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The transcription factor GATA-3 is necessary for the formation of a mammary gland and the maintenance of mammary-cell differentiation. The loss of GATA-3 function in a fully formed mammary gland generates oestrogen receptor-negative, proliferating cells that lack expression of myoepithelial markers. Cells with similar characteristics in breast cancer are associated with poor prognosis.

"Cell differentiation is based almost certainly on the regulation of gene activity, so that for each state of differentiation a certain set of genes is active in transcription and other genes are inactive." So wrote Roy Britten and Eric Davidson in their classic paper of 1969 speculating on the mechanistic basis of differentiation[1]. In the ensuing years much work has been done to unravel the regulatory networks that are necessary for proper development and differentiation in multiple model organisms and a plethora of cell-fate specifications. The ductal branches of the mammary gland are encased in myoepithelial cells on the exterior and lined with ductal luminal cells along its length. At the ends of these ducts are structures, terminal end buds (TEB), containing cells believed to have progenitor properties. Two important recent reports focus on the development and differentiation of the luminal cells of the mammary gland, not only for the inherent interest in understanding how a major organ of the body induces and maintains differentiation, but also because the study of mammary luminal cells has important clinical ramifications in breast carcinogenesis and therapy[2,3]. Both groups found that Gata-3, a transcription factor, was necessary for development and differentiation of luminal cells.

Zena Werb and colleagues used a microarray strategy to identify genes induced during the development of the mammary gland following puberty[2]. *Gata-3* distinguished itself as being one of the most highly expressed genes in both the ductal and TEB structures, accompanied by a distinct lack of expression in myoepithelial cells. On the other hand, on page 201, Jane Visvader and colleagues focused on *Gata-3* as a gene that is known to exhibit restricted expression in the luminal lineage and demonstrates altered expression in breast cancer[3]. It is well known that Gata-3 has important functions beyond the mammary gland. Gata-3 is an essential transcription factor that was first identified as a regulator of immune cell function and is now known to be involved in the differentiation of several tissue types[4]. Targeted disruption of *Gata-3* results in embryonic lethality and *Gata-3* gene activity has been reported to be important in the development of T-cells, the nervous system, kidneys, fetal liver haematopoiesis and the hair follicle[4].

Both groups conditionally deleted *Gata-3* function in the mammary gland at a number of developmental stages. In embryonic cells, loss of *Gata-3* abrogated formation of a proper gland and was associated with aberrant TEB formation, aberrant invasion into the mammary fat pad and lack of branching morphogenesis, as well as a lack of luminal epithelial cells within the mammary ducts. Furthermore, the Werb group showed that loss of *Gata-3* expression in already established luminal cells resulted in a loss of differentiation through a two-phase response: the first phase manifested as an expanded and disorganized, multilayered epithelium; the second phase progressed to cell detachment and release into the ductal lumen, disruption of the ductal architecture and cell death.

Interestingly, epithelial cells engineered for *Gata-3* deficiency did not transdifferentiate into myoepithelial cells. Transcription factors often exhibit transcriptional cross-antagonism, where a given transcription factor blocks the ability of a cell to adopt an alternate cell fate[5]. However, in this regulatory system, Gata-3 does not seem to act as a transcriptional cross-antagonist. Instead, luminal markers (CK18, oestrogen receptor α (ERα), β-casein, whey acidic protein and E-cadherin) are diminished in an expanding population of cells, which ultimately die. Thus, acute loss of Gata-3 results in an expansion of an epithelial population that lacks markers of luminal or myoepithelial differentiation. The loss of luminal markers from already established luminal epithelial cells indicates that Gata-3 expression is needed for active maintenance of the differentiated luminal phenotype. Finally, both groups demonstrated that lack of Gata-3 during pregnancy impaired lactogenesis. Thus, the conditional elimination of Gata-3 activity at different stages of development demonstrated that Gata-3 has multiple morphological and functional roles during mammary development and pregnancy.

Given the data implicating Gata-3 in development of luminal epithelium and maintenance of the differentiated phenotype, it would be informative to investigate expression of Gata-3 in a stem and/or progenitor population. Identification of a luminal progenitor population has recently been reported[6,7] and expression of luminal markers (such as ERα) is restricted to this $CD29^{lo}$ $CD24^{+}$ $CD61^{+}$ population[3]. In *Gata-3*-deficient mice this luminal progenitor pool increases significantly[3], consistent with the interpretation that loss of Gata-3 leads to a block in the differentiation of luminal progenitors as well as relieving a block to proliferation. Correspondingly, engineered expression of Gata-3 in a mammary stem cell-enriched population induced expression of milk proteins, both in the absence and presence of lactogenic hormones.

These data suggest that a hierarchy of differentiation controlled by Gata-3 exists within the mammary gland. An early bipotent progenitor population gives rise to a luminal progenitor population and a distinct myoepithelial progenitor population[3]. Expression of Gata-3 in the luminal progenitor population permits the expression of differentiated functions and the institution of a proliferative block. On the other hand, the loss of Gata-3 function results in a dramatic decrease in ERα-positive cells. The clinical ramifications of this observation could be profound — designating a human breast cancer as oestrogen receptor-positive or -negative is a major decision point for prognosis and treatment. For prognosis, one of the most informative components of tumour classification systems is designation of grade.

