Embryo implantation triggers dynamic spatiotemporal expression of the basement membrane toolkit during uterine reprogramming

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Abstract

Basement membranes (BMs) are specialized extracellular scaffolds that influence behaviors of cells in epithelial, endothelial, muscle, nervous, and fat tissues. Throughout development and in response to injury or disease, BMs are fine-tuned with specific protein compositions, ultrastructure, and localization. These features are modulated through implements of the BM toolkit that is comprised of collagen IV, laminin, perlecan, and nidogen. Two additional proteins, peroxidasin and Goodpasture antigen-binding protein (GPBP), have recently emerged as potential members of the toolkit. In the present study, we sought to determine whether peroxidasin and GPBP undergo dynamic regulation in the assembly of uterine tissue BMs in early pregnancy as a tractable model for dynamic adult BMs. We explored these proteins in the context of collagen IV and laminin that are known to extensively change for decidualization. Electron microscopic analyses revealed: 1) a smooth continuous layer of BM in between the epithelial and stromal layers of the preimplantation endometrium; and 2) interrupted, uneven, and progressively thickened BM within the pericellular space of the postimplantation decidua. Quantification of mRNA levels by qPCR showed changes in expression levels that were complemented by immunofluorescence localization of peroxidasin, GPBP, collagen IV, and laminin. Novel BM-associated and subcellular spatiotemporal localization patterns of the four components suggest both collective pericellular functions and distinct functions in the uterus during reprogramming for embryo implantation.

Introduction

Basement membranes (BMs) are complex extracellular scaffolds of proteins that circumscribe cell populations, modulate cell behaviors, and provide architectural and mechanical support to tissues [1,2]. Basement membranes play active roles in regulating tissue biology by being positioned adjacent to key

cell types: 1) between the epithelial or endothelial cells and their underlying stromal compartment [3,4]; and 2) encapsulating cells such as myocytes [5–7], adipocytes [8,9], Schwann cells [10,11], and decidua cells [12–15]. These scaffolds activate signal transduction by directly interacting with transmembrane cell receptors, modulate paracrine signaling by regulating chemokine gradients and immobilizing growth factors, and establish tissue tension and integrity by bearing compression and expansion tissue forces [16–19]. Basement membranes are comprised of a group of proteins, including collagen IV, laminin, perlecan, and nidogen, that are together termed the BM toolkit [20–22]. Emerging concepts argue that BMs undergo dynamic physiological adaptation by being fine-tuned with specific composition and localization throughout the life of organisms [23–25], though the fundamental mechanisms remain unknown.

Two additional proteins have emerged as potential members of the toolkit: peroxidasin and Goodpasture antigen-binding protein (GPBP). Peