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Evolutionary origins of the placental expression of chromosome 19 cluster galectins and their complex dysregulation in preeclampsia

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ABSTRACT

Introduction: The dysregulation of maternal-fetal immune tolerance is one of the proposed mechanisms leading to preeclampsia. Galectins are key regulator proteins of the immune response in vertebrates and maternal-fetal immune tolerance in eutherian mammals. Previously we found that three genes in a Chr19 cluster encoding for human placental galectin-13 (PP13), galectin-14 and galectin-16 emerged during primate evolution and may confer immune tolerance to the semi-allogeneic fetus.
Materials and Methods: This study involved various methodologies for gene and protein expression profiling, genomic DNA methylation analyses, functional assays on differentiating trophoblasts including gene silencing, luciferase reporter and methylation assays. These methods were applied on placental specimens, umbilical cord blood cells, primary trophoblasts and BeWo cells. Genomic DNA sequences were analyzed for transposable elements, transcription factor binding sites and evolutionary conservation.
Results and Discussion: The villous trophoblastic expression of Chr19 cluster galectin genes is developmentally regulated by DNA methylation and induced by key transcription factors of villous placental development during trophoblast fusion and differentiation. This latter mechanism arose via the co-option of binding sites for these transcription factors through promoter evolution and the insertion of an anthropoid-specific L1PREC2 transposable element into the 5' untranslated region of an ancestral gene followed by gene duplication events. Among placental Chr19 cluster galectin genes, the expression of *LGALS13* and *LGALS14* is down-regulated in preterm severe preeclampsia associated with SGA. We reveal that this phenomenon is partly originated from the dysregulated expression of key transcription factors controlling trophoblastic functions and galectin gene expression. In addition, the differential DNA methylation of these genes was also observed in preterm preeclampsia irrespective of SGA.
Conclusions: These findings reveal the evolutionary origins of the placental expression of Chr19 cluster galectins. The complex dysregulation of these genes in preeclampsia may alter immune tolerance mechanisms at the maternal-fetal interface.

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[1] Dedication: This manuscript is dedicated to the memory of Dr. Hans Bohn, whose scientific legacy, contribution to placentology and to this study, and inspiration will always be remembered.

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1. Introduction

Preeclampsia is a heterogeneous obstetrical syndrome with various etiologies and subforms, which affects 3–5% of pregnancies and is a chief cause of short- and long-term morbidity and mortality of the mother and her offspring [1–7]. Preterm or early-onset preeclampsia has often severe clinical presentation [2,6–8], and it is frequently associated with defective remodeling of the decidual and myometrial segments of maternal spiral arteries by invasive trophoblasts in early pregnancy [9–11], with oxidative stress of the placenta and the consequent placental release of toxic substances that promote the development of hypertension, proteinuria and an exaggerated systemic maternal inflammation [7,12–28], and with impaired fetal growth that often necessitates preterm delivery [6–8,28]. In this context, we found the decreased placental expression of galectin-13 (Placental Protein 13, PP13) in preterm preeclampsia [29,30] and also low first trimester maternal serum concentrations of trophoblast-secreted galectin-13, especially in cases associated with IUGR [31,32]. This phenomenon may be key in the early pathology in preterm preeclampsia, since galectin-13 and other placental galectins in a Chr19 cluster induce T cell apoptosis and may confer additional immune tolerance mechanisms in hemochorial placentation [33,34]. These observations are important from an evolutionary point of view since this galectin cluster emerged in anthropoid primates, a clade of primates consisting of New and Old World monkeys and apes, and galectin-13 evolved in Old World monkeys and apes [33,34], which have intense spiral artery remodeling by the invasive trophoblast [35–39] that is impaired in preeclampsia [10,40,41]. In fact, a recent study found that in normal pregnancies galectin-13 forms perivenous aggregates associated with leukocyte-containing zones of necrosis in early pregnancy decidua [42]. It was suggested that syncytiotrophoblast-secreted galectin-13 drains through the decidual veins, and then decidual galectin-13 aggregates attract and activate maternal immune cells, diverting them away from maternal spiral arteries and preventing them from attacking invasive semi-allogeneic trophoblasts [42].

Galectin-13 and placental Chr19 cluster galectins are predominantly expressed by the syncytiotrophoblast [29,30,32–34,43–45], a multinucleated syncytium that is in direct contact with maternal immune cells in maternal blood [29,30,33,34,42–44,46]. This cell layer is generated by the categorical reprogramming of the trophoblastic transcriptional program mainly governed by cAMP, and the consequent biochemical and morphological differentiation and fusion of the underlining villous cytotrophoblasts into this syncytium [44,46–53]. The unique transcriptomic activity of the syncytiotrophoblast [54] is responsible for the production of a large set of steroid and peptide hormones, immune proteins and other placental proteins chiefly expressed by the placenta and characteristically detectable in the maternal circulation during pregnancy [46,55]. These and non-secreted molecules enable key functions of the syncytiotrophoblast in the maintenance of pregnancy including feto-maternal gas, nutrient and waste exchange, hormonal regulation of fetal development, and the generation of an immunological barrier between the mother and the semi-allogeneic fetus [44,55]. Defects in syncytiotrophoblast formation have been implicated in the development of preeclampsia. For example, the decreased placental expression of the molecular machinery of trophoblast fusion, including GCM1 transcription factor, fusogenic retroviral proteins and their receptors have been observed in severe preeclampsia [56–62]. It was even presumed that the whole villous trophoblastic differentiation program is severely disturbed in preeclampsia [63], a hypothesis that could not be confirmed by whole-genome transcriptomic studies [49,51].

