MCE Special Edition: Animal Models of Endocrine Neoplasia

GATA factors in endocrine neoplasia

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Abstract

GATA transcription factors are structurally-related zinc finger proteins that recognize the consensus DNA sequence WGATAA (the GATA motif), an essential *cis*-acting element in the promoters and enhancers of many genes. These transcription factors regulate cell fate specification and differentiation in a wide array of tissues. As demonstrated by genetic analyses of mice and humans, GATA factors play pivotal roles in the development, homeostasis, and function of several endocrine organs including the adrenal cortex, ovary, pancreas, parathyroid, pituitary, and testis. Additionally, GATA factors have been shown to be mutated, overexpressed, or underexpressed in a variety of endocrine tumors (e.g., adrenocortical neoplasms, parathyroid tumors, pituitary adenomas, and sex cord stromal tumors). Emerging evidence suggests that GATA factors play a direct role in the initiation, proliferation, or propagation of certain endocrine tumors via modulation of key developmental signaling pathways implicated in oncogenesis, such as the WNT/ β -catenin and TGF β pathways. Altered expression or function of GATA factors can also affect the metabolism, ploidy, and invasiveness of tumor cells. This article provides an overview of the role of GATA factors in endocrine neoplasms. Relevant animal models are highlighted.

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1. Overview of GATA factors in development and disease

1.1. Members of the vertebrate GATA family

By regulating cell fate specification and differentiation, GATA transcription factors play important roles in eukaryotic development. The vertebrate GATA family comprises six members (GATA1-6) named in order of discovery (Patient and McGhee, 2002). All bind to the consensus DNA element, WGATAA, known as the GATA motif (Bresnick et al., 2012). With the exception of *Gata5*, homozygous null mutations in genes encoding GATA family members result in embryonic lethality in mice, underscoring the vital roles that these transcription factors play in development [reviewed in (Molkentin, 2000; Viger et al., 2008)]. Human diseases associated with germline loss-of-function mutations in GATA factors are summarized in Table 1.

All vertebrate GATA proteins contain a pair of conserved zinc finger domains (Patient and McGhee, 2002). The C-terminal finger is essential for DNA binding, whereas the N-terminal finger physically interacts with other transcriptional regulators such as the “Friend of GATA” factors FOG1 (ZFPM1) and FOG2 (ZFPM2) (Bresnick et al., 2012). GATA motifs are common in the genomes of vertebrates, but global chromatin immunoprecipitation studies in various cell types suggest that GATA factors occupy less than 1% of the consensus sites (Bresnick et al., 2012). This implies the existence of mechanisms that discriminate among these abundant motifs.

GATA factors can act as either transcriptional activators or repressors depending on the context (Viger et al., 2008). Although all GATA factors bind to the same DNA element, there is surprisingly little functional redundancy among these proteins (Zheng and Blobel, 2010). Individual GATA factors elicit distinctive functions through cooperative interactions with other transcriptional regulators. Functional diversity is also achieved via post-translational modifications (e.g., phosphorylation, acetylation, and sumoylation) that alter the intrinsic activity of different GATA factors (Viger et al., 2008).

During the differentiation of a particular lineage, a GATA motif in the promoter/enhancer of a gene may be occupied sequentially by more than one GATA factor. For example, as erythroid progenitors

mature the stem cell factor GATA2 is displaced from specific sites by the terminal differentiation factor GATA1, a phenomenon termed a “GATA switch” (Bresnick et al., 2010). A similar switch is presumed to occur in intestinal epithelium, where GATA6 maintains stem/progenitor cells and GATA4 promotes terminal differentiation into mature enterocytes (Beuling et al., 2011). GATA switches are often associated with altered transcriptional output, emphasizing that different GATA factors can exert distinct functions at the same binding site (Bresnick et al., 2012). GATA switches occur not only during normal development but also in disease states, such as endometriosis. The transformation of endometrial stroma into endometriotic stroma is accompanied by an epigenetically-mediated switch from expression of *GATA2* to *GATA6* (Dyson et al., 2014).

1.2. GATA factors as tumor markers

Since GATA factors regulate genetic networks that can expand stem/progenitor cell populations or drive differentiation, it comes as no surprise that alterations in the expression or function of GATA factors have been linked to neoplastic transformation. Indeed, GATA factor genes have been shown to be mutated, overexpressed, or underexpressed in a wide range of solid tumors and leukemias [reviewed in (Ayanbule et al., 2011; Bresnick et al., 2012; Viger et al., 2008; Zheng and Blobel, 2010)].

GATA factors can serve as tumor markers that shed light on the developmental origins, clinical behavior, and pathogenesis of certain neoplasms, as illustrated by studies of GATA3 in breast cancer and GATA2 in prostate cancer. GATA3 is abundantly expressed in luminal cells of the mammary epithelium but not their multipotential progenitors (Chou et al., 2010). Conditional deletion studies in the mouse have shown that *Gata3* is required for branching morphogenesis and terminal differentiation of luminal epithelial cells [reviewed in (Chou et al., 2010; Zheng and Blobel, 2010)]. Interestingly, loss of *Gata3* in adult mammary epithelium triggers de-differentiation of luminal cells, increased cell proliferation, and disorganization of ducts, features reminiscent of neoplastic transformation. In primary breast tumors low or absent *GATA3* expression is associated with shorter patient survival and a host of negative prognostic indicators (primary tumor size, lymph node metastases, lack of estrogen receptor and progesterone

receptor expression, etc.) (Chou et al., 2010). GATA2 is expressed in both benign prostatic epithelium and prostate cancer, but levels of GATA2 are significantly higher in the latter and correlate with the risk of cancer progression and metastasis (He et al., 2014). Androgen receptor (AR) signaling is a key driver of prostate cancer, and GATA2 has emerged as a critical regulator of AR expression and activity in this malignancy (Chiang et al., 2014; He et al., 2014; Wu et al., 2014).

1.3. GATA factors interface with signaling pathways involved in both normal development and tumorigenesis

Signaling pathways that control stem cell self-renewal, terminal differentiation, and cell survival, such as the WNT/ β -catenin and TGF β pathways, are often co-opted during tumorigenesis. GATA factors have been shown to interface with developmental signaling pathways implicated in oncogenesis, as evidenced by studies of GATA6 in colorectal tumors. Constitutive activation of WNT/ β -catenin signaling and inhibition of bone morphogenetic protein (BMP) signaling are the principal genetic alterations associated with colorectal tumor formation (Whissell et al., 2014). GATA6 plays a key role in colorectal tumorigenesis by driving expression of *LGR5*, which enhances WNT/ β -catenin signaling and enables tumor stem cell renewal, and by inhibiting expression of BMPs, which promote terminal differentiation (Tsuji et al., 2014; Whissell et al., 2014). GATA factors have also been shown to regulate genes involved in apoptosis of normal and tumor cells. For example, GATA4 has been shown to protect cardiomyocytes from doxorubicin-induced apoptosis by upregulating anti-apoptotic members of the BCL2 protein family (Aries et al., 2004; Kobayashi et al., 2010) and to protect ovarian tumor cells from an extrinsic apoptosis inducing ligand TRAIL (Kyrönlahti et al., 2010) (see Section 2.2).

