Published in final edited form as:

J Cell Physiol. 2010 January ; 222(1): 42–49. doi:10.1002/jcp.21943.

GATA3 in Development and Cancer Differentiation: Cells GATA Have It!

Jonathan Chou^{1,2}, Sylvain Provot¹, and Zena Werb^{1,2,*}

¹Department of Anatomy, University of California, San Francisco, California

²Program in Biomedical Sciences, University of California, San Francisco, California

Abstract

There is increasing evidence that the numerous mechanisms that regulate cell differentiation during normal development are also involved in tumorigenesis. In breast cancer, differentiation markers expressed by the primary tumor are routinely profiled to guide clinical decisions. Indeed, numerous studies have shown that the differentiation profile correlates with the metastatic potential of tumors. The transcription factor GATA3 has emerged recently as a strong predictor of clinical outcome in human luminal breast cancer. In the mammary gland, GATA3 is required for luminal epithelial cell differentiation and commitment, and its expression is progressively lost during luminal breast cancer progression as cancer cells acquire a stem cell-like phenotype. Importantly, expression of GATA3 in GATA3-negative, undifferentiated breast carcinoma cells is sufficient to induce tumor differentiation and inhibits tumor dissemination in a mouse model. These findings demonstrate the exquisite ability of a differentiation factor to affect malignant properties, and raise the possibility that GATA3 or its downstream genes could be used in treating luminal breast cancer. This review highlights our recent understanding of GATA3 in both normal mammary development and tumor differentiation.

One of the classical “hallmarks of cancer” is the ability for the tumor to invade and metastasize (Hanahan and Weinberg, 2000). This complex process includes several steps, including recruiting blood vessels, intravasation into the circulation, scattering to distant tissues, extravasation into the parenchyma of a new organ, and subsequent colonization and growth (Nguyen et al., 2009). Pathologists have long recognized the intimate connection between tumor progression and its differentiation status. Well-differentiated tumors are generally less advanced and carry a better prognosis whereas poorly differentiated tumors are generally more aggressive and carry a worse prognosis (Kufe and Bast, 2003). It has been postulated that cancer cells selectively turn on the expression of embryonic morphogenesis regulators to undergo the epithelial-mesenchymal transition (EMT) and concomitantly turn off programs that maintain their differentiated state (Yang and Weinberg, 2008). Interestingly, in addition to gaining motility, the acquisition of an EMT-like state generates cells with properties of stem cells (Mani et al., 2008). In recent years, these embryonic factors have been found to confer malignant traits such as invasiveness and resistance to apoptosis to neoplastic cells (Gupta et al., 2009). This observation has led to the hypothesis that there might exist less-differentiated stem-like cells within solid tumors, which have been referred to as cancer stem cells or tumor-initiating cells capable of self-renewal and giving rise to the entire tumor (Visvader and Lindeman, 2008). These

considerations illustrate the fact that the key regulatory mechanisms controlling normal embryonic development (EMT, stem cell differentiation and others) are critical players during tumor progression. They also underscore the importance of identifying the overlapping molecular programs that are shared in these two cellular processes in order to understand how cancers develop and metastasize.

A fundamental aspect of development is the specification and maintenance of differentiated cell types arising from multipotent progenitor cells. The specification of cell fate is mediated in part by hierarchical networks of transcription factors and *cis*-regulatory elements that control their expression. Transcription factors are often organized in multi-gene families and play essential roles in activating target genes of specific cell fates and in repressing target genes of alternate cell fates. The GATA family of transcription factors, of which there are six in mammals, are such master regulators. The GATA factors share common features: they contain two transactivation domains at the amino terminus, two zinc fingers at the carboxyl terminus and a conserved basic region that is located immediately after each zinc finger motif (Fig. 1). GATA family members bind to a consensus DNA sequence (A/T)GATA(A/G) in the promoters of genes to directly activate or repress expression of target genes. In addition, GATA factors recruit chromatin remodeling complexes to remodel gene loci (Takemoto et al., 2002; Zhou and Ouyang, 2003; Yamashita et al., 2004). At the amino acid level, the family members share varying degrees of homology. For example, while GATA2 and GATA3 are about 55% homologous, GATA3 and GATA4 are only 20% homologous. However, the zinc finger motifs are about 80% homologous among all the six members.

Each specific GATA member is also highly conserved across vertebrate species. GATA3 homologs are found in human, chimpanzee, dog, mouse, rat, chicken, zebrafish, frog, and fruit fly. Between human and mouse, GATA3 shares 97% amino acid identity. In addition, structural analysis of GATA3 suggests that the GATA members can homo- or heterodimerize to bind DNA, or directly bind DNA via its two zinc fingers (Bates et al., 2008).

GATA factors are expressed in a tissue-specific manner. GATA1 and GATA2 are expressed primarily in hematopoietic cells, whereas GATA4, GATA5, and GATA6 are expressed in mesoderm- and endoderm-derived tissues such as the heart, liver and intestines. GATA3 is present in both hematopoietic (e.g., T cells) and non-hematopoietic tissues, including the kidney, central nervous system, skin and mammary gland (Kaufman et al., 2003; Kourou-Mehr et al., 2006; Grote et al., 2008). Although these transcription factors have a restricted expression pattern, their functions are partially interchangeable. For example, the *Gata1* null defects in erythroid cells can be partially rescued by knocking in *Gata3* into the endogenous *Gata1* locus (Tsai et al., 1998; Takahashi et al., 2000). In addition, GATA1, -2, -3, and -4 can all activate expression of interleukin-4 (IL-4) and IL-5, a GATA3 target gene in T cells, as well as repress interferon- γ (IFN γ) (Ranganath and Murphy, 2001). This limited capacity for GATA factors to substitute functionally for each other suggests that the cellular context in which each GATA factor is expressed is important. However, since GATA3 is unable to fully rescue the *Gata1*-null phenotype, it suggests that the redundancy is incomplete, and that despite being highly homologous proteins, each GATA factor retains distinct functions.

Numerous studies using loss- and gain-of-function approaches have shown that GATA family members are crucial transcriptional regulators during the development of a variety of tissues. With the exception of *Gata5*, null mutations for each of the *Gata* genes results in embryonic lethality in mice, underscoring their pivotal roles during development. Furthermore, it was recently shown that GATA4, in combination with another transcription factor Tbx5 and a chromatin-remodeling protein Baf60c, can direct ectopic differentiation of mouse mesoderm into beating cardiomyocytes (Takeuchi and Bruneau, 2009). These studies

all point to the importance of GATA family members in various aspects of development and cell differentiation.

In this review, we highlight the recent understanding of GATA3 both in mammary gland development as well as in breast cancer differentiation and metastasis. While we will briefly touch on aspects of GATA3 in other systems such as the immune system and skin, we refer the reader to other recently published reviews for a more in depth discussion on those topics (Ho and Pai, 2007; Ho et al., 2009).

GATA3 Contributes to the Normal Development of a Variety of Tissues

GATA3 is a critical regulator in both mouse and human development. *Gata3* null embryos die between E11 and E12 due to internal bleeding, and display growth retardation, deformities in the brain and spinal cord, and gross aberrations in fetal liver hematopoiesis, suggesting that this gene is important in the development of various systems (Pandolfi et al., 1995). Haploinsufficiency of *GATA3* results in Barakat syndrome in humans, characterized by familial hypoparathyroidism, sensorineural deafness and renal dysplasia (also known as HDR syndrome), and can be caused by mutations in *GATA3* that render it physically or functionally inactive. Indeed, several mutations causing Barakat syndrome have been mapped to the critical zinc fingers and adjacent regions that mediate binding to DNA (Van Esch et al., 2000). Interestingly, mutations that abrogate the DNA-binding ability of *GATA3* are also found in human breast cancer specimens (Usary et al., 2004).

GATA3 can undergo several post-translational modifications. The KRRLSA motif found in between the two zinc fingers has sites for acetylation and phosphorylation. The finding that the hypoacetylated KRR mutant of *GATA3* functions as a hypomorph underscores the importance of these residues. Additionally, the Ras-ERK MAPK cascade regulates *GATA3* stability in T cells through inhibition of the Mdm2 E3 ligase and the ubiquitin-proteasome system (Yamashita et al., 2005).

For *GATA3* to regulate gene expression, it must translocate from the cytoplasm into the nucleus to access its target genes. *GATA3* contains a classical nuclear import signal, and is transported into the nucleus by importin- α (Yang et al., 1994). The affinity of *GATA3* to importin- α is regulated by phosphorylation, which is mediated by p38 mitogen-activated protein kinase (MAPK) and serves to enhance nuclear transport (Goldfarb et al., 2004; Maneechotesuwan et al., 2007). Interestingly, corticosteroids, which are commonly used to treat allergic disease, have a potent inhibitory effect on *GATA3* in T cells by competing for importin- α , and by inducing the expression of a p38 MAPK inhibitor (Maneechotesuwan et al., 2009).

Given that *GATA3* is expressed in such a wide variety of tissues, it is not surprising that the context of *GATA3* expression is critical. This is in part mediated by interactions with other protein partners, which may help direct *GATA3* to cell-specific targets or modify *GATA3* function. These proteins include Smad3, a component of the TGF β signaling pathway, which is important for *GATA3* to regulate the expression of T helper cell 2 (T_H2) cytokines (Blokzijl et al., 2002). In addition, in thymocytes, *GATA3* binds to the Friend-of-GATA proteins 1 and 2 (FOG1 and FOG2) and PU.1 (Zhou et al., 2001; Nesbit et al., 2004; Chang et al., 2005). These partners may function as co-factors to facilitate *GATA3* activity, or function to sequester *GATA3* from binding DNA. Because many of these studies have been conducted in T cells, it is unknown whether these regulatory mechanisms and binding partners can be generalized to other cell types, or whether other tissue-specific binding partners exist.

The function of GATA3 has been most extensively studied in T cell development. These studies demonstrate that GATA3 is involved in various aspects of thymocyte development, and emphasize the notion that GATA3 levels are carefully titrated throughout thymocyte development (Ho et al., 2009). Levels that are too low result in developmental failure, while levels that are too high are cytotoxic. Furthermore, inappropriate expression of GATA3 during thymocyte development can divert the development of thymocyte progenitors into alternative lineages (i.e., mast cells) in a Notch-dependent manner (Taghon et al., 2007).

During their development, thymocytes are specified to be single-positive CD4⁺ (helper T cells, or T_H cells) or CD8⁺ (cytotoxic T cells). The T_H cell population is then further subdivided into T_H1 and T_H2 cells (although other effector cell types such as T_H17 cells have recently been characterized). Importantly, the specification of T_H2 fate depends on GATA3, whereas the T box family transcription factor T-bet specifies the T_H1 lineage (Grogan and Locksley, 2002). Recently, *Gata3* was also shown to be a downstream target of Notch; Notch-mediated differentiation of T_H2 cells depends on GATA3. In fact, in the absence of GATA3, Notch turns from a T_H2 inducer into a powerful inducer of T_H1 differentiation (Amsen et al., 2007; Fang et al., 2007). GATA3 also plays a role in chromatin remodeling to allow T_H2 transcriptional factors access to the DNA locus (Lee et al., 2001; Avni et al., 2002). In addition, GATA3 and T-bet cross-antagonize each other to repress the alternate cell fate. For example, GATA3 inhibits the expression of IL-12-induced *Ifn γ* , a classical T_H1 cytokine, and directly transactivates *Il-5* and *Il-13* to reinforce the T_H2 cell choice (Ouyang et al., 1998; Ansel et al., 2006). These studies suggest that GATA3 has a fundamental role in thymocyte developmental lineage choice, survival and maintenance.

Enforced expression of *Gata3* during T cell development induces CD4⁺CD8⁺ double-positive (DP) T cell lymphoma. The malignant transformation involves cooperation with c-Myc. The lymphoma cells also exhibit activating Notch1 mutations, which result in high expression of Notch targets. Therefore, *Gata3* over-expression converts DP thymocytes into a pre-malignant state, characterized by high c-Myc expression, whereby subsequent induction of Notch1 signaling leads to fully transformed thymocytes (van Hamburg et al., 2008). This example illustrates the dual role of GATA3 in both physiologic development and cancer biology, and underscores the importance of carefully titrating GATA3 levels during development.

GATA3 also plays important roles in skin and hair follicle development. In the skin, epithelial cells undergo an upward differentiation process to give rise to the different hair follicle lineages such as the medulla, cortex and cuticle of the hair shaft and the inner root sheath (IRS) (Fuchs, 2007). GATA3 promotes differentiation of the IRS cell lineage, and loss of GATA3 in the skin using a lacZ knock-in that disrupts the *Gata3* allele results in an expansion of IRS precursors and a paucity of differentiated IRS cells (Kaufman et al., 2003). Two other groups have deleted *Gata3* specifically in the epidermis and hair follicles using keratin-14-Cre (K14-Cre). These conditional knockout (CKO) mice display delayed hair growth and maintenance, abnormal hair follicle organization, and defects in skin differentiation (de Guzman Strong et al., 2006; Kurek et al., 2007). The *Gata3*-CKO mice have desiccated skin that leads to a defective skin barrier and also exhibit an increase in basal epidermal cell proliferation, even though the mice are bald. Transcriptional profiling of *Gata3*-CKO mice shows a defect in lipid biosynthesis due to loss of the lipid acyltransferase gene *Agpat5*, which was identified as a direct GATA3 target.

In addition to its roles in T cell and skin development, GATA3 has different functions in several other tissues. In particular, GATA3 also plays important roles in the development of the nervous system (Lim et al., 2000; Moriguchi et al., 2006; Hong et al., 2008; Zhao et al., 2008; Jones and Warchol, 2009), kidney (Grote et al., 2008), lens fiber cells in the eye

(Maeda et al., 2009), and the mammary gland. For example, inactivation of *Gata3* in the nephric duct leads to ectopic ureter budding during development, resulting in a spectrum of urogenital malformations. In addition, embryonic lethality due to loss of *Gata3* has been hypothesized to be caused by noradrenaline deficiency in the sympathetic nervous system (Lim et al., 2000). These diverse roles of GATA3 are summarized in Table 1. Its role in the development of the mammary gland is discussed in detail in the following section.

