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GATA factors: Master regulators of gene expression in trophoblast progenitors

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Abstract

Mammalian reproduction is critically dependent on trophoblast cells, which ensure embryo implantation and placentation. Development of trophoblast cell lineages is a multi-step process and relies upon proper spatial and temporal gene expression, which is regulated by multiple transcription factors. However, most of the transcription factors that are implicated in trophoblast development regulate gene expression at a specific developmental stage or in a specific trophoblast subtype. In contrast, recent studies from our group and other laboratories indicate that conserved GATA family of transcription factors, GATA2 and GATA3, are important to regulate gene expression at multiple stages of trophoblast development. Furthermore, our conditional gene deletion studies revealed that functional redundancy of GATA2 and GATA3 ensures both self-renewal of trophoblast stem and progenitor cells and their differentiation to trophoblast cells of a matured placenta. Together these findings indicate that GATA2/GATA3 are the master orchestrators of gene expression in trophoblast cells and they fine tune gene regulatory network to establish distinct trophoblast cell types during placentation.

Keywords

Trophoblast progenitor; Placenta; GATA2; GATA3; Transcriptional regulation; Pregnancy

1. Introduction

Molecular mechanisms that dictate different stages of trophoblast development are largely understood from studies with mouse models. These studies indicate that trophoblast development is governed by establishment of distinct gene regulatory networks in trophoblast progenitors and differentiated trophoblast cells. The distinct gene expression programs in trophoblast cells are often orchestrated by conserved transcription factors, which are implicated in both mouse and human trophoblast development. For example, Caudal Type Homeobox 2 (CDX2) has been implicated in the development of both mouse

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and human trophoblast progenitors [1]. CDX2 is important for proper development of the trophoblast in a preimplantation mouse embryo and trophoblast stem cell (TSC)-like progenitor cells (TSPCs, which differentiate to all specialized trophoblast cells of a matured placenta) within an early post-implantation embryo [1]. CDX2 is also selectively expressed in the cytotrophoblast (CTB) progenitors of a human first trimester placenta [1]. Similarly, Glial Cells Missing Homolog 1 (GCM1) has been implicated in the development and function of syncytiotrophoblasts (SynTBs) in rodents and humans [1]. The GATA family transcription factors are conserved in mammals and have been shown to be intricately involved in the early mammalian development process [2]. Interestingly, two of the GATA family members, GATA2 and GATA3, are selectively expressed within trophoblast progenitors during early mouse development and directly regulate key genes [3,4], which in turn control trophoblast development in both pre- and postimplantation embryos. In this review we will focus on our current understanding of GATA2/GATA3-dependent transcriptional mechanisms in trophoblast cells and future research perspectives to understand the importance of GATA2/GATA3-mediated gene regulation in human trophoblast progenitors.

2. Multi-stage trophoblast development during mouse and human placentation

Successful development of the trophoblast cell lineage is dependent upon specification of stem/progenitor populations and proper balance of their self-renewal vs. differentiation during placentation. This intricate process begins with the establishment of the TE lineage in blastocysts. In an early postimplantation mouse embryo [embryonic day (E)5.5-E7.5] TSCs/TSPCs arise within the extra-embryonic ectoderm (ExE) and can be identified from expression of TSC-specific genes, including *Cdx2* and *Eomesodermin* (*Eomes*) (Fig. 1A). Later, ~E8.5, lineage-specific trophoblast progenitors arise within the chorion and ectoplacental cone (EPC) (Fig. 1A). The chorion attaches to the allantois and gives rise to Glial Cell Missing-1 (GCM1) expressing TSPCs [5] (Fig. 1A). These progenitors differentiate into labyrinth trophoblast progenitors (LTPs), which express high levels of Epithelial Cellular Adhesion Molecule (EpCAM^{Hi}) [6], Fig. 1A). Subsequently, trophoblast cell syncytialization leading to formation of the SynTB layer [7] within the labyrinth zone.

In the EPC, Achaete-scute Complex Homolog 2 (ASCL2)+, PR Domain Zinc Finger Protein 1 (PRDM1)+ and Trophoblast Specific Protein Alpha (TPBPA)+ progenitors arise [8–10] (Fig. 1A). These progenitors subsequently differentiate to specialized trophoblast subtypes of the junctional zone, which includes trophoblast giant cells (TGCs), Spongiotrophoblasts (SpTs), Glycogen trophoblasts (GlyTs), which are characterized with accumulation of glycogen storage. Three subtypes of secondary TGCs were classified within the mouse junctional zone and the decidua [10,11]; (i) the maternal arterial canal associated TGCs (c-TGCs), (ii) parietal-TGCs (p-TGCs) lining the implantation site and (iii) spiral artery associated TGCs (SpA-TGCs), which invade the uterine wall and maternal vessels [12–14]. Along with these endovascular SpA-TGCs, a population of glycogen cells also invades interstitially into the maternal decidua. Thus, the SpA-TGCs and the glycogen cells constitute the invasive trophoblast cell population at the mouse maternal-fetal interface.

