Galectin-7 Impairs Placentation and Causes Preeclampsia: Features in Mice

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Abstract—Preeclampsia is a serious pregnancy-induced disorder unique to humans. The etiology of preeclampsia is poorly understood; however, poor placental formation is thought causal. Galectin-7 is produced by trophoblast and is elevated in first-trimester serum of women who subsequently develop preeclampsia. We hypothesized that elevated placental galectin-7 may be causative of preeclampsia. Here, we demonstrated increased galectin-7 production in chorionic villous samples from women who subsequently develop preterm preeclampsia compared with uncomplicated pregnancies. In vitro, galectin-7 impaired human first-trimester trophoblast outgrowth, increased placental production of the antiangiogenic sFlt-1 splice variant, sFlt-1-e15a, and reduced placental production and secretion of ADAM12 (a disintegrin and metalloprotease12) and angiotensinogen. In vivo, galectin-7 administration (E8–E12) to pregnant mice caused elevated systolic blood pressure, albuminuria, impaired placentation (reduced labyrinth vascular branching, impaired decidual spiral artery remodeling, and a proinflammatory placental state demonstrated by elevated IL1β, IL6 and reduced IL10), and dysregulated expression of renin-angiotensin component in the placenta, decidua, and kidney, including angiotensinogen, prorenin, and the angiotensin II type 1 receptor. Collectively, this study demonstrates that elevated galectin-7 during placental formation contributes to abnormal placentation and suggests that it leads to the development of preeclampsia via altering placental production of sFlt-1 and renin-angiotensin system components. Targeting galectin-7 may be a new treatment option for preeclampsia. (Hypertension. 2020;76:00-00. DOI: 10.1161/HYPERTENSIONAHA.120.15313.) • Data Supplement

Key Words: angiotensinogen • disintegrin • mice • placentation • pre-eclampsia

Preeclampsia is a serious pregnancy-induced disorder unique to humans. With a worldwide incidence of 4.6% of pregnancies,1 over 4 million women develop preeclampsia each year, claiming the lives of 100,000 women and 500,000 babies2 and increasing long-term chronic disease risk in both mother and child.1

Preeclampsia manifests clinically as a complex multi-system disease1 diagnosed by sudden onset hypertension (>20 weeks gestation) and at least one associated complication (proteinuria, other maternal organ dysfunction or fetal growth restriction).3 Poor placentation during the first-trimester is thought the underlying cause of preeclampsia; however, its etiology in relation to time of disease onset is unclear.1,4 During placentation, extravillous trophoblast (EVT) invade from anchoring placental villi into the decidua, remodeling uterine spiral arteries to create high flow, low resistance vessels. This process is maximal in the first-trimester but continues until ~18 weeks gestation.5 The placental villi are bathed in maternal blood into which they release a wealth of factors which reflect placental function.1 If the placenta is abnormal it can release toxic factors which damage maternal vasculature.6 The abnormal placenta also has dysregulated expression of renin-angiotensin system (RAS) components which in turn may lead to activation of the maternal intrarenal RAS and failure of the...
circulating renin-angiotensin-aldosterone-system (RAAS) to respond appropriately to the homeostatic demands of pregnancy.6

Galectins are animal (soluble) lectins abundantly expressed at the maternal-fetal interface.7 Galectins bind to surface glycoproteins (preferentially β-galactoside) and have many functions critical for placenta including cell invasion and immune tolerance: dysregulated expression of galectins-1, 3, 9, and 13 is associated with preeclampsia.7–11

Galectin-7 is expressed by first-trimester syncytiotrophoblast and EVT, but the function of galectin-7 during placentation is unknown. Galectin-7 has many functions including roles in cell adhesion,14 migration,15–17 and immune cell regulation,18 all key functions during placentation. Lgals7-deficient mice are fertile and give rise to normal and fertile offspring.19 Galectin-7 acts intracellularly, via interactions with Ras20 or Bel-2,21 and extracellularly via paracrine mechanisms to induce gene transcription.22,23 Galectin-7 is abnormally elevated in first-trimester serum from women who subsequently develop preeclampsia.12 We hypothesized that elevated placental galectin-7 may play a causative role in the development of preeclampsia.

Methods
The authors declare that all supporting data are available within the article, the Data Supplement and the mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE24 partner repository with the dataset identifier PXD019331.