1.4. GATA factor dysregulation can affect tumor cell migration, invasiveness, and ploidy

GATA factor dysregulation can impact tumor cell biology in other ways. In breast cancer cells GATA3 promotes differentiation, limits cell migration, and suppresses metastasis by inducing expression of a microRNA (miR-29b) that downregulates expression of genes involved in angiogenesis, collagen

remodeling, and proteolysis (Chou et al., 2013). Ovarian carcinomas are thought to arise from ovarian surface (germinal) epithelium or neighboring oviduct epithelium (Nezhat et al., 2015). Approximately 50% of ovarian carcinomas lack *GATA6* expression entirely, and an additional 40% show abnormal *GATA6* immunostaining that is either weak or cytoplasmic rather than nuclear (Cai et al., 2009). Loss of *GATA6* in germinal epithelial cells triggers their de-differentiation, manifested as the loss of expression of proteins required for epithelial organization (Cai et al., 2009; Capo-chichi et al., 2011; Capo-chichi et al., 2009). Loss of *GATA6* expression in these cells also leads to deformation of the nuclear envelope and a failure of cytokinesis, resulting in aneuploidy (Capo-chichi et al., 2009). The link between *GATA6* deficiency and aneuploidy appears to be a generalized phenomenon, because mouse peritoneal macrophages lacking *Gata6* expression also exhibit changes in ploidy along with metabolic derangements (Gautier et al., 2014; Rosas et al., 2014).

1.5. Focus of this article

This article reviews the role of GATA factors in neoplasias of various endocrine tissues. The expression patterns of GATA factors in developing endocrine organs and in their corresponding neoplasms are described. The use of GATA factors as endocrine tumor markers in both preclinical and clinical settings is discussed. Relevant animal models, such as the mouse, ferret, dog, and goat, are highlighted. Endocrine-related tumors, such as breast and prostate cancer, are not a focus of this review.

Dozens of putative GATA target genes have been identified in endocrine tissues. Unfortunately, space constraints do not allow us to cite all the original research papers characterizing these target genes. Instead, the reader is referred to review articles that summarize GATA target genes in endocrine tissues (LaVoie, 2003; Röhrig et al., 2014; Tevosian, 2014; Viger et al., 2008).

2. Ovarian neoplasms

2.1. Roles of GATA4 and GATA6 in ovarian development and function

GATA4 and GATA6 are the predominant GATA factors expressed in the developing ovary (Fig 1) (LaVoie, 2014; Viger et al., 2008). At embryonic day (E) 10.5 in the mouse, *Gata4* expression is evident in the genital ridge (Hu et al., 2013), and by E13.5 GATA4 is found in most ovarian somatic cells (Anttonen et al., 2003; Efimenko et al., 2013; Heikinheimo et al., 1997; Kyrönlahti et al., 2011b). In the adult ovary, GATA4 is present in theca cells and in granulosa cells of primary, preantral, and antral follicles, but not in primordial follicles or luteal cells (LaVoie et al., 2004; Viger et al., 2008). Like *Gata4*, *Gata6* is expressed in somatic cells of the prenatal ovary and in theca and granulosa cells of large follicles in the adult ovary; however, in contrast to *Gata4*, *Gata6* is expressed in corpora lutea (Heikinheimo et al., 1997; Miyamoto et al., 2008). Both GATA4 and GATA6 are found in ovarian surface epithelium (Capo-chichi et al., 2003).

Ovarian expression of GATA factors is controlled by an array of endocrine and paracrine factors, including gonadotropins and members of the TGF β superfamily [reviewed in (Viger et al., 2008)]. For example, treatment of juvenile mice with eCG enhances follicular expression of *Gata4* and *Gata6* (Heikinheimo et al., 1997), while treatment of cultured preovulatory human granulosa cells with hCG upregulates GATA6 expression (Laitinen et al., 2000). In granulosa cell tumors, TGF β treatment increases GATA4 levels (Anttonen et al., 2006). In ovarian somatic cells and other endocrine tissues, GATA factors serve to integrate input from signaling pathways including the cyclic AMP/protein kinase A (cAMP/PKA) and mitogen-activated protein kinase (MAPK) pathways [reviewed in (Viger et al., 2008)]. Activation of these two signaling pathways in gonadal cells results in phosphorylation of GATA4 at distinctive sites that mediate synergistic interactions with other transcriptional regulators [reviewed in (Viger et al., 2008)]. Promoter analyses have identified a number of GATA-dependent genes in ovarian steroidogenic cells, including *Star*, *Cyp11a1*, *Cyp19a1*, *Hsd17b1*, and *Inha* [reviewed in (Viger et al., 2008)]. Targeted mutagenesis studies in the mouse have delineated the importance of *Gata4* and *Gata6* in follicular development and ovarian function (Table 2) [reviewed in (Tevosian, 2014)].

2.2. Granulosa cell tumors

Granulosa cell tumors (GCTs), the most common sex-cord stromal tumors, are subclassified in two forms: an adult-type (AGCT), that typically occurs in perimenopausal women, and a rare juvenile form that affects mostly children and adolescents (Schumer and Cannistra, 2003). GCTs are steroidogenically active and can cause precocious puberty, disturbances in the menstrual cycle, and endometrial hyperplasia (Jamieson and Fuller, 2012; Schumer and Cannistra, 2003).

AGCTs retain the biological features of normal proliferating granulosa cells of preovulatory follicles (Fuller et al., 2002). Thus, the molecular pathogenesis of AGCT is hypothesized to entail disrupted expression of signaling pathways that regulate granulosa cell proliferation and apoptosis. A somatic missense mutation (p.C134W) in *FOXL2*, a transcription factor required for normal murine granulosa cell differentiation and ovarian maintenance (Schmidt et al., 2004; Uhlenhaut et al., 2009), is present in ~95% of AGCTs, suggesting that it is pathognomonic for this tumor (Jamieson et al., 2010; Kim et al., 2010b; Shah et al., 2009). In contrast, juvenile GCTs lack the p.C134W mutation (Kalfa et al., 2007).

Current evidence suggests that a key event in AGCT pathogenesis is a failure of the mutant *FOXL2* to form specific protein-protein interactions, leading to subtle changes in the transcription of target genes (L'Hote et al., 2012). Recent studies suggest that *GATA4* cooperates with *FOXL2* during granulosa cell tumorigenesis (Fig 2). The majority of AGCTs express *GATA4* at levels comparable to normal preovulatory granulosa cells (Laitinen et al., 2000), and high *GATA4* expression in these tumors predicts both increased risk of recurrence and shorter disease specific survival (Färkkilä et al., 2014). *GATA4* expression in AGCTs also correlates with the intrinsic apoptotic pathway inhibitor *BCL2* and proliferative *CCND2* expression, suggesting that *GATA4* may act as an anti-apoptotic factor in adult AGCTs (Kyrönlähti et al., 2008). *GATA4*, *SMAD3*, and *FOXL2* physically interact and modulate gene expression, cell viability, and apoptosis in AGCTs (Anttonen et al., 2014). *GATA4* is also expressed in juvenile GCTs (Virgone et al., 2012); however, increased expression of *GATA4* does not correlate with aggressive behavior as seen in adults. In contrast to *GATA4*, *GATA6* expression in AGCTs is inversely

correlated with tumor size, suggesting that GATA6 may suppress proliferation in this cell type (Anttonen et al., 2005).

A number of transgenic mouse models have been generated to examine the pathogenesis of AGCTs (Table 3). Aberrant expression of GATA factors accompanies tumorigenesis in several of these mouse models. Collectively, these models reinforce the importance of SMADs and GATA factors in the genesis of AGCTs.