Thus, formation of a mature placenta in mouse is a multi-step process, which involves proper specification, self-renewal and differentiation of distinct progenitor populations.

On the other hand, early stages of human placentation are associated with the development of two different types of chorionic villi from the chorionic plate, (i) the anchoring villi, which attach to uterine decidua and (ii) the floating villi, which do not attach to the uterine decidua (Fig. 1B and C). Recent studies have indicated presence of two different types of proliferating CTB progenitors in these chorionic villi. The outer layers of both floating and anchoring villi contain SynTBs (Fig. 1B), established from fusion of cell cycle arrested villous CTB (vCTB) progenitors. The anchoring villi attach to the uterine decidua via formation of a cell column. The cell column in anchoring villi is derived from proliferating column CTB (cCTB) progenitors. cCTB progenitors differentiate to extravillous trophoblasts (EVTs), which invade into the maternal decidua (Fig. 1C). Subsequently, a group of EVT cells invades and remodels the uterine artery to establish the endovascular trophoblasts. Interestingly, analyses of marker expression showed that a few vCTB progenitors within an early (<week 8) first trimester human placenta express TSC/TSPC marker CDX2 [15], whereas CDX2 expression is lost in cCTB progenitors. However, both vCTB progenitors and cCTB progenitors maintain expression of NOTCH1, which promotes cCTB proliferation and survival [16]. Thus, it appears that TSPC compartment during early human placentation is comprised of the CDX2(+)/NOTCH1(+) vCTB cells, which adapt two distinct differentiation fates. In both floating and anchoring villi they differentiate to form the outer SynTB layer. In addition, in the anchoring villi they differentiate to develop the CDX2(-)/NOTCH1(+) cCTB progenitors, which proliferate to form the CTB cell columns to attach to the uterine decidua and subsequently differentiate into the EVT and the endovascular trophoblast cells.

3. Concept of trophoblast cell-autonomous master regulators

As discussed above, both rodent and human placentations are multi-step processes, where trophoblast cells of specialized function arise from distinct progenitors. It is well known that extracellular signaling mechanisms regulate distinct stages of trophoblast development. For example, FGF4 and TGF- β signaling promotes the trophoblast stem state in mouse [17,18], whereas HGF-cMet signaling regulates development and maintenance of the EpCAM^{Hi} LTP population during placental labyrinth formation. The extracellular signaling components induce distinct cell fate by promoting activation or repression of transcription factors and epigenetic regulators that are specific to a particular developmental stage. For example, expression of CDX2 marks TSC/TSPC-fate and suppression of CDX2 and induction of GCM1 promotes labyrinth trophoblast fate. Thus, the stem vs. progenitor vs. differentiation fate of trophoblast cells during successful placentation depends on balanced gene expression patterns, in which a group of key factors are repressed and another group of key factors are activated in a trophoblast progenitor to dictate self-renewal vs. differentiation (Fig. 2A). Impairment of this gene expression balance leads to either impairment of TSC/TSPC self-renewal or their differentiation, leading to defective placentation (Fig. 2B and C).

The simultaneous induction and repression of key genes in a trophoblast progenitor indicate existence of trophoblast cell-autonomous master regulators that coordinate gene expression

balance downstream to extra cellular signaling pathways, thereby dictating specific cell fate. We propose that these master regulators should have three unique properties; (i) they should be conserved in trophoblast cells across mammals, (ii) they should directly regulate both activation and repression of a trophoblast gene and (iii) they should be constitutively expressed in all trophoblast during the course of trophoblast cell types during placenta development. Interestingly, GATA2 and GATA3 fulfill all of these three criteria. GATA2 and GATA3 are conserved in trophoblast cells across placental mammals [3,4,19,20]. Both GATA2 and GATA3 are induced during TE-specification in preimplantation embryos and subsequently maintained in trophoblast cells throughout the course of placental development [3], indicating a constitutive expression pattern. Also, being pioneer transcription factors [21], GATA2/GATA3 could instigate activation of silent genes. Furthermore, by interacting with various corepressors they could repress transcription of an active gene [22]. These properties uniquely positions these two GATA factors as master regulators of gene expression in all trophoblast progenitors, including TSCs/TSPCs and lineage-specific progenitors; a fact confirmed by functional analyses in rodent, bovine and human trophoblast progenitors [3,19,23].

