Breast Cancer RESEARCH

VIEWPOINT

Slugging their way to immortality: driving mammary epithelial cells into a stem cell-like state

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Abstract

Delineating the molecular factors that define and maintain the mammary stem cell state is vital for understanding normal development and tumourigenesis. A recent study by Guo and colleagues identifies two master transcriptional regulators of mammary stem cells, *Slug* and *Sox9*, ectopic expression of which confers stem cell attributes on differentiated mammary epithelial cells. *Slug* and *Sox9* expression was also shown to determine *in vivo* metastatic potential of human breast cancer cell lines. Understanding these factors in the context of normal lineage differentiation is an important step toward elucidating the mammary epithelial cell hierarchy and the origins of cancer stem cells.

Background

The mammary gland is a highly dynamic tissue, undergoing significant morphological change during puberty, pregnancy, lactation and involution. Flow cytometry using multiple markers has enabled the prospective isolation of mammary epithelial subpopulations suggesting a hierarchical organisation of stem, progenitor and differentiated cells. Utilising the cleared mammary fat pad transplantation assay, developed from the pioneering work of Deome and colleagues [1], mammary stem cells (MaSCs) have been defined as highly enriched in the $CD24^{+/Low}Sca1^{-}CD49f^{High}CD29^{High}$ population [2-5]. A single cell from this population is able to recapitulate the entire gland upon transplantation [3,5], showing defining stem cell characteristics of multi-differentiative potential and self-renewal. Despite much work to define factors necessary for MaSC function, molecular mechanisms regulating MaSCs are poorly defined. Epithelial–mesenchymal transition (EMT), a key developmental programme in the embryo that confers mesenchymal cell traits on epithelial cells, has recently been linked to the MaSC state [6,7]. EMT has also been implicated in tumour invasion and metastasis, and a potential role in self-renewal strengthens known associations between normal tissue stem cells and cancer cells [8].

Despite ongoing controversy over the existence and origin of cancer cells, the cancer stem cell model – in which tumours are maintained by a population of stem-like cancer cells – provides an attractive framework to understand metastatic potential and tumour heterogeneity in response to treatment. Irrespective of the origins of these cells, their exact nature and nomenclature and their operational detection, it is unlikely that neoplastic (stem-like) cell populations invent a novel programme to drive their sustained proliferation. Instead, cancer cells adopt the self-renewal programme active in the antecedent stem cell population and exploit this to organise the complex tissues observed at various stages of neoplastic progression. Delineating the molecular factors that define the mammary epithelial cell hierarchy and maintain the MaSC state is therefore an important step towards understanding both normal development and tumourigenesis.

Slug and Sox9 as master regulators of the mammary stem cell state

A recent study by Guo and colleagues identified two master transcriptional regulators of the mammary stem cell state: the EMT-associated transcription factor Slug, and the SRY-box transcription factor Sox9 [9]. Ectopic co-expression of *Slug* and *Sox9* in primary mouse mammary cells *in vitro* for 5 days was sufficient to convert differentiated luminal cells into MaSCs, as demonstrated by a competitive cleared mammary fat pad transplantation assay. Differentiated luminal cells expressing *Slug* and *Sox9* were able to reconstitute the entire mammary gland upon serial transplantation, exhibiting defining stem cell characteristics of multi-potency and self-renewal. These factors were shown to act co-operatively as expression of each factor individually was

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not sufficient to confer stem cell traits on fully differentiated cells.

Ectopic *Sox9* expression alone in differentiated cells produced cells with luminal progenitor characteristics, being able to proliferate *in vitro* and form hollow acini in three-dimensional culture conditions. Ectopic expression of *Slug* was able to convert luminal progenitor cells, which endogenously express *Sox9*, into cells with stem cell activity. In contrast, stem cell activity was enhanced in basal cells, which endogenously express *Slug*, by forced expression of *Sox9*. Interestingly, when *Sox9* was ectopically expressed in basal cells at the same time as *Slug* was knocked down, the cells acquired a luminal progenitor-like phenotype *in vitro*. Gene expression analysis of differentiated cells ectopically expressing *Slug* or *Sox9* showed upregulation of basal or luminal progenitor-associated genes, respectively, and both signatures were upregulated upon Slug/Sox9 co-expression. The authors postulated that Slug and Sox9 regulate basal and luminal lineage programmes, respectively. Each confers distinct biological properties on the cell, but both are required for MaSC function.

Given the important potential link between MaSCs and breast cancer stem cells, the study next looked at the effects of Slug and Sox9 on tumour-initiating potential and metastasis. The human breast cancer cell line MDA-MB-231 expresses both *Slug* and *Sox9*, forms tumours upon subcutaneous injection and metastasises to the lung upon tail vein injection into NOD/SCID mice. Knockdown of *Sox9* reduced the tumour-initiating potential of the cells by over 70-fold following subcutaneous injection. In contrast, knockdown of *Slug* did not affect tumour initiation but the resulting tumours were sixfold smaller than those in controls. In a metastasis assay, *Slug* or *Sox9* knockdown reduced lung metastases by fivefold and 40-fold, respectively. In non-metastatic MCF7ras human breast cancer cells implanted orthotopically into NOD/SCID mice, there was a significant increase in macrometastases from primary tumours ectopically co-expressing *Slug* and *Sox9*. Finally, in a tissue microarray of 306 clinical breast cancer samples, patients with primary tumours expressing high levels of both *Slug* and *Sox9* had a significantly lower overall survival rate. The authors conclude that human breast cancer stem cells are controlled by the same master regulators governing the murine MaSC state.

Stem cell enhancement or altered cell fate?

Many studies have now demonstrated that transplantation potential in the cleared mammary fat pad assay is most highly enriched in the basal cell layer of the mammary epithelium [2-4]. If this transplantation potential can be serially propagated, we call this mammary epithelial stem cell potential. However, both the potential plasticity of the normal mammary epithelium and the inductive potential of the mammary environment are increasingly well recognised.

The work of Guo and colleagues is a very important step forward in understanding the molecular regulation of this plasticity and how it can promote mammary stem cell potential, but it will be difficult to disentangle these phenomena. *Slug* promotes the basal phenotype when ectopically expressed in luminal progenitors. *Sox9* promotes luminal progenitor activity in differentiated luminal cells and stem cell activity in differentiated basal cells. Together, *Slug* and *Sox9* determine stem cell-like activity in the mammary epithelium. Whether the basal phenotype is required for this activity (perhaps by determining response to the microenvironment) or whether changes in underlying self-renewal processes are independent of phenotypic changes remains unclear. Perhaps both scenarios are occurring, with *Sox9* priming cellular self-renewal to respond to niche interactions that are made possible by the transcriptional programme driven by *Slug* activity.

Finally, as Guo and colleagues note themselves, in the mammary epithelium 'basal' does not necessarily mean 'stem cell'; and indeed the majority of basal mammary cells are not stem cells but rather are myoepithelial cells. Myoepithelial cells are, in terms of both function and identity, a cross between epithelial cells and mesenchymal cells and they express numerous EMT markers as part of their normal biology [10]. It is therefore wrong to characterise a luminal to basal phenotypic switch as EMT. Rather, expression of 'mesenchymal markers' is part of the normal differentiative programme of the mammary epithelium, and this paper must be understood in that context.

Abbreviations

EMT, epithelial–mesenchymal transition; MaSC, mammary stem cell; NOD/SCID, nonobese diabetic/severe combined immunodeficiency.

Competing interests

The authors declare that they have no competing interests.

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