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Short communication

Galectin-7 serum levels are altered prior to the onset of pre-eclampsia

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ABSTRACT

Galectins regulate many cell functions important for placental development, however, the localization and role of galectin-7 is unknown. We hypothesized galectin-7 would be expressed by the placenta and detected in serum. Galectin-7 immunolocalized to syncytiotrophoblast, extravillous trophoblast and glandular epithelium in 1st trimester placenta/decidua and to syncytiotrophoblast and endothelial cells in term placenta, but in pre-eclamptic placentas endothelial staining was absent. Galectin-7 serum concentration was significantly elevated in women (weeks 10–12 and 17–20) who subsequently developed pre-eclampsia compared to women with healthy pregnancies. Galectin-7 is a promising prospective serum biomarker for pre-eclampsia and likely has important functions in placentation.

1. Introduction

During the establishment of pregnancy, the human blastocyst implants into the uterine endometrium to facilitate the formation of a functional placenta. Implantation involves the blastocyst apposing then adhering to the uterine luminal epithelium before the primitive syncytium and subsequently the 'extravillous trophoblast' (EVT) invades through the decidua to engraft maternal spiral arteries. Impaired implantation and placentation are thought to lead to first trimester miscarriage, placental insufficiency and other obstetric complications [1,2].

Galectins bind to surface glycoproteins and have many and varied roles in reproduction [3,4]. They regulate many cell functions important for placental development [5–8]. Dysregulated placental expression of galectins-1, 3 and 13 is associated with pre-eclampsia [3,4,9]. Galectin-7 [6,10] was not identified in term placenta by RT-PCR [11], however, we recently identified galectin-7 protein in 1st trimester syncytiotrophoblast and cell column trophoblast [12].

We hypothesized that galectin-7 would be detectable in maternal serum and that it would be altered in pre-eclampsia. We aimed to localize galectin-7 to maternal and placental cells in the 1st and 3rd trimesters of pregnancy and measure galectin-7 levels in maternal serum from normal and pre-eclamptic pregnancies.

2. Methods

2.1. Human samples

This study was approved by the Monash Health Human Research and Ethics Committee and The University of Tokyo and Musashion Red Cross Hospital. Written and informed consent was obtained from each patient.

First trimester placental tissue was collected from healthy women undergoing termination of pregnancy for psychosocial reasons (amenorrhoea 6–12 weeks).

Maternal venous blood was obtained from women undergoing uncomplicated, normotensive pregnancies (weeks 6–37; $n = 57$; Table 1) or pregnancies complicated by pre-eclampsia (defined in Table 1) prior to the onset of disease (weeks 10–12 [$n = 4$–6/group], 17–20 [$n = 3$–6/group]) or soon after the manifestation of the disease but before commencing any medication (weeks 21–37 [$n = 18$]) (Tables 1 and 2). Sera were separated by centrifugation and stored at −70 °C.

2.2. Galectin-7 immunohistochemistry

Galectin-7 immunohistochemistry was performed as previously described [12] using formalin-fixed 1st trimester placental villous ($n = 4$), decidua ($n = 6$) or term placenta from normal or pre-eclamptic pregnancies ($n = 3$–4/group).

2.3. Galectin-7 and HLAG immunofluorescence

Formalin-fixed decidua 5 μm sections ($n = 5$) were treated as described above except: non-immune serum diluted in and washes performed in phosphate buffered saline; galectin-7 and HLAG (Pharmingen, 557577) primary antibody concentration 1 μg/ml; secondary antibody incubation (Donkey α mouse alexa fluor 488 and Donkey α goat alexa fluor 594; both 1:200) in non-immune serum for 2 h at RT; following further washes, sections were mounted using Vectastain containing DAPI (DAKO).

2.4. Galectin-7 ELISA

Serum concentration of galectin-7 were assayed using the RayBio® Human Galectin-7 ELISA (ELH-Galectin7-001) according to the manufacturer's instructions. Serum samples were diluted 1:4. The minimum detection limit for galectin-7 was 41.5 pg/ml.

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Table 1
Maternal characteristics of women providing serum samples through pregnancy.

Serum samples		Maternal age	Pre-eclampsia diagnosis (weeks gestation)	Gestation at delivery (weeks)[b]	Birth weight (g)[c]	Blood collected (weeks gestation)[b]
6–37 weeks	Normal pregnancy ($n = 45$)	32.9 ± 0.7 ($n = 44$) (19–42)	na	38.48 ± 0.3 ($n = 30$) (35–41)	3024 ± 68 ($n = 28$) (2130–3854)	17.0 ± 1.4 (6–37)
21–37 weeks	Normal pregnancy ($n = 16$)[a]	34.3 ± 1.1 ($n = 13$) (27–40)	na	38.4 ± 0.4 ($n = 10$)* (36–40)	3013 ± 77 ($n = 10$)$^{\hat{}}$ (2590–3845)	30.2 ± 1.2 (24–37)
	Pre-eclampsia[d] ($n = 18$)	32.5 ± 1.2 ($n = 13$) (24–38)	29.4 ± 1.0 (22–36)	30.9 ± 2.0 ($n = 9$)* (22–41)	1602 ± 337 ($n = 9$)$^{\hat{}}$ (534–3576)	At time of diagnosis
	Early onset ($n = 16$)	32.9 ± 1.3 ($n = 11$) (24–38)	29.4 ± 1.0 (22–36)	28.4 ± 1.5 ($n = 7$) (22–34)	1142 ± 172 ($n = 7$) (534–1803)	At time of diagnosis
	Late onset ($n = 2$)	30.5 ± 4.5 (26–35)	35.5 ± 0.5 (35–36)	39.5 ± 1.5 (38–41)	3210 ± 366 (2844–3576)	At time of diagnosis