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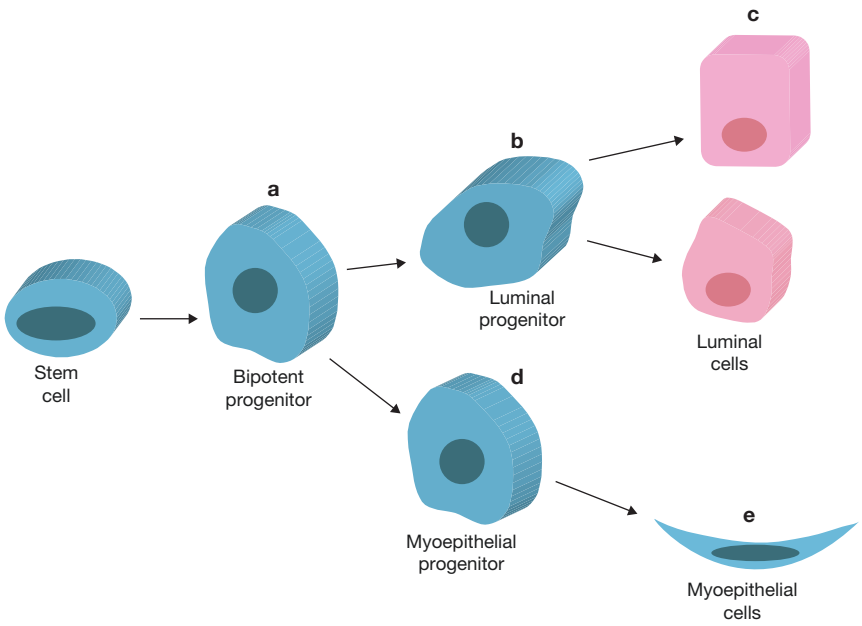

Figure 1 A schematic representation of a proposed epithelial hierarchy for the generation of oestrogen receptor-negative tumours. Cells shaded blue represent oestrogen receptor-negative populations within a normal mammary gland. Cells shaded pink represent populations that can become oestrogen receptor-negative if they lose GATA-3 function. Staining for myoepithelial markers would distinguish **e** from **a**–**c** and staining for CD61 would distinguish **b** from **c**. These insights may provide tools for further stratification of oestrogen receptor-negative tumours.

This component is essentially an estimation of the extent of differentiation within a tumour and has important implications for the future course of the disease. Tumours that are highly differentiated have a more favourable prognosis than those that are less differentiated. Recently, it was reported that tumours expressing oestrogen- and GATA-3 regulated genes exhibit a good prognosis and show significant differences in relapse-free and overall survival compared with tumours that express cell proliferation and anti-apoptosis genes, which have a poor prognosis[8]. Similarly, a recent meta-analysis of published breast cancer cDNA data sets found that low *GATA-3* expression correlated with invasive carcinomas with poor clinical outcome[9]. The authors demonstrated that low GATA-3 expression was associated with higher histologic grade, positive nodes, larger tumour size, oestrogen receptor and progesterone receptor negativity, HER2–neu overexpression and greater risk for recurrence or metastasis. Most importantly, their analysis showed that GATA-3 had independent prognostic significance, above and beyond conventional variables, and suggested that immunohistochemical analysis of GATA-3 may be the basis for a new clinically applicable test to predict tumour recurrence early in the progression of breast cancer[9].

In addition to prognostic utility, understanding the mechanistic basis for the generation of ERα-negative tumours could provide therapeutic targets to some of the most clinically recalcitrant human breast tumours that exist. Currently, our most effective therapies address tumours that are classified as ERα-positive and interrupt oestrogen-dependent functions in proliferation and survival. Any agent that would shift a greater proportion of breast cancers into the oestrogen receptor-positive category or, alternatively, allow us to prevent the formation of ERα-negative tumours could aid in containing this disease.

So how might ERα-negative breast tumours arise? ERα is normally expressed in the luminal epithelial cells of the breast and not the myoepithelial compartment[10]. As loss of Gata-3 function is embryonic lethal (at least in mice) and precludes formation of a mammary gland, it is safe to assume that most ERα-negative tumours are not the result of an initial lack (congenital mutation) of GATA function within the entire gland. Therefore, ERα-negative populations probably result from a focal expansion of a variety of different possible candidates, which include an uncommitted progenitor that has neither luminal nor myoepithelial markers (Fig. 1a), a committed luminal progenitor that has not yet differentiated (Fig. 1b), a fully formed luminal epithelial cell that has lost GATA-3 function and thus lost ERα and luminal markers (Fig. 1c), or myoepithelial lineages (Fig. 1d, e). In most of these cases, incapacitation of apoptotic mechanisms would be necessary to allow the survival of each population. This predicts that ERα-negative tumours would exhibit heterogeneity in the expression of informative markers associated with the different candidate groups mentioned above. In support of this speculation, it was recently reported that myoepithelial markers are expressed in less than one third of ERα-negative invasive breast cancers[10]. This observation is consistent with the hypothesis that these cancers arose from myoepithelial cells (Fig. 1e) and suggests that the remaining two thirds may arise from abrogation in GATA-3 function in the progenitors (Fig. 1a, b, d) or luminal cells (Fig. 1c) . This remains to be tested.

Mutation of *GATA-3* in human breast tumours is an infrequent event occurring in approximately 5% of human tumours[11]. However, inactivation of GATA function can be achieved in a number of fashions: in other types of cancer, *GATA-3* is silenced by DNA hypermethylation of the promoter sequence[12] or histone methylation of the gene[13]. Additionally, post-translational modification of the protein can result in decreased protein stability[14] or increased protein stability[15]. Further studies will determine whether these mechanisms modulate GATA-3 in the generation of ERα-negative breast cancers and whether they warrant a different therapeutic approach.

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