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GATA-3 and the regulation of the mammary luminal cell fate

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Summary

The GATA family of transcription factors plays essential roles in the specification and maintenance of differentiated cell types. GATA-3 was identified in a microarray screen of the mouse mammary gland as the most highly expressed transcription factor in the mammary epithelium and is expressed exclusively in the luminal epithelial cell population. Targeted deletion of GATA-3 in mammary glands leads to profound defects in mammary development and inability to specify and maintain the luminal cell fate in the adult mouse. In breast cancer, GATA-3 has emerged as a strong and independent predictor of tumor differentiation, estrogen receptor status and clinical outcome. GATA-3 maintains tumor differentiation and suppresses tumor dissemination in a mouse model of breast cancer. This review explores our current understanding of GATA-3 signaling in luminal cell differentiation, both in mammary development and breast cancer.

Introduction

A central feature of development is the specification, restriction and maintenance of cell fates from multipotent stem and progenitor cells. From studies in organisms as diverse as sea urchins, flies, and mice, it is becoming clear that the specification of cell fates occurs in part through the activation of gene regulatory networks [1,2]. These networks are made up of hierarchical sets of transcriptional activators and repressors that activate the effector genes of a given cell fate while repressing the gene products of alternate cell fates. A transcription factor at a given node in the network is activated when a combinatorial set of cis-regulatory elements are bound to its promoter, essentially operating as logic gates in the network [3,4]. In embryonic stem cells, these transcription factors are normally quiescent but poised for activation as a result of specific patterns of histone methylation and transcriptional repression [5,6]. The initial specification of cell fate arises in part through juxtacrine and paracrine signaling pathways, such as Wnt and Notch, that activate the initial nodes in the network [1]. These transcription factors then autoactivate in positive-feedback loops and activate the other members in the network in order to establish the differentiated state.

The GATA transcription factors are critical players in the gene regulatory networks that govern the specification of cell fates. The GATA family is composed of six highly conserved transcription factors that bind the DNA sequence (A/T)GATA(A/G) via two zinc-finger domains with the consensus sequence CX₂CX₁₇CX₂C [7]. GATA-1, GATA-2 and GATA-3 are linked to the specification of hematopoietic cell fates, while GATA-4, GATA-5 and GATA-6 play critical roles in the specification of endodermal tissues, including heart and lung. These include the role of GATA-1 in erythropoiesis, GATA-3 in T-cell and Th2 differentiation,

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GATA-4 in cardiac and gastric epithelial differentiation, and GATA-6 in lung epithelial differentiation [8-13]. In several systems, GATA factors specify the fate of a specific cell lineage while repressing alternate lineages. For example, GATA-1 and PU.1 transcription factors are necessary for the specification of the erythroid and myeloid lineages, respectively. These factors exhibit transcriptional cross-antagonism, in that GATA-1 represses PU.1 by interacting with its co-factor c-Jun, while PU.1 represses GATA-1 DNA-binding activity [12,14]. This transcriptional cross-antagonism reinforces cell fate decisions and commits the cells to their respective fates. In T-helper cells, GATA-3 and T-bet specify the Th-2 and Th-1 cell fates, respectively [13,15]. The development of these cell fates depend on the relative levels of these transcription factors, which are reinforced by cell-type specific cytokines and positive-feedback regulatory loops that auto-activate with cell division [16].

GATA-3 in the mammary gland

The mammary gland is a tubular organ that develops postnatally through branching morphogenesis, whereby a branched ductal network extends into a stromal fat pad to form a branched ductal tree [17]. The structure responsible for the branching process is the terminal end bud (TEB), which consists of epithelial progenitor cells. The differentiated mammary gland is composed of two epithelial cell types, the luminal epithelial and myoepithelial cells. It was recently shown that GATA-3 is necessary for the specification and maintenance of the luminal cell fate [18•,19•]. In a microarray screen in which the gene expression profile of pubertal mammary epithelium was compared to epithelium-free stroma, GATA-3 emerged as the most highly enriched transcription factor in the mammary epithelium [18•,20•]. GATA-3 protein was restricted to the luminal cells and was absent in myoepithelial cells. The targeted loss of GATA-3 via MMTV-Cre mediated deletion of floxed-GATA-3 led to profound defects in mammary branching morphogenesis due to the failure of TEB formation, indicating that GATA-3 is necessary for mammary development. Further, the inducible deletion of GATA-3 in adult mammary glands using Cre/lox recombination led to the proliferation of luminal cells with loss of differentiation markers, such as E-cadherin and β -casein. The GATA-3 null cells ultimately detached from the basement membrane and underwent caspase-mediated cell death in the ductal lumen, indicating that GATA-3 is necessary to maintain luminal cell differentiation in the adult mammary gland. As a result, GATA-3 null mammary glands showed profound defects in lobulo-alveolar development and significantly decreased milk protein expression and lactation [18•,19•].

