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Review

Reprogramming non-mammary and cancer cells in the developing mouse mammary gland

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ABSTRACT

The capacity of any portion of the murine mammary gland to produce a complete functional mammary outgrowth upon transplantation to an epithelium-divested fat pad is unaffected by the age or reproductive history of the donor. Likewise, through serial transplantations, no loss of potency is detected when compared to similar transplantations of the youngest mammary tissue tested. This demonstrates that stem cell activity is maintained intact throughout the lifetime of the animal despite aging and the repeated expansion and depletion of the mammary epithelium through multiple rounds of pregnancy, lactation and involution. These facts support the contention that mammary stem cells reside in protected tissue locales (niches), where their reproductive potency remains essentially unchanged through life. Disruption of the tissue, to produce dispersed cells results in the desecration of the protection afforded by the "niche" and leads to a reduced capacity of dispersed epithelial cells (in terms of the number transplanted) to recapitulate complete functional mammary structures. Our studies demonstrate that during the reformation of mammary stem cell niches by dispersed epithelial cells in the context of the intact epithelium-free mammary stroma, non-mammary cells, including mouse and human cancer cells, may be sequestered and reprogrammed to perform mammary epithelial cell functions including those ascribed to mammary stem/progenitor cells.

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1. Introduction

Schofield [1] was the first to propose the idea of a specific location defined by specific cells and cellular signals controlling stem cell function (a stem cell niche) for hematopoietic stem cells. This theory was proposed to explain why stem cells from aged mice were functionally as capable of long-term engraftment of young recipients as hematopoietic stem cells from young donors. His idea was that stem cells were essentially "immortal" so long as they resided in their niche but when removed from these sites then "immortality" was lost. He defined this stem cell "niche" as a specific anatomical site where stem cells were sustained and could reproduce; where differentiation of the stem cell was inhibited and most importantly a site where reversion to a stem cell phenotype might be induced in a more (slightly) mature cell type. Schofield's concept remained hypothetical and without direct evidence until the late 1990s when work from Spradling and his colleagues validated each of Schofield's predictions about a stem cell niche in the ovary of *Drosophila melanogaster* [2–4]. A similar validation shortly

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followed from the study of the testes in *Drosophila* and later in *Caenorhabditis. elegans* [5]. Even the most far-reaching of Schofield's concept namely that a more mature cell could be induced to acquire stem cell attributes by interaction with the niche was validated in these invertebrate models.

2. Use of conditional reporter models to test for niche signals

We were inspired to test this final point of Schofield's niche concept in the regenerating mammary gland because we had successfully rescued mammary stem/progenitor cells (Fig. 1) from transgenic mammary tissues where regenerative capacity had been obliterated by the ectopic expression of the transgene by simply mixing the incompetent epithelial cells with normal wild type mammary epithelium prior to introduction into the epithelium-free mammary fat pad [6]. In two models, (WAP-Notch4/Int3 X WAP-Cre/Rosa26R and WAP-TGFβ1 X WAP-Cre/Rosa26R), where lacZ-reporter marked cells (PI-MEC) were present in mammary epithelial populations incapable of growth and reconstitution of mammary epithelium in vivo, we found that interaction with normal wild type epithelial cells allowed them to produce progeny during mammary gland regeneration. These results suggested that the mammary epithelial cells themselves in combination with the mammary fat pad and its stroma, along with extrinsic growth and hormonal factors were components essential to the mammary stem cell niche.

It is known that signals from progesterone receptor positive (PR) mammary epithelial cells are essential to secretory alveolar development [7] and that signaling from estrogen receptor alpha-positive (ERα) epithelium is needed for mammary duct growth and expansion [8]. The growth factor, amphiregulin (AR), has been identified as an important mediator of ERα+ signaling for duct elongation and development [9]. Gata3 has been shown to be essential for luminal epithelial differentiation in the ducts [10] and Beta1 integrin expression for full development of the secretory alveoli [11]. Other regulatory factors present or generated in the mammary stroma have also been identified such as transforming growth factor beta (TGFβ) [12], fibroblast growth factor (FGF), heregulin (HGF), insulin growth factors (IGFs) and the RANKL/RANK interaction [13–15]. Thus the mammary microenvironment that supports and maintains mammary epithelial homeostasis and the capacity for regeneration upon transplantation consists of local signals emanating from both the stroma and the existing epithelium and circulating host factors. Adhesiveness and cell-to-cell contact also plays an important role in mammary structure and function [16–18].

