when recipient mice became pregnant and began lactation the transgenic male cells differentiated into secretory mammary epithelial cells producing and secreting milk proteins. These experiments demonstrated the power of the normal niche over somatic stem cells indicating it is not the stem cells that dictate surrounding cellular behavior but instead the surrounding normal cells of the niche direct the resident stem cells.

Additional experiments demonstrated that stem cells isolated from the central nervous system (CNS), bone marrow (BM), and embryonic stem cells (ESC) participate in the regeneration of mammary outgrowths when applied in the same model [25–28]. It has been established that somatic stem cells of ectodermal and mesodermal origin along with ESCs respond to intercellular signaling cues originating from the normal mammary microenvironment [24–27]. The controlling signals emanate from the epithelial component of the microenvironment. When the non-mammary cells are transplanted alone, without the epithelial component, no mammary outgrowth is observed. In the case of ESC transplantation without the MEC component, teratomas form at the transplantation sites [24–27]. This indicates that signals from the mammary stroma alone are not sufficient to direct non-mammary stem cell growth and differentiation. The different cell origins that undergo phenotype switching are summarized in Table 1.

## Cancer Cell Redirection by the Normal Niche

Having demonstrated that the normal mammary niche can control stem cells of non-mammary origin the effects of the normal niche were investigated on cancer cells. In order to study this effect, transgenic mice that express wild type *neu* under transcriptional regulation of the mouse mammary tumor

virus-long terminal repeat (MMTV-LTR) promoter were bred with WAP-Cre/Rosa26R mice [28]. Overexpression of *neu* oncogene is associated with increased rates of tumor formation in breast cancer patients [29]. The MMTV-*neu* mouse model is an established and accepted animal model for study into HER2<sup>+</sup> human breast cancer [30, 31].

Tumor cells isolated from mammary carcinomas of triple transgenic WAP-Cre/Rosa26R/MMTV-*neu* mice continually express *lacZ* [28]. When transplanted alone into cleared mammary fat pads *lacZ*<sup>+</sup> mammary tumors formed within 7 months regardless of the number of cancer cells transplanted [32]. When the tumor-derived cells were co-transplanted with normal MECs in ratios of 2:1, 1:1, and 1:10 cancer cells to MECs *lacZ*<sup>+</sup> mammary tumors formed. However, when the tumor-derived WAP-Cre/Rosa26R/MMTV-*neu* cells were co-transplanted with normal MECs in a 1:50 ratio no *lacZ*<sup>+</sup> mammary tumors formed. This phenomenon has been termed “Cancer cell redirection.” The once tumorigenic cells lose their tumor-forming capacity. Instead mammary ductal trees formed that consisted of both *lacZ*<sup>−</sup> and *lacZ*<sup>+</sup> mammary epithelial cells indicating that the *lacZ*<sup>+</sup> mammary cancer cells were incorporated into the growing mammary ducts. Furthermore, *lacZ*<sup>+</sup> cells were found to have differentiated into keratin 8<sup>+</sup> (K8) luminal epithelial cells and myoepithelial cells based on positive expression of smooth muscle actin and keratin 14 (K14). Additionally, when recipient mice were allowed to complete a full-term pregnancy the incorporated WAP-Cre/Rosa26R/MMTV-*neu* cells expressed and secreted milk proteins including β-casein. In second generation mammary transplants the tumor-derived did not form mammary tumors when transplanted as tissue fragments. Only when the WAP-Cre/Rosa26R/MMTV-*neu* cells were dissociated and sorted apart from the MECs did the tumor-derived cells regain their tumor forming capacity.

Mammary tumors that form in MMTV-*neu* mice do not express the hormone receptors estrogen receptor (ER) or progesterone receptor (PR) [30]. No expression of ER or PR was found in either tumors formed by transplanted WAP-Cre/Rosa26R/MMTV-*neu* cells nor in mammary outgrowth formed by WAP-Cre/Rosa26R/MMTV-*neu* cells and MECs [32]. This observation suggests that cancer cell redirection does not result in total reprogramming of the cancer cell genotype, but does result in a change of phenotype.