2.4. Sertoli-Leydig cell tumors of the ovary

Sertoli-Leydig cell tumors (STLCs) are rare ovarian sex cord-stromal tumors characterized by proliferation of Sertoli and Leydig cells of varying degrees of differentiation (Zhang et al., 2014). Sertoli cells, not Leydig cells, are thought to constitute the neoplastic component of these tumors (Nouriani et al., 2002). The majority of SLCTs are diagnosed in adolescents or young adults. One-third of patients exhibit hyperandrogenic manifestations (acne, male-pattern baldness, etc.) (Zanotti, 2002; Zhang et al., 2014). A small percentage of SLCTs have estrogenic manifestations, such as menometrorrhagia or postmenopausal bleeding.

SLCTs are one of the characteristic tumors in the pleuropulmonary blastoma familial tumor predisposition syndrome, which is caused by heterozygous germline mutations in the microRNA maturation gene *DICER1* (Schultz et al., 2014). More than half of SLCTs harbor mutations in *DICER1* (Witkowski et al., 2013). Conditional deletion experiments in mice have established that *Dicer1* regulates gonadal somatic cell function (Huang and Yao, 2010; Kim et al., 2010a; Lei et al., 2010; Nagaraja et al., 2008), but sex cord stromal tumors have not been reported in germline or conditional *Dicer1* knockout mice.

Based on its established role in testicular somatic cell differentiation (see section 3.1), GATA4 is hypothesized to have a role in pathogenesis of ovarian SLCTs. A series of studies have shown that GATA4 and its cofactor FOG2 are expressed in some but not all SLCTs (Ketola et al., 2000; Mosbech et

al., 2014; Siltanen et al., 1999; Virgone et al., 2012). Currently, however, there is no genetic or epigenetic data linking altered expression or function of GATA factors to SLCTs.

2.5. Thecoma-fibroma tumors of the ovary

Thecoma–fibroma is a group of benign ovarian sex cord-stromal tumors. These rare neoplasms are composed of varying amounts of theca cells and fibroblasts (Chen et al., 2003). This group of tumors is subdivided into three categories: 1) thecoma, containing lipid-laden theca cells without fibroblasts, 2) thecoma–fibroma, containing both theca cells and fibroblasts, and 3) fibroma, composed almost entirely of fibroblasts. These tumors can occur at any age, but menopausal and postmenopausal women account for most of the cases (Chen et al., 2003). Patients typically present with an abdominal mass and attendant pain; functional tumors may be associated with irregular menstrual bleeding.

Young women with Gorlin (nevoid basal cell carcinoma) syndrome, a tumor predisposition disorder associated with excessive hedgehog signaling due to heterozygous mutations in *PTCH1*, develop bilateral ovarian fibromas (Morse et al., 2011). One boy with Gorlin syndrome and a testicular thecoma-fibroma has been reported (Ueda et al., 2010). Loss of heterozygosity at 9q22.3, which harbors the *PTCH1* gene, is observed in 40% of sporadic ovarian thecoma-fibroma cases (Tsuji et al., 2005), supporting a pathogenic association between aberrant hedgehog signaling and thecoma-fibroma development. Abundant expression of GATA4 and its cofactor FOG2 have been reported in two pediatric cases of thecoma-fibroma, including a case of bilateral ovarian fibroma associated with Gorlin syndrome (Virgone et al., 2012).

3. Testicular neoplasms

3.1. GATA factors implicated in testicular development and function

During fetal testicular development *Gata4* is expressed in Sertoli cells, fetal Leydig cells, and peritubular myoid cells (Table 4) (Bielinska et al., 2007; Mazaud-Guittot et al., 2014). In the adult testis

Gata4 is expressed in Sertoli cells, Leydig cells, and putative stem Leydig cells (Ketola et al., 2002; Ketola et al., 1999; Kilcoyne et al., 2014; Landreh et al., 2014; McCoard et al., 2001; Oreal et al., 2002). Like GATA4, GATA6 is found in testicular somatic cells. In the mouse, testicular *Gata6* expression begins in Sertoli cells at E14.5 (Robert et al., 2002) and in fetal Leydig cells shortly thereafter (Tevosian, 2014). Postnatally, Sertoli cells continue to express *Gata6* through to adulthood (Anttonen et al., 2003; Imai et al., 2004; Ketola et al., 2002; Ketola et al., 1999; Ketola et al., 2003; Oreal et al., 2002). *Gata1* is also expressed in mouse Sertoli cells, albeit in a stage-specific manner (stages VI-IX of the seminiferous epithelial cycle) that is dependent on the presence of maturing germ cells (Ketola et al., 2002; Yomogida et al., 1994). Both GATA4 and GATA6 have been detected in adult Leydig cells (Bielinska et al., 2007; Ketola et al., 1999; Ketola et al., 2003), although GATA4 is the predominant GATA factor in this steroidogenic cell type.

Promoter analyses and related studies have identified several groups of putative target genes for GATA4 in testis, including genes associated with sex determination (*Sry*, *Sox9*, *Dmrt1*), peptide hormone production (*Inha*, *Inhb*, *Amh*), gonadotropin signaling (*Fshr*, *Lhcgr*), steroid synthesis (*Star*, *Cyp11a1*, *Cyp17a1*), and cell-cell interactions (*Clmp*, *Cldn11*, *Cx30.2*) [reviewed in (Tevosian, 2014; Viger et al., 2008)]. In both Sertoli and Leydig cells, GATA4 activity is modulated via cooperative interactions with other transcriptional regulators/cofactors including SF1, liver receptor homolog 1 (LRH-1/NR5A2), FOG1, and FOG2 [reviewed in (Viger et al., 2008)]. FOG proteins do not bind directly to DNA, but they can function as either enhancers or repressors of GATA transcriptional activity depending on the cell and promoter context studied; on gonadal promoters, however, FOG proteins appear to play a strictly repressive role (Tevosian, 2014). As in ovarian cells, GATA4 is a target for post-translational modifications such as phosphorylation (Tremblay and Viger, 2003; Viger et al., 2008).

Analysis of genetically-engineered mice has shown that interactions between GATA4 and its cofactor, FOG2, regulate the differentiation and function of fetal and adult Sertoli cells [reviewed in (Tevosian, 2014)]. *Fog2*^{-/-} mice and *Gata4*^{ki/ki} mice, which bear a knock-in mutation that abrogates the interaction of GATA4 with FOG cofactors, exhibit similar testicular phenotypes including decreased

testicular *Sry* expression, aberrant differentiation of Sertoli cells, and sex reversal (Bouma et al., 2007; Manuylov et al., 2007; Tevosian et al., 2002). More recently, conditional mutagenesis studies have shown that functional GATA4 is required for genital ridge development, testis cord morphogenesis, and Sertoli cell function (Hu et al., 2015; Hu et al., 2013; Kyrönlahti et al., 2011a; Manuylov et al., 2011).