With the *in vivo* importance of GATA-3 established, it was then shown that GATA-3 is necessary for the specification of luminal cells from mammary stem/progenitor cells. The cell surface markers CD24, CD29, and CD61 enrich for mammary stem cells and luminal progenitor cells [21,22]. The targeted deletion of GATA-3 in mammary glands led to a profound expansion in the progenitor population, indicating that luminal cell differentiation is blocked in the absence of GATA-3. The retroviral introduction of GATA-3 in mammary stem cells was sufficient to induce markers of luminal differentiation, including whey acidic protein and β -casein [19•]. This indicated that GATA-3 is necessary and sufficient for luminal cell specification (Figure 1A). To uncover additional transcription factors that may cooperate with GATA-3 in its gene regulatory network, data mining was performed on the mammary epithelial microarray data set. This yielded several epithelial-specific transcription factors, including FOXA1, MSX2, FOXP4, TRPS1, ELF5, EHF, RUNX1, and members of the Id, Irx, Sox, and TCFAP-2 transcription factor families [18•]. Of these, FOXA1 showed a strong positive correlation with GATA-3 in four independent human breast cancer microarray datasets, suggesting that GATA-3 and FOXA1 may be coordinately regulated. Indeed, promoter analysis and chromatin immunoprecipitation indicated that FOXA1 contained a functional GATA-3 binding site, which suggests that FOXA1 may a downstream effector of GATA-3 signaling [18•] (Figure 1B). Other work showed that GATA-3 represses adipocyte differentiation by

inhibiting PPAR γ [23•]. Thus, in addition to activating luminal cell genes, GATA-3 likely represses the gene products of non-luminal cell types in the mammary gland (Figure 1B).

Other transcription factors that are necessary for mammary development and luminal cell differentiation may similarly cooperate with GATA-3 in its gene regulatory network. The homeobox genes *Msx1* and *Msx2* are co-expressed in the post-natal mammary epithelium, and deficiency of *Msx2* leads to arrest in mammary sprouting at E16.5, similar to the mammary phenotype of conditional GATA-3 null mice [24,25]. The Friend of GATA (FOG) transcriptional effectors are multi-zinc finger proteins that bind to the N-terminal zinc finger of GATA factors and regulate their activity [26]. FOG-1 and FOG-2 are necessary for the functions of GATA-1 and GATA-4 during erythropoiesis and cardiac morphogenesis, respectively [26,27]. FOG-1 affects the temporal and transcriptional activity of GATA-1, making it a key regulator of GATA-1 in the erythroid gene regulatory network [28]. In the mammary gland, FOG-2 is upregulated during pregnancy and lactation. The mammary-specific deletion of FOG-2 using WAP-Cre led to accelerated involution of the lactating mammary gland, suggesting that the GATA-3/FOG-2 interaction may be necessary for lobulo-alveolar development [29]. The upregulation of FOG-2 during pregnancy may modify the transcriptional activity of GATA-3 in luminal cells, thereby modifying effector gene activation during lobulo-alveolar development (Figure 1B). Other transcriptional regulators are upregulated in the lactating mammary gland and are necessary for lobulo-alveolar development, including *C/EBP β* , *Id2*, *Stat5a*, *HoxA9*, *HoxB9*, and *HoxD9* [30]. Further work will be necessary to define the interactions between GATA-3 and these transcriptional regulators and to further characterize the GATA-3 gene regulatory network in the mammary gland.