GATA3 Is Critical for the Normal Development of the Mammary Gland

The mammary gland is composed of mammary epithelial cells, as well as various stromal cells such as adipocytes, fibroblasts, macrophages and mast cells. The epithelium consists of a dual layer of epithelial cells that originate from a common progenitor but are specified by distinct pathways, similarly to the T_H1/T_H2 system. The luminal epithelial cells line the ductal epithelium, secrete milk proteins and express GATA3. These cells are surrounded by a basal layer of myoepithelial cells, which do not express GATA3 (Fig. 2A). These differentiated cell types arise from a multipotent progenitor population that has been recently characterized (Shackleton et al., 2006; Stingl et al., 2006). Interestingly, the GATA3-negative basal cell population contains the progenitor cell pool, among other cell types (Asselin-Labat et al., 2007). This is consistent with the model that less committed progenitors do not express GATA3, a factor that promotes luminal cell differentiation and maintenance.

In addition to the epithelial cells, GATA3 is expressed in the white adipocyte precursors present in the mammary gland. But in contrast to the positive role of GATA3 in thymocyte and epithelial cell differentiation, down-regulation of GATA3 in these cells leads to adipocyte differentiation, while constitutive GATA3 expression suppresses adipocyte differentiation and prevents cells from developing beyond the preadipocyte stage. This is mediated in part through direct suppression of peroxisome proliferator-activated receptor γ (*Ppar* γ). Thus, GATA3 regulates the preadipocyte-adipocyte developmental transition (Tong et al., 2000).

Prior to puberty, the mammary gland is a rudimentary organ consisting of a primitive network of ductal epithelium. Shortly after the onset of puberty, specialized structures known as terminal end buds (TEBs) develop at the invading epithelial tips of the mammary epithelium (Fig. 2A). TEBs contain an outer layer of cap cells, which are believed to be myoepithelial progenitors, and a multilayered inner core of body cells, which contains the luminal cell progenitors. The TEBs proliferate, bifurcate, and invade into the fatty stroma of the mammary gland in a process known as branching morphogenesis. In the mouse, this process continues until 10–12 weeks of age, when the mature ductal tree is established and completely fills the length of the fat pad (Smalley and Ashworth, 2003; Sternlicht et al., 2006).

Microarray profiling of the TEBs versus mature epithelial ducts versus the stroma shows that GATA3 is the most highly expressed transcription factor in the mammary epithelium (Kouros-Mehr and Werb, 2006). Using a mammary epithelium-specific knockout of *Gata3*, we and others have shown that GATA3 is necessary for mammary gland development (Kouros-Mehr et al., 2006; Asselin-Labat et al., 2007). Early deletion of *Gata3* specifically in the mammary epithelium using the murine mammary tumor virus (MMTV) promoter-Cre recombinase (MMTV-Cre) shows that prior to puberty, the rudimentary mammary gland is similar to wild-type littermates. However, with the onset of puberty, the mammary glands of *Gata3*-CKO mice fail to develop TEBs. In addition, the epithelium fails to invade into the stroma (Fig. 2B). When examined 5 weeks later, the outgrowths display gross structural defects, including irregular luminal diameters and deficiencies in side branching. Taken

together, this suggests a role for GATA3 in ductal elongation and branching. Furthermore, the ductal epithelium that forms contains regions that lack luminal cells and regions that contain a multilayered luminal epithelium. Surprisingly, these outgrowths, which express Cre recombinase and have undergone recombination, retain a functional *Gata3* allele, suggesting that there is a selective pressure to maintain GATA3 expression during development (Kouros-Mehr et al., 2006; Asselin-Labat et al., 2007).

Deletion of *Gata3* in the adult mammary gland after development has taken place using a doxycycline-inducible system reveals severe cellular defects in the luminal epithelium, including de-differentiation of the luminal cells, disorganization of the duct, a decrease in cell-cell adhesion and an increase in cell proliferation. These characteristics are strongly reminiscent of cancer cell properties important for cell invasion and metastasis. In this case, cell detachment into the lumen is followed by widespread cell death, likely due to the lack of basement membrane-derived survival signals. However, acute loss of *Gata3* leads to an expansion of a de-differentiated luminal cell population prior to cell death. Further analysis shows that these *Gata3*-deleted cells retain luminal character and do not transdifferentiate into myoepithelial cells. Long-term loss of *Gata3*, however, leads to caspase-mediated luminal cell death and lactational insufficiency. Remarkably, introduction of *Gata3* into a purified mammary progenitor-enriched population induces luminal cell differentiation. This suggests that GATA3 is necessary in the adult mammary gland to maintain the integrity and function of the luminal epithelium and sufficient to specify the luminal cell fate (Kouros-Mehr et al., 2006; Asselin-Labat et al., 2007).

How acute loss of *Gata3* in the mammary gland might lead to expansion of a de-differentiated epithelial cell population remains unknown, but this feature is also observed in the skin and the lens (Kurek et al., 2007; Maeda et al., 2009). These studies suggest that there is a complex relationship between differentiation and regulation of the cell cycle. One might expect that GATA3 normally represses the cell cycle so that acute loss of GATA3 results in increased proliferation. However, a recent study shows that in contrast, GATA3 normally represses the cyclin inhibitor *p18^{INK4C}* (Pei et al., 2009). Low GATA3-expressing cells such as luminal progenitors express a high level of *p18^{INK4C}*, which functions to restrain progenitor cell proliferation. Thus, the mechanisms of how a GATA3-negative pool of cells expands and how GATA3 might regulate aspects of the cell cycle remain open questions.

Several direct downstream targets of GATA3 in the luminal epithelium have been identified. They include genes such as *Foxa1*, an important regulator of estrogen receptor (ER) expression, mucin, which may play a role in epithelial polarity, and the cyclin inhibitor *p18^{INK4C}*, which has been suggested to restrain luminal cell progenitors (Abba et al., 2006; Kouros-Mehr et al., 2006; Pei et al., 2009). Bioinformatic analysis of a mammary epithelial microarray dataset reveals additional epithelial-specific transcription factors that may cooperate with GATA3 in its gene regulatory network, which include MSX2, FOXP4, TRPS1, ELF5, EHF and RUNX1 (Kouros-Mehr and Werb, 2006).

GATA3 in Tumor Differentiation and Metastasis

Given the fundamental role of GATA3 in maintaining the differentiation and adhesion of the luminal epithelial cell, we and others hypothesized that loss of *Gata3* is causally involved in the pathogenesis of breast cancer. In the clinical setting, breast tumors are commonly subdivided into estrogen receptor positive (ER+) and negative (ER-) tumors. Whereas ER+ tumors tend to be morphologically well differentiated and exhibit a relatively good prognosis, ER- tumors are poorly differentiated and exhibit a poor prognosis. To date, in a series of eleven independent microarray gene expression profiling studies of ER+ and ER-

breast tumors, GATA3 emerges as a strong prognostic indicator of breast cancer. Low GATA3 expression is strongly associated with higher histologic grade, poor differentiation, positive lymph nodes, ER- and progesterone receptor (PR) negative status, and HER2/neu overexpression, all indicators of poor prognosis (Perou et al., 2000; Sorlie et al., 2001; Jøssens et al., 2002; Sorlie et al., 2003; Mehra et al., 2005). While two tissue microarray studies have suggested that GATA3 status has independent prognostic significance in breast cancer (Mehra et al., 2005; Dolled-Filhart et al., 2006), this matter is still controversial. One study shows that while GATA3 expression has a strong association with ER, in a multivariate analysis of over 3,100 cases of invasive ductal carcinoma, GATA3 lacks independent prognostic value (Voduc et al., 2008), despite being associated with ER+ expression (Hoch et al., 1999). Nonetheless, the highest levels of GATA3 are observed in the “luminal A” subtype of breast cancer, which expresses luminal differentiation markers such as ER/PR and has the best prognostic outcome. Given the various subtypes of cancer, it is possible that GATA3 plays a different role in basal versus luminal type tumors.

GATA3 levels in various human breast cancer cell lines inversely correlate with their metastatic capability. Metastatic cell lines such as the MDA-MB-231 cells have low GATA3 levels, whereas non-metastatic cell lines such as the MCF7 cells have high GATA3 levels (Kouros-Mehr et al., 2008). Furthermore, in several mouse models, including the MMTV-PyMT (polyoma middle T antigen) and MMTV-Neu models which develop luminal breast cancer, loss of GATA3 correlates with loss of differentiation genes, the transition from adenoma to early carcinoma and the onset of tumor dissemination. The MMTV-PyMT model recapitulates many characteristics of human disease including the progression to metastasis. (Lin et al., 2003). Late carcinomas and metastases invariably do not express GATA3. Interestingly, using a tumor transplant model, our laboratory showed that re-introduction of *Gata3* using a retrovirus into MMTV-PyMT late carcinoma cells that are then transplanted orthotopically into the mammary fat pad is sufficient to differentiate the tumor cells (Fig. 3). The tumor cells not only form lumens, but also express β -casein and basement membrane components such as perlecan, suggesting that the cells exhibit apical-basal polarity. Strikingly, re-introduction of *Gata3* suppresses tumor metastases to the lungs by over 25-fold, linking GATA3 to both tumor differentiation and metastasis (Kouros-Mehr et al., 2008). In addition, mutations in the zinc finger domains of GATA3, which diminish or abolish the ability of GATA3 to bind DNA, have also been identified in a subset of human breast cancers, further underscoring the importance of GATA3-regulated genes in breast cancer (Usary et al., 2004).

Whether GATA3 suppresses organ-specific metastasis is another topic of interest. We and others have shown that GATA3 suppresses pulmonary metastases from mouse and human mammary tumors (Kouros-Mehr et al., 2008; Dydensborg et al., 2009). To extend these studies from the MMTV-PyMT mouse model to human cells, Dydensborg and colleagues expressed *GATA3* in the human-derived MDA-MB-231 LM2 cell line, which was generated by repeated rounds of in vivo selection for lung tropism (Minn et al., 2005). These authors demonstrate that while expression of GATA3 does not affect the number of spontaneous lung metastases when the cells are implanted orthotopically in the mammary fat pad, there is a reduction in the number of cells that survive in the lungs when the cells are injected intravenously (IV) into the tail vein. In accordance with our previous work, this suggests that GATA3 affects tumor cell survival at distant sites such as the lung. However, in this experimental model, the authors did not notice an increase in differentiation genes. This is possibly due to the use of MDA-MB-231 cells, which are an undifferentiated, aneuploid, triple negative (ER-/PR-/HER2-) cell line. To further understand the effects of GATA3 on the LM2 cells, the authors performed microarray analysis to identify genes that are up- and down-regulated in the MDA-MB-231 control tumors versus the *GATA3*-expressing tumors. Interestingly *ID1* and *ID3*, which previously were shown to promote breast cancer

metastasis by facilitating sustained proliferation during early stages of metastatic colonization (Gupta et al., 2007), are significantly down-regulated in *GATA3*-expressing cells. Whether *GATA3* directly controls the expression of these genes, however, remains to be determined. Additional work will be required to determine if *GATA3* suppresses metastases to other organs commonly affected in humans such as the bone or brain.

Does the loss of *GATA3* expression in breast cancer induce tumor de-differentiation so that cells expand and acquire EMT-like characteristics? Since the loss of *GATA3* in the mammary gland causes luminal cell proliferation and basement-membrane detachment, similar mechanisms may exist in breast cancer to accelerate malignant conversion and gain metastatic capability. However, when *Gata3* is deleted in early, well-differentiated tumors in the MMTV-PyMT model, the cells undergo caspase-mediated cell death, similar to deletion in normal epithelium. This suggests that premature loss of *GATA3* is not sufficient to promote malignant progression, and is not tolerated in early tumors. Instead, a *GATA3*-negative, stem cell-like tumor population persisting in early tumors expands during tumor progression and is likely responsible for the transition to the *GATA3*-negative state. Consistent with this notion, adenoma and carcinoma cells are progressively enriched for cell-surface markers of mammary stem-like cells. In addition, this stem-like population appears to be more motile when compared to the differentiated cells. Thus, although progression to a *GATA3*-negative state underlies the onset of tumor dissemination, further events are likely necessary for successful formation of metastases from disseminated cells. These data indicate that *GATA3* is a crucial regulator of tumor differentiation and suppressor of tumor metastasis.

In luminal progenitor cells, *GATA3* directly represses *p18^{INK4C}* to regulate the cell cycle, and therefore, the levels of *GATA3* must be carefully titrated in these cells (Pei et al., 2009). Low *p18^{INK4C}* and high *GATA3* expression, which would result in increased cell proliferation, are simultaneously observed in luminal A breast cancer. Mice deficient for *p18^{INK4C}* have an expanded luminal progenitor population throughout life and develop ER+ luminal tumors at a high penetrance. Because *GATA3* is expressed, however, these cells differentiate into luminal epithelial cells, resulting in a well-differentiated tumor with a good prognosis (Pei et al., 2009). In contrast, the absence of *GATA3* in a tumor results in a slowly proliferating but undifferentiated cancer. Thus, *GATA3* is required not only for differentiation, but also to regulate cell proliferation in the mammary gland. However, the relationship between cell proliferation and *GATA3* is likely more complex, and may depend on the differentiation status of the cell. Interestingly, luminal progenitors have also been suggested to be the candidate target cell population for basal tumor development in *BRCA1* mutation carriers (Lim et al., 2009).

In addition to regulating cell differentiation, adhesion and proliferation, *GATA3* may influence tumor progression and metastasis in an indirect mechanism by affecting the microenvironment. The microenvironment plays important roles during both normal and cancer development (Coussens and Werb, 2002). For example, macrophages found in the stroma immediately adjacent to the TEB help form and are often associated with collagen fibers to facilitate ductal branching; ablation of macrophages delays outgrowth and branching. Macrophages are also found within the TEB, where they clear apoptotic epithelial cells as the lumen is formed (Pollard, 2009). In several mouse models of breast cancer including the MMTV-PyMT model, the loss of *GATA3* coincides with the onset of angiogenesis and an increased number of tumor-associated macrophages (TAMs). Analysis of MMTV-PyMT mammary tumors reveals a progressive increase in macrophages during tumor development. These macrophages regulate the angiogenic switch to promote tumor angiogenesis (Lin et al., 2006; DeNardo et al., 2009). These observations are interesting given the pro-tumorigenic roles of TAMs in a number of cancers; a high density of TAMs in

human tumors correlates with poor prognosis in more than 80% of cases (Bingle et al., 2002). In accordance with this, experimental evidence shows that suppressing macrophage growth factors such as CSF1 can delay tumor progression and inhibit metastasis (Lin et al., 2001). In light of these studies, and the observation that GATA3 may repress components of the interferon response signature associated with metastasis (Dydensborg et al., 2009), one could speculate that GATA3 may affect tumorigenesis and metastasis by influencing the tumor microenvironment. However, an understanding of whether this is a primary or secondary effect (e.g., due to inducing differentiation) awaits further study.

Identifying GATA3-Regulated Targets

An important goal to understanding the diverse roles of GATA3 on various cell types remains to identify downstream targets that mediate the effects of GATA3. How does GATA3 control cell fate decisions and what downstream targets are important for it to do so? One essential step will be to conduct a comprehensive analysis of GATA3 binding sites by chromatin-immunoprecipitation (ChIP) followed either by microarray profiling (ChIP on chip) or by deep sequencing (ChIP-Seq).

One potential set of GATA3 targets is microRNAs (miRNAs). miRNAs are small, non-coding RNAs that serve to modulate gene expression post-transcriptionally by either inhibiting translation of their specific targets or causing degradation of the target mRNAs. miRNAs play a role in the maintenance of mouse mammary epithelial progenitor cells, and miRNAs such as let-7 promote mammary differentiation (Ibarra et al., 2007). Not surprisingly, miRNAs are also important regulators of tumor progression and metastasis, both in promoting and suppressing metastasis. For example, miR-10b is highly expressed in metastatic breast cancer cells and promotes cell migration and invasion. Overexpression of miR-10b in otherwise non-metastatic breast tumors initiates robust invasion and metastasis, and the level of miR-10b expression in primary breast carcinomas correlates with clinical progression (Ma et al., 2007). Other studies have identified miRNAs that inhibit breast cancer metastasis (Ma and Weinberg, 2008; Tavazoie et al., 2008; Valastyan et al., 2009).

Recently, two studies have shown that GATA1 regulates erythropoiesis by controlling miRNAs (Dore et al., 2008; Pase et al., 2009). Using a microarray screening approach, miR-144 and miR-451 were identified as GATA1-regulated miRNAs. The authors demonstrate that these two miRNAs are important for erythrocyte development, and show that in the zebrafish, one important function of miR-451 is to down-regulate *gata2*. These studies reveal that miRNAs lie directly downstream of GATA factors, uncovering a new mechanism by which GATA factors specify cell fate, and that regulation of miRNA loci may be key components to how these transcription factors function. Future studies investigating the link between GATA3 and miRNAs may provide valuable new insight into mammary gland development and the pathogenesis of breast cancer, which may ultimately reveal novel therapeutic strategies.

Conclusions

The role of GATA3 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. GATA3 promotes the differentiation of luminal cells, while repressing other cell types in the mammary gland such as adipocytes. A better understanding of how GATA3 regulates luminal cell differentiation will be important in breast cancer therapy and shed further light on its role as a prognostic factor. GATA3 defines a distinct class of cancer genes that are differentiation factors rather than conventional tumor suppressor genes, which affect the malignant phenotype by enforcing

differentiation. This concept forms the basis for using high-dose retinoic acid therapy to restore tumor differentiation and achieve remission in acute promyelocytic leukemia patients, who carry a translocation that blocks myeloid differentiation. Therefore, uncovering paracrine or juxtacrine signals that activate GATA3 expression during luminal cell specification, as well as understanding the downstream targets of GATA3 will be critical to our understanding of tumor differentiation in breast cancer.

Acknowledgments

This study was supported by grants from the National Institutes of Health (CA129523 and ES012801 to Z.W.) and funds from the UCSF Medical Scientist Training Program and a California Breast Cancer Research Program Pre-doctoral Fellowship (to J.C.). We thank Charina Choi for helpful discussion and critical reading of this manuscript, and Pengfei Lu and Kai Kessenbrock for help with figures.

Literature Cited

- Abba MC, Nunez MI, Colussi AG, Croce MV, Segal-Eiras A, Aldaz CM. GATA3 protein as a MUC1 transcriptional regulator in breast cancer cells. *Breast Cancer Res* 2006;8:R64. [PubMed: 17078870]
- Amsen D, Antov A, Jankovic D, Sher A, Radtke F, Souabni A, Busslinger M, McCright B, Gridley T, Flavell RA. Direct regulation of Gata3 expression determines the T helper differentiation potential of Notch. *Immunity* 2007;27:89–99. [PubMed: 17658279]
- Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and Il4 locus accessibility. *Annu Rev Immunol* 2006;24:607–656. [PubMed: 16551261]
- Asselin-Labat ML, Sutherland KD, Barker H, Thomas R, Shackleton M, Forrest NC, Hartley L, Robb L, Grosveld FG, van der Wees J, Lindeman GJ, Visvader JE. Gata-3 is an essential regulator of mammary-gland morphogenesis and luminal-cell differentiation. *Nat Cell Biol* 2007;9:201–209. [PubMed: 17187062]
- Avni O, Lee D, Macian F, Szabo SJ, Glimcher LH, Rao A. T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. *Nat Immunol* 2002;3:643–651. [PubMed: 12055628]
- Bates DL, Chen Y, Kim G, Guo L, Chen L. Crystal structures of multiple GATA zinc fingers bound to DNA reveal new insights into DNA recognition and self-association by GATA. *J Mol Biol* 2008;381:1292–1306. [PubMed: 18621058]
- Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: Implications for new anticancer therapies. *J Pathol* 2002;196:254–265. [PubMed: 11857487]
- Blokzijl A, tenDijke P, Ibanez CF. Physical and functional interaction between GATA-3 and Smad3 allows TGF-beta regulation of GATA target genes. *Curr Biol* 2002;12:35–45. [PubMed: 11790301]
- Chang HC, Zhang S, Thieu VT, Slee RB, Bruns HA, Laribee RN, Klemsz MJ, Kaplan MH. PU.1 expression delineates heterogeneity in primary Th2 cells. *Immunity* 2005;22:693–703. [PubMed: 15963784]
- Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420:860–867. [PubMed: 12490959]
- de Guzman Strong C, Wertz PW, Wang C, Yang F, Meltzer PS, Andl T, Millar SE, Ho IC, Pai SY, Segre JA. Lipid defect underlies selective skin barrier impairment of an epidermal-specific deletion of Gata-3. *J Cell Biol* 2006;175:661–670. [PubMed: 17116754]
- DeNardo DG, Barreto JB, Andreu P, Vazquez L, Tawfik D, Kolhatkar N, Coussens LM. CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell* 2009;16:91–102. [PubMed: 19647220]
- Dolled-Filhart M, Ryden L, Cregger M, Jirstrom K, Harigopal M, Camp RL, Rimm DL. Classification of breast cancer using genetic algorithms and tissue microarrays. *Clin Cancer Res* 2006;12:6459–6468. [PubMed: 17085660]
- Dore LC, Amigo JD, Dos Santos CO, Zhang Z, Gai X, Tobias JW, Yu D, Klein AM, Dorman C, Wu W, Hardison RC, Paw BH, Weiss MJ. A GATA-1-regulated microRNA locus essential for erythropoiesis. *Proc Natl Acad Sci USA* 2008;105:3333–3338. [PubMed: 18303114]