The role of GATA4 in Leydig cell development, however, has remained unclear, because gene targeting experiments in mice have not shown a consistent phenotype [reviewed in (Tevosian, 2014)]. For example, *Gata4*^{-/-} progenitors exhibit an impaired capacity to differentiate into fetal Leydig cells in the testis of chimeric mice (Bielinska et al., 2007). In contrast, conditional ablation of *Gata4* in Leydig cells as early as E12.5 does not cause an overt impairment in the expression of Leydig cell differentiation markers in the fetal or adult testis (Manuylov et al., 2011). Interpreting the results of gene targeting experiments in the mouse testis is challenging because of context-dependent effects, compensatory responses, alternative pathways of differentiation, and functional redundancy (Tevosian, 2014). To circumvent these limitations, the impact of *Gata4* deficiency on Leydig cell function has been analyzed in less complicated experimental models: two immortalized mouse Leydig tumor cell lines (MA-10, mLTC-1) and primary cultures of adult mouse Leydig cells (Bergeron et al., 2015; Schrade et al., 2015). Using siRNA and related knockdown approaches, *Gata4* deficiency has been shown to have profound effects on specific metabolic pathways, notably steroidogenesis and glycolysis.

A missense mutation in the human *GATA4* gene has been linked to abnormal testicular development in one kindred, although the precise impact of this mutation on somatic cell function is unclear (Lourenco et al., 2011). More recently, mutations in *FOG2* have been demonstrated in unrelated individuals with 46,XY gonadal dysgenesis (Bashamboo et al., 2014).

Despite the intriguing, stage-specific expression pattern of GATA1 in testis, gene targeting experiments in the mouse suggest that GATA1 is not essential for Sertoli cell function (Lindeboom et al., 2003).

3.2. *GATA4* is expressed in large-cell calcifying Sertoli tumors

GATA4 expression has been reported in large-cell calcifying Sertoli tumors (LCCSCT), one of the sex cord tumors of testis (Fig 3) (Ketola et al., 2000). LCCSCTs produce estrogen and are associated with gynecomastia and advanced skeletal maturation (Gourgari et al., 2012). Most cases of LCCSCT are sporadic, but about 40% are associated multiple neoplasia syndromes such as Peutz–Jeghers syndrome (PJS) or Carney complex (CNC). PJS is caused by loss-of-function mutations in the *STK11* gene, which inhibit AMP-activated protein kinase, resulting in increased activity of the mammalian target of rapamycin (mTOR) (Gourgari et al., 2012). Dysregulation of the mTOR pathway has been linked to tumorigenesis in various tissues, including endocrine tissues (de Joussineau et al., 2014). CNC is caused by *PRKARIA* mutations, the gene encoding regulatory subunit type 1 of protein kinase A. This leads to excessive cAMP and mTOR signaling (de Joussineau et al., 2014; Sahut-Barnola et al., 2010). Whether signaling activation in these tumor disposition syndromes is associated with altered phosphorylation of GATA4 is unknown. Lending credence to this possibility, increased expression of GATA4 is evident in the adrenal glands of *Prkar1a* knockout mice signaling (Sahut-Barnola et al., 2010).

3.3. *GATA4* is expressed in canine testicular tumors

A comprehensive survey of canine testicular tumors documented strong GATA4 immunoreactivity in all Sertoli cell tumors and the vast majority of (27/28) Leydig (interstitial) cell tumors (Ramos-Vara and Miller, 2009). Mixed germ cell sex cord-stromal tumors (MGST) in this species also expressed GATA4.

3.4. *Gata6* is downregulated in an experimental model of Leydig cell adenoma

Heterozygous loss-of-function mutations in *Men1*, encoding a chromatin remodeling gene, predispose mice to the development of multiple endocrine tumors, recapitulating the human *MEN1* cancer predisposition syndrome. Additionally, *Men1*^{+/-} mice develop gonadal somatic cell tumors, a feature not typical of humans with this cancer predisposition syndrome. Female *Men1*^{+/-} mice develop GCTs that underexpress *Gata6* (Table 3), while their male counterparts develop Leydig cell tumors that

underexpress *Gata6* (Mould et al., 2009). Loss of heterozygosity at the *Men1* locus is evident in these gonadal tumors, suggesting a direct link between *Men1* gene inactivation and tumorigenesis in this model (Bertolino et al., 2003; Hussein et al., 2007). Decreased expression of *Gata6* represents an attractive candidate for mediating gonadal somatic cells tumorigenesis in this model because: 1) GATA6 affects TGF β signaling in other tumors such as colorectal neoplasms (see Section 1.4), 2) dysregulated TGF β superfamily signaling accompanies Leydig cell tumorigenesis in the *Men1*^{+/-} mice (Hussein et al., 2008), and 3) targeted mutagenesis of genes involved in TGF β signaling (e.g., *Inha*, *Amh*, *Amhr2*) have been linked to testicular and ovarian somatic cell tumors in mice (Behringer et al., 1994; Matzuk et al., 1995; Mishina et al., 1996) (Table 3).

3.6. Testicular adrenal rest tumors

Leydig cells in the adult testis can arise from different populations of stem/progenitor cells, including undifferentiated mesenchymal cells in the testicular interstitium, vascular progenitors, and peritubular cells (Davidoff et al., 2004; Landreh et al., 2014; Mendis-Handagama and Ariyaratne, 2001). Men with disrupted adrenocortical function due to CYP21 or CYP11B1 deficiency develop neoplastic nodules of hormonally-active adrenocortical tissue in the testis (testicular adrenal rest tumors, TARTs), thought to arise from one of these reservoirs of pluripotential stem/progenitor cells (Reisch et al., 2013; Val et al., 2006). TARTs express adrenocortical-specific genes (*CYP11B1*, *CYP11B2*, and *MC2R*) at much higher levels than adjacent testicular tissue (Smeets et al., 2015). In addition, TARTs express the Leydig cell markers *INSL3* and *HSD17B3*. These findings reinforce the premise that TARTs may arise from a totipotent embryonic cell type in response to hormonal dysregulation. The endocrine and paracrine factors that drive TART growth are not fully understood. A longitudinal analysis of men with CYP21 deficiency found no association between the presence of TARTs and parameters of disease control with exogenous glucocorticoids $\pm$ mineralocorticoids (Reisch et al., 2013). *Gata4/Gata6* double knockout mice generated with *Sfl*-cre develop TART-like cells that produce glucocorticoids (Padua et al., 2015). This

GATA-deficient mouse model may be useful for exploring the signals that drive TART formation in humans (Heikinheimo et al., 2015).

4. Adrenocortical neoplasms

4.1. GATA6 and GATA4 have been implicated in adrenocortical development and function

GATA6 is the principal GATA factor expressed in the adrenal cortex. *Gata6* is expressed diffusely in the adrenal cortex of the fetal mouse (Kiiveri et al., 2002). Postnatally, expression of *Gata6* in the mouse adrenal is limited to capsular and subcapsular cells (Pihlajoki et al., 2013). In primates, *GATA6* is expressed in the zona reticularis, where it is thought to regulate androgen biosynthesis (Jimenez et al., 2003; Nakamura et al., 2007; Nakamura et al., 2009). By comparison, GATA4 has a more restricted pattern of expression during adrenocortical development and is presumed to have a more limited role in the function of this organ (Kiiveri et al., 2002). During human development, GATA4 mRNA is evident in the fetal zone of the adrenal, but there is only weak expression of this transcript in the adrenal cortex postnatally. Similarly, *Gata4* is transiently expressed in the mouse adrenal cortex during fetal but not postnatal development.

Consistent with its proposed role in the biosynthesis of adrenocorticoids and androgens, GATA6 has been shown to enhance the transcription of *CYP11A1*, *CYP17A1*, *CYP5*, *SULT2A1*, and *HSD3B2* in cell lines [reviewed in (Röhrig et al., 2014)]. GATA4 can substitute for GATA6 in *trans*-activation studies of the *CYP17A1* promoter (Flück and Miller, 2004), suggesting that GATA4 may serve to augment *CYP17A1* expression during fetal development.

