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Extracellular Matrix and Absent Decidua Dysregulate the Epithelial to Mesenchymal Transition in Extravillous Trophoblast in Placenta Accreta Spectrum

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Abstract

Our study purpose was to describe the extracellular matrix (ECM) surrounding the placental implantation site and the interaction between ECM and trophoblast to understand causal mechanisms of placenta accreta spectrum (PAS). Utero-placental tissue from PAS cases and normal myometrium was compared with non-PAS postpartum hysterectomy placental bed. HTR8/SVneo cells were used to investigate first trimester invasion. Evaluation was performed immunohistochemical staining (IHC) and Transwell invasion assays. Our outcome measures were histological scores of IHC and percent invasion for transwell assays. PAS specimens had absent decidua and placental villi in direct myometrial contact. EVT were encased within ECM containing Type IV collagen, fibronectin and Type III collagen rather than Type I Collagen. Positive staining was present for all wound remodeling factors tested in PAS EVT with differential ECM expression between PAS and non-PAS samples. Transwell assays showed Type III collagen promoting cell invasion whereas other ECM was inhibitory. Absence of mature Type I Collagen suggests that over previous cesarean scar, the area of trophoblast invasion behaves like an active wound. The invasion assays confirm that ECM differences result in altered trophoblast behavior. The extracellular matrix itself, as well as the absence of decidua impact the invasive behavior of placental trophoblast cells.

Introduction

The U.S. maternal mortality rate[1] is partially attributable to a 3.6-fold increased risk associated with caesarean delivery (CD)[2]. The CD rate increased 5-fold in the last 40 years[3], paralleled by complications in subsequent pregnancies including abnormal implantation/placentation[4–9]. Uterine scar is associated with formation of placenta accreta spectrum (PAS), a highly morbid abnormal attachment of placental trophoblast cells to a myometrial scar without intervening decidua that is now estimated to impact 0.17% of pregnancy.[4,10–14] Suggested mechanisms for accreta formation include tropism of trophoblast into scar, absence of decidual signaling preventing trophoblast invasion, and scar micro-fissures or dehiscence, permitting an inappropriate trophoblast invasion[10–14]. Added is the possibility of aberrant trophoblast function, interacting with the scar to enhance invasion.[14] The etiology of abnormal placentation is poorly understood, in part because of a lack of mechanistic understanding of normal uterine wound healing[15]. Regardless of etiology, PAS has emerged as a chief contributor to perinatal surgical morbidity and mortality due to hemorrhage and associated complications, particularly when undiagnosed prior to delivery.[4,8,14]

Wound healing normally follows a predictable trajectory[16–19] and involves an epithelial-to-mesenchymal transition (EMT), a conserved molecular mechanism which transforms epithelial cells into motile mesenchymal cells. This enables mobilization and recruitment of cells to damaged tissue[20–22]. A proliferative phase of tissue remodeling, collagen deposition and angiogenesis follows, characterized by dominance of mature Type I over immature Type III collagen[16, 18, 19]. The time course of maturation has not been delineated in myometrial or endometrial scars of normal or pathologic pregnancy[15]. In general, wound healing and scar maturation in epithelial tissues takes up to 24 months but is less well characterized in smooth muscle organs, since most do not undergo repetitive scarring occurs with CD.

Placental invasion is also mediated by a form of EMT, in which epithelial cytotrophoblast differentiate into motile, extravillous trophoblast cells (EVT), which invade the decidua[24,25]. This invasion involves integrin - ECM binding between trophoblast and decidua, particularly integrin α6 – laminin binding, and integrin α5 – fibronectin binding[21–23]. The trophoblast EMT is characterized by the predominance of either α5 or α6, which impacts

trophoblast migration/ invasiveness[20, 24–30]. Other external factors influence invasion. Uterine secreted BMP-7 initiates reversal of the EMT, a process known as the mesenchymal-to-epithelial transition (MET)[31, 32]. There is evidence that the decidual components, including soluble factors, decidual natural killer cells (dNK) and other cell types guide and eventually terminate invasion.[23–34] The invasive nature of trophoblast in ectopic pregnancies, characterized like PAS by the absence of decidua, supports this concept[35,36].

The objective of this study was to describe uterine scar wound healing at the site of trophoblast invasion. Our hypothesis was that abnormal ECM signaling and the absence of decidual mediators alters trophoblast invasion in PAS. We hypothesized that ECM type present at the utero-placental junction could modulate EVT invasiveness and that the presence of immature collagen types would increase cellular invasive behavior. We investigated this by examining ECM components, wound healing factors and differentiation signals at the junction between the placenta and uterus in normal and PAS pregnancies. We sought to validate this concept *in vitro* using a Transwell trophoblast invasion model.

Results

The clinical characteristics of the subjects comprising the retrospective and prospective samples are given in Table 1. The gestational age for PAS cases ranged 31–38 weeks, while controls were 36–40 weeks. Data regarding prior cesarean was available, however data regarding additional prior uterine procedures including dilation and curettage were not reliably available and thus not included.

Table 1

Subject	Reason for hyst	Maternal age (years)	G	P	Prior CD	Inter-pregnancy Interval	GA (weeks)	Previa	PAS type	Samples obtained
Retro/PAS										
1	PAS	37	3	2	2	84 mos	34	+	Increta	Invasion site/ Remote Myo
2	PAS	31	5	3	1	24 mos	31	+	Increta	Invasion site/ Remote Myo
3	PAS	36	7	2	2	48 mos	32	+	Accreta	Invasion site/ Remote Myo
4	PAS	32	4	2	2	12 mos	32	+	Accreta	Invasion site/ Remote Myo
5	PAS	36	4	3	3	36 mos	36	+	Increta	Invasion site/ Remote Myo
6	PAS	37	6	5	3	NA	33	+	Percreta	Invasion site/ Remote Myo
7	PAS	47	1	0	0	-	36	-	Accreta	Invasion site/ Remote Myo
8	PAS	40	5	2	2	NA	37	-	Accreta	Invasion site/ Remote Myo
Pro/PAS										
9	PAS	31	6	5	5	23 mos	34	+	Percreta	Invasion site
10	PAS	24	4	2	2	24 mos	38	+	Increta	Invasion site
11	PAS	32	5	3	3	17 mos	33	+	Accreta	Invasion site

Hyst = hysterectomy G = Gravidity, P = Parity, Retro = retrospective samples, Pro = prospective samples, mos = months, NA = Data not available, PAS = Placenta Accreta Spectrum, Myo = myometrium

Extracellular matrix components

All PAS specimens demonstrated the presence of placental villi (pv) and an absence of decidua (Fig. 1A). Illustrations of stained tissue shown in the paper are representative and reflect similar conditions in all the other samples. EVT were encased within an ECM consisting of basement membrane Type IV collagen, fibronectin and immature Type III collagen (Fig. 1E,G,I). By contrast, Type I collagen was observed in ECM bundles surrounding the control myometrium (myo) (Fig. 1D), along with fibronectin, Type III and Type IV collagen (Fig. 1F,G,J). Laminin was not appreciated either surrounding the EVT or in normal myometrial ECM bundles (Fig. 1K,L). The relative quantities of these ECM components are shown in Fig. 1M. Semi-quantitative histologic (H-) scoring on a 0–3 scale was utilized rather than quantitative colorimetric scoring to differentiate ECM and cell types within a single image. The relative abundance of immature collagen types encasing PAS EVT relative to control myometrium basement membrane suggests the substrate into which EVT invades, rather than the classical assumption of myometrial invasion, is in fact into the ECM of the local scar.

Wound remodeling factors

Scoring for the staining of wound remodeling factors, TNF-alpha, FGF-2, CTGF, MMP-2, MMP-8, and Smad2 [18–23], was performed for the remote myometrium (myo), and in PAS EVT and placental villi (PV) to demonstrate which of these factors are present at baseline in non-scarred ECM. Staining for the wound remodeling factors in the myometrium was near-absent for both PAS and control, except for CTGF (Fig. 2E,F), for which high levels were detected in both PAS and control. The EVT (designated by HLA-G, Fig. 2M,N) in PAS samples were positive for all the wound remodeling factors tested; (Fig. 2A-L). Two factors, FGF2 and MMP-8, were further increased in EVT relative to villous cytotrophoblast (Fig. 2O). This suggests that EVT itself contributes to ongoing ECM remodeling at the invasion site.

Integrins

Invasion involves integrin - ECM binding between trophoblast and decidua, particularly integrin α6 – laminin binding, and integrin α5 – fibronectin binding[21–23]. The trophoblast EMT is characterized by the predominance of either α5 or α6, which impacts trophoblast migration/ invasiveness[20, 24–30] Integrin α5 was abundantly present on syncytiotrophoblast (placental villi, pv) and EVT surfaces (Fig. 3A,B, with Fig. 3C demonstrating via cytokeratin confirmation of EVT location within the placental bed site). Integrin α6 was present on decidua cell surfaces and to a less degree the EVT of only the normal term placental bed specimens (Fig. 3E with 3F demonstrating decidual location via vimentin staining); there was no appreciable staining on PAS specimens (Fig. 3D). In combination with the abundance of α5 binding substrate fibronectin and absence of α6 binding substrate laminin demonstrated in Fig. 1, these data indicate that a pro-mesenchymal/migratory phenotype in PAS EVT might be mediated by a lack of decidual integrin α6.

Differentiation signals

Other external factors influence invasion through the EMT. Uterine secreted BMP-7 initiates reversal of the EMT, a process known as the mesenchymal-to-epithelial transition (MET)[31, 32]. In the non-pathologic placental bed, BMP-7 was present throughout the decidua whereas in PAS samples, BMP-7 was only present well beyond the area of trophoblast invasion (Fig. 4D), within the myometrium. This may suggest that BMP-7, a factor which normally

reverses cellular migration, is present in deeper myometrium but not within ECM of scar, preventing cessation of migration.

Prior research has shown that decidual natural killer (dNK) cells may play a role in placentation by interacting with EVT cells and releasing cytokines such as IL-8 and IFN-10 to promote trophoblast invasion and spiral artery remodeling[33]. dNK cells are postulated to release metalloproteinases that assist in the breakdown of extracellular matrix allowing for trophoblastic invasion[34]. In the normal placental bed, but not in PAS, abundant dNK staining for the HLA-G-specific KIR2DL4 receptor was present in the region of trophoblast invasion (Fig. 4H) without any CD56 + dNK cell staining identified (Fig. 4J). In PAS samples, neither CD56 nor KIR2DL4 dNK cell staining was seen at the site of placental invasion (Fig. 4G,I). dNK cells have been shown in decidua to modulate trophoblast behavior, suggesting that the absence of dNK cells in the ECM may also be responsible for failure to arrest trophoblast invasion.

In vitro Transwell Assay

From the results above, we would expect Transwell invasion assays would yield increased invasion relative to control (100%) with immature Collagen Type III. In our *in vitro* validation, the Transwell invasion assays showed a significant difference in cell invasion among the extracellular matrix components ($p = 0.015$). Promotion of HTR8/SVneo cell invasion was driven by Type III collagen substrate, whilst laminin and fibronectin both exerted an inhibitory effect (Fig. 5). This confirmed that the ECM substrate which the placental cells encounter at implantation mediates 1st trimester trophoblast behavior.