Primary Tissue Isolation and Culture
Human placental tissue was collected under appropriate Human Research and Ethics Committee approvals (Monash Health and the Royal Women’s Hospital, Melbourne #09317B; King’s College Hospital, London REC:03-04-070). Written and informed consent was obtained from each patient before surgery. First- and second-trimester placental villous and decidua tissue was donated by healthy women undergoing pregnancy termination for psychosocial reasons (amniorrhca 6–22 weeks; n=82). First-trimester placenta were cultured as described in the Data Supplement. Briefly, EVTs were isolated from cytotrophoblast for gelatin zymography, villous explants were cultured for RT-qPCR or mass spectrometry or extracellularly via paracrine mechanisms.29 Chorionic villous samples (CVS, n=20) taken from women undergoing screening for fetal chromosomal abnormalities were snap-frozen immediately after collection. Patient characteristics are shown in Methods and Tables S1 and S2 in the Data Supplement.

In Vivo Mouse Experiments
All procedures were approved by the Monash Medical Centre (B) (#MMCB2016/07) and Melbourne University (#1814607) Animal Ethics Committees. This study followed the NHMRC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Recombinant Galectin-7 Administration
Mated female C57BL/6 mice received subcutaneous injections of 400 µg/kg per day galectin-7 or vehicle control from E8 (E, embryonic day; plug detection, E0) to E12, or 5 consecutive days in nonpregnant mice. Systolic blood pressure was measured by tail-cuff plethysmography. Pregnant mice were killed on E13, E17, or allowed to pup (n=5–6/group). Tissues, urine, and serum collected were subjected to gene array, RT-qPCR, ELISA, placental morphometry, histology, and immunohistochemistry as detailed in the Data Supplement.

Statistics
Statistical analyses were performed by GraphPad Prism version 8.3.1. P<0.05 was considered significant. Data were tested for normality and statistical tests (indicated in figure legends) chosen according to experimental design.

Results
Galectin-7 Is Elevated in Human Placenta From Pregnancies That Subsequently Develop Preterm Preeclampsia
Galectin-7 is produced by first- and second-trimester placental villi and decidua (Figure S1A and S1B). Galectin-7 production did not change across gestation, except for a significant increase in placental villi LGALS7 expression at 10 weeks gestation (Figure S1A and S1B). Elevated placental galectin-7 was found in CVS from women who subsequently developed preterm preeclampsia compared with uncomplicated controls (Figure 1A and 1B). Galectin-7 immunolocalized predominantly to syncytiotrophoblast cytoplasm (Figure 1B).

Galectin-7 treatment inhibited EVT outgrowth from first-trimester placental villi (Figure 1C) and increased production of SFLT-1-E15A (Figure 1D), a primate, placental-specific splice variant of sFlt-1 augmented during preeclampsia.31 There was no effect of galectin-7 on the conserved full-length FLT-1 (Figure 1E).

Galectin-7 Induced the Features of Preeclampsia in Mice
The human data presented above strongly implicate galectin-7 in the etiology of preeclampsia. Therefore, we investigated whether elevating galectin-7 during placental formation in mice induced features of preeclampsia.

Because galectin-7 has not previously been localized to mouse implantation sites, we first examined galectin-7 production in implantation sites across gestation. Galectin-7 protein increased across gestation peaking in the decidua and metrial lymphoid aggregate of pregnancy (MLAp) compartment at E15 and E17 compared with E6 implantation site (Figure S2A) and was significantly higher in E15 and 17 decidua and MLAp compared with E15 and 17 placenta (Figure S2A). Galectin-7 immunolocalization was predominantly intracellular and strongly localized to E6 myometrium and E13/16 MLAp and fetus and weakly immuno-localized to E13/16 labyrinth (Figure S2B).

To model the profile of elevated serum galectin-7 only during early pregnancy (the period of maximal placenta) of women who subsequently develop preeclampsia,22 we injected galectin-7 to pregnant mice from E8 to E12, to elevate galectin-7 during the period of maximal placenta in mice (E9-E14). This significantly increased serum galectin-7 concentration at E13 but not E17 (Figure S3A). There was no change in placental or decidual galectin-7 levels (Figure S3B).

This transient augmentation of circulating galectin-7 elevated systolic blood pressure (Figure 2A) and increased umbilical creatinine ratio (Figure 2B) in pregnant mice but had no effect in nonpregnant mice. Galectin-7 treatment had no effect on serum or placental Flt-1 (Figure 2C; Figure S3C, respectively) or serum sEndoglin (Figure S3D).