## Redirecting Human Cancer Cells

In order to address the question of whether cancer redirection can occur in human cancer cells, cancer cells from two human cancer types were incorporated into the animal model of cancer cell redirection. Two cell lines derived from triple negative breast cancers (TNBC), MDA-MB-231 and MBA-MB-468, were used [33]. When the TNBC cells were transplanted alone

or in a 1:1 ratio with MECs mammary tumors formed. When the TNBC cells were transplanted in a 1:50 ratio with MECs the cancer cells were redirected to form normal mammary ductal trees. The redirected TNBC cells differentiated into K8<sup>+</sup> luminal epithelial cells and K14<sup>+</sup> myoepithelial cells. During lactation the chimeric mammary outgrowths comprised of redirected TNBC cells and MECs produced human milk proteins ( $\alpha$ -lactalbumin and lysozyme) and mouse milk proteins ( $\beta$ -casein) respectively.

Cells derived from a human embryonal testicular carcinoma, NTERA-2 cl (NT2), have also been redirected by the normal mouse mammary microenvironment [34]. When transplanted alone, or in a 1:1 ratio with MECs, the NT2 cells formed mammary tumors, similar to the mouse mammary tumor-derived cells and the human TNBC cells. However, chimeric mammary outgrowths of human NT2 cells and mouse MECs formed when the two cell types were co-transplanted in 1:10 and 1:50 ratios suggesting that the embryonal carcinoma cells have a higher level of plasticity than the TNBC and MMTV-neu cells. The NT2 cells differentiated into luminal, secretory, and myoepithelial cells like the TNBC and MMTV-neu cells [32–34]. These results indicate that cancer cell redirection induced by the normal mouse mammary microenvironment is not restricted to only cells of mouse origin, nor is the phenomenon restricted to only cells of mammary origin. The results of redirecting cancer cells by the normal mammary microenvironment are summarized in Table 2.

**Table 2** Summary of results where cancer cells are redirected by the normal mammary microenvironment

Cells transplanted	#positive takes/ #implants	#cancer cell <sup>+</sup> takes/ #positive takes	%tumors
Mouse erbB2 <sup>+</sup> cancer cells alone	0/18	0/0	100 (18/18)
Mouse erbB2 <sup>+</sup> cancer cells w/MECs (1:5)	7/8	5/7	62.5 (5/8)
Mouse erbB2 <sup>+</sup> cancer cells w/MECs (1:50)	15/16	15/15	6.25 (1/16)
Human testicular carcinoma cells alone	0/6	0/0	33.3 (2/6)
Human testicular carcinoma cells w/MECs (1:5)	5/6	5/5	0.0
Human testicular carcinoma cells w/MECs (1:50)	10/12	10/10	0.0
Human TNBCs w/MECs (1:5)	5/20	5/5	50.0 (10/20)
Human TNBCs w/MECs (1:50)	10/16	10/10	0.0