The impact of GATA6 on adrenocortical development and physiology has been assessed through conditional gene deletion using *Sfl*-cre (Pihlajoki et al., 2013). *Gata6* conditional knockout mice exhibit a pleiotropic phenotype that includes: 1) a thin, cytomegalic adrenal cortex, 2) decreased expression of the zG differentiation marker *Cyp11b2*, 3) blunted production of glucocorticoids in response to exogenous ACTH, 4) the spontaneous accumulation of non-steroidogenic cells expressing gonadal-like markers, 5)

ectopic chromaffin cells, and 6) absence of an adrenal X-zone. Based on analogous conditional deletion studies of *Gata6* in pulmonary (Tian et al., 2011; Zhang et al., 2008) and intestinal epithelia (Beuling et al., 2012; Beuling et al., 2011), GATA6 is hypothesized to regulate the balance between stem/progenitor cell expansion and differentiation in the adrenal cortex. Targeted ablation of *Gata4* in SF1⁺ cells has no significant impact on adrenocortical development, but *Gata4/Gata6* double mutant mice exhibit adrenocortical aplasia (Padua et al., 2015).

4.2. Gonadectomy-induced adrenocortical neoplasia

Gonadectomy (GDX) triggers the appearance of gonadal-like neoplasms in the adrenal cortex of mice (Röhrig et al., 2014). This phenomenon, termed GDX-induced adrenocortical neoplasia, is thought to reflect the metaplastic differentiation of stem cells in the adrenal capsule/subcapsule in response to the hormonal changes that accompany GDX (↑LH, ↓inhibin, etc.). The neoplastic cells express *Gata4*, *Lhcgr*, *Inha*, and enzymes required for sex steroid biosynthesis (*Cyp17a1*, *Hsd17b3*, *Cyp19a1*) (Bielinska et al., 2006; Schillebeeckx et al., 2015). Prototypical adrenocortical markers, such as *Gata6* and adrenocorticoid biosynthetic enzymes (*Cyp21a1*, *Cyp11b1*, *Cyp11b2*), are downregulated in the neoplastic adrenal tissue (Bielinska et al., 2006). In the mouse GDX-induced adrenocortical neoplasia is strain dependent, and chimera studies suggest that strain susceptibility to GDX-induced neoplasia is cell-intrinsic and resides in the stem/progenitor compartment (Röhrig et al., 2014). The genetic basis of strain susceptibility, however, remains unclear. Linkage analysis of crosses between susceptible (DBA/2J) and non-susceptible (C57Bl/6) mouse strains has shown that GDX-induced adrenocortical neoplasia is a complex trait (Bernichtein et al., 2007).

Loss- and gain-of-function studies have established that GATA4 directly modulates GDX-induced adrenocortical neoplasia. Constitutive and conditional mutations in *Gata4* mitigate the accumulation of gonadal-like neoplastic cells and the expression of sex steroidogenic markers in the adrenal cortex of gonadectomized female mice (Krachulec et al., 2012). Transgenic expression of *Gata4* in the adrenal cortex using a *Cyp21a1* promoter induces adrenocortical neoplasia in a non-susceptible

strain (C57Bl/6) (Chrusciel et al., 2013). Fate mapping studies suggest that GATA4⁺ neoplastic cells arise from a distinctive pool of WT1⁺ progenitors in the adrenal capsule (Bandiera et al., 2013). Under basal conditions, these cells give rise to normal steroidogenic cells in the adrenal cortex; GDX activates these WT1⁺ progenitors and triggers their differentiation into gonadal-like steroidogenic tissue. Thus, WT1⁺ capsular cells represent a reserve stem/progenitor cell population that can be mobilized in response to extreme physiological demand (i.e., GDX-induced hormonal changes). These WT1⁺ capsular cells are presumed to be the progenitors of GDX-induced adrenocortical neoplasms. Whereas GATA4 drives GDX-induced adrenocortical neoplasia, GATA6 appears to inhibit the process. Conditional deletion of *Gata6* using *Sfl*-cre augments the GDX-induced expression of gonadal-like markers in mice (Pihlajoki et al., 2013).

GDX-induced adrenocortical neoplasia is a well documented phenomenon in not only mice but also hamsters, ferrets, goats, and other domesticated species (Beuschlein et al., 2012; Bielinska et al., 2009). Male Angora goats are routinely gonadectomized to enhance mohair production, and these castrate animals have an increased incidence of adrenocortical adenomas (Altman et al., 1969). GDX-induced adrenocortical neoplasia is a major cause of morbidity and mortality in pet ferrets, affecting up to 20% of these animals. The neoplastic cells that accumulate in the adrenal glands of gonadectomized ferrets express GATA4 (Fig 4) and other gonadal-like markers and secrete sex steroids (Bielinska et al., 2006; Schillebeeckx et al., 2015).

There are related mouse models in which GDX triggers the accumulation of gonadal-like cells in adrenal cortex. Following GDX, *Inha*-Tag mice develop malignant gonadal-like tumors, a process that is thought to entail a feed-forward signaling loop involving *Gata4* and *Lhcgr* (Rahman et al., 2004). Similarly, *Inha*^{-/-} mice develop adrenocortical tumors in response to GDX (Matzuk et al., 1992). The resultant tumors exhibit increased expression of *Gata4* and other gonadal-like markers and a reciprocal downregulation of *Gata6* (Looyenga and Hammer, 2006). Enforced expression of LH enhances adrenocortical neoplasia in *Inha*^{-/-} mice (Beuschlein et al., 2003), whereas ablation of *Smad3* attenuates tumor growth in this model (Looyenga and Hammer, 2007).

4.3. Dysregulation of GATA factors in other genetically-engineered mouse models of adrenocortical neoplasia

Several mouse models of adrenocortical neoplasia exhibit abnormal expression of GATA factors, even in the absence of GDX. Mice harboring multiple copies of the steroidogenic factor-1 (*Sf1*) genetic locus, mimicking the amplification of *SF1* seen in childhood adrenocortical carcinoma, develop adrenocortical neoplasms that express gonadal-like markers including *Gata4* (Doghman et al., 2007). Activation of WNT/ β -catenin signaling is a hallmark of human adrenocortical tumors (Assie et al., 2014; Tissier et al., 2005). Constitutive activation of β -catenin signaling, a hallmark of human adrenocortical tumors, triggers the accumulation of GATA4⁺ cells in the subcapsule of mice (Berthon et al., 2010). Similarly, overexpression of *Igf2*, a characteristic of human adrenocortical carcinomas, in the adrenal cortex of mice leads to the accumulation of subcapsular cells that express *Gata4* (Drelon et al., 2012). Collectively, these results suggest that deregulation of GATA factors is probably a general feature of adrenal tumorigenesis (at least in rodents), irrespective of whether it is triggered by GDX or genetic alterations also found in patients.

4.4. Expression of GATA4 and GATA6 in human adrenocortical tumors

The vast majority (>90%) of human adrenocortical adenomas and carcinomas express *GATA6* (Fig 5) and approximately one-third express *GATA4* (Kiiveri et al., 2004). Diminished expression of *GATA6*, *SF1*, and their target gene *INHA* in adrenocortical carcinomas correlates with poor outcome (Parviainen et al., 2013). Overexpression of *GATA6* in adrenocortical tumor cells enhances BMP signaling, which inhibits cell proliferation and viability (Johnsen et al., 2009). Although global DNA methylation surveys of human adrenocortical neoplasms have yielded inconsistent results, hypermethylation and downregulation of both *GATA6* and *GATA4* have been reported (Fonseca et al., 2012; Rechache et al., 2012).

5. Parathyroid Neoplasms

5.1. Role of GATA3 in parathyroid development

The parathyroid glands regulate calcium balance in the body through the secretion of parathyroid hormone (PTH). *GATA3* expression has been reported in the second and third branchial arches, which harbor the progenitors of the parathyroid glands (Debacker et al., 1999; Grigorieva et al., 2010). The importance of *GATA3* in parathyroid function has emerged from characterization of patients with hypoparathyroidism, sensorineural deafness, and renal anomaly (HDR) syndrome, a condition caused by heterozygous loss-of-function mutations in *GATA3* (Ali et al., 2007; Nesbit et al., 2004; Van Esch et al., 2000). Using mouse models of HDR syndrome, investigators have shown that *GATA3* is involved in the embryonic development of the parathyroid glands and in adult parathyroid cell proliferation (Grigorieva et al., 2010). *Gata3*^{-/-} and *Gata3*^{+/-} embryos have smaller parathyroid-thymus primordia, and the parathyroid glands of adult *Gata3*^{+/-} mice do not show increased proliferation or enlargement in response to hypocalcemia induced by a low calcium/vitamin D diet. These studies identified *Gcm2*, a gene required for proper parathyroid development, as a target of activation by *GATA3*.

5.2. GATA3 is a marker of parathyroid neoplasms

In two recent surveys of parathyroid tumors, *GATA3* immunoreactivity was demonstrated in all parathyroid adenomas and carcinomas examined (Betts et al., 2014; Ordonez, 2014). As a tumor marker, *GATA3* was found to be comparable in sensitivity and specificity to PTH, the marker that has traditionally been used in the diagnosis of parathyroid tumors.

6. Pancreatic Neoplasms

6.1. Role of GATA4 and GATA6 in pancreatic development

The pancreas is a mixed endocrine and exocrine gland that arises from the amalgamation of dorsal and ventral buds of foregut endoderm. A number of transcription factor genes, including *Pdx1* and *Ptf1a*, have been shown to be essential for the early stages of pancreatic development in the mouse (Oliver-Krasinski and Stoffers, 2008). GATA4 and GATA6 exhibit overlapping patterns of expression in multipotential progenitor cells within the pancreatic anlagen (Decker et al., 2006; Ketola et al., 2004; Ritz-Laser et al., 2005). As development proceeds, GATA4 expression predominates in pancreatic acinar cells, while GATA6 predominates in the ductal compartment and a subpopulation of endocrine cells (Fig 6, 7) (Decker et al., 2006; Ketola et al., 2004).

GATA4 is able to transactivate the glucagon (*Gcg*) gene promoter *in vitro* (Ritz-Laser et al., 2005). The same study demonstrated that mutation of the GATA motif in the *Gcg* promoter reduces its basal promoter activity in glucagon producing cells. GATA6, but not GATA4, has been shown to physically interact with NKX2.2, an essential islet transcription factor (Decker et al., 2006).

The importance of GATA factors to pancreatic development was underscored when an international consortium of investigators demonstrated that heterozygous mutations in human *GATA6* cause a spectrum of pancreatic developmental defects ranging from agenesis to neonatal diabetes and adult-onset diabetes (Bonnefond et al., 2012; De Franco et al., 2013; Lango Allen et al., 2012; Yorifuji et al., 2012). Subsequently deletions or mutations of *GATA4* were shown to be a monogenic cause of neonatal and childhood-onset diabetes with variable exocrine phenotypes (Shaw-Smith et al., 2014). The roles of GATA4 and GATA6 in pancreatic development and disease have been modeled in the mouse (Carrasco et al., 2012; Decker et al., 2006; Martinelli et al., 2012; Watt et al., 2007; Xuan et al., 2012). Expression of a GATA6-Engrailed dominant repressor fusion protein in pancreatic progenitors using a *Pdx1* promoter caused pancreatic hypoplasia (Decker et al., 2006). Analysis of embryos derived by tetraploid complementation of *Gata4*^{-/-} ES cells demonstrated a complete absence of the ventral but not the dorsal pancreas; *Gata6*^{-/-} embryos displayed a similar, albeit less dramatic, phenotype (Watt et al., 2007). Conditional mutagenesis of either *Gata4* or *Gata6* in multipotent pancreatic progenitors (using *Pdx1*-cre) has minimal impact on pancreatic development or function, whereas mutagenesis of both genes

results in pancreatic agenesis and diabetes (Carrasco et al., 2012; Xuan et al., 2012). The double mutant mice exhibit impaired proliferation of pancreatic progenitor cells, aberrant branching morphogenesis, and a subsequent failure to induce the differentiation of progenitor cells expressing *Cpa1* and *Neurog3*. The prevailing hypothesis, based on the established roles of GATA4 and GATA6 in other foregut derivatives such as the lung and intestine (Beuling et al., 2012; Zhang et al., 2008), is that GATA proteins, in combination with other transcription factors, regulate the balance between stem cell expansion and differentiation in the developing pancreas. Although GATA6 is preferentially expressed in endocrine pancreas, conditional knockout mouse studies using *Ptf1a*-cre have shown that GATA6 has direct effects on survival of acinar cells in the exocrine pancreas (Martinelli et al., 2012). More recently, investigators have generated β -cell specific knockouts of *Gata4* or *Gata6* and concluded that these factors have important but nonessential roles in promoting endoplasmic reticulum integrity and β -cell survival, which may contribute to the pathogenesis of type 1 diabetes (Sartori et al., 2014). The precise function of GATA4 in mature pancreatic acinar cells is unclear.

6.2. *GATA6 is downregulated in insulinomas triggered by MEN1 deficiency in the mouse*

Mice harboring loss-of-function mutations in *Men1* develop multiple endocrine neoplasias (see Section 3.4) including insulinomas (Serewko-Auret et al., 2010). Transcriptome analysis of isolated control, normal, hyperplastic, and adenomatous islets showed that *Gata6* downregulation accompanies tumor formation. It has been proposed that *Gata6* dysregulation plays a fundamental role in tumor formation and progression in this model by modulating TGF β superfamily or WNT/ β -catenin signaling, as in other systems (see Section 1.3).

6.3. *Roles of GATA6 and GATA4 in pancreatic ductal adenocarcinoma*

Although not an endocrine tumor, pancreatic ductal adenocarcinoma (PDA) sheds light on the role of GATA factors in organogenesis and oncogenesis. PDA has a complex genomic landscape characterized by frequent point mutations and copy number changes. Common genetic changes include

activating mutations of *KRAS2* and inactivating mutations in the cell cycle regulator *CDKN2A*, the tumor suppressor *TP53*, and *SMAD4* (Hong et al., 2011; Jones et al., 2008). *GATA6* amplification and overexpression are hallmarks of PDA (Collisson et al., 2011; Fu et al., 2008; Kwei et al., 2008). Enforced expression of *GATA6* in pancreatic cancer cell lines enhances proliferation and growth in soft agar, whereas inhibition of *GATA6* impairs growth of pancreatic cancer cell lines. *GATA6* activates signaling in pancreatic cancer by negatively regulating the WNT antagonist Dickkopf-1 (*DKK1*) (Zhong et al., 2011). Smoking is a major risk factor for pancreatic cancer, and a recent study showed that nicotine promotes progression of *Kras*-induced pancreatic adenocarcinoma via *Gata6*-dependent dedifferentiation of acinar cells (Hermann et al., 2014). *GATA4* is frequently overexpressed and infrequently methylated in PDA, whereas *GATA5* is generally hypermethylated in these neoplasms (Fu et al., 2007). A separate study documented *GATA4* immunoreactivity in a majority of infiltrating pancreatic adenocarcinomas (Karafin et al., 2009).