A fundamental topic to be explored is the mechanism by which GATA-3 is activated during the luminal cell specification. In other systems, Notch and Wnt are critical mediators of cell fate decisions and activators of GATA expression. The development of endomesoderm in sea urchins arises through the activation of the Wnt-8/Frizzled/ β -catenin pathway in veg2 endomesodermal progenitor cells. The Wnt pathway activates three transcription factors, including a GATA factor (*gatae*), which autoactivate in positive-feedback loops and then repress Wnt8, making the Wnt pathway a transient specification signal for veg2 cells [1]. In T helper cells, Notch signaling is necessary for the induction of GATA-3 expression and the differentiation of Th2 cells. Notch directly binds to the GATA-3 promoter and synergizes with GATA-3 to promote Th2 effector gene expression [31,32]. In the mammary gland, a role for canonical Wnt signaling has been implicated in mammary epithelial specification. Canonical Wnt signaling is present in mesenchymal cells at 12.5 dpc during the early stages of mammary placode formation and subsequently appears in the developing epithelial buds between 12.5 and 15.5 dpc [33]. Mice deficient in *Lef1*, a member of the *Lef/Tcf* family of canonical β -catenin signaling effectors, have a significantly reduced ability to form mammary epithelial placodes [33,34]. Further work is needed to determine if GATA-3 lies downstream of Wnt signaling in this process and which Wnt factors are responsible for placode formation. Candidate Wnt factors include Wnt-2, Wnt-5a, and Wnt-7b, which are enriched in mammary terminal end buds, while Wnt-4, Wnt-5b, and Wnt-6 are expressed throughout the mammary epithelium [20•,35].

GATA-3 in breast cancer

Breast cancers are luminal epithelial neoplasias that are clinically subdivided on the basis of several tumor markers, including estrogen receptor (ER), progesterone receptor (PR), and HER2/Neu. A series of expression profiling studies of human breast tumors have identified additional tumor markers that predict tumor behavior and clinical outcome. Six independent microarray studies of 369 primary breast tumors found that positive GATA-3 expression is

among the best predictors of ER positive status [36•-41•]. In two studies, artificial neural network and correlative gene analysis identified GATA-3 as the third best and best predictor of ER status, respectively [36•,40•]. Additional microarray studies showed that human breast cancers can be classified into five distinct sub-groups, including the Luminal A, Luminal B, ERBB2, basal-like, and normal breast sub-classes [41•-43•]. In these studies, GATA-3 expression was strongly correlated with the Luminal A sub-type of breast tumors, which expressed luminal differentiation markers and carried the best prognostic outcome among the sub-classes. Meta-analysis of four microarray datasets indicated that GATA-3 was a strong predictor of clinical outcome in breast tumors, with independent prognostic significance above conventional variables [44•]. Low GATA-3 expression was strongly predictive of poor clinical outcome, high tumor grade, positive lymph node status, and large tumor size. Log-rank analysis of a microarray dataset revealed that GATA-3 expression was the second best predictor of clinical outcome among 8024 genes, with its low expression indicating a poor prognosis [45•]. Additionally, two tissue microarray studies of 389 primary breast tumors showed that GATA-3 status carried strong prognostic significance in breast cancer [44•,46]. Rare functional mutations of GATA-3 have also been reported in primary breast tumors [47]. These studies have implicated a role for GATA-3 in breast cancer biology and prognosis, but functional studies that define that role are lacking.

Using the MMTV-PyMT mouse model of breast cancer, we have investigated the role of GATA-3 in breast cancer pathogenesis and progression [48•]. MMTV-PyMT mice develop luminal breast cancers that progress from hyperplasias to carcinomas, which reliably model human breast cancer [49,50]. We isolated focal, GFP-positive hyperplasias from a 3-week-old MMTV-PyMT mouse and transplanted them into wild-type mice to follow tumor progression. Between 5 and 8 weeks post-transplant, tumor outgrowths displayed histologic progression from adenoma to carcinoma and onset of tumor dissemination to distant sites. Between 15 and 18 weeks post-transplant the outgrowths progressed to late carcinoma and acquired metastatic capability. This model allowed the separation of the processes of tumor dissemination and metastasis and allowed the dissection of the individual steps during malignant progression.