TNBC triple negative breast cancer

## In Vitro Redirection

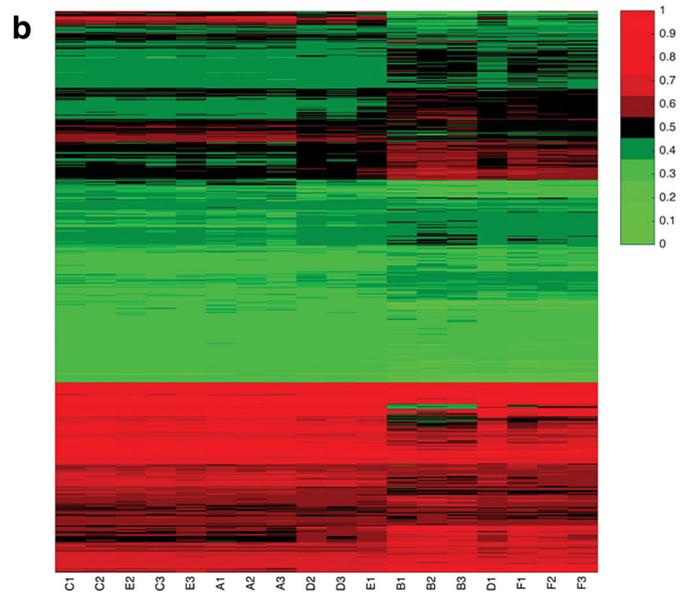
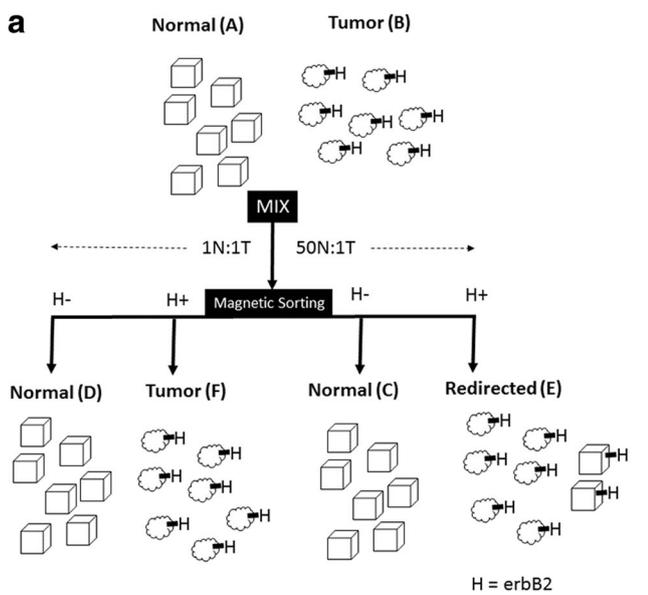
When we redirected WAP-Cre/Rosa26R/MMTV-neu tumor-derived cells in vivo we discovered that even though erbB2 was overexpressed due to the constitutive expression of the neu oncogene driven by MMTV, phosphorylation of erbB2 in the redirected cells was absent. ErbB2 was phosphorylated in the mammary tumors formed by transplantation of the cancer cells alone but was absent in the redirected outgrowths [32]. We have leveraged this observation into the formation of an in vitro model of cancer cell redirection [35].

Using the same ratios of cancer cells to MECs, we found that erbB2 phosphorylation can be attenuated in vitro [35]. We use the absence of erbB2 activity as a marker of cancer cell redirection. The use of our in vitro system cuts the time and cost of experiments from a minimum of 8 weeks in vivo to 4–6 days in vitro.

Based on our observation that erbB2 is continually expressed in redirected MMTV-neu mammary tumor-derived cells we sorted co-cultures of MMTV-neu and normal mouse mammary epithelial cells (COMMA-D $\beta$ geo) cells into erbB2<sup>+</sup> and erbB2<sup>-</sup> fractions (Fig. 1a). Each sorted fraction was designated an identifier (A, B, C, etc.) (Fig. 1a). Gene expression profiles were developed from the mouse mammary tumor cells, the normal epithelial cells, and erbB2<sup>+</sup> and erbB2<sup>-</sup> fractions sorted from 1:1 and 1:50 (cancer:normal) co-cultures (Fig. 1b) [36]. We used mutual information relationship analyses on the dataset of over 35,000 gene expression measurements spread over 13,000 curated gene sets. From those >35,000 genes, we were able to reduce the number to a set of 20 molecular marker signatures of significance that totaled 906 unique loci. From the 906 unique loci we refined the gene set to 120 core redirection biomarkers. The 20 molecular signatures included such pathways as a Notch signaling pathway, a p53 pathway, an EMT pathway, and multiple cell death and differentiation pathways.