Galectin-7 treatment had no effect on gestation length (Figure S3E), fetal number (Figure S3F), or fetal body weight (Figure 2D). The placental and decidual unit size was significantly reduced at E13 (not E17; Figure 2E), but the fetal:placenta and decidua unit ratio was unchanged (Figure 2F). Although no fetal growth restriction was observed, reduced pup bodyweight (P7 to P21) was found in pups born from galectin-7-treated dams (Figure 2D).
Galectin-7 Altered Renin-Angiotensin System Components

Using a mouse preeclampsia gene expression array (QIAGEN), we found galectin-7 treatment altered decidual and kidney Agtr1a (angiotensin II type 1 receptor; Tables S3 through S5). We therefore determined whether galectin-7 treatment altered production of major RAS components Ace, Ace2, Agt (angiotensinogen), Agt1a, Atp6ap2 (prorenin receptor), Mme (neprilysin), and Renin (Prorenin) (Figure S4). Galectin-7 treated mice showed significantly reduced placental Agt expression at E13 (Figure 3A), increased Agtr1a expression in the kidney at E13 and placenta and heart at E17 (Figure 3B), altered Renin expression at E13 in the decidua (reduced) and kidney (increased; Figure 3C) and increased kidney Atp6ap2

Figure 1. Galectin-7 was elevated in human chorionic villous samples (CVS) from pregnancies that developed preterm preeclampsia. A, LGALS7 expression in CVS. One-way ANOVA, n=3–9/group. B, Galectin-7 immunostaining in CVS (insert shows negative control) and quantification. One-way ANOVA, n=3–5/group. C, Gal7 (Galectin-7) treatment reduced first-trimester placental outgrowth (area within dotted line, normalized to length of outgrowth) compared with vehicle control (Con). Paired t-test, n=3. D, sFlt-1-e15α and E, sFlt-1 expression in first-trimester placental villous cultured with galectin-7 or vehicle control. Student t-test, n=3–6/group. Data presented as mean±SEM; *P<0.05. PPE indicates preterm preeclampsia; TPE, term preeclampsia; and Un, uncomplicated.

Figure 2. Gal7 (Galectin-7) administration (E8–12 or 5 day equivalent in nonpregnant [NP] mice) induced hypertension and albuminuria in pregnant mice. A, Systolic blood pressure (sBP). Mixed-effects model (Sidak multiple comparison test), n=6–12; B, Urinary albumin/creatinine ratio. Mixed-effects model (Sidak’s multiple comparison test), n=6–10; C, Serum Flt-1 concentration. n=3–6; D, Fetal and pup bodyweight. Two-way ANOVA (Sidak multiple comparison test), n=5–6; E, Placenta and decidua unit weight. Student t-test, n=4–6; F, Fetal: placenta and decidua unit weight ratio n=4–6. Data presented as mean±SEM; *P<0.05. E indicates embryonic day; and P, postnatal day.
expression at E13 (Figure 3D). Using human first-trimester placental villi, we confirmed that galectin-7 downregulated angiotensinogen expression (RT-qPCR; Figure 3E) and secretion (mass spectrometry; Figure 3F; Table S8).

Galectin-7 Impaired Placental Formation in Mice

Galectin-7 administration impaired placental development compared with vehicle control (Figure 4A and 4B): at E13, the labyrinth and junctional zones were smaller and at E17 the decidua was larger (Figure 4A). Labyrinth vascular branching was significantly reduced at E13 in placentas from galectin-7 treated mice. Vascular branching counts fell significantly at E17 compared with E13, and there was no difference between treated and control mice at E17 (Figure 4C).

Trophoblast invasion of decidual spiral arteries was not identified in galectin-7 treated E13 implantation sites, with no CK7-positive trophoblast visible in α-SMA stained decidual spiral arteries (Figure 4D). Correspondingly, vascular smooth muscle cells were retained around decidual arteries in galectin-7-treated mice (Figure 4E), suggesting galectin-7 significantly impaired decidual spiral artery remodeling. There was no effect of galectin-7 on uterine Natural Killer cell number in the decidua (Figure 4F), suggesting although uterine Natural Killer cells are the main mediators of spiral artery remodeling in mice, reduced spiral artery remodeling seen here was due to impaired trophoblast invasion. We did not however investigate uterine Natural Killer phenotype or function.

Placentas from galectin-7-treated mice had elevated Il1β (Figure 5A) at E13 and elevated Il6 (Figure 5B) and reduced Il10 (Figure 5C) at E17.