Microarray analysis revealed that loss of luminal differentiation genes highly correlated with tumor progression in the model. Among these genes, GATA-3 expression was lost early in tumor progression during the transition from adenoma to early carcinoma and the onset of tumor dissemination. The retroviral restoration of GATA-3 in late carcinomas was sufficient to induce luminal differentiation markers, such as β -casein [48•], similar to observations in mammary epithelial cells and cell lines [19•,47]. The GATA-3-infected tumors showed a significantly reduced ability to disseminate to distant sites. Significantly, the inducible deletion of GATA-3 in well-differentiated tumors led to caspase-mediated cell death, suggesting that loss of GATA-3 is not sufficient to promote malignant progression. Instead, a GATA-3 negative stem cell-like tumor cell population persisting in early tumors expanded during tumor progression and was responsible for the transition to the GATA-3 negative state. These data indicate that GATA-3 is a critical regulator of tumor differentiation and suppressor of tumor dissemination and that its lack of expression underlies the early stages of breast cancer progression.

A central question to be addressed is how GATA-3 and ER cooperate in mammary development and breast cancer. Though GATA-3 and ER are strongly correlated in breast cancers, ER is expressed in 40-50 percent of luminal cells and in fibroblasts and other stromal cells of the mammary gland [51]. Estradiol stimulation of mammary epithelial cells is not sufficient to induce GATA-3, suggesting that GATA-3 is not a downstream target of ER [39•]. However, GATA-3 is necessary for ER α signaling in the T47D cell line [52]. The targeted deletion of ER in mice led to the inability to form TEBs, similar to the GATA-3 conditional knockout mice. However, luminal differentiation genes were still expressed in the absence of ER α .

[53]. Further, ER activity induces cellular proliferation, while GATA factors are implicated in cellular quiescence [18••,54,55]. It appears that the GATA-3 and ER pathways may have non-overlapping functions in mammary luminal cells, and these functions may be functionally linked by transcriptional regulators. A candidate linker is FOXA1, which is necessary for the transcriptional activity of ER α and its binding to estrogen response elements in target gene promoters [56,57]. As FOXA1 may also be a downstream effector of GATA-3, FOXA1 may be a bridge between the GATA-3 and ER pathways [18••] (Figure 1B). Further work will be necessary to define the interactions between these transcriptional regulators and to delineate their roles in the gene regulatory network of the luminal cell.

Perspectives

The role of GATA-3 in the differentiation of the mammary luminal cell adds to the growing body of evidence implicating the GATA family of transcription factors as key regulators of cell fate specification and maintenance. GATA-3 promotes the differentiation of luminal cells, while repressing other cell types in the mammary gland, such as adipocytes. The transcription factors that promote the differentiation of myoepithelial cells in the mammary gland have yet to be discovered. Compared to other systems, the mammary gland offers several advantages for the further study of these processes, including the simplicity of the organ, the presence of only two epithelial cell types, and the ease of experimental manipulation. Understanding how GATA-3 regulates luminal cell differentiation will shed further light on its role in breast cancer, where its importance as a prognostic factor and predictor of tumor grade and differentiation status has been underscored by a multitude of gene expression profiling studies. GATA-3 defines a distinct class of cancer related genes that are differentiation factors, rather than conventional tumor suppressor genes. It maintains the differentiated state in the pre-cancerous state and its activation enforces differentiation into a luminal cell fate, reversing the malignant phenotype. Uncovering the paracrine or juxtacrine signals that activate GATA-3 during luminal cell specification may offer therapeutic strategies for inducing GATA-3 and cellular differentiation in mammary adenocarcinomas. The prospect of a differentiation therapy of breast cancer awaits further mechanistic understanding of GATA-3 in mammary luminal cells.

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Figure 1.

(A) Schematic representation of the role of GATA-3 in specifying and maintaining the luminal cell fate in the mammary gland.

(B) A simplified gene regulatory network depicting the known interactions between GATA-3 and other transcriptional regulators in the mammary gland. Not shown are transcription factors that are essential for lobulo-alveolar development, including C/EBP β , Id2, Stat5a, HoxA9, HoxB9, and HoxD9.