Distributions of gene expression levels showed a remarkable pattern. On one side of the distribution visualization following classic multidimensional scaling (CMDS) are the normal epithelial cells and on the other side are the cancer cells (Fig. 2a). Of note is the position of the 1:50 erbB2<sup>+</sup> fraction, the redirected cells. Instead of appearing close to the cancer cells, the genetic profile of the redirected fraction now appears next to the normal epithelial cell profiles. Additionally, the expression profile distribution of the redirected fractions has changed from the histogram plot observed in the cancer cells to biphasic distributions similar to the normal epithelial cells (Fig. 2b). Visualization of the gene expression profiles reveals that redirected cancer cells are more similar to profiles of normal epithelial cells than the profiles generated from the cancer cells.

When we investigated individual differential expression (DE) gene between the fractions we found numerous growth



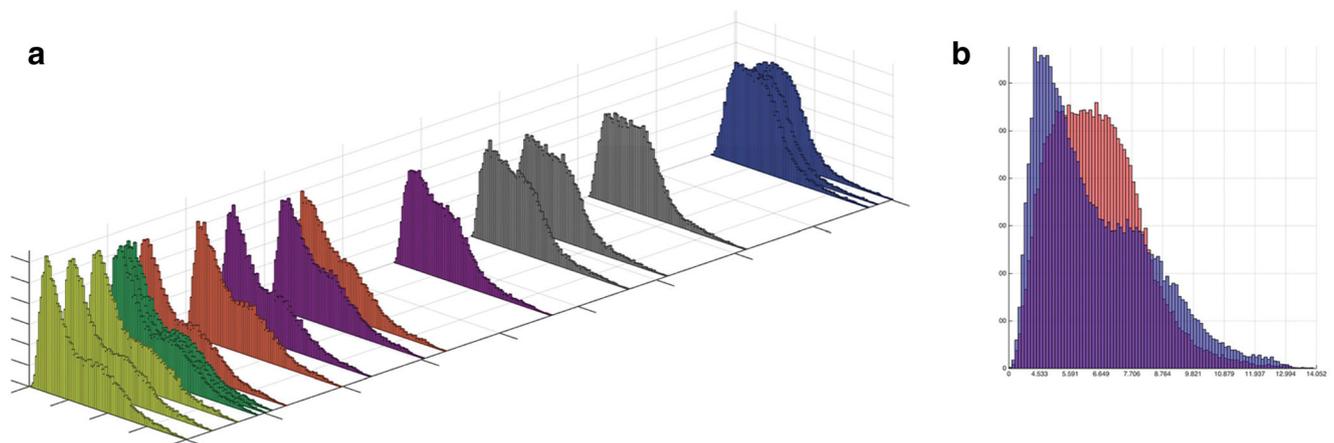
**Fig. 1** The Experimental System and Global Gene Expression Pattern. **a** The experimental system consists of six cell groups; normal (A) and tumor (B) cells were mixed in either a 1:1 or 50:1 (normal:tumor) ratio, then magnetically sorted based on expression of erbB2 into four resultant groups: normal (D) or tumor (F) from the 1:1 mixing and normal (C) and

redirected tumor (E) from the 50:1 mixing; **b** Heatmap of all transcripts present in the microarray, log<sub>2</sub> expression values normalized by column; columns are identified at the bottom of the figure with their cell group classification and replicate number

factor and cytokine family members DE between the normal, cancer, and redirected cell populations. We looked at 251 genes from growth factor and cytokine families and found 110 genes DE between the groups [37]. The families investigated were the EGF, FGF, TGFβ, Notch, Wnt and Hedgehog growth factor and receptor families and chemokine ligands and receptors. Genes of interest DE between the cancer cells and redirected cells include *IL-6*, *β-catenin*, *CXCL12*, *SMO*, *Notch2*, and *FGFR1*. All of the pathways and families investigated have been demonstrated to be involved in normal mammary development, homeostasis, or mammary tumor development [37].

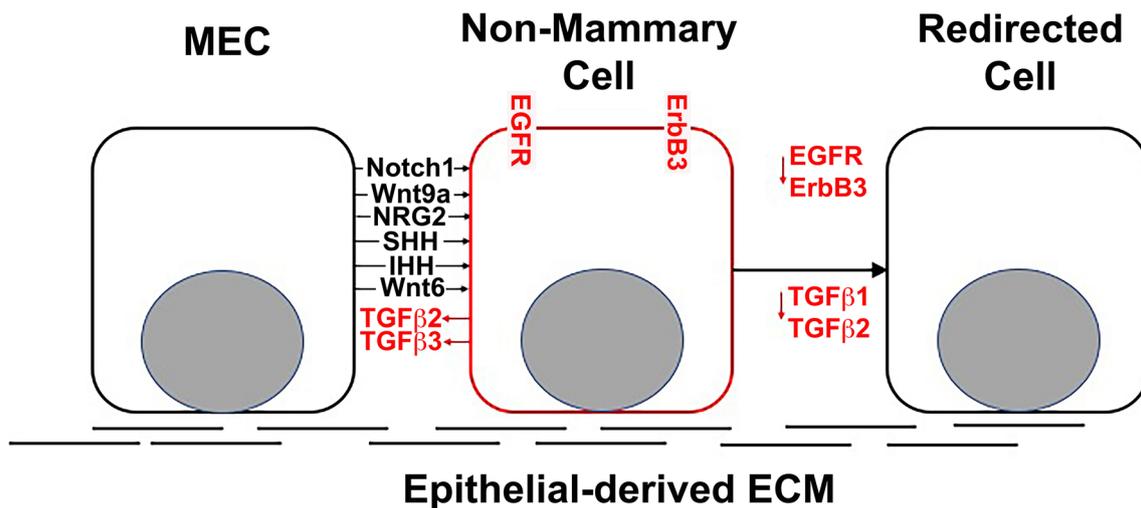
### Role of ECM in Phenotype Determination

In all of the studies cited previously phenotype determination of exogenous stem cells and cancer cells was due to interactions with MECs either in vivo or in vitro. Recently it was observed that components of the ECM found in normal mammary glands can induce a phenotype redirection of non-mammary stem cells [38]. Factors within ECMs are specific to their tissue or organ of origin and contribute to normal microenvironments and niches [19, 39]. When acellular ECM components were isolated, purified, and co-transplanted with *lacZ*<sup>+</sup> testicular derived stem cells or *lacZ*<sup>+</sup>



**Fig. 2** Distribution of Expression Levels for Each Cell Group. **a** Expression level distributions ordered in one dimension on the basis of dissimilarity by mutual information. Yellow = normal (cell group A), orange = redirected (E), blue = tumor (B). Order of cell group-replicates,

from bottom-left to top-right: A<sub>3</sub>, A<sub>1</sub>, A<sub>2</sub>, C<sub>3</sub>, C<sub>2</sub>, C<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub>, D<sub>3</sub>, D<sub>2</sub>, E<sub>1</sub>, D<sub>1</sub>, F<sub>3</sub>, F<sub>2</sub>, F<sub>1</sub>, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>. **b** Expression level distributions for all cell group E replicates (blue) superimposed on distributions for all B replicates (red)



**Fig. 3** Proposed model for induced phenotype switching. Normal MECs produce ECM and select intercellular signals (Notch2, Wnt9a, NRG2, SHH, IHH, Wnt6) that induce changes in the non-mammary cell that

induces a switch in phenotype. The non-mammary cell down regulates expression of TGFβ2 and TGFβ3 and the EGFR and ErbB3 receptors

ESCs into cleared mammary fat pads normal mammary outgrowths were generated comprised of *lacZ*<sup>+</sup> and *lacZ*<sup>-</sup> epithelial cells [38]. This result indicates that *lacZ*<sup>+</sup> cells contributed to the outgrowths and that *lacZ*<sup>-</sup> cells from the host mouse also contributed. No exogenous MECs were used in the transplants indicating that the *lacZ*<sup>-</sup> cells that were incorporated into the mammary outgrowths originated in the host animal. Adipose-derived stem cells can form acinar structures that express the epithelial surface marker keratin 18 and epithelial genes *CDH1* and *KRT18* [40].