- Dydensborg AB, Rose AA, Wilson BJ, Grote D, Paquet M, Giguere V, Siegel PM, Bouchard M. GATA3 inhibits breast cancer growth and pulmonary breast cancer metastasis. *Oncogene* 2009;28:2634–2642. [PubMed: 19483726]
- Fang TC, Yashiro-Ohtani Y, Del Bianco C, Knoblock DM, Blacklow SC, Pear WS. Notch directly regulates Gata3 expression during T helper 2 cell differentiation. *Immunity* 2007;27:100–110. [PubMed: 17658278]
- Fuchs E. Scratching the surface of skin development. *Nature* 2007;445:834–842. [PubMed: 17314969]
- Goldfarb DS, Corbett AH, Mason DA, Harreman MT, Adam SA. Importin alpha: A multipurpose nuclear-transport receptor. *Trends Cell Biol* 2004;14:505–514. [PubMed: 15350979]
- Grogan JL, Locksley RM. T helper cell differentiation: On again, off again. *Curr Opin Immunol* 2002;14:366–372. [PubMed: 11973136]
- Gregory BD, O'Malley RC, Lister R, Urich MA, Tonti-Filippini J, Chen H, Millar AH, Ecker JR. A link between RNA metabolism and silencing affecting Arabidopsis development. *Dev Cell* 2008;14:854–866. [PubMed: 18486559]
- Grote D, Boualia SK, Souabni A, Merkel C, Chi X, Costantini F, Carroll T, Bouchard M. Gata3 acts downstream of beta-catenin signaling to prevent ectopic metanephric kidney induction. *PLoS Genet* 2008;4:e1000316. [PubMed: 19112489]
- Gupta GP, Perk J, Acharyya S, de Candia P, Mittal V, Todorova-Manova K, Gerald WL, Brogi E, Benenzra R, Massague J. ID genes mediate tumor reinitiation during breast cancer lung metastasis. *Proc Natl Acad Sci USA* 2007;104:19506–19511. [PubMed: 18048329]
- Gupta PB, Onder TT, Jiang G, Tao K, Kuperwasser C, Weinberg RA, Lander ES. Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell* 2009;138:1–15.
- Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70. [PubMed: 10647931]
- Ho IC, Pai SY. GATA-3 - not just for Th2 cells anymore. *Cell Mol Immunol* 2007;4:15–29. [PubMed: 17349208]
- Ho IC, Tai TS, Pai SY. GATA3 and the T-cell lineage: Essential functions before and after T-helper-2-cell differentiation. *Nat Rev Immunol* 2009;9:125–135. [PubMed: 19151747]
- Hoch RV, Thompson DA, Baker RJ, Weigel RJ. GATA-3 is expressed in association with estrogen receptor in breast cancer. *Int J Cancer* 1999;84:122–128. [PubMed: 10096242]
- Hong SJ, Choi HJ, Hong S, Huh Y, Chae H, Kim KS. Transcription factor GATA-3 regulates the transcriptional activity of dopamine beta-hydroxylase by interacting with Sp1 and AP4. *Neurochem Res* 2008;33:1821–1831. [PubMed: 18338249]
- Ibarra I, Erlich Y, Muthuswamy SK, Sachidanandam R, Hannon GJ. A role for microRNAs in maintenance of mouse mammary epithelial progenitor cells. *Genes Dev* 2007;21:3238–3243. [PubMed: 18079172]
- Jenssen TK, Kuo WP, Stokke T, Hovig E. Associations between gene expressions in breast cancer and patient survival. *Hum Genet* 2002;111:411–420. [PubMed: 12384785]
- Jones JM, Warchol ME. Expression of the Gata3 transcription factor in the acoustic ganglion of the developing avian inner ear. *J Comp Neurol* 2009;516:507–518. [PubMed: 19673002]
- Kaufman CK, Zhou P, Pasolli HA, Rendl M, Bolotin D, Lim KC, Dai X, Alegre ML, Fuchs E. GATA-3: An unexpected regulator of cell lineage determination in skin. *Genes Dev* 2003;17:2108–2122. [PubMed: 12923059]
- Kouros-Mehr H, Werb Z. Candidate regulators of mammary branching morphogenesis identified by genome-wide transcript analysis. *Dev Dyn* 2006;235:3404–3412. [PubMed: 17039550]
- Kouros-Mehr H, Slorach EM, Sternlicht MD, Werb Z. GATA-3 maintains the differentiation of the luminal cell fate in the mammary gland. *Cell* 2006;127:1041–1055. [PubMed: 17129787]
- Kouros-Mehr H, Bechis SK, Slorach EM, Littlepage LE, Egeblad M, Ewald AJ, Pai SY, Ho IC, Werb Z. GATA-3 links tumor differentiation and dissemination in a luminal breast cancer model. *Cancer Cell* 2008;13:141–152. [PubMed: 18242514]
- Kufe DW, Bast RC. *Cancer medicine*. Vol. 2 v.. Hamilton, Ont, London: BC Decker; 2003. p. xxivp. 2699p. 2640

- Kurek D, Garinis GA, van Doorninck JH, van der Wees J, Grosveld FG. Transcriptome and phenotypic analysis reveals Gata3-dependent signalling pathways in murine hair follicles. *Development* 2007;134:261–272. [PubMed: 17151017]
- Lee GR, Fields PE, Flavell RA. Regulation of IL-4 gene expression by distal regulatory elements and GATA-3 at the chromatin level. *Immunity* 2001;14:447–459. [PubMed: 11336690]
- Lim KC, Lakshmanan G, Crawford SE, Gu Y, Grosveld F, Engel JD. Gata3 loss leads to embryonic lethality due to noradrenaline deficiency of the sympathetic nervous system. *Nat Genet* 2000;25:209–212. [PubMed: 10835639]
- Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, Asselin-Labat ML, Gyorki DE, Ward T, Partanen A, Feleppa F, Huschtscha LI, Thorne HJ, Fox SB, Yan M, French JD, Brown MA, Smyth GK, Visvader JE, Lindeman GJ. Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. *Nat Med* 2009;15:907–913. [PubMed: 19648928]
- Lin EY, Nguyen AV, Russell RG, Pollard JW. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med* 2001;193:727–740. [PubMed: 11257139]
- Lin EY, Jones JG, Li P, Zhu L, Whitney KD, Muller WJ, Pollard JW. Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. *Am J Pathol* 2003;163:2113–2126. [PubMed: 14578209]
- Lin EY, Li JF, Gnatovskiy L, Deng Y, Zhu L, Grzesik DA, Qian H, Xue XN, Pollard JW. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res* 2006;66:11238–11246. [PubMed: 17114237]
- Lu P, Werb Z. Patterning mechanisms of branched organs. *Science* 2008;322:1506–1509. [PubMed: 19056977]
- Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 2007;449:682–688. [PubMed: 17898713]
- Ma L, Weinberg RA. Micromanagers of malignancy: role of microRNAs in regulating metastasis. *Trends Genet* 2008;24:448–456. [PubMed: 18674843]
- Maeda A, Moriguchi T, Hamada M, Kusakabe M, Fujioka Y, Nakano T, Yoh K, Lim KC, Engel JD, Takahashi S. Transcription factor GATA-3 is essential for lens development. *Dev Dyn* 2009;238:2280–2291. [PubMed: 19623612]
- Maneechotesuwan K, Xin Y, Ito K, Jazrawi E, Lee KY, Usmani OS, Barnes PJ, Adcock IM. Regulation of Th2 cytokine genes by p38 MAPK-mediated phosphorylation of GATA-3. *J Immunol* 2007;178:2491–2498. [PubMed: 17277157]
- Maneechotesuwan K, Yao X, Ito K, Jazrawi E, Usmani OS, Adcock IM, Barnes PJ. Suppression of GATA-3 nuclear import and phosphorylation: A novel mechanism of corticosteroid action in allergic disease. *PLoS Med* 2009;6:e1000076. [PubMed: 19436703]
- Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Briskin C, Yang J, Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008;133:704–715. [PubMed: 18485877]
- Mehra R, Varambally S, Ding L, Shen R, Sabel MS, Ghosh D, Chinnaiyan AM, Kleer CG. Identification of GATA3 as a breast cancer prognostic marker by global gene expression meta-analysis. *Cancer Res* 2005;65:11259–11264. [PubMed: 16357129]
- Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, Viale A, Olshen AB, Gerald WL, Massague J. Genes that mediate breast cancer metastasis to lung. *Nature* 2005;436:518–524. [PubMed: 16049480]
- Moriguchi T, Takako N, Hamada M, Maeda A, Fujioka Y, Kuroha T, Huber RE, Hasegawa SL, Rao A, Yamamoto M, Takahashi S, Lim KC, Engel JD. Gata3 participates in a complex transcriptional feedback network to regulate sympathoadrenal differentiation. *Development* 2006;133:3871–3881. [PubMed: 16943277]
- Nesbit MA, Bowl MR, Harding B, Ali A, Ayala A, Crowe C, Dobbie A, Hampson G, Holdaway I, Levine MA, McWilliams R, Rigden S, Sampson J, Williams AJ, Thakker RV. Characterization of GATA3 mutations in the hypoparathyroidism, deafness, and renal dysplasia (HDR) syndrome. *J Biol Chem* 2004;279:22624–22634. [PubMed: 14985365]