Galectin-7 Altered Production of Key Regulators of Trophoblast Invasion

Galectin-7 enhances invasion via MMP9 in other cells. Here, despite increased MMP9 production by human placental villi (Figure 6A and 6), galectin-7 inhibited EVT outgrowth (Figure 1C), suggesting that galectin-7 inhibits trophoblast invasion via a different mechanism. This hypothesis is supported by our in vivo data, where murine placental production of MMP9 was not significantly altered (Figure S5A), although galectin-7 is active in mouse cells, demonstrated by induction of Mmp9 in vitro (Figure S5B). We have previously shown a similar difference in regulation of invasion between trophoblast and cancer cells with IL11. To identify the mechanism by which galectin-7 impaired trophoblast invasion, we screened factors known to regulate EVT invasion. Galectin-7 significantly inhibited human first-trimester placental villous ADAM (a disintegrin and metalloproteinase)12 expression (Figure 6C), increased PAPPA (Pappalysin)2 expression (Figure S5C) but had no effect on IL11 expression (Figure S5D). Galectin-7 treatment inhibited human first-trimester villous secretion of ADAM12S (Figure 6D) and correspondingly, reduced murine placental and decidual Adam12 expression (Figure 6E) and placental ADAM12S production (Figure 6F).

Discussion

We provide evidence that placental galectin-7 was elevated in women who subsequently develop preterm preeclampsia. Augmented galectin-7 during the period of placental formation in mice caused hypertension and albuminuria, likely by disrupting placentation and altering placental expression.
of angiogenic factors, regulators of trophoblast invasion and cytokines, all known to be involved in the etiology of preeclampsia.

CVS offer an unprecedented opportunity to investigate placental alterations and function before the onset of preeclampsia. Our CVS data indicate that galectin-7 production is increased in placentas of women who go on to develop preterm preeclampsia and that galectin-7 alters placental expression of genes found in multiple pathways which are associated with preeclampsia. Increased syncytial production of galectin-7 provides strong evidence supporting increased placental release of galectin-7 into maternal blood as we previously found. The impact of even slightly increased galectin-7 production may be substantial and sustained: galectin proteins are highly stable because of protease resistance and increased stability following ligand binding. Moreover, elevated galectin-7 expression is enhanced by an autocrine amplification loop in various epithelial cell types.

Whether decidual production of galectin-7 is likewise altered in preeclampsia is unknown: our CVS samples contained no decidua. A previous microarray study utilizing CVS containing decidua did not investigate *lgals7*. It is of note that we found no change in galectin-7 production in placental or decidual tissue from weeks 6 to 22, except in week 10 placental villous and interestingly, this was found only in a subgroup of placentas. This tissue is obtained from terminations,
so the pregnancy outcome is unknown. It is possible that placentas with elevated galectin-7 at week 10 may have developed preeclampsia; however, clinical characteristics which may indicate high risk of preeclampsia were not recorded.

Galectin-7 administration induced hypertension and albuminuria in pregnant mice yet did not alter serum sFlt-1 concentration. It is unsurprising that galectin-7 did not regulate sFlt-1 in mice, as in human placental villi galectin-7 regulated only sFlt-1-e15a, the sFlt-1 splice variant present only in the placenta of higher-order primates.31 sFlt-1-e15a is predominantly produced by the placenta (as opposed to, eg, sFlt-1-i13, which is predominantly endothelial) and is proposed as the primary sFlt-1 variant associated with preeclampsia.31 Mirroring our finding that galectin-7 was elevated only in CVS from preterm preeclampsia pregnancies, sFlt-1-e15a is significantly elevated in maternal plasma from women with early onset preeclampsia.38

Our observation that sFlt-1 is not required to induce preeclampsia features in mice has precedence: models generated by TNFα infusion39 and loss of PlGF40 show no effect on circulating sFlt-1; moreover, nicotinamide rescues preeclampsia features without altering sFlt-1 levels in 2 mouse models of preeclampsia.41 Taken together, this suggests that at least in mouse models of preeclampsia, hypertension is modulated independent of sFlt-1. Whether sFlt-1/PlGF levels are causal factors in the development of preeclampsia or reflect placental cellular stress is of debate.40,42

In a healthy pregnancy, the maternal renal RAS and circulating RAAS are activated to expand the cardiovascular system, maintain blood pressure, and increase renal blood flow. In established preeclampsia alterations to the circulating RAAS and tissue (placental, decidual, renal), RAS are clear6,43: prorenin, angiotensinogen, ACE (angiotensin-converting enzyme), and the AT1R (angiotensin II type 1 receptor) are all upregulated in the placenta44–46; however, the specific alterations to the RAS during placental formation and the effect of these alterations in the pathogenesis of preeclampsia are unknown, in part due to the lack of appropriate models. Galectin-7 treatment altered the expression of multiple RAS genes, including placental Agt before preeclampsia onset, and placental Agtr1a in established preeclampsia. We hypothesize that the initial reduction in Agt may reduce angiotensin II production, thus inhibiting trophoblast invasion and spiral artery remodeling early in pregnancy. Conversely, increased placental AT1R in established preeclampsia may be in response to oxidative stress and further compromise uteroplacental blood flow. Further experiments, particularly activity assays (eg ACE) are required to prove that the RAAS and tissue RAS are dysregulated in galectin-7-treated mice. We also found that galectin-7 reduced Agt expression and angiotensinogen secretion in human first-trimester placental

Figure 5. Galectin-7 administration (E8–12) altered mouse placental inflammatory cytokine production. A. IL1β B. IL6 C. IL10. Student’s t-test, n=4-6, Data presented as mean±SEM, *P<0.05. E indicates embryonic day.
villi, supporting a role for galectin-7 in regulating RAS components in human placenta.