7. Pituitary neoplasms

7.1. Role of *GATA2* in pituitary development and function

Among differentiated hormone-secreting cell types found in the pituitary gland, both gonadotrope and thyrotrope cells express *Gata2* from E10.5 onward in the mouse. The secretory products of thyrotrope and gonadotrope cells are heterodimers that share a common α -glycoprotein subunit (α GSU) and a specific β -subunit (FSH β , LH β , and thyrotropin- β) [reviewed in (Viger et al., 2008)]. The genes encoding α GSU (*Cga*) and thyrotropin- β (*Tshb*) are targets of activation by *GATA2* (Gordon et al., 1997; Steger et al., 1994). This transcription factor has also been implicated in the regulation of the GnRH receptor gene (Schang et al., 2013).

Analyses of transgenic and knockout mice have established that *GATA2* is involved in both gonadotrope and thyrotrope development (Charles et al., 2006; Dasen et al., 1999; Scully and Rosenfeld, 2002). Interactions between *GATA2* and another transcription factor, *PIT1*, are critical determinants of

pituitary cell fate (Dasen et al., 1999; Scully and Rosenfeld, 2002). In gonadotropes, where GATA2 is expressed in the absence of PIT1, GATA2 promotes the expression of gonadotrope-specific genes. In thyrotropes, where GATA2 and PIT1 are coexpressed, thyrotrope-specific genes are up-regulated by the binding of both factors to adjacent DNA *cis*-elements. PIT1 interacts via its homeodomain with a zinc finger of GATA2, modulating target gene transactivation (Dasen et al., 1999). Conditional ablation of *Gata2* in the anterior pituitary reduces gonadotrope and thyrotrope cell numbers at birth and impairs the secretory capacity of these cells in the adult (Charles et al., 2006).

7.2. *GATA2 is a marker of pituitary neoplasms*

Consistent with its established role in gonadotrope and thyrotrope development GATA2 is found in most α GSU-positive and thyrotropin-secreting human pituitary adenomas (Umeoka et al., 2002; Wang et al., 2009).

8. Summary and outlook

Studies over the past two decades have established that GATA factors are required for the proper development, differentiation, and function of endocrine tissues. More recently, GATA factors have been implicated in forms of endocrine neoplasia (Table 5), although the molecular mechanisms involved are not fully understood. Altered GATA factor expression or function owing to acquired genetic (mutations, deletions, amplifications) or epigenetic changes (e.g., DNA methylation) has been linked to tumor formation. GATA factors can impact tumorigenesis through modulation of key developmental signaling pathways implicated in oncogenesis, such as the WNT/ β -catenin and TGF β signaling pathways. In addition to affecting signaling pathways, GATA factor dysregulation can have effects on tumor cell metabolism, ploidy, and invasiveness.

Traditionally, transcription factors have been considered poorly druggable, but recent studies offer hope that GATA factors can be targeted pharmacologically in endocrine neoplasms. A small molecule inhibitor of GATA2 has been shown to suppress AR expression and exert anticancer activity

against prostate cancer cell lines (He et al., 2014). It may be feasible to adopt similar approaches for inhibition of GATA factors in other tumors.

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Table 1. Human diseases associated with germline loss-of-function mutations in GATA factors

Gene	Human disease phenotype	References
<i>GATA1</i>	<ul style="list-style-type: none"> • X-linked dyserythropoietic anemia and thrombocytopenia • X-linked Diamond-Blackfan anemia 	(Crispino and Weiss, 2014)
<i>GATA2</i>	<ul style="list-style-type: none"> • Monocytopenia with mycobacterial infections (MonoMAC) • Dendritic cell, B, and natural killer (NK) lymphoid deficiency • Familial MDS/AML • Emberger syndrome (primary lymphedema with MDS) 	(Bresnick et al., 2012; Spinner et al., 2014)
<i>GATA3</i>	<ul style="list-style-type: none"> • HDR syndrome (hypoparathyroidism, deafness, and renal dysplasia) 	(Ali et al., 2007; Nesbit et al., 2004; Van Esch et al., 2000)
<i>GATA4</i>	<ul style="list-style-type: none"> • Congenital heart disease • Congenital diaphragmatic hernia • Monogenic diabetes • Aberrant testicular development 	(Lourenco et al., 2011; Prediville et al., 2014; Shaw-Smith et al., 2014; Yu et al., 2013)
<i>GATA5</i>	<ul style="list-style-type: none"> • Congenital heart disease 	(Shi et al., 2014)
<i>GATA6</i>	<ul style="list-style-type: none"> • Congenital heart disease • Pancreatic agenesis and/or monogenic diabetes • Biliary tract abnormalities • Congenital diaphragmatic hernia 	(Allen et al., 2012; Bonnefond et al., 2012; Maitra et al., 2010; Prediville et al., 2014; Yu et al., 2014)

Table 2. Ovarian phenotypes of *Gata4* and *Gata6* conditional knockout mice

Gene(s)	Cre	Phenotype	References
<i>Gata4</i>	<i>Sf1</i> -cre	Impaired granulosa/theca cell proliferation, depletion of primordial follicles, ovarian cyst formation, and sterility	(Efimenko et al., 2013)
	<i>Amhr2</i> -cre	Cystic ovarian changes, impaired fertility, and a blunted response to exogenous gonadotropins	(Kyrönlahti et al., 2011b)
<i>Gata6</i>	<i>Sf1</i> -cre	No overt phenotype	(Pihlajoki et al., 2013)
	<i>Amhr2</i> -cre	Early embryonic lethal owing to leaky expression in yolk sac endoderm	Wilson, unpublished observations
<i>Gata4</i> & <i>Gata6</i>	<i>Cyp19</i> -cre	Impaired formation of large pre-ovulatory follicles and corpora lutea, an abnormal estrous cycle, and infertility; impaired expression of <i>Fshr</i> , <i>Lhcgr</i> , <i>Inha</i> , and <i>Inhbb</i>)	(Bennett et al., 2013; Bennett et al., 2012)
	<i>Sf1</i> -cre	Aberrant folliculogenesis and impaired expression of genes involved in early ovarian development, including <i>Foxl2</i> and <i>Fst</i>	(Padua et al., 2014)