In parous females WAP-Cre/Rosa26-lacZ, lacZ expression marks cells that survive after lactation and involution (PI-MEC). Experiments where WAP-Cre/Rosa26-lacZ reporter glands from nulliparous females were incubated as explant fragments in various combinations of growth factors and hormones demonstrated that milk induction in the epithelial cells was not necessary to active the Rosa26-lacZ reporter [19]. This indicated that the PI-MEC were already present in nulliparous mammary tissue and were subsequently identified following pregnancy, lactation and involution. In other experiments, PI-MEC were marked by the expression of GFP in WAP-Cre/Chicken-actin gene promoter (CAG)-flox-stop-flox-GFP parous females. In these studies GFP^+ PI-MEC were fluorescent activated cell sorted (FACS) and found to be virtually 100% present in the $CD49f^{hi}$ population. This population was shown earlier to possess essentially all of the mammary repopulating activity. Subsequent transplantation of GFP+/$CD49f^{hi}$ positive PI-MEC and the GFP-/CD49flo epithelial cells into epithelium-divested mammary fat pads indicated that all the repopulating activity was associated with the GFP^+ fraction.

Encouraged by these observations, we set out to determine if cells from non-mammary tissues could be altered from their initial cell fate lineage to adopt mammary epithelial characteristics upon interaction with mammary epithelial cells during reconstitution of mammary epithelium in regenerating mammary tissue in vivo.

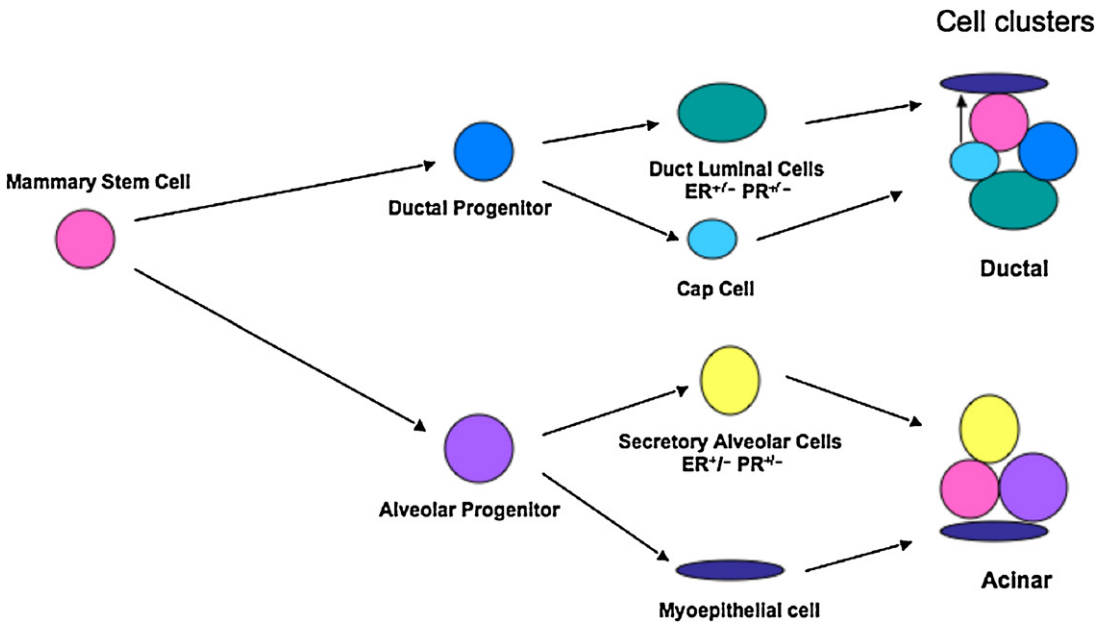

Fig. 1. Mouse mammary stem/progenitor functional hierarchy. A multi-potent stem cell gives rise to two lineage limited progenitor cells, ductal and alveolar progenitors. Both lineage-limited progenitors are multi-potent as they are capable of producing both $ER^{+/-}$ and $PR^{+/-}$ luminal epithelial cells as well as myoepithelial cells. Ductal progenitors give rise to specialized cap cells in the terminal end buds, which in turn become the myoepithelial cells of the subtending ducts. Alveolar progenitors proliferate during pregnancy to produce the luminal and myoepithelial cells of developing secretory lobules.