Data provided as mean ± sem with range given in brackets. Where data are unavailable, the *n* for that information is indicated in brackets also.
Superscript symbols (*, ^, #, +) denote significant difference between pairs.
[a] samples are subset of 6–37 weeks normal pregnancy.
[b] Statistical test used: non-parametric one-way ANOVA.
[c] Statistical test used: one-way ANOVA.
[d] Pre-eclampsia was diagnosed by the presence of hypertension (an absolute blood pressure ≥140 mmHg systolic and/or 90 mmHg diastolic after 20 weeks of gestation) with proteinuria (≥300 mg/24-h). Patients with pre-eclampsia did not have any prior history of hypertension or renal disease. Women with pre-eclampsia were classified as having either early (diagnosis ≤34 weeks gestation) or late (diagnosis ≥35 weeks gestation) onset pre-eclampsia. All control women showed no clinical or pathological signs of pre-eclampsia, infections, or any other maternal or placental disease.

2.5. Statistics

All statistical analyses were performed using GraphPad Prism (GraphPad, San-Diego, CA, US). $P < 0.05$ was considered statistically significant.

3. Results

Galectin-7 immunolocalized to syncytiotrophoblast but not cytotrophoblast, cell column trophoblast, glandular epithelium, invasive and endothelial EVTs and non-EVTs, likely immune cells in 1st trimester placental villi and decidua (Fig. 1A–D). In term placenta, galectin-7 localized to syncytiotrophoblast and endothelial cells within the villous (Fig. 1E) but in placentas from women with pre-eclampsia endothelial staining was absent (Fig. 1F).

Galectin-7 was detected in serum from pregnant women (Fig. 2A). Galectin-7 serum concentration was highly variable during the first 4 weeks of the 2nd trimester. Serum galectin-7 was significantly elevated during weeks 13–16 compared to weeks 6–12, 17–26 and 27–37 (Fig. 2A; $p < 0.05$).

Galectin-7 serum concentration was significantly higher in women with pre-eclampsia compared to uncomplicated, gestation-matched pregnancies (Fig. 2B; $p < 0.05$). There was no association between the time of onset of disease and galectin-7 serum concentration (Fig. 2C).

Prospective sampling (weeks 10–12, 17–20) of galectin-7 showed significantly elevated levels in women who went on to develop pre-eclampsia compared to women who had normal pregnancies (Fig. 2D; $p < 0.05$). Both early and late onset pre-eclampsia were predicted at weeks 10–12 of gestation (Fig. 2E; $p < 0.05$).

4. Discussion

Here we demonstrate for the first time galectin-7 serum concentration was significantly elevated during weeks 10–12 and 17–20 of gestation in women who went on to develop pre-eclampsia, compared to women with normal pregnancies. The tissue localization of galectin-7 suggests possible roles in placentation, syncytial formation, EVT function and feto-maternal immune interactions.

Overall, galectin-7 shows promise as a biomarker for pre-eclampsia which is altered as early as 10 weeks of gestation. However, a small number of women were tested here and further testing on a much larger cohort is required to determine galectin-7's potential as a predictive biomarker for pre-eclampsia. It is unlikely that any one marker will predict all cases of pre-eclampsia. Ideally, a predictive test for pre-eclampsia could combine serum concentration of galectin-7 with other proteins, in particular other galectin family members which are altered in pre-eclampsia such as galectins-1, 3 and 13 [3,13].

The origin of serum galectin-7 levels is likely from placental and non-placental sources. We speculate from our immunohistochemistry data that elevated serum galectin-7 found during weeks 13–16 of gestation is of placental,

Table 2
Maternal characteristics of women providing prospective serum samples.

Serum samples	Maternal pathology	Maternal age (years)[c]	Pre-eclampsia diagnosis (weeks gestation)	Gestation at delivery (weeks)[c]	Birth weight (g)[d]	Blood collected (weeks gestation)[c]
10–12 weeks	Normal pregnancy ($n = 6$)	34.0 ± 2.0 (24–37)	na	39.2 ± 0.9 (35–41)	2858 ± 153$^{\#}$ (2130–3150)	11.0 ± 0.4 (10–12)
	Early onset ($n = 4$)[a]	38.5 ± 1.2 (37–42)	29.0 ± 2.5 (23–33)	31.5 ± 3.4 (25–40)	1530 ± 596$^{\#}$ (534–3104)	11.0 ± 0.4 (10–12)
	Late onset ($n = 6$)[b]	37.5 ± 2.0 (32–44)	36.7 ± 0.6 (35–38)	37.5 ± 0.5 (36–39)	2644 ± 192 (2045–3198)	10.8 ± 0.3 (10–12)
17–20 weeks	Normal pregnancy ($n = 6$)	31.2 ± 0.7* (28–33)	na	39.1 ± 0.3$^{\hat{}}$ (38–40)	3105 ± 168^{+} (2252–3600)	18.7 ± 0.4 (17–20)
	Early onset ($n = 3$)[a]	35.3 ± 2.2 (31–38)	30.0 ± 3.5 (23–34)	31.7 ± 3.4 (25–36)	1728 ± 669^{+} (534–2846)	19.3 ± 0.5 (18–20)
	Late onset ($n = 5$)[b]	37.6 ± 2.1* (33–44)	37.0 ± 0.9 (35–39)	37.6 ± 0.7 (36–39)	2772 ± 287 (2045–3432)	18.6 ± 0.5 (17–20)

Data provided as mean ± sem with range given in brackets.
Superscript symbols (*, ^, #, +) denote significant difference between pairs.
[a] Two women provided samples at both weeks 10–12 and 17–20.
[b] Three women provided samples at both weeks 10–12 and 17–20.
[c] Statistical test used: non-parametric one-way ANVOA.
[d] Statistical test used: one-way ANOVA.