Table 3. Genetically-engineered mouse models of GCT

Genotype	Phenotype	Tumor penetrance (%)	References
<i>Inha-TAg</i>	Infertile, GCT formation at age of 5-7 mo, serum inhibin B ↑, serum gonadotropins ↓	100	(Kananen et al., 1995)
<i>Inha</i> ^{-/-}	Bilateral, mixed, or incompletely differentiated sex cord-stromal tumors coupled with cachexia-like syndrome at age of 4 wks	100	(Matzuk et al., 1992)
<i>Smad1/5</i> double knockout	Infertile, poorly differentiated, metastatic, uni- or bilateral GCT at age of 3 mo, histopathological features similar to human juvenile GCT	100	(Pangas et al., 2008) (Middlebrook et al., 2009)
<i>Smad1/5/8</i> triple knockout	Infertile, poorly differentiated, metastatic, uni- or bilateral GCT at age of 3 mo	100	(Pangas et al., 2008)
<i>Catnb</i> ^{fl_{ox(ex3)}/+} ; <i>Amhr2</i> ^{cre/+}	Subfertile, follicle-like lesions that evolve into GCT at 7.5 mo of age	57	(Boerboom et al., 2005)
<i>Men1</i> ^{+/-}	Develop GCTs, a feature not typical of humans with the MEN1 cancer predisposition syndrome; <i>Gata6</i> is downregulated in GCTs that arise in the ovaries of these mice	70	(Mould et al., 2009)

Table 4. Testicular phenotypes of *Gata4* and *Gata6* conditional knockout mice

Gene(s)	Cre	Phenotype	References
<i>Gata4</i>	<i>Sfl</i> -cre	Decreased expression of <i>Dmrt1</i> ; germ cell attrition	(Manuylov et al., 2011)
	<i>Amhr2</i> -cre	Late onset impairment of Sertoli cell function with loss of fertility	(Kyrönlähti et al., 2011b)
	<i>Wtl</i> -creERT2	Conditional ablation at E10.5 leads to a block in testicular differentiation and male-to-female sex reversal	(Manuylov et al., 2011)
	<i>CAG</i> -creER	Impaired formation of the genital ridge and subsequent licensing of primordial germ cells	(Hu et al., 2015; Hu et al., 2013)
<i>Gata6</i>	<i>Sfl</i> -cre	No overt phenotype	(Pihlajoki et al., 2013)
	<i>Amhr2</i> -cre	Early embryonic lethal owing to leaky expression in yolk sac endoderm	Wilson, unpublished observations
<i>Gata4</i> & <i>Gata6</i>	<i>Sfl</i> -cre	Impaired spermatogenesis, absence of Leydig cells, and ectopic appearance of adrenocortical-like cells	(Padua et al., 2015)

1175 **Table 5. GATA factors in endocrine cell types and their corresponding neoplasms**

Tissue	GATA factor	Cell type	Tumor
Ovary	GATA4	Granulosa cells Theca cells	Human: AGCT, SLCT, thecoma-fibroma
	GATA6	Granulosa cells Theca cells Corpus luteum	
Testis	GATA1	Sertoli cell	
	GATA4	Sertoli cells Leydig cells Peritubular cells	Human: LCCSCT Canine: SCT, LCT, MGSC
	GATA6	Sertoli cells Leydig cells	
Adrenal cortex	GATA4	Fetal cortex	Mouse, ferret: GDX-induced neoplasms Mouse: <i>Inha</i> -Tag and <i>Inha</i> ^{-/-} gonadal-like tumors Human: Minority of adenomas and carcinomas
	GATA6	Mouse: Fetal cortex Adult (sub)capsule Human: Fetal cortex zR	Human: Majority of adenomas, carcinomas
Parathyroid gland	GATA3	Parathyroid cells	Human: Adenomas and carcinomas
Pancreas	GATA4	Multipotential progenitor cells Acinar cells	Human: Pancreatic ductul adenocarcinoma
	GATA6	Multipotential progenitor cells Islet cells	Human: Pancreatic ductul adenocarcinoma
Pituitary	GATA2	Gonadotrope cells Thyrotrope cells	Human: adenomas

1176
1177 **Abbreviations:** AGCT, adult-type granulosa cell tumor; GDX, gonadectomy; LCCSCT, large-cell
1178 calcifying Sertoli tumor; LCT, Leydig (interstitial) cell tumor; MGSC, mixed germ cell sex cord-stromal
1179 tumor; SCT; Sertoli cell tumor; SLCT, Sertoli-Leydig cell tumor; zR, zona reticularis.

Figure Legends

Figure 1. Expression of *Gata4* and *Gata6* in developing mouse ovary. (A-D) Cryosections of E18.5 mouse were subjected to *in situ* hybridization for GATA4 or GATA6 using [³³P]-labelled riboprobes and counterstained with hematoxylin and eosin (H&E). Shown are corresponding brightfield (A,B) and darkfield (C,D) photomicrographs. GATA4 and GATA6 mRNA are evident in fetal ovary (ov) and small intestine (si). Additionally, GATA6 mRNA is seen in the mesovarium (mo) but not in the Müllerian duct (md). Bars = 100 µm. (E,F) Immunoperoxidase staining of ovary from a parous female (P90) mouse. GATA4 and GATA6 protein are evident in granulosa cells (gc) and germinal epithelium (ge). Bars = 50 µm.

Figure 2. Interplay among GATA4, FOXL2, and SMAD3 during human granulosa cell tumor development. Sections of formalin-fixed AGCT were subjected to immunoperoxidase staining for A) GATA4, B) FOXL2, and C) SMAD3. Bar = 50 µm. Normal granulosa cell growth is modulated by cooperative interactions among wild-type FOXL2, GATA4, and SMAD3 (D). The C134W mutation in *FOXL2* disrupts this balance leading to malignant cell growth (E).

Figure 3. Immunohistochemical staining of GATA4 in a human large cell calcifying Sertoli cell tumor. The box in panel A is shown at higher magnification in panel B. The asterisk highlights a calcified granule in the Sertoli cell tumor. Note that GATA4 is abundantly expressed in Sertoli (arrowheads) tumor cells. The adjacent normal Sertoli cells (open arrowheads) also express GATA4. Abbreviations: nl, normal seminiferous tubule; tu; tumor. Bar = 50 µm.

Figure 4. Immunohistochemical staining of GATA4 in a GDX-induced adrenocortical carcinoma of the ferret. Serial sections of formalin-fixed tissue were stained with A) hematoxylin & eosin, or B) GATA4 antibody, followed by hematoxylin counterstaining. Bar = 50 µm.

Figure 5. Immunohistochemical staining of GATA6 in human adrenocortical tumors.

Representative samples of nonfunctional (Nonf), Conn's (aldosterone-producing), and Cushing's (cortisol-producing) adenomas (A, C, E, respectively) and carcinomas (B, D, F, respectively) are shown.

Immunoreactive cells (arrowheads) exhibit brown nuclear staining. Sections were counterstained with hematoxylin. Bar = 50 μ m.

Figure 6. Immunohistochemical staining of GATA4 and GATA6 in the developing mouse pancreas.

Three developmental stages are illustrated: E15.5, newborn (postnatal day 1), and adult (3 months). The expression patterns reveal association of GATA4 (A, G, M) primarily with exocrine and GATA6 (B, H, N) with endocrine cells (arrows). Bar = 150 μ m.

Figure 7. GATA4 immunostaining distinguishes normal exocrine pancreas from a well-

encapsulated insulinoma of the ferret. Serial sections of formalin-fixed tissue were stained with A)

hematoxylin & eosin (H&E), or B) GATA4 antibody, followed by hematoxylin counterstaining. Note that

pancreatic acinar cells exhibit GATA4 immunoreactivity, whereas insulinoma cells do not. Bar = 50 μ m.

Figure 1

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Figure 7