Decidualization resistance in the origin of preeclampsia

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Introduction

Preeclampsia is a major obstetrical complication with short- and long-term life-threatening consequences for both mother and child. Shallow cytотrophoblast invasion through the uterine decidua into the spiral arteries is implicated in the pathogenesis of preeclampsia, although the cause of deficient arterial invasion remains unknown. Research that is focused on the “soil”—the maternal decidua—highlights the importance of this poorly understood but influential uterine layer. Decidualization of endometrial cells regulates embryo invasion, which is essential for spiral artery remodeling and establishing the maternal-fetal interface. Exploration of the association between impaired decidualization and preeclampsia revealed suboptimal endometrial maturation and uterine natural killer cells present in the decidua before preeclampsia development. Furthermore, decidualization defects in the endometrium of women with severe preeclampsia, characterized by impaired cytотrophoblast invasion, were detected at the time of delivery and persisted 5 years after the affected pregnancy. Recently, a maternal deficiency of annexin A2 expression was found to influence aberrant decidualization and shallow cytотrophoblast invasion, suggesting that decidualization resistance, which is a defective endometrial cell differentiation during the menstrual cycle, could underlie shallow trophoblast invasion and the poor establishment of the maternal-fetal interface. Based on these findings, the transcriptional signature in the endometrium that promotes decidualization deficiency could be detected before (or after) conception. This would serve to identify women at risk of developing severe preeclampsia and aid the development of therapies focused on improving decidualization, perhaps also preventing severe preeclampsia. Here, we discuss decidualization deficiency as a contributor to the pathogenesis of pregnancy disorders with particular attention to severe preeclampsia. We also review current diagnostic strategies and discuss future directions in diagnostic methods based on decidualization.

Key words: cytотrophoblast, decidua, decidualization resistance, endometrium, placenta, preconception care, pregnancy complications, severe preeclampsia, uterus

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Late-onset PE may result from stressors associated with maternal disorders such as metabolic and immune diseases and chronic hypertension.\textsuperscript{20,21}

Inadequate placental perfusion is also associated with other pregnancy complications. Pregnancies with intrauterine growth-restricted infants and one-third of preterm births involve defective vascular remodeling resulting from shallow trophoblast invasion.\textsuperscript{22} Therefore, inadequate placental perfusion is not sufficient to cause PE. The contribution of maternal-impaired decidualization to the pathophysiology of PE is receiving increasing attention in the field of reproductive medicine.\textsuperscript{23–26} Decidualization is essential for the regulation of CTB invasion and thus establishment of maternal-fetal interface during placentation. Accordingly, a deficiency in decidualization could lead to shallow CTB invasion implicated in PE. Here, we present the latest advances in the maternal-fetal interface during placentaion. We also discuss current diagnostic strategies and how a decidualization perspective could inform diagnoses.

The Placental Perspective

By postconception day 10 in a normal pregnancy, the human embryo is completely enveloped within the endometrial lining. The blastocyst is nourished by secretions from the endometrial glands (via histotrophic support) under hypoxic and hypoglycemic conditions until the maternal-placenta circulation is fully established.\textsuperscript{27} At approximately 8 to 10 weeks’ gestation, the placental extravillous trophoblasts (EVTs) transform into invasive cells through a partial epithelial-mesenchymal transition, in which the epithelial-like adhesion molecules are replaced by vascular-like adhesion molecules and the EVT mimic a vascular adhesion phenotype.\textsuperscript{28}

After this transition, EVT invade the full thickness of the decidualized endometrium and reach the inner third of the myometrium.\textsuperscript{29}

This invasion replaces smooth muscle cells and elastin in arteries with inert fibrinoid material.\textsuperscript{30} Subsequently, trophoblast cells enter the lumen of the spiral arteries, aggregate, and plug the arteries.\textsuperscript{16,31} Then, EVT migrate toward the myometrium reaching the inner third, where they form multinucleated giant cells.\textsuperscript{17} These migrations occur through the decidua and are likely controlled by this compartment.\textsuperscript{32,33} This process, known as the second wave of trophoblast invasion, is usually complete by 18 weeks’ gestation and is critical for the establishment of definitive uteroplacental circulation. As pregnancy progresses, the 120 to 140 small maternal spiral arteries that supply the placenta dilate to accommodate the increasing demands of the fetoplacental unit (Figure 1).\textsuperscript{34} The placenta is a high-volume, low-resistance organ, and at term, almost one-fifth of the maternal cardiac output (approximately 800 mL) passes through the placenta each minute.\textsuperscript{35}

