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Galectin-7 acts as an adhesion molecule during implantation and increased expression is associated with miscarriage

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ABSTRACT

Introduction: Galectins are expressed at the fetal–maternal interface and have multiple roles including during blastocyst implantation. The expression of galectin-7 however has not been investigated in the uterus. We aimed to localise galectin-7 to the endometrium of women with normal fertility and with a history of miscarriage and prospectively determine whether serum levels are altered in women who subsequently miscarry. We also investigated the role of galectin-7 on trophoblast–endometrial epithelial cell adhesion.

Methods: Immunohistochemistry localised galectin-7 to endometrium throughout the menstrual cycle in women (normal fertility or with history of miscarriage) and in first trimester implantation sites. Galectin-7 serum levels were determined by ELISA. We used both endometrial epithelial–trophoblast cell lines and primary cells for cell–cell adhesion experiments.

Results: Galectin-7 immunolocalized to endometrial luminal and glandular epithelium in normally fertile women and was upregulated in epithelium and stroma of women with a history of miscarriage. Similarly, gelectin-7 serum levels were elevated at 6 weeks gestation in women who subsequently miscarried compared to gestation matched controls. Exogenous galectin-7 reduced endometrial epithelial–trophoblast adhesion in cell-line and primary cell assays. However, when endometrial epithelial cells were isolated from women with endometrial disorders, galectin-7 increased epithelial –trophoblast adhesion.

Conclusions: Galectin-7 is produced by endometrial epithelium and is abnormally elevated in the endometrium of women with a history of miscarriage. Serum levels may be useful as a predictive biomarker of miscarriage. Our data suggests that galectin-7 facilitates adhesion of the embryo to the endometrium and elevated galectin-7 may result in abnormal adhesion.

1. Introduction

During the initiation of pregnancy, the human blastocyst must implant into the uterus in order to facilitate the formation of a functional placenta. Implantation involves the blastocyst apposing then adhering to the uterine luminal epithelium before trophoblast cells migrate and invade through the endometrial decidua to engraft and remodel maternal spiral arteries. Inadequate, or inappropriate implantation can lead to recurrent miscarriage, placental insufficiency and other obstetric complications [1,2].

Implantation is initiated during the 'window of implantation', a specific period during the menstrual cycle where the uterus is receptive to the blastocyst [3]. To become receptive, the luminal and glandular epithelium undergo significant changes, particularly with respect to their secretory capacity and adhesion molecule expression [3], facilitating the adhesion of two epithelial surfaces; the blastocyst trophectoderm and the uterine luminal epithelium. Adhesion molecules such as mucins, glycoconjugates, trophinin, cadherins and integrins are all regulated during the window of implantation [3].

While implantation failure results in infertility, impaired implantation results in inadequate placentation and associated conditions including early miscarriage [4]. More recently, it has been proposed that recurrent miscarriage [defined as 3 or more

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consecutive miscarriages [5]] is associated with abnormalities in endometrial receptivity that lead to inappropriate implantation and subsequent miscarriage [6]. Certainly, both the endometrial epithelium and decidua are altered in women with recurrent miscarriage, including aberrant expression of adhesion molecules by the endometrial epithelium [5,7] and impaired decidualization [8].

Galectins are animal lectins which bind to surface glycoproteins, with preferential binding to β-galactoside [9]. Galectins regulate many cell functions important for implantation such as cell-adhesion and migration, signal transduction, apoptosis, immune cell activation, differentiation and hormone production [9–12]. The galectin family has many and varied roles in reproduction [13,14], including during implantation. For example, galectin-3 is upregulated in the human endometrial epithelium during the secretory phase [15] and in mice decreased implantation is found following tissue specific knockdown of galectin-3 [16]. Further, an association with spontaneous abortion and a splice variant of galectin-9 is found in both mice and women [17], indicating critical roles for at least these lectins in implantation.

Galectin-7 is a 15 kDa protein originally identified in the epidermis. It is generally expressed by epithelial cells and localizes to areas of cell–cell contact [9]. It is a prototype galectin found as both a monomer and a dimer [18]; unlike most mammalian galectins it is known to exist as a monomer in solution [18]. Galectin-7 has recently been identified in 1st trimester human endometrial epithelial cells and the syncytiotrophoblast and extravillous trophoblast (Menkhorst et al. under review) however to date it has not been investigated in the cycling endometrium.

Galectin-7 is secreted by keratinocytes [19]. However, like all galectins its sequence has no typical secretion signal peptide. It is released out of cells through an unusual route which requires intact carbohydrate-binding activity of the secreted protein [12]. Galectin-7 has a well characterized role in wound healing; it accelerates re-epithelialisation of corneal wounds more efficiently than most known growth factors [20,21].

We hypothesized that as an adhesion molecule, epithelial galectin-7 would be important in facilitating blastocyst implantation. We aimed to identify galectin-7 expression in the endometrium during the menstrual cycle of normally fertile women and women who have a history of miscarriage, to see whether tissue and serum levels of galectin-7 are associated with previous miscarriage or predictive of subsequent miscarriage and to investigate the function of galectin-7 during implantation.

2. Methods

2.1. Ethical approvals

This study was approved by the Monash Health Human Research and Ethics Committee (#09317B; #06014C) and the Mercy Hospital for Women Ethics Committee (R03/23). Written and informed consent was obtained from each patient.

2.2. Endometrial samples

Endometrial samples (Table 1) from normally fertile women (3–6 samples per stage of the menstrual cycle), women who have a history of miscarriage (3–5 samples per stage of the menstrual cycle except late secretory) and women with unexplained primary infertility (1–3 samples per stage of the menstrual cycle except mid-secretory) were obtained from women undergoing unrelated surgical procedures. The average age of the women was not different between groups (Table 1). The median number of miscarriages that the miscarriage cohort had undergone was 3.0 and the range was 2–12. In 3 cases the women had experienced 2 miscarriages.

2.3. First trimester placenta samples

First trimester placental tissue was collected from healthy women undergoing termination of pregnancy for psychosocial reasons (amenorrhoea 6–12 weeks). The tissues were examined by an experienced pathologist at Monash Health and showed no pathologies. Implantation sites – sites where the placental villous was adhered to the decidua, were chosen for immunohistochemical analysis ($n = 4$).

Table 1
Characteristics of non-pregnant participants.

	Normally fertile ($n = 17$)	Miscarriage ($n = 11$)	Unexplained infertility ($n = 6$)
Maternal age (years)	35.0 (28–40)	32.0 (25–44)	31.5 (24–38)
Gravidity[a]	2.0 (2,3)	5.0 (2–12)	0
Parity[b] median [%(n)]	2.0 (2–4)	1.0 (0–2)*	0
0	0	33.3 (3)	100 (6)
1	0	44.4 (4)	0
2	63.6 (7)	22.2 (2)	0
≥3	36.3 (4)	0	0
Number of previous miscarriages[c]	0	3.0 (2–12)	0

Data provided as the median, with range given in brackets.
*$p < 0.05$ compared to normally fertile.
[a] Data unavailable for 10 fertile patients; 2 miscarriage patients.
[b] Data unavailable for 7 fertile patients; 2 miscarriage patients.
[c] Data unavailable for 2 miscarriage patients – defined as recurrent miscarriage only.

2.4. Serum samples from pregnant women

The serum used in this study was from a Biobank of serum samples prospectively collected from the mid to late first trimester specifically to investigate miscarriage (Table 2). Serum was collected from pregnant women between weeks 6–10 of gestation as previously described [22]. Importantly, fetal cardiac activity was confirmed by ultrasound at the time of blood collection. From the biobank of samples, 109 controls were chosen from pregnancies that went on to have a healthy live birth and 18 miscarriage samples were chosen. Miscarriage was defined as the loss of the pregnancy at less than 20 weeks gestation [22].

2.5. Galectin-7 immunohistochemistry

Formalin-fixed cycling endometrium or placental villous 5 μm sections on poly-L-lysine (Sigma) coated glass slides were dewaxed in histosol and rehydrated in ethanol. Antigen retrieval was performed in 0.01 M citrate buffer (pH 6) before endogenous peroxidise activity was quenched by incubation in 3%H_2O_2/Methanol for 10 min. Sections were blocked in non-immune serum (10% normal horse serum, 2% normal human serum in Tris buffered saline [TBS]) for an hour before application of the primary antibody in non-immune serum (galectin-7, R&D Systems, AF1339, 1 μg/ml; Goat IgG, R&D Systems, 1 μg/ml) overnight at 4 °C. Slides were washed in 0.6% Tween-20 (Sigma) in TBS before incubation in the secondary antibody (Biotinylated horse anti-goat, 1:200) for 30 min at RT. Slides were again washed before addition of streptavidin-biotin complex/HRP (Vector) for 30 min at RT. Sections were stained with the substrate 3′3-diamionbenzidine (K3466, DAKO) and counterstained with haematoxylin. Quality controls were included in each run.

Table 2
Characteristics of pregnant participants.

	Controls ($n = 109$)	Miscarriage ($n = 18$)
Maternal age (years)	30.0 (21–44)	30 (19–43)
Gravidity [%(n)]		
Primiparous	34.8 (38)	27.7 (5)
Multiparous	65.2 (71)	72.3 (13)
Parity [%(n)][a]		
0	54 (59)	62.5 (10)
1	34 (37)	25 (4)
≥2	12 (13)	12.5 (2)
Gestation at sampling (wks + days)	8 + 2 (6 + 0–10 + 6)	7 + 4 (6 + 2–9 + 2)*
Gestation at 1st symptoms (wks + days)[b]	n/a	11 + 0 (7 + 0–14 + 5)
Gestation when the miscarriage was confirmed by ultrasound (wks + days)	n/a	11 + 0 (7 + 0–18 + 0)
Gestation at delivery (wks + days)	39 + 1 (32 + 0–42 + 0)	n/a
Birth weight (g)	3475 (1785–4680)	n/a

Data provided as the median, with range given in brackets.
*$p < 0.05$ compared to control.
[a] Data unavailable for two miscarriage patients.
[b] Data unavailable for four miscarriage patients.