- Nguyen DX, Bos PD, Massague J. Metastasis: From dissemination to organ-specific colonization. *Nat Rev Cancer* 2009;9:274–284. [PubMed: 19308067]
- Ouyang W, Ranganath SH, Weindel K, Bhattacharya D, Murphy TL, Sha WC, Murphy KM. Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. *Immunity* 1998;9:745–755. [PubMed: 9846495]
- Pandolfi PP, Roth ME, Karis A, Leonard MW, Dzierzak E, Grosveld FG, Engel JD, Lindenbaum MH. Targeted disruption of the GATA3 gene causes severe abnormalities in the nervous system and in fetal liver haematopoiesis. *Nat Genet* 1995;11:40–44. [PubMed: 7550312]
- Pase L, Layton JE, Kloosterman WP, Carradice D, Waterhouse PM, Lieschke GJ. miR-451 regulates zebrafish erythroid maturation in vivo via its target gata2. *Blood* 2009;113:1794–1804. [PubMed: 18849488]
- Pei XH, Bai F, Smith MD, Usary J, Fan C, Pai SY, Ho IC, Perou CM, Xiong Y. CDK inhibitor p18(INK4c) is a downstream target of GATA3 and restrains mammary luminal progenitor cell proliferation and tumorigenesis. *Cancer Cell* 2009;15:389–401. [PubMed: 19411068]
- Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. *Nature* 2000;406:747–752. [PubMed: 10963602]
- Pollard JW. Trophic macrophages in development and disease. *Nat Rev Immunol* 2009;9:259–270. [PubMed: 19282852]
- Ranganath S, Murphy KM. Structure and specificity of GATA proteins in Th2 development. *Mol Cell Biol* 2001;21:2716–2725. [PubMed: 11283251]
- Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, Wu L, Lindeman GJ, Visvader JE. Generation of a functional mammary gland from a single stem cell. *Nature* 2006;439:84–88. [PubMed: 16397499]
- Smalley M, Ashworth A. Stem cells and breast cancer: A field in transit. *Nat Rev Cancer* 2003;3:832–844. [PubMed: 14668814]
- Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein Lonning P, Borresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001;98:10869–10874. [PubMed: 11553815]
- Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lonning PE, Brown PO, Borresen-Dale AL, Botstein D. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci USA* 2003;100:8418–8423. [PubMed: 12829800]
- Sternlicht MD, Kouros-Mehr H, Lu P, Werb Z. Hormonal and local control of mammary branching morphogenesis. *Differentiation* 2006;74:365–381. [PubMed: 16916375]
- Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D, Li HI, Eaves CJ. Purification and unique properties of mammary epithelial stem cells. *Nature* 2006;439:993–997. [PubMed: 16395311]
- Taghon T, Yui MA, Rothenberg EV. Mast cell lineage diversion of T lineage precursors by the essential T cell transcription factor GATA-3. *Nat Immunol* 2007;8:845–855. [PubMed: 17603486]
- Takahashi S, Shimizu R, Suwabe N, Kuroha T, Yoh K, Ohta J, Nishimura S, Lim KC, Engel JD, Yamamoto M. GATA factor transgenes under GATA-1 locus control rescue germline GATA-1 mutant deficiencies. *Blood* 2000;96:910–916. [PubMed: 10910904]
- Takemoto N, Arai K, Miyatake S. Cutting edge: The differential involvement of the N-finger of GATA-3 in chromatin remodeling and transactivation during Th2 development. *J Immunol* 2002;169:4103–4107. [PubMed: 12370337]
- Takeuchi JK, Bruneau BG. Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors. *Nature* 2009;459:708–711. [PubMed: 19396158]
- Tavazoie SF, Alarcón C, Oskarsson T, Padua D, Wang Q, Bos PD, Gerald WL, Massagué J. Endogenous human microRNAs that suppress breast cancer metastasis. *Nature* 2008;451:147–152. [PubMed: 18185580]