Defective remodeling of maternal spiral arteries may result in a narrow lumen, a thick muscle layer, wide or thin-walled arteries, or funnel-shaped vessels, all of which are pathologic hallmarks of PE and are termed shallow endovascular invasion.\textsuperscript{15} This pathologic remodeling results from the failure of EVT to mimic a vascular adhesion phenotype.\textsuperscript{28} Consequently, the fetoplacental unit outgrows its blood supply, resulting in dysfunctional placental perfusion and ultimately clinical disease.\textsuperscript{22} However, this is not sufficient to cause PE, because other pregnancy complications such as intravascular growth restriction and one-third of preterm births involve defective vascular remodeling.\textsuperscript{22} Defective remodeling causes oxidative stress in the syncytiotrophoblast and the release of proinflammatory cytokines, such as tumor necrosis factor alpha and interleukin-1, and antiangiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFLT1).\textsuperscript{36,37} The disruption of maternal endothelial function with an exaggerated inflammatory response likely results in the clinical syndrome of PE.\textsuperscript{18}

Interestingly, assessing changes associated with PE at a genetic level provides a second perspective. CTBs freshly isolated from preeclamptic placentas were found to present a different transcriptional profile compared with control placentas.\textsuperscript{38} When CTBs were cultured for 48 hours, enabling differentiation and invasion, the abnormal pattern of gene expression in preeclamptic cells was reverted to control levels. This surprising finding pointed to the relevance of the maternal decidua in influencing CTB invasiveness.\textsuperscript{38} Key questions in the role of the decidua in PE remain, including why shallow CTB endovascular invasion occurs and how the maternal decidua induces shallow CTB invasion. Answers to these questions will enable the development of preventative measures and possible cures for PE.

The Decidua Perspective

Remarkably, removing the decidua in the immediate postpartum period by uterine curettage accelerates the speed of recovery in patients with PE.\textsuperscript{39,40} Decidual removal was first explored in 1993 in a pilot study with 32 patients with sPE. Significantly decreased mean arterial pressure at each 2-hour point for the first 24 hours after delivery was observed compared with patients who were not curedtted. The platelet count increased in the curettage group, whereas the noncurettage group had decreased platelet count.\textsuperscript{39} In 2013, Ragab et al\textsuperscript{40} consolidated the concept of accelerated recovery in a randomized controlled study involving 420 women with PE or eclampsia who underwent immediate postpartum curettage (n = 220) compared with patients without this intervention (n = 200). This study showed significant improvement in mean arterial blood pressure in the curettage group for the first 6, 12, and 24 hours after delivery. Likewise, there was an improvement in the platelet count in the curettage group compared with the control group.\textsuperscript{40}

In humans, decidualization does not depend on the presence of a conceptus.\textsuperscript{41} The process involves the differentiation of human endometrial stromal cells (hESCs) in response to hormonal stimuli beginning in the midsecretory phase of menstrual cycle.\textsuperscript{42} This differentiation includes changes at the morphologic and secretory level. Morphologically, it is characterized by the transformation of elongated fibroblast-like cells into an enlarged
Extravillous trophoblast cells (EVTs) transform into invasive cells through a partial epithelial-mesenchymal transition in which epithelial-like adhesion molecules are replaced by vascular-like adhesion molecules. EVT migrations occur through the decidua until reaching the inner third of the myometrium to invade the maternal spiral artery. Optimal EVT invasion is required to adapt the maternal vasculature to pregnancy and maintain blood flow to the placenta.

Decidualization forms the decidua, a tissue with the capacity to detect and respond to placental and embryonic stimuli, protecting the fetus from the maternal immune system and oxidative stress. The main functions of decidualized endometrium are (1) stopping epithelium proliferation, which is needed to become receptive to implantation; (2) protecting the embryo in the maternal-fetal interface from the maternal immune response and from oxidative stress; (3) regulating the local immune response to control the trophoblast invasion; and (4) allowing angiogenesis to prepare the maternal vasculature to support embryonic development during pregnancy. Overall, the decidua plays a vital role in coordinating embryo implantation and subsequently EVT interactions within the uterus, making decidualization essential for establishing the maternal-fetal interface.