Preeclampsia is associated with increased sensitivity to Ang II. Alterations in circulating angiotensin peptides may alter vascular sensitivity to Ang II or activate other maternal RASs such as the intrarenal RAS: indeed, kidney RAS gene expression was altered in galectin-7-treated mice. To the best of our knowledge, this is the only in vivo model of preeclampsia which displays alterations to the RAS without imposing direct changes on RAS components or the uterine vasculature. This model could be useful to understand the role of the RAS in the etiology of preeclampsia.

While we found no effect of galectin-7 on the development of hypertension or albuminuria in nonpregnant mice, RAS gene expression was altered in the peripheral organs of pregnant mice. Future studies could determine whether galectin-7 is upregulated in hypertension or cardiovascular disease.

Intriguingly, in women with established preeclampsia, the placenta at the time of delivery is very often not morphologically abnormal: here, we found that although the E13 placenta was morphologically abnormal, the E17 placenta was morphologically normal with restored weight and labyrinth vascular branching. Despite this, our gene expression data demonstrated that placental function likely remained altered, as evidenced by elevated IL6 and Agtr1a and reduced Il10 expression. Elevated placental IL1β, IL6, and reduced IL10 is found in women with preeclampsia and likely reflects a proinflammatory placental state. Galectin-7 is reported to induce Th1 cell polarization toward Th1, including reduced IL10 production; however, to our knowledge, this is the first report of galectin-7 stimulating IL1β and IL6 expression.
Galectin-7 is a well-established regulator of cell movement, promoting migration/invasion in many epithelial and epithelial cancer cells. Here, we found galectin-7 treatment impaired trophoblast invasion/outgrowth in vivo and in vitro. Galectin-7 inhibition of invasion has only previously been reported in prostate cancer. In this study, we have identified for the first time that galectin-7 impairs human trophoblast invasion likely via ADAM12 and PAPPA2. ADAM proteins are multidomain molecules, which have multiple critical functions including promoting cell proliferation, survival, migration, and invasion. ADAM12 has 2 alternatively spliced variants, ADAM12L, a transmembrane isoform and ADAM12S, a secreted isoform. ADAM12 localizes to human villous and extravillous trophoblast and promotes outgrowth in vitro. Whether ADAM12 regulates trophoblast invasion in mice is unknown; however, reduced trophoblast invasion in galectin-7-treated placentas was associated with lower placental ADAM12 production. ADAM12 promotes murine endometrial stromal cell decidualization; however, we saw no effect of galectin-7 treatment on the decidualization or decidual production of ADAM12S, likely as galectin-7 treatment did not begin until E8, when decidualization is essentially complete.

Reduced circulating ADAM12S is found in women who subsequently develop preeclampsia. Circulating ADAM12S at 20 weeks gestation is a better predictor of subsequent preeclampsia in pregnancies with a male fetus than a female. The preterm CVS samples were all from pregnancies with male fetuses and 5/6 term CVS samples were from pregnancies with female fetuses. Whether placental sex has an effect on galectin-7 production in preeclampsia is unknown but should be considered as other galectins show sex-dependent expression patterns in intrauterine growth restriction and we previously found that galectin-7 is also upregulated in prospective sera from women who developed term preeclampsia.

Perspectives

Overall, this study demonstrates that galectin-7 may play a significant role in the initiation of preeclampsia: via impaired placental formation, placental inflammation, and placental release of anti-angiogenic factors. As galectin-7-induced hypertension and albuminuria only in pregnant mice, we hypothesize that in women, galectin-7 acts via the placenta to induce the systemic features of preeclampsia. There are few mouse models of preeclampsia driven by the placenta, and none that display RAS dysregulation without direct alterations to RAS components or uterine vasculature, thus this in vivo model of preeclampsia will be of significant utility to understand the mechanisms leading to preeclampsia and to test therapeutics in preclinical trials. Galectin-7 may be a therapeutic target to normalize placental function during mid-gestation, and in combination with other risk factors, a novel biomarker to identify women at risk of developing preeclampsia.

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Disclosures

None.

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