4. Current understanding of GATA factor function in trophoblast cells

The GATA family of transcription factors bind to W(A/T) GATAR(A/G) motifs (GATA motifs) of regulatory elements and control developmental processes by regulating tissue-specific gene expression [22]. An earlier study analyzed *Gata3* mRNA expression in postimplantation mouse embryos and showed that *Gata3* mRNA is most abundantly expressed in trophoblast cells prior to E9.5 [24]. However, the first direct evidence of gene regulation by GATA factors in rodent trophoblast cells came from the studies of Linzer laboratory [25,26], which showed that GATA2 and GATA3 are important to regulate expression of placental lactogen I (*Prl3d1*) and proliferin (*Prl2c2*) in TGCs. We first reported that both GATA2 and GATA3 are highly expressed in mouse TSCs [27] and GATA3 is selectively upregulated within the TE lineage in a blastocyst [4]. We also showed that depletion of GATA3 in pre-implantation mouse embryos inhibits their development to the fully expanded blastocyst stage [4]. Therefore, we proposed that GATA3 is important for TE lineage development and to maintain TSC self-renewal [4]. This concept was further established by studies from other laboratories, which confirmed the critical role of GATA3 in establishment of trophoblast fate during early embryonic development [28] and to derive inducible TSCs from differentiated cells [29,30].

The importance of GATA factors in trophoblast gene regulation has also been confirmed in bovine and human trophoblasts [19,20,23]. Both *GATA2* and *GATA3* mRNAs are highly expressed in the human TE [23]. We showed that GATA2 and GATA3 proteins are highly expressed in vCTB progenitors [3] and in cCTBs and EVT within an anchoring villous (Figs. 2C and 3). Furthermore, expression analyses of GATA3 (Fig. 2C) and GATA2 (Fig. 3) showed that they are highly expressed in cCTB progenitors as well cCTB-derived nascent EVT population that are poised to invade the maternal decidua. Thus, similar to that in mouse, GATA2 and GATA3 are constitutively expressed in all trophoblast cell types of a developing human placenta. Not surprisingly, GATA2 and GATA3 are implicated in

regulation of human chorionic gonadotropin alpha-subunit (CGA), Syncytin, HLA-G and a number of other genes in human trophoblast cells [20,23,31,32].

Despite the important role of GATA2 and GATA3 in trophoblast cells, studies with knock out mice showed that placentas develop in *Gata2* as well as *Gata3*-null embryos [26]. These findings indicated that individual function of either GATA2 or GATA3 is dispensable for trophoblast progenitor development. Therefore, it was hypothesized that GATA2 and GATA3 might have functional redundancy and one GATA factor compensates loss of the other during trophoblast development. Our recent study with dual *Gata2/Gata3* conditional knockout mice confirmed importance of functional redundancy of GATA factors in trophoblast progenitors [3]. We showed that a number of key trophoblast genes are direct targets of both GATA factors. Furthermore, mechanistic analyses revealed that GATA factors dynamically repress or activate their transcription in a developmental stage-specific manner [3].

5. Future perspectives

Our recent study provided genetic evidence that GATA2 and GATA3 functions are essential to ensure trophoblast development in both pre- and postimplantation mouse embryos. However, a number of questions remain unanswered. For example:

5.1. Do GATA factors orchestrate common gene expression program in mammalian trophoblast progenitors?

Although we have identified global GATA2/GATA3 targets in TSCs [3], global GATA targets in human vCTB or cCTB progenitors are yet to be identified. Also, we do not know whether a common gene regulatory network exists in rodent TSPCs and human vCTB progenitors. Due to the conserved nature of GATA2 and GATA3 expression in both mouse and human trophoblast progenitors, we should employ an integrative approach, in which regulatory mechanisms in primary trophoblast progenitors from both rodents and human pregnancies will be studied simultaneously. This integrative approach will be foundational to gain insights about conserved regulatory mechanisms during trophoblast development.

5.2. How GATA factors fine-tune temporal gene expression during trophoblast development?

Snapshots of gene expression in *Gata2*- and *Gata3*-double knockout trophoblast progenitors indicated that GATA2/GATA3 fine-tune expression of a gene depending on the developmental stage. For example, GATA factors repress expression of *Prl3b1* and *Ascl2* in the TE but activate their expression in differentiated trophoblast cells [3]. So, to better understand development and differentiation of trophoblast progenitors, we need to understand how GATA factors fine-tune temporal gene expression. As GATA factors could interact with coactivators, corepressors and epigenetic modulators, they could establish different nucleoprotein complexes at the same chromatin domain in response to different extracellular signaling, leading to alternative transcriptional outcomes. Thus, it will be interesting to identify GATA-interactomes at different stages of trophoblast development and how signaling components alter that interactome.

