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GATA2 is expressed at critical times in the mouse uterus during pregnancy

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ABSTRACT

In mammals, such as mouse and human, timely production of the progesterone receptor (PR) in the proper uterine compartments is critical for preparing the uterus for the initiation and maintenance of pregnancy. Developmentally, the expression of GATA2, a member of the six member zinc-finger family of transcription factors, has been shown to be necessary for multiple non-related tissues, such as the hematopoietic system, adipose maturation and the urogential system. We recently identified *Gata2* as a potential progesterone target gene in the mouse uterus; however, the expression of the GATA genes in the mouse uterus during pregnancy has not been demonstrated. In the present study, we examined the expression of GATA2 protein during the phases of pregnancy, including early pregnancy where progesterone (P4) signaling is critical in order to facilitate the window of receptivity for embryo implantation and during the decidualization of the uterine stroma, a process of cellular proliferation and differentiation which is necessary for maintenance of the invading embryo until placentation occurs. Here, we report that GATA2 protein is expressed in the uterine luminal and glandular epithelium pre-implantation, spatio-temporally co-localizing with that of the PR. Additionally, GATA2 continues to be expressed in the decidualized stroma throughout early pregnancy indicating a role in the maintenance of decidual cells. Based on these findings, we conclude that GATA2 is expressed during critical phases of early pregnancy, similar to that of the PR, and that it may play a major role in mediating P4 signaling in the mouse uterus.

The uterus is a critical organ for mammalian reproduction and the maintenance of a healthy, developing fetus to term. It consists of heterogeneous cell types including the outer layer of myometrium and inner endometrium which is itself composed of the luminal and glandular epithelium and fibroblastic stroma. During pregnancy the precise spatial and temporal production of the ovarian hormones estrogen (E2) and progesterone (P4) acting through their receptors are required to initiate and maintain a healthy pregnancy as reviewed by (Rubel et al., 2010; Franco et al., 2008). Though the involvement of P4 in pregnancy has been known for years (Allen, 1970), the critical role of the progesterone receptor (PR) was determined through the use of the progesterone receptor knock-out mouse (PRKO), which demonstrated multiple reproductive defects such as anovulation, infertility, lack of implantation and decidualization and mammary gland defects (Lydon et al., 1995). There are two well characterized isoforms of the PR which have distinct roles depending on tissue context, including the uterus (Mulac-Jericevic et al., 2000, 2003). The PR is transiently expressed in early pregnancy during the pre-implantation period, with peak expression in the luminal epithelium on day 2.5 (0.5 the day of the plug) in the mouse, with similar expression in other mammals, and quickly dropping on subsequent days (Tan et al., 1999; Spencer et al., 2004). At this time expression of stromal PR occurs and lasts throughout decidualization. This peak expression in turn triggers the expression of downstream P4 target genes which are critical for embryo implantation and subsequent decidualization of the uterus (Ma et al., 1998; Lee et al., 2006, 2007). In addition to the critical timing of P4 expression and target gene induction, is the communication of these genes that occur via epithelial–stromal interactions which prepares the uterus for receptivity (Rubel et al., 2010). Elucidating further the gene networks that coordinate P4 signaling in the uterus through the PR is an important task in order to understand the mechanism of maintaining a healthy endometrium.

Microarray analysis performed in our lab utilizing ovariectomized mice acutely treated with P4 for 4 h revealed many potential uterine genes regulated in a ligand-dependent manner by the progesterone receptor (Jeong et al., 2005). One gene of interest that was found to be increased upon P4 injection was the transcription factor *Gata2*. *Gata2* belongs to a six-member family of transcription factors that share homology in their DNA binding zinc-finger region (Lowry and Atchley, 2000), which bind to a WGATAR consensus sequence. They are divided into a hematopoietic group (*Gata1–3*) (Weiss and Orkin, 1995) and those expressed in endodermal and mesodermal tissue (*Gata4–6*) (Molkentin, 2000),

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though differences in their DNA binding specificities (Ko and Engel, 1993; Merika and Orkin, 1993) allow for their regulation in a wider range of tissues. Further underlying the dynamic expression of *Gata2* seen throughout mammalian tissues is that in addition to its critical role in regulating hematopoiesis, *Gata2* has been shown to be critical in regulation of the prostate, adipose, pituitary and urogential formation (Zhou et al., 1998; Perez-Stable et al., 2000; Tong et al., 2000; Suh et al., 2002). Given the clear role *Gata2* has adapted in divergent tissues to regulate processes of differentiation and development, coupled with its identification as a potential P4 target gene in the uterus, we aim to examine if *Gata2* is expressed during the critical stages of pregnancy initiation and maintenance.

In mammals, the expression and functions of GATA2 protein during pregnancy has not been investigated. Microarray data from human endometrium during the pre-receptive phase versus the window of implantation have demonstrated that the expression of *GATA2* is down regulated during the window of implantation, expression which is concomitant with that of PR, providing evidence of its potential role as a P4 target gene and its importance in mammalian pregnancy (Kao et al., 2002; Borthwick et al., 2003; Mirkin et al., 2005). Similar microarrays from mouse uterine epithelium confirm similar *Gata2* expression during the peri-implantation period (Campbell et al., 2006) and mRNA expression has been confirmed in the uterus during a limited period of early pregnancy (Jeong et al., 2005). However, uterine and cellular compartmentalization of protein expression throughout pregnancy has not yet been determined. This will be critical in order to further define potential protein interactions and the relevant biological context of uterine expression of GATA2. To explore the possible function of GATA2 during pregnancy in the mouse, we evaluate in detail the expression of GATA2 from the onset of pregnancy, through the peri-implantation period and embryo invasion into the uterine stroma and subsequent decidualization process. We report for the first time that expression of GATA2 during pregnancy correlates with that of the PR *in vivo* during pregnancy, providing the first report of a possible strong role for this transcription factor in mammalian pregnancy.

1. Results and discussion

1.1. Confirmation of Gata2 as a progesterone target gene in the murine uterus

To verify *Gata2* as a uterine P4 target gene we utilized both *in vitro* and *in vivo* methods. Ligand induced nuclear receptor regulation of gene expression, including that of the PR, acts by translocation into the nucleus and interacting with the chromatin through the receptors' DNA binding domain and subsequent recruitment of co-regulators (Li and O'Malley, 2003). We utilized *in vitro* cell culture to investigate the ability of the PR to regulate expression of an approximately 1 kb region of the mouse *Gata2* proximal promoter through transient transfection analysis in human endometrial epithelial Hec-1A cells. We observed a significant increase in expression of luciferase with the addition of either PR isoform (PRA and PRB) upon treatment with the P4 analog R5020 (Fig. 1A). Transfection analysis allows the use of a closed system in which control is asserted over potential regulatory elements which may be directing gene transcription. We provide evidence that is consistent with the hypothesis that regulation of *Gata2* expression by P4 is mediated through direct interaction of PR with the proximal promoter region of the *Gata2* gene.

It has previously been established that an acute dose of P4 injected into ovariectomized mice elicit induction of early up and down regulated P4 target genes, and that this induction is absent in PRKO mice (Jeong et al., 2005). P4 was injected into both wild-type and PRKO ovariectomized mice for 6 h and uteri removed for RNA isolation and quantitative PCR. Acute P4 treatment resulted in significant induction of *Gata2* mRNA in the wild-type mice but not in the PRKO mice as previously demonstrated (Fig. 1B), providing further confirmation that *Gata2* is a uterine P4 target gene which requires the presence of the PR to mediate its expression. To verify induction of GATA2 protein following acute P4 treatment, uterine cross-sections of wild-type mice prepared from identical treatments were fluorescently labeled for the presence of either PR or GATA2 (Fig. 1C). Acute P4 treatment is known to down regulate its own receptor in the luminal epithelium in ovariectomized mice, and our immunofluorescence demonstrates consistency with this observation underlying intact P4 signaling. Corroborating our results showing an increase in *Gata2* mRNA following P4 treatment, we see an increase of GATA2 protein after P4 treatment. Interestingly, in ovariectomized mice, expression of GATA2 is present in both the luminal and glandular epithelium; whereas PR expression is significantly lower in the glandular epithelial compartment. The change in expression of both PR and GATA2 protein following P4 injection is clearly shown in the right panel of Fig. 1C where expression of PR is present at elevated levels in the absence of P4 and subsequently decreases following acute treatment. Expression of GATA2 in ovariectomized mice follows a paradigm opposite of the PR, with low expression in the absence of ligand activated PR and subsequent increase following acute P4 treatment.

1.2. Expression of GATA2 in the uterine epithelium of the pseudopregnant mouse

To functionally verify the presence of GATA2 in the mouse uterus during the peri-implantation period of early pregnancy, 8 week old female wild-type mice were mated with vasectomized males. This mating condition allowed for examination of gene regulation and P4 signaling in early pregnancy in the uterus without interference from contributing factors secreted by the implanting embryo. Following observation of the uterine plug deposited during mating and designated day 0.5, mice were sacrificed on the mornings of days 0.5, 1.5, 2.5, 3.5 and 4.5. Evaluation of the peri-implantation period for relevant gene expression is limited to these time points following from an entry back into the mouse estrus cycle by day 5.5 due to lack of embryo and loss of P4 signaling. In order to better evaluate the functional changes in GATA2 protein expression, dual immunofluroescence was performed in order to confirm changes in expression during early pregnancy and visualize differences in uterine compartmental expression. As seen in Fig. 2a, d, g, j, and m, PR expression increases from day 0.5 in the luminal epithelium to reach peak levels on day 2.5. Expression of PR is maintained in the luminal epithelium following day 2.5, however, expression levels rapidly decline beginning from day 3.5 and continuing through day 4.5. During this period, PR levels are also present beginning on day 1.5 in the sub-epithelial stroma and increase in expression throughout the stroma following day 2.5 and remain steady until the time of implantation on day 4.5, consistent with previous PR expression data (Diao et al., 2011; Tan et al., 1999). Remarkably, expression of GATA2 in the luminal epithelium mimics that seen of the PR with precision, as expression of GATA2 rises until it peaks on day 2.5 followed by a rapid decrease in epithelial expression (Fig. 2b, e, h, k, and n). Additionally, as observed with stromal PR expression, the levels of GATA2 protein appear in the stroma from the day of the plug and maintain their level of expression through day 4.5. A phenomenon not seen with PR expression is the observation that GATA2 protein is present in the glandular epithelium throughout the peri-implantation period, with a noticeable peak in expression seen on day 2.5. Expression of both proteins can be easily visualized in the merged images