Decidualization resistance is an aberrant response of the endometrium to hormone-driven differentiation signals. This defect can lead to complications at any pregnancy stage, including late-onset conditions, even though decidualization failure occurs before implantation. In intrauterine growth restriction, failures in decidualization result in the uterovascular remodeling disruptions observed in this complication. In recurrent pregnancy loss, there is evidence of an aberrant decidualization related to premature senescence. Aberrant decidua may not support the dramatic changes in oxidative stress produced in response to active maternal perfusion of the placenta, and miscarriage may occur.

These and other pregnancy disorders are typically classified into distinct categories, but much of this classification is arbitrary because these conditions occur along a continuum and have common and interrelated risk factors with overlapping biomarkers. Deficient spiral artery remodeling is well associated with PE and is also described in a spectrum of obstetrical syndromes, including intrauterine growth restriction, preterm labor, premature rupture of membranes, miscarriage, and fetal death. The common thread among these adverse pregnancy events may be impaired decidualization.

**Decidualization Resistance in Severe Preeclampsia**

Epidemiologic studies support a maternal contribution to PE by reporting risk factors for PE associated with the mother. Some examples are family history of PE, nulliparity, cesarean delivery in a first pregnancy, maternal high body mass index and age of >40 years, and chronic hypertension. Major risk factors for PE are having developed PE in the first pregnancy, previous intrauterine growth restriction, and placental abruption.

Healthy pregnancy is likely established before implantation and placentation and influenced by the correct formation of the decidua. Decidualization resistance, entailing the inability of the maternal “soil” to undergo tissue modifications, leads to aberrations in placental invasion and adverse pregnancy outcomes. Modifications in optimal decidualization are usually cellular changes in hESCs (morphologic, biochemical, and secretory phenotypes) and the recruitment of immune cells (uNK and regulatory T cells). In decidualization resistance, the process...
fails at some point resulting in a deficient decidua, impaired EVT invasion, and arterial remodeling. Thus, decidualization resistance could be considered a maternal risk factor for pregnancy complications, including PE. As a consequence, the contribution of impaired decidualization to the pathogenesis of PE is receiving increasing attention.\textsuperscript{23,25} Below, we review the evolution of decidualization deficiency theory.

This hypothesis for the involvement of decidualization failure in the pathogenesis of PE started as a logical deduction based on the cross-talk between the EVT and the decidua during placentation.\textsuperscript{24,61} However, uncovering any potential etiological factors occurring early in pregnancy, such as inadequate EVT invasion, would require an assessment of the relevant tissue, and obtaining first-trimester placenta and decidua is difficult. Thus, many studies are performed using third-trimester placentas and basal plate decidua. Transcriptional analysis of chorionic villous samples with normal endometrial maturation before and after implantation.\textsuperscript{63} The expression 112 of these was obtained from stromal cells isolated from decidual samples mentioned earlier. The conditioned medium from sPE patient cells did not support EVT invasion, suggesting that decidualization resistance is associated with shallow EVT invasion, a common feature in sPE.\textsuperscript{25}

Global transcriptional profiling revealed alterations in gene expression during in vitro decidualization of hESCs from former patients with sPE. In particular, annexin A2 (ANXA2) expression was lower\textsuperscript{22}; ANXA2 is abundantly expressed in the placenta, and its impaired activity could cause fibrinolytic deficiency associated with increased thrombosis, predisposing to PE.\textsuperscript{64} We investigated the relevance of ANXA2 in decidualization defects to assess its value as a putative preconception maternal biomarker with translational potential for sPE risk prediction.\textsuperscript{26} We assessed endometrial deficiency using a small interfering RNA and a knockout mouse model. This model produced a decidualization resistance phenotype with a shallow trophoblast invasion into the decidua, causing placenta invasion into the decidua, causing placental invasion into the decidua, maintaining a high economic burden. Healthcare costs of short-term complications developed during the first year after delivery reach $2.18 billion in the United States.\textsuperscript{65} An Irish study estimated that healthcare systems shoulder an average cost of €6.5 million per year owing to PE assuming a prevalence of 5%.\textsuperscript{66} These costs increase further with long-term complications.