- Tong Q, Dalgin G, Xu H, Ting CN, Leiden JM, Hotamisligil GS. Function of GATA transcription factors in preadipocyte-adipocyte transition. *Science* 2000;290:134–138. [PubMed: 11021798]
- Tsai FY, Browne CP, Orkin SH. Knock-in mutation of transcription factor GATA-3 into the GATA-1 locus: Partial rescue of GATA-1 loss of function in erythroid cells. *Dev Biol* 1998;196:218–227. [PubMed: 9576834]
- Usary J, Llaca V, Karaca G, Presswala S, Karaca M, He X, Langerod A, Karsen R, Oh DS, Dressler LG, Lonning PE, Strausberg RL, Chanock S, Borresen-Dale AL, Perou CM. Mutation of GATA3 in human breast tumors. *Oncogene* 2004;23:7669–7678. [PubMed: 15361840]
- Valastyan S, Reinhardt F, Benaich N, Calogrias D, Szász AM, Wang ZC, Brock JE, Richardson AL, Weinberg RA. A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. *Cell* 2009;137:1032–1046. [PubMed: 19524507]
- Van Esch H, Groenen P, Nesbit MA, Schuffenhauer S, Lichtner P, Vanderlinden G, Harding B, Beetz R, Bilous RW, Holdaway I, Shaw NJ, Fryns JP, Van de Ven W, Thakker RV, Devriendt K. GATA3 haplo-insufficiency causes human HDR syndrome. *Nature* 2000;406:419–422. [PubMed: 10935639]
- van Hamburg JP, de Bruijn MJ, Dingjan GM, Beverloo HB, Diepstraten H, Ling KW, Hendriks RW. Cooperation of Gata3, c-Myc and Notch in malignant transformation of double positive thymocytes. *Mol Immunol* 2008;45:3085–3095. [PubMed: 18471881]
- Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: Accumulating evidence and unresolved questions. *Nat Rev Cancer* 2008;8:755–768. [PubMed: 18784658]
- Voduc D, Cheang M, Nielsen T. GATA-3 expression in breast cancer has a strong association with estrogen receptor but lacks independent prognostic value. *Cancer Epidemiol Biomarkers Prev* 2008;17:365–373. [PubMed: 18268121]
- Yamashita M, Ukai-Tadenuma M, Miyamoto T, Sugaya K, Hosokawa H, Hasegawa A, Kimura M, Taniguchi M, DeGregori J, Nakayama T. Essential role of GATA3 for the maintenance of type 2 helper T (Th2) cytokine production and chromatin remodeling at the Th2 cytokine gene loci. *J Biol Chem* 2004;279:26983–26990. [PubMed: 15087456]
- Yamashita M, Shinnakasu R, Asou H, Kimura M, Hasegawa A, Hashimoto K, Hatano N, Ogata M, Nakayama T. Ras-ERK MAPK cascade regulates GATA3 stability and Th2 differentiation through ubiquitin-proteasome pathway. *J Biol Chem* 2005;280:29409–29419. [PubMed: 15975924]
- Yang J, Weinberg RA. Epithelial-mesenchymal transition: At the crossroads of development and tumor metastasis. *Dev Cell* 2008;14:818–829. [PubMed: 18539112]
- Yang Z, Gu L, Romeo PH, Bories D, Motohashi H, Yamamoto M, Engel JD. Human GATA-3 trans-activation, DNA-binding, and nuclear localization activities are organized into distinct structural domains. *Mol Cell Biol* 1994;14:2201–2212. [PubMed: 8114750]
- Zhao GY, Li ZY, Zou HL, Hu ZL, Song NN, Zheng MH, Su CJ, Ding YQ. Expression of the transcription factor GATA3 in the postnatal mouse central nervous system. *Neurosci Res* 2008;61:420–428. [PubMed: 18554735]
- Zhou M, Ouyang W. The function role of GATA-3 in Th1 and Th2 differentiation. *Immunol Res* 2003;28:25–37. [PubMed: 12947222]
- Zhou M, Ouyang W, Gong Q, Katz SG, White JM, Orkin SH, Murphy KM. Friend of GATA-1 represses GATA-3-dependent activity in CD4+ T cells. *J Exp Med* 2001;194:1461–1471. [PubMed: 11714753]

Fig. 1.

Functional domains in the mouse GATA-binding protein 3. GATA3 is composed of 443 amino acids, and contains two amino terminal transactivation domains, TA1 and TA2, and two zinc-finger motifs, ZF1 and ZF2, which are followed immediately by a conserved basic region. The distal zinc-finger motif (ZF2) binds to DNA containing the canonical GATA motif, (A/T)GATA(A/G). The proximal zinc-finger motif (ZF1) seems to have broader specificity. Mutation of the amino acids KRR, which lies in the first basic region in between ZF1 and ZF2, confers dominant negative or hypomorphic function. The second basic region contains two important motifs: the YxKxHxxxRP motif (in which x denotes any amino acid) mediates DNA binding and the NRPL motif forms the interface between two GATA molecules, indicating that GATA3 may homo- or heterodimerize. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Fig. 2. GATA3 in normal mammary gland development. **A:** A schematic representation of the mammary epithelium and stroma during mammary gland development. The luminal epithelial cells, highlighted in yellow, express GATA3 while the myoepithelial cells, highlighted in red, express very low levels of GATA3. The terminal end bud (TEB) invades through the stroma during pubertal development, and consists of both GATA3-negative cap cells and GATA3-positive cells. The stroma consists of a heterogeneous cell population of fibroblasts, adipocytes, macrophages, eosinophils, and mast cells, and plays an important role in facilitating branching and ductal elongation. Arrow points to the direction of TEB migration. **B:** Whole-mount carmine red staining of mouse mammary glands from 5-week-

old wild-type (left) and GATA3 conditional knock-out (CKO) (right) mice outlines the epithelium. In the wild-type mammary gland, the epithelium has invaded into the stroma (from left to right) past the lymph node (LN), with multiple TEBs formed. Inset shows bifurcating TEBs. In contrast, the GATA3-CKO mammary gland shows a defect in epithelial invasion into the stroma, without prominent TEBs formed. Inset shows a lack of TEBs at the distal end of the epithelium. Scale bar corresponds to 3 mm. Part (A) is modified from Lu and Werb (2008) and (B) is reprinted from Kouros-Mehr et al. (2006) with permission from Elsevier. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Fig. 3.

GATA3 is sufficient to induce differentiation in carcinoma cells. Primary cultures of adenocarcinomas from MMTV-PyMT mice were transduced with retrovirus containing either empty vector (control) or GATA3 and transplanted into wild-type mice. Tumors were grown for 6 weeks. H&E staining (A,B) and immunocytochemistry for β -casein (C,D) show that tumor cells expressing GATA3 differentiate and form milk proteins. Schematic shows that GATA3 tumors not only form lumens (E,F), but also express differentiation markers and basement membrane components such as perlecan (marked in blue). Parts (A–D) are reprinted from Kouros-Mehr et al. (2008) with permission from Elsevier. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 1

The function of GATA3 in various tissues

Tissue	Functions of GATA3	References
T cell	T _H 2 cell specification	Grogan and Locksley (2002), van Hamburg et al. (2008), Ho et al. (2009)
	Cell survival during β -selection	
	Transforms T cells in cooperation with c-Myc and Notch	
Skin	Differentiation of the inner root sheath precursors	Kaufman et al. (2003), de Guzman Strong et al. (2006), Kurek et al. (2007)
	Decreases basal cell proliferation	
	Role unknown in skin cancer	
Mammary Gland	Specifies and maintains differentiation, adhesion, proliferation of luminal cells	Abba et al. (2006), Kouros-Mehr et al. (2006), Kouros-Mehr et al. (2008), Dydensborg et al. (2009), Pei et al. (2009)
	Low GATA3 expression correlates with worse breast cancer prognosis	
	Re-introduction into carcinoma cells suppresses metastasis to lung	
Kidney	Prevents ectopic metanephric kidney duct formation	Grote et al. (2008)
	Haploinsufficiency results in renal dysplasia	
	Role unknown in kidney cancer	
Lens	Differentiation of lens fiber cells	Maeda et al. (2009)
	Regulates cell cycle to suppress cellular proliferation	
Neurons	Maintains noradrenaline levels during development by up-regulating dopamine β -hydroxylase	Lim et al. (2000), Moriguchi et al. (2006), Hong et al. (2008), Zhao et al. (2008), Jones and Warchol (2009)
	Differentiation of sympathoadrenal and auditory ganglion neurons	
	Expressed in multiple areas of the brain, though function yet unknown	
Adipocyte	Antagonizes adipocyte differentiation	Tong et al. (2000)
	Loss of function associated with obesity	

GATA3 contributes to the differentiation and normal development of epithelial and non-epithelial tissues. Studies in T cells and the mammary gland show that GATA3 also plays an important role in tumorigenesis.