Discussion

Our data suggest that although by definition PAS trophoblast are juxtaposed to myometrium without intervening decidua, the placental invasion site in PAS is functionally most similar to the extracellular matrix seen in immature scars. Additionally, invading PAS EVT demonstrate multiple markers of wound remodeling, markers that are near-absent in the myometrium of both PAS and control. Together these findings suggest an interaction between the EVT and the components of the ECM substrate into which they are embedded, which promote ongoing EMT. The absence of Type I collagen in the materno-fetal junctional zone ECM of PAS suggests that rather than a fully healed CD scar, the area of abnormal trophoblast invasion is an active wound, with extracellular matrix components of fibronectin and Type IV collagen providing a literal pathway for ongoing cellular migration, and tissue remodeling/disruption. This hypothesis is supported by our results showing that ECM components differentially influence early trophoblast invasive behavior.

Our hypotheses are supported by the available literature. The role of integrins in the promotion of invasion was recognized by Loke et al [23]. The inhibition of the EMT (and promotion of the MET) by BMP-7 is a generally recognized phenomenon [31]. BMP-7 is over-expressed in placentas from preeclampsia, an under-invasion pathology[41], which would predict under-expression in PAS, and confirmed by our results. However, the role of the EMT-MET transition is poorly understood developmentally as relates to placentation and pregnancy. The role of dNK is complex[40], but persistence of these cells in normal third trimester pregnancy[42] contrasts with the loss of dNK noted in PAS[43] suggesting that their absence may relate to the increased invasiveness of the EVT in PAS. This connection provides avenues for additional study of endometrial/decidual optimization to reduce the effect of non-traditional contributors to PAS such as IVF. Furthermore, improved understanding of uterine scarring may assist in

surgical conduct of cesarean to prevent formation of scars or niches with a predisposition towards abnormal trophoblast invasion.

Abnormal levels of tissue remodeling factors were demonstrated in the EVT of PAS specimens, with our specimens being collected in the later third trimester. This suggests that the remodeling process in PAS may be continuous throughout pregnancy. This process *in vivo* likely influences trophoblast depth and behavior beyond the normal cessation point in the second trimester. The enigmatic fibrinoid observed in histologic samples may represent the byproduct of this ongoing tissue remodeling [37]. Although a recent etiologic proposal suggests that PAS represents primarily a “dehiscence” with placenta mechanically dissecting through scar, the placenta may be, instead, disintegrating the scar from the inside out[38]. This hypothesis is supported by a recent report that evidence of scar was only found in 23–31% of PAS histologic evaluation.[39]

Integrin α6, a normal adhesion protein in decidua, was absent at the placental-myometrial junction in PAS and suggests that decidual integrin α6 may serve as an anti-invasive modulating signal. Its absence in PAS at the junction of placental and myometrial tissue would promote persistent trophoblast migration and the continued invasion observed in PAS. The notion that decidual factors modulate EVT is stronger for BMP-7, which stimulates arrest of EMT and transition to the MET [31]. In PAS we found BMP-7 only present in areas significantly beyond the invading EVT. The absence of CD56 + decidual natural killer cells in PAS specimens, supported by previous findings[40], also suggests that this key immune cell in the decidua may similarly serve as an arresting/modulating signal in normal placentation. The combination of these factors supports that the absence of decidua plays a functional role in trophoblast behavior, rather than merely an absent feature in PAS histology. However, whether surgical scarring causes failed decidual formation, abnormal/absent endometrial decidualization, or whether the abnormal EVT themselves destroy and replace decidua with fibrinoid is unknown and unable to be determined by our third trimester findings.

PAS has remained an enigmatic pathology. The strong associations between PAS and CD scar and placenta previa may be partially explained by our findings. However, our findings have implications for other contributors to PAS, including smaller areas of scarring from prior uterine surgeries, devascularization procedures such as uterine artery embolization, and immune functions which may assist in understanding contributions of risk factors such as *in vitro* fertilization (IVF)[4]. We have previously suggested that PAS is mediated by the combination of a scarred myometrium and the absence of decidua, influencing trophoblast behavior and vascular remodeling [11]. Our current findings continue to support and enhance this model (Fig. 6, adapted from Duzyj et al.[11] BJOG 2018).

The most significant limitation of our study is the small sample size for the immunohistochemical studies. However, consistent findings were found across all specimens, and we have high confidence that the specimens were maximally representative of PAS based on our methodology. Additionally, although sampling was not at a uniform location in retrospective subjects due to study design, the findings from the retrospective samples were confirmed prospectively when location of tissue collection was controlled. Finally, our samples were heterogeneously representative across placenta accreta spectrum which may alter histologic results, although given that this disease is felt to represent a spectrum rather than discrete categoric pathologies (accreta vs. increta vs. percreta), we feel that our results do represent the spectrum of disease.

The other primary limitation of our study was the semi-quantitative nature of the scoring system used to differentiate between specimens. However, attempts at quantitative computerized scoring were problematic in that extracellular matrix scoring was difficult to compare across images due to dominance of specific staining, and

conversely too small to quantify when observed only in rare individual cells. Given that multiple cell types were quantified in each figure, human analysis and differentiation was required to appropriately categorize which cell types were positively stained. This problem was primarily managed by blinding trained and consistent reviewers to the identity of the slides being assessed, a methodology we have previously used[11,12]. The principal strengths of our study are the application of an *in vitro* verification of the hypothesis suggested by the immunohistochemical findings, as well as the broad investigation of multiple factors implicated in the EMT.

Our study suggests that placenta accreta spectrum arises from direct trophoblast embedding into an immature extracellular matrix in the absence of a multi-modal decidual stop signal. This matrix then provides a substrate for aberrant trophoblast behavior and inappropriate persistence of the epithelial to mesenchymal transition, resulting in the phenotypic depth of placental invasion and tissue disruption observed in PAS.

Methods

Specimens were acquired both retrospectively and prospectively. The protocols were approved by the Institutional Review Board at Rutgers Robert Wood Johnson Medical School in New Brunswick, NJ (protocol #s Pro20150001436, Pro20150002328), and all methods were carried out in accordance with relevant guidelines and regulations. Retrospective samples were obtained from archived, paraffin embedded utero-placental tissue from CD/hysterectomies. De-identified placental bed and remote myometrium sections from non-accreta postpartum hysterectomies, due to atony (n = 3), scar dehiscence (n = 1) and cervical dysplasia (n = 1), were selected as control samples with confirmation of EVT presence in decidua. Retrospective PAS pregnancies were identified by a pathologist and retrieved from archived materials where “placenta accreta/increta/percreta” appeared in the pathology diagnosis (n = 8). Histologic specimens were reviewed and sections demonstrating maximum EVT invasion/thinnest residual myometrial thickness were selected for analysis by a single pathologist (author MC), as well as normal myometrium from a site remote from placental invasion. Normal placental bed was not collected from pathologic samples due to the possibility that all decidua was pathologic and not a suitable control. Additional clinical factors were not used to exclude subjects from review.

Prospective subjects were characterized by a prior low transverse CD and antenatal sonographic suspicion for PAS. Subjects were approached during a prenatal visit or in the pre-operative area for study participation, and informed consent was obtained from all subjects prior to surgery per Rutgers IRB approved consent processes. Because of this method, only elective cases were enrolled, and additional clinical factors were not used for exclusion criteria. The PAS CD/hysterectomy specimens were obtained immediately following hysterectomy (n = 3). The specimen was brought directly to the pathology laboratory, and a sample was obtained from the area of maximal placental invasion/minimal myometrial thickness. Confirmation of histologic accreta was subsequently performed by the pathologist for the remainder of the specimen. Fresh tissue was oriented to represent full-thickness slices from placental villi through the myometrial junction to serosa, formalin fixed for 48 hours, and paraffin embedded. Serial sections were cut at 5μm thickness for immunohistochemistry. There was no public involvement and patient involvement was limited to the informed consent process.

Immunohistochemistry:

Immunohistochemical staining was performed for extracellular matrix components: types I (Novus NBP1-30054, 1:100), III (Abcam ab7778, 1:100) and IV (Abcam ab6586, 1:100) collagen, fibronectin (Genway GWB-1C597A, 1:1000) laminin (R&D P3H9-2 10ug/mL); adhesion proteins: integrin α5 (Abcam EPR7854, 1:75) and α6 (Abcam

EPR18124, 1:300); wound remodeling factors: tumor necrosis factor-alpha (Novus NB600-1422, 1:75), fibroblast growth factor 2 (Genway 2H5G2C11, 1:200), connective tissue growth factor (CTGF, Abcam ab6992, 1:100), matrix metalloproteinase 2 (Abcam ab110186, 1:100), and 8 (Abcam ab81286, 1:100), Smad-2 (1:75), HLA-G (Abcam 4H84, 1:25), and bone morphogenic protein 7 (Abcam ab54904, 1:100); decidual natural killer cell markers: KIR2DL4, a receptor on dNK cells known to bind to HLA-G (ab154386, 1:100) and CD56, a neural adhesion molecule (NCAM-1) as a marker of dNK cells (ab133345, 1:100). Masson trichrome staining was performed (Sigma-Aldrich) to delineate scar and fibrinoid location relative to villous trophoblast and myometrium. Chromogen for detection was either Vector Red or DAB. For extracellular matrix components, staining was performed through the Rutgers Biospecimen Repository and Histopathology Service to ensure use of validated antibodies. Three representative images were taken for each specimen. Semiquantitative histological scores were assigned by two independent investigators. Strength of staining was quantified on a 0–3 scale in each representative image for myometrial cells, extravillous trophoblast, extracellular matrix elements and decidua. Because semiquantitative scores are ordinal variables, the Mann Whitney U test was used to detect differences between samples, assigning significance to those comparisons with a p value < 0.05.

In vitro Transwell assay

In vitro Transwell invasion assays were conducted by plating HTR8/SVneo cells (ATCC) on Transwell membranes coated with extracellular matrix components of relevance for this study. Extracellular matrix components polymerized on transwell surfaces for 5–6 hours prior to cell seeding. Cells were serum starved for 6 hours prior to the experiment in RPMI media with 0.5% FBS. Cell proliferation was then arrested with Mitomycin C (10 µg/ml) in 0.5% FBS for 2 hr. prior to plating cells in the Transwell inserts (Millicell). The Transwell inserts were coated with either collagen I, collagen III, collagen IV, fibronectin, or laminin at a concentration of 5 $\mu g/cm^2$ or DMEM buffer for control. Cells (5×10^4) were plated in RPMI media with 0.5% FBS in the upper chamber of the Transwell insert, and RPMI media containing 5% FBS was added in the lower chamber as chemoattractant. After incubation at 37°C for 24 hr., the Transwell insert was removed from the well and cells were fixed with 4% paraformaldehyde (10 min). Cells on the top surface of the membrane were removed with a moistened sterile cotton swab, and membranes were excised from the inserts with a blade. The membranes on the bottom surface of the insert were cells were stained with crystal violet then imaged at 100X magnification. Four random fields were counted to calculate percent invasion in the ECM-coated wells compared to uncoated wells. Student's t-test and ANOVA with Tukey's post hoc analysis were used to detect differences between groups with a minimum of three independent experiments performed in triplicate. DMEM buffer without ECM served as 100% invasion for control.

Declarations

Competing interests:

The authors declare no competing interests.

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Author Contribution

Christina Duzyj conceived of the project, planned and carried out the experiments, analyzed the data, and wrote the manuscript.Haylea S Patrick was involved in sample collection, experimental conduct, data analysis and manuscript conception and editing.Pooja Desai and Seth J Barishansky were involved in experiment conduct and image analysis for data collection, as well as manuscript approval.Marina A Chekmareva was involved in sample identification and analysis, and manuscript editing.Sonia C DaSilva-Arnold, Bingbing Wang,, Todd Rosen and Stacy Zamudio were involved in experimental design, oversight of research conduct and manuscript editingNicholas P Illsley served as a primary research mentor for the study, involved in project planning, experimental design, and manuscript editing.

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Data Availability

The datasets generated during the current study principally apply to the semiquantitive histologic scoring from IHC, and the percent invasion from the invasion assays. There are available from the corresponding author on reasonable request.

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Figures

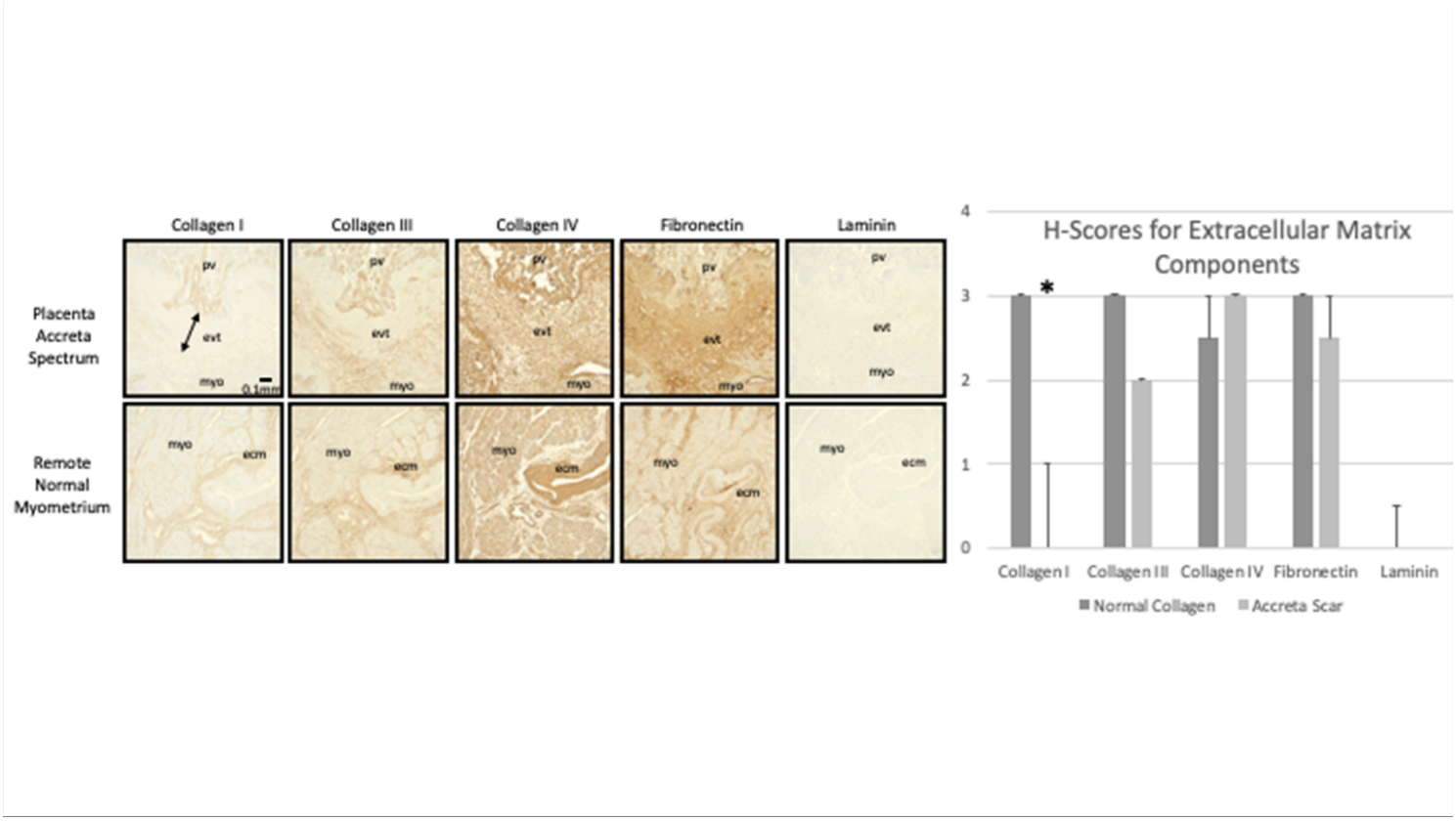


Figure 1

Representative section of a placenta accreta spectrum placental invasion site demonstrating placental villi (pv), and extravillous trophoblast (evt) invasion into the uterus within a band of extracellular matrix (ECM), absent decidua, and thin residual myometrium (myo) (1A), which appears different than the appearance of normal myometrium (myo) encased in extracellular matrix bundles (ECM) by Masson trichrome staining (1B). This band of matrix demonstrated on serial sections (arrowhead), and the encased evt (chevron) within did not stain comparably to normal myometrium or its encircling collagen bundles for any other matrix element (Figure 1C-N). All development for matrix elements was performed with Vector NovaRed. Semi-quantitative H-scores differed statistically ($p<0.05$) for Type I, III and IV Collagens between normal myometrium and PAS (Figure 1M).

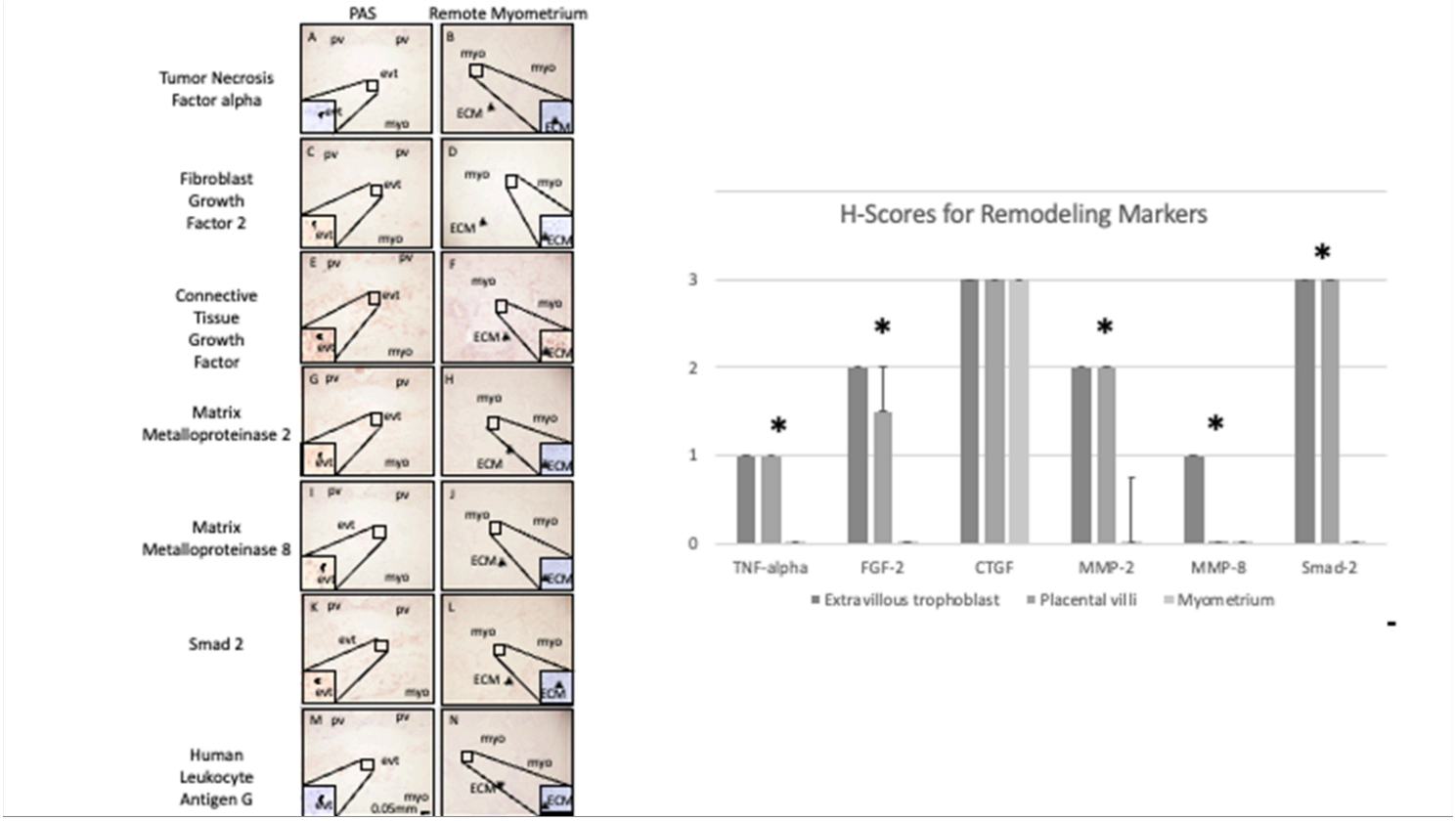


Figure 2

Wound remodeling factors were found to be expressed by PAS extravillous trophoblasts (evt, chevrons on magnified view) themselves (confirmed by human leukocyte antigen G staining, Figure 2M,N). None of these factors were found within control myometrium (myo). Fibroblast growth factor 2 and matrix metalloproteinase 8 were statistically elevated within the evts themselves related to normal expression in placental villi (pv) (Figure 2O). All development for matrix elements was performed with Vector NovaRed.