Our group demonstrated that defective decidualization is associated with sPE\textsuperscript{65} by analyzing the decidualization process in hESCs isolated from nonpregnant donors with a previous sPE diagnosis compared with control women with a previous normal pregnancy. Samples were collected between 1 and 5 years after pregnancy, and decidualization of hESCs was induced in the culture. The ability to differentiate was analyzed by stage-specific antigens, morphology, and transcriptional profile and revealed that hESCs from patients with previous sPE were unable to decidualize. Transcriptional analysis identified 129 genes differentially expressed in sPE compared with controls (Figure 2, A). We also investigated whether impaired decidualization resistance present at the time of delivery in women with sPE by isolating portions of decidua (basalis and parietalis) from maternal-fetal interface sections using laser microdissection. Samples were collected at the time of delivery from women with sPE and women who had a spontaneous preterm birth with no signs of infection. Transcriptional profiling revealed >200 dysregulated genes in sPE related to decidual dysfunctionality. Isolated decidual cells from patients with sPE dedifferentiated in vitro and failed to redifferentize in the culture. Finally, we explored whether decidualization resistance might affect EVT invasion in a model mimicking aspect of the uterine environment in PE. This model was performed using CTBs isolated from second-trimester human placentas and cultured on a Matrigel substrate. The conditioned medium for these cultures was obtained from maternal-fetal interface sections of decidua (basalis and parietalis) overlapped with normal endometrial maturation before and after implantation. Furthermore, 116 of the 154 DEGs overlapped with DEGs related to decidualization in the absence of EVT. The expression 112 of the 154 DEGs was in the opposite direction from the expression expected in normal decidualization, including 16 DEG down-regulated in decidual natural killer cells. These findings motivated further inquiry into the concept that deficiency or defects in predecidualization gene expression and uNK cells are precursors to PE.\textsuperscript{23}

Placenta- or Decidua-Based Diagnostic Strategies

PE carries a high economic burden. Healthcare costs of short-term complications developed during the first year after delivery reach $2.18 billion in the United States.\textsuperscript{65} An Irish study estimated that healthcare systems shoulder an average cost of €6.5 million per year owing to PE assuming a prevalence of 5%.\textsuperscript{66} These costs increase further with long-term complications. Early identification of women at risk of PE is necessary to mitigate both the short- and long-term adverse effects. The challenge to prevention and diagnostic strategies is that the etiology of PE is widely separated in time from the onset of disease symptoms. Thus, diagnostic strategies based on biomarkers of placental dysfunction have limited success, and no single marker or model combining markers has performed well enough to be suitable for routine. The most promising screening methods with a high sensitivity and specificity are with combined
biomarkers sFLT1 and placental growth factor (PIGF). A meta-analysis of 15 studies with 534 cases of PE and 19,587 controls after 19 weeks' gestation evaluated the diagnostic value of the sFlt-1 to PlGF ratio as a screening method of PE and found a sensitivity of 80% and a specificity of 92%. However, there was a considerable heterogeneity between studies, including a lack of uniformity in the cutoff to rule out disease risk. In a multicenter study, a cutoff sFLT1 to PIGF ratio was established in 38, representing a sensitivity of 66.2% and a specificity of 83.1% for the diagnosis of PE within 4 weeks in patients at 24 to 37 weeks' gestation. The same ratio was used to rule out the disease within 1 week with a negative predictive value of 99.9%. Finally, multiparametric models were developed to detect pregnancies at risk of PE by screening at 11 to 13 weeks' gestation. These models combine data from medical history with biophysical and biochemical markers. However, when applied to different populations, they showed poor performance. A multivariate Gaussian distribution model was recently proposed including maternal factors, early PlGF determination, and biophysical variables, but this model is not validated in different populations.

The goal is obtaining an effective early screening for prevention and prediction because placental alterations become evident in the second trimester of pregnancy. Current therapies are based on careful monitoring, antihypertensive therapy, low-dose aspirin, healthy diet, exercise, and stress reduction, all of which are done in the hopes of prolonging pregnancy, not to cure the condition. Significant findings have already been made in the maternal contribution to PE and in the role of decidualization resistance in its pathophysiology. However, further investigations are needed to better understand and evaluate the detection of decidualization resistance before pregnancy in women without a history of PE. In particular, research on the underlying mechanisms that lead to deficient decidualization would be especially valuable. Decidua-based diagnostic strategies for PE could be applied even before pregnancy, because decidualization is a process that occurs during

**FIGURE 2**

Decidualization resistance: the maternal contribution to severe preeclampsia

A, Women with preeclampsia present decidualization failure years after childbirth. hESCs isolated from patients with PE and cultured in vitro to promote decidualization showed an inert transcriptomic profile and were unable to decidualize. Among the 129 dysregulated genes, ANXA2 was down-regulated. B, In an in vitro model, ANXA2 was silenced in hESCs isolated from women with previous severe preeclampsia. The consequences of ANXA2 deficiency were confirmed with the use of an ANXA2-null mouse model. Together, these results suggest that a deficiency in ANXA2 is associated with the decidualization resistance observed in preeclamptic patients, highlighting the maternal contribution in the pathogenesis of severe preeclampsia.

ANXA2, annexin A2; DEG, differential expression genes; hESCs, human endometrial stromal cells; siRNA, small interfering RNA.

The menstrual cycle, regardless of the presence of a conceptus. Developing a molecular test for application to the endometrial tissue at the time of decidualization—before pregnancy—could enable a prospective risk screening for this pathologic condition. Such testing would have promising clinical potential in the characterization of risk, which is the first step in achieving effective prevention (Figure 3).

Conclusion

Decidualization resistance in the pathophysiology of PE solidifies a maternal contribution to the development of the condition. However, further investigations are needed to determine how to detect decidualization resistance before pregnancy in women without a history of PE. In particular, research on the underlying mechanisms leading to deficient decidualization would be especially valuable. Ideally, decidual-based diagnostic strategies for PE could be applied before pregnancy, because decidualization occurs during the menstrual cycle regardless of the presence of conceptus. Such testing would have promising clinical potential in the characterization of risk.

ACKNOWLEDGMENTS

We thank PhD student Irene Muñoz (Instituto de Investigación Sanitaria INCLIVA-Igenomix Foundation, Valencia, PhD grant PRE2019-090770 from Spanish Ministry of Science and Innovation) for assistance in compiling data. This work was supported by a fellowship Program grant FDEGENET/2019/008 from the Generalitat Valenciana (to N.C.-M.); an Institute of Health Carlos III Grant PI19/01659 (to T.G.-G); and the Spanish Ministry of Science and Innovation Grant RTI2018-094946-B-100 (to C.S).

REFERENCES

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<tr>
<th>Abbreviation</th>
<th>Term</th>
<th>Definition</th>
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<tr>
<td>CTB</td>
<td>Cytotrophoblast</td>
<td>Unfused trophoblast cells that cover the implanting blastocyst surface. During implantation, CTBs invade the decidua and this invasion is shallow in preeclampsia.</td>
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<tr>
<td>CVS</td>
<td>Chorionic villous samples</td>
<td>Villi that sprout from the chorion to provide maximal contact area with maternal blood can be sampled to assess gene expression.</td>
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<tr>
<td>DEGs</td>
<td>Differentially expressed genes</td>
<td>Genes showing statistically significant different expression between 2 conditions.</td>
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<tr>
<td>EVTs</td>
<td>Extravillous trophoblasts</td>
<td>Cells that invade the maternal spiral arteries forming a chimeric vasculature. In preeclampsia, this invasion is shallow.</td>
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<tr>
<td>hESCs</td>
<td>Human endometrial stromal cells</td>
<td>Fibroblast-like cells in the endometrial stroma that differentiate during decidualization, an essential process for decidua formation.</td>
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<tr>
<td>PE</td>
<td>Preeclampsia</td>
<td>A major obstetrical complication with short-term and long-term life-threatening consequences for both mother and child.</td>
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<tr>
<td>PIGF</td>
<td>Placental growth factor</td>
<td>An angiogenic factor whose concentration is low in patients who subsequently develop preeclampsia.</td>
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<tr>
<td>sFLT1</td>
<td>Soluble fms-like tyrosine kinase-1</td>
<td>An antiangiogenic factor whose concentration in maternal plasma is high before and at the time of preeclampsia diagnosis.</td>
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<tr>
<td>—</td>
<td>Single-cell resolution</td>
<td>Sequence information from individual cells with optimized next-generation sequencing.</td>
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<tr>
<td>sPE</td>
<td>Severe preeclampsia</td>
<td>Preeclampsia with severe symptoms and signs.</td>
</tr>
<tr>
<td>—</td>
<td>Transcriptomics</td>
<td>The study of transcriptome, which is the complete set of RNA molecules within a cell.</td>
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