Since indirect evidence suggested that placental Chr19 cluster galectin expression is related to villous trophoblast differentiation [33,43,44], here we aimed to study how the trophoblastic expression of these galectins is related to villous trophoblast differentiation, and whether the trophoblastic expression of this galectin cluster is dysregulated in preeclampsia in relation to an altered villous trophoblast fusion or differentiation program.

2. Materials and methods

Human samples were retrieved from the Bank of Biological Specimens or were collected prospectively at the Perinatology Research Branch and Wayne State University (Detroit, MI, USA). Written informed consent was obtained from women before the collection of samples, and the research was approved by the Institutional Review Boards of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development and Wayne State University. Frozen placentas were used for RNA isolation and qRT-PCR, and for laser capture microdissection, genomic DNA isolation and bisulfite sequencing (Zymo Research Corporation, Irvine, CA, USA). Umbilical cord blood cells were used for genomic DNA isolation and bisulfite sequencing. Formalin-fixed paraffin embedded placentas were applied for tissue microarray and immunostainings with anti-PP13 (Behringwerke AG, Marburg/Lahn, Germany [64]) and anti-galectin-14 (AbD Serotec, Martinsried/Planegg, Germany) antibodies, and for *in situ* hybridization of three galectin genes. Primary trophoblasts isolated from normal term placentas and BeWo cells were used for functional experiments, including trophoblast differentiation, luciferase reporter and methylation assays, and immunostainings with anti-PP13 and anti-galectin-14 antibodies. Methylated DNA immunoprecipitation and DNA methylation arrays were performed at the University of Iowa (Iowa City, IA, USA). Genomic coordinates of genes, pseudogenes and transposable elements (TEs) in their 5' untranslated regions (5'UTRs) were retrieved from the UCSC Genome Browser (http://genome.ucsc.edu). Coding sequences were aligned and the gene tree was generated using the neighbor joining algorithm implemented in MEGA version 3.1 (www.megasoftware.net). The evolutionary conservation of transcription factor (TF) binding sites in the 5'UTRs were investigated by 'phylogenetic shadowing' using eSHADOW (http://eshadow.dcode.org). The Transcriptional Element Search System (TESS) (www.cbil.upenn.edu/tess) and the Transfac Database of the BIOBASE Biological Databases (www.biobase-international.com) were used to predict putative TF binding sites in the 5'UTRs. Demographics data were analyzed using SPSS version 12.0 (SPSS Inc., Chicago, IL), all other data were analyzed in the R statistical environment (www.r-project.org). All methods are described in detail in the Supplementary Information; additional data are shown in Supplementary Tables 1–4 and Supplementary Figs. 1–5.

3. Results and discussion

3.1. Villous trophoblast differentiation drives placental Chr19 cluster galectin expression

First, we characterized villous trophoblastic Chr19 cluster galectin gene expression in the placenta. *In situ* hybridization on normal term placentas showed *LGALS13*, *LGALS14* and *LGALS16* expression in the terminally differentiated syncytiotrophoblast but not in the cytotrophoblasts (Fig. 1A). In accord, the syncytiotrophoblast but not the cytotrophoblasts was immunopositive for PP13/galectin-13 and galectin-14 (Fig. 1B). Then, we examined how the expression of galectin genes are related to trophoblast syncytialization and differentiation *in vitro* by their parallel expression profiling with syncytin-1 (*ERVWE1*) and human chorionic gonadotropin (*CGB3*), markers of trophoblast syncytialization and differentiation [49,65–69] (Fig. 1C). We found increasing mRNA expression of galectin genes peaking on Days 2–3 in primary trophoblasts, following *ERVWE1* (Day 2) and preceding *CGB3* (Days 3–4) expression peaks, in parallel with the fusion of 80–90% of trophoblastic cells into syncytiotrophoblasts. The increasing mRNA expression of galectin genes peaked on Day 4 in differentiating BeWo cells, in parallel with that of *CGB3* and *ERVWE1*. When looking at the time-course of protein expression of galectins for which specific antibodies were available, cytoplasmic galectin-13 and galectin-14 immunopositivity appeared in differentiating primary trophoblasts on Day 3 and peaked on Day 7 (Fig. 1D).