These results have also been translated into in vitro models as well. ECM derived from human breasts supports the growth and differentiation of normal human breast epithelial cells and the growth of human breast cancer cell lines when incorporated into a 3D bioprinted model [41–43]. Interestingly, the TGFβ, Hedgehog, Notch, Wnt, and p53 signaling pathways were found to be active in this in vitro model [42]. These genes are also involved in the redirection of cancer cells in vitro [37]. The breast cancer cell lines investigated were derived from ER<sup>+</sup> or TNBC tumors but not HER2<sup>+</sup> tumors. This suggests that the pathways involved in growth in normal mammary microenvironments are conserved between cancer cells, normal epithelial cells, and redirected non-mammary stem cells and redirected cancer cells. A potential model for phenotype switching induced by the normal mammary gland microenvironment that includes results from in vitro models using MECs and ECM is illustrated in Fig. 3.

### Requirement of MECs in Phenotype Determination

The results cited above indicate that mouse mammary ECM components are sufficient to induce changes in phenotype in

mouse cells of non-mammary origin in vivo and ECM derived from human breast supports the growth of normal human breast epithelial cells and human breast cancer cells. When cells of non-mouse origin are transplanted into cleared mouse mammary fat pads no mammary ductal formation occurs. Non-tumorigenic immortalized human breast epithelial cells formed small transient nodules that regressed and disappeared when transplanted [44]. Similar results were observed when bovine mammary epithelial cells were transplanted into cleared mouse mammary fat pads [45]. Hollow spheres consisting of bovine epithelial cells formed but no invasion of the surrounding stroma was observed. These results match those observed when testes-derived cells, CNS cells, ESCs, and bone marrow-derived mouse cells were transplanted alone [24–27]. Without the co-transplantation of normal mouse MECs no ductal formation occurs, and the non-mammary cells exist in situ for a period of time before dying and removal by the host. In order to produce human or bovine mammary structures in a mouse mammary fat pad fibroblasts of the desired species are introduced prior to the addition of mammary epithelial cells [45, 46]. In the case of using human fibroblasts, the mouse mammary gland is “humanized” and the introduction of human breast epithelial cells results in the formation of human breast terminal ductal lobule units (TDLUs) [46].

Dispersed bovine mammary epithelial cells were co-transplanted with normal mouse MECs into cleared mammary fat pads [45]. The resulting outgrowths demonstrated two morphologically distinct structures. The first structure resembled bovine mammary architecture seen when the cells were transplanted alone without the mouse MECs and the second structure resembled TEBs present in normal mouse mammary development [45]. Interestingly, the mammary structures formed by the co-transplantation of human

testicular carcinoma cells and mouse MECs more closely resembled human TDLUs and not mouse TEBs [34]. These results suggest that mammary ductal structures are species specific.

## Conclusions

The normal mouse mammary microenvironment has the capacity to induce phenotype switching in normal stem/progenitor cells of non-mammary origin. The epithelial component, or at least epithelial-derived ECM, is required for successful stem cell reorientation. This has been confirmed multiple times as when the non-mammary cells are transplanted into the mammary fat pad devoid of any epithelial components no phenotype realignment is observed. Furthermore, the normal mouse mammary microenvironment can redirect cancer-derived cells to adopt a normal mammary epithelial phenotype thus attenuating the tumor-forming capacity of the tumor-derived cells. When the cancer cells are redirected in vitro a shift in gene expression profile from cancer cell towards a normal mammary epithelial profile is achieved. Taken together this indicates that intercellular signals that originate in the epithelial compartment of the normal mouse mammary microenvironment are capable of inducing phenotype guidance for cells of both mammary and non-mammary origins as well as tumorigenic cells.

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