5.3. Is early pregnancy loss associated with impairment of GATA factor function?

It is estimated that ~70% of all human conceptions do not lead to live birth [33]. Most of these losses are preclinical and occur prior to blastocyst implantation or within 3–4-weeks of implantation. However, ~15% of all conceptions are associated with early (before 20 week of gestation) clinical pregnancy loss (miscarriage) [33,34]. Although sporadic miscarriage is the most common complication, 1–3% of pregnant women suffer from recurrent miscarriage, defined as the occurrence of two or more consecutive pregnancy losses [33,34]. While fetal chromosomal abnormalities are responsible for majority of (~70%) of sporadic miscarriages, only a smaller fraction of recurrent miscarriages are associated with chromosomal abnormalities. Furthermore, almost half of the recurrent miscarriages are considered idiopathic, as they are not associated with chromosomal anomalies or other common maternal disorders [33–35]. Thus, it is recognized that, in addition to common risk factors, recurrent miscarriages, especially primary recurrent miscarriages (with no successful pregnancies) might be associated with genetic susceptibility (86, 87). Interestingly, GATA2 and GATA3 mutations have been linked to pregnancy disorder and miscarriage [36]. In that context, GATA2 and GATA3 functions have been linked with proper decidualization and immune system modulation. However, it is still unknown whether specific mutations in *GATA2* or *GATA3* genes in trophoblast cells cause predisposition to miscarriages. We found that GATA2/GATA3-mediated gene regulation in TSPCs establishes proper embryonic-extraembryonic signaling cross-talk during mouse development. Due to the conserved nature of GATA factor expression in human trophoblasts, it stands to reason that a similar mechanism may also be functional during human embryonic development. Thus, it will be intriguing to test whether pregnancy disorders are associated with specific GATA mutations.

5.4. What mechanisms regulate adaptation/plasticity in human trophoblast progenitors?

Our understanding of transcriptional mechanisms, which are associated with altered trophoblast function in pathological pregnancies, including preeclampsia and IUGR, is at its infancy. Human trophoblast cells are largely categorized based on their spatial and temporal appearances and function. However, protein expression studies show heterogeneity of GATA factor expression within a trophoblast sub-population, including cCTB progenitors and nascent EVT_s (Figs. 2C and 3). This heterogeneity supports plasticity in trophoblast cells, which allows them to adapt with challenges within the maternal environment [37,38]. We propose that plasticity in human trophoblast progenitors is regulated by subtle changes in gene expression. We also propose that GATA2 and GATA3 actively contribute to these dynamic gene expression mechanisms and impairment of this GATA-mediated gene regulation contributes to pathological pregnancies. Thus, it will be intriguing to test whether adaptation/plasticity in human trophoblast progenitors are characterized with alteration in GATA-dependent transcriptional mechanisms.

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Fig. 1. Trophoblast progenitors in developing mouse and human placentae.

(A) Schema of postimplantation trophoblast lineage development in mouse. In early post-implantation embryos TSCs/TSPCs exist within the ExE, which is developed from the expansion and differentiation of the polar TE. In contrast, mural TE cells differentiate to form the primary TGC layer. EPC and chorionic ectoderm that are developed from the EXE, contain distinct TSPC populations, which can be distinguished based on the GCM1 expression. Afterwards, TSPCs within EPC and chorion differentiate into lineage-specific trophoblast progenitors. Finally, specialized trophoblast subtypes like TGCs, SpTs GlyTs, and SynTBs arise to form a mature placenta. B, Expression of CDX2 in vCTBs (green

arrows) within early first trimester placental villi marks a mouse TSPC like progenitor population. In floating villi, these progenitors differentiate to outer SynTBs (red arrows). C, A 10 week human placentation site was stained with anti-GATA3 antibody. Images show GATA3 expression in trophoblast cells within anchoring villi. GATA3 is expressed in all cell types; in cCTB progenitors within the cell column (green arrows), in nascent EVT, arising from the CTB columns (red arrows) and in EVTs that invade into the decidua (black arrows). Heterogeneity within cCTB progenitors and nascent EVTs are evident from cells that lack GATA3 expression or have reduced level of expression (red circles).

Fig. 2. Models depicting trophoblast development leading to normal vs. defective placentation. A, Balance between self-renewal and differentiation of trophoblast progenitors is maintained by different extracellular signaling. Pioneer transcription factors, GATA2 and GATA3, which are conserved and constitutively expressed in trophoblast cells orchestrate stage-specific gene expression downstream to extracellular signaling, leading to normal placentation. (B) Impaired self-renewal of trophoblast progenitors could lead to premature placenta or trophoblast insufficiency. (C) Defective differentiation of progenitors will also lead to

defective placentation due to arrested development of a placenta primordial. Alternatively, this could lead to trophoblast progenitor hyperplasia.

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Fig. 3.

An anchoring villous of a 9 week human placenta showing GATA2 expression in trophoblast cells. Heterogeneity of cells within a trophoblast column is shown by high (red arrows), low (green arrows) or absence (blue arrows) of GATA2.