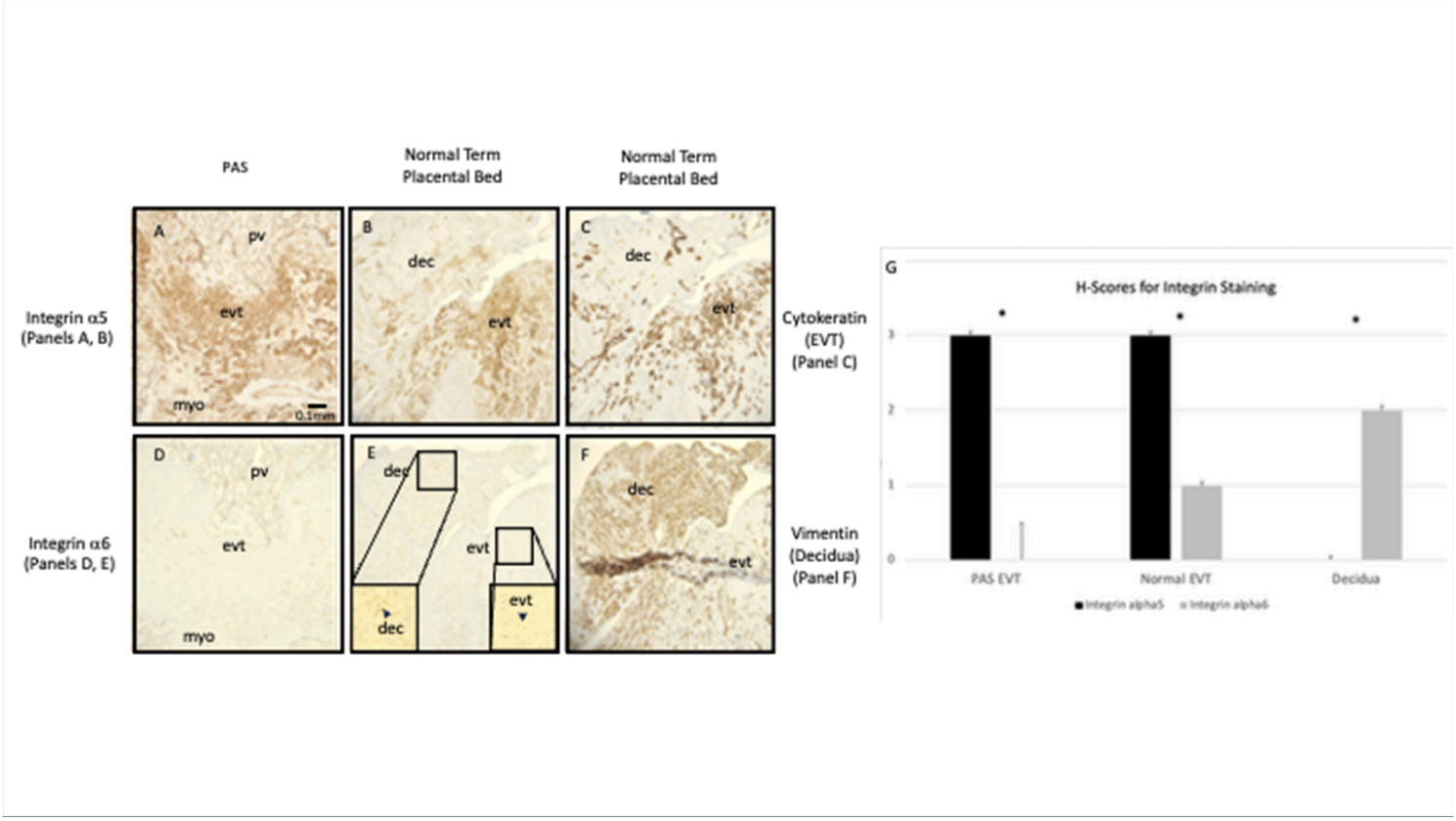


Figure 3

Integrin alpha 5 was found to be expressed by PAS and non-pathologic extravillous trophoblast (evt) within normal term placental bed (Figure 3A,B). Integrin alpha 6 was found to be present within decidual cells (dec, arrow) and to a lesser degree normal EVT (Figure 3E, arrow), but completely absent from the area of evt invasion in accreta samples (Figure 3D). Images collected using DAB as a chromogen. All differences were $p < 0.05$ (Figure 3G).

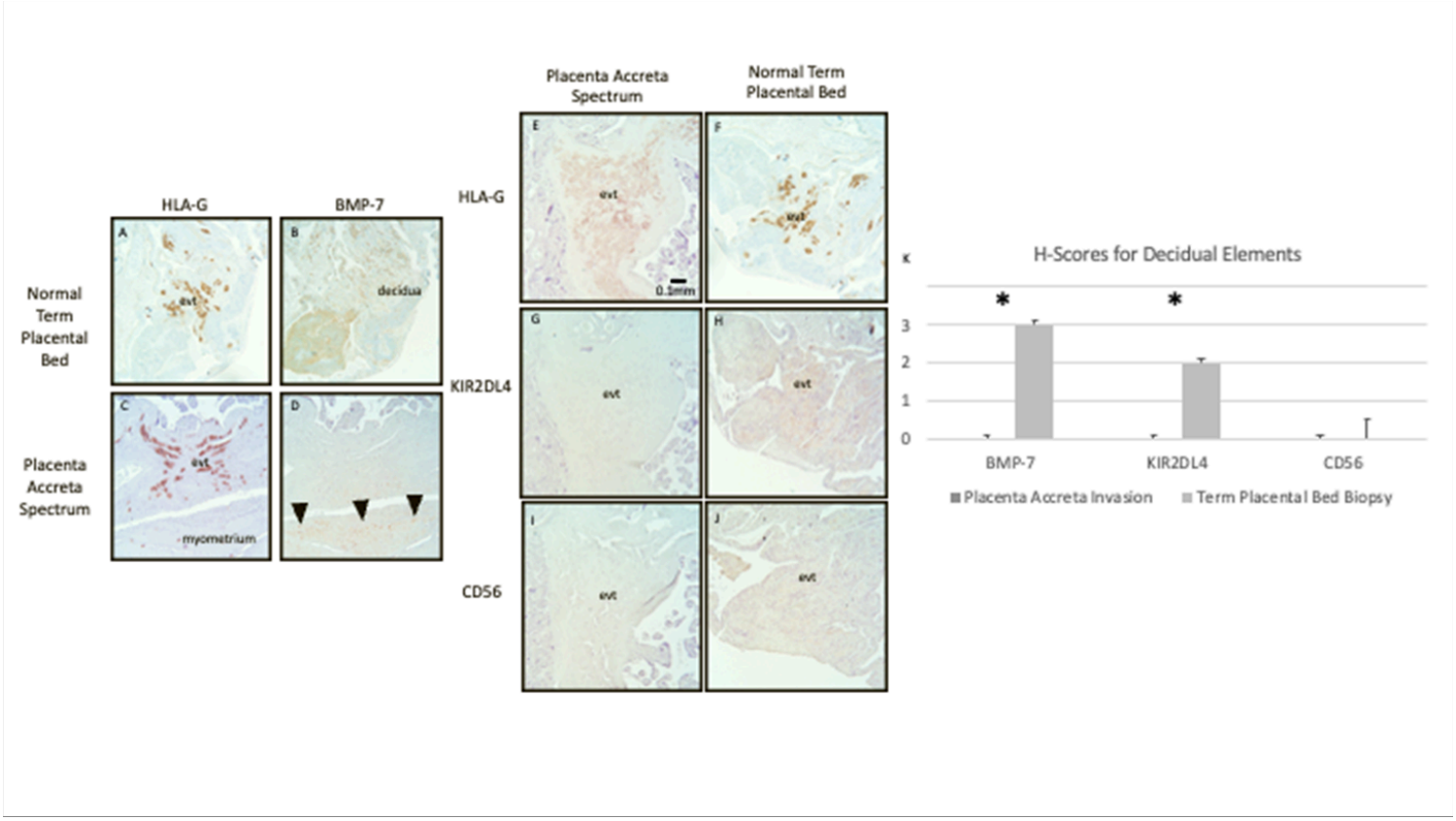


Figure 4

Absence of decidua in placenta accreta samples resulted in absence of normally seen bone morphogenic protein 7 (BMP-7) and KIR2DL4 (decidual natural killer cells with receptors for HLA-G) in areas of extravillous trophoblast (evt) invasion in placenta accreta samples (Figure 4D,G). CD56 uterine natural killer cells were also not appreciated in normal third trimester placental bed biopsies (Figure 4I,J). All development was performed with Vector NovaRed. Differences between PAS BMP 7 and KIR2DL4 dNK cells were $p < 0.05$.

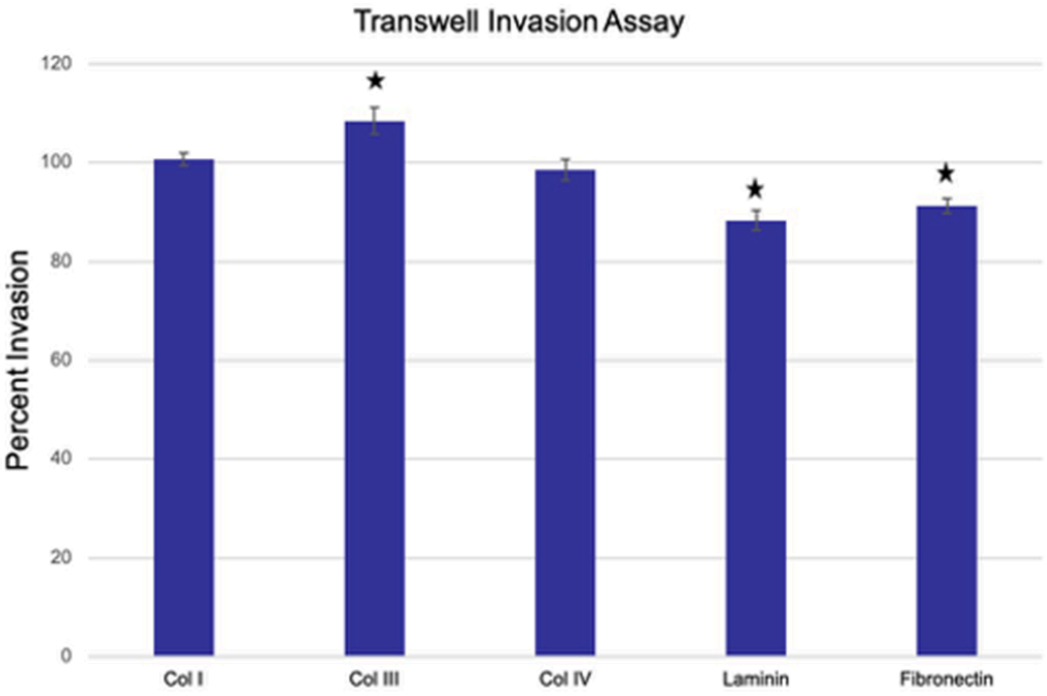


Figure 5

Differential immortalized first trimester trophoblast invasion is seen based upon which extracellular matrix element is utilized, with Type III collagen promoting trophoblast invasion, and both fibronectin and laminin causing an inhibitory effect, $p<0.05$.

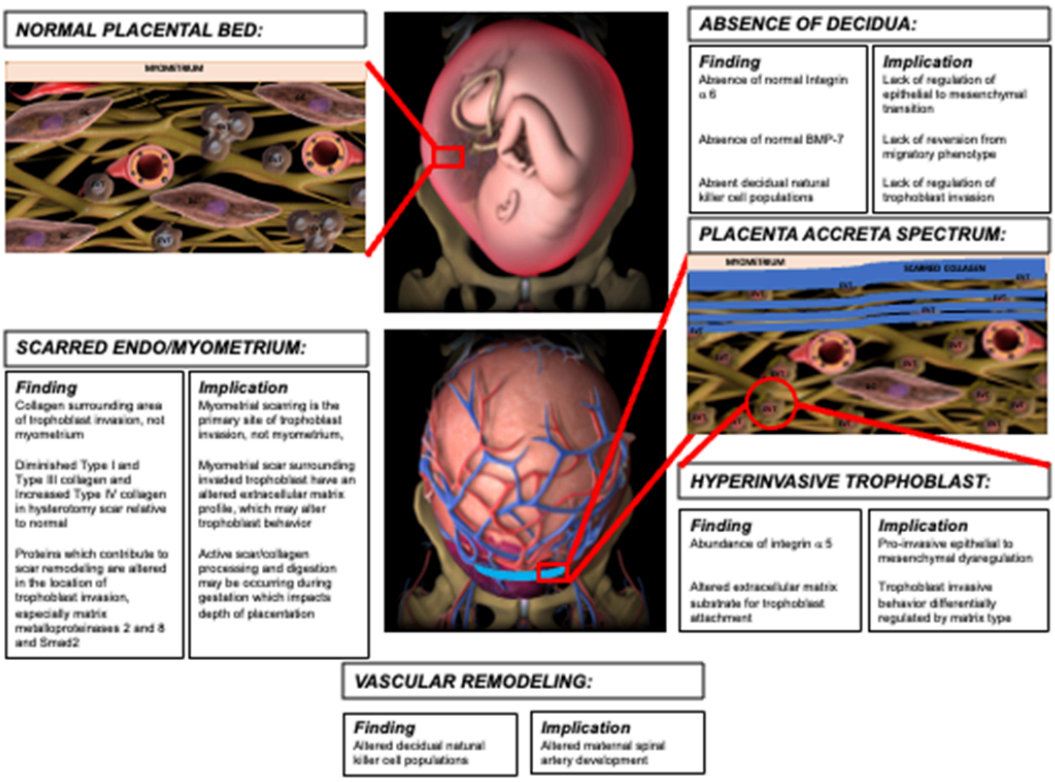


Figure 6

We postulate an update to our previously proposed model of placenta accreta pathogenesis, adapted from Duzyj et al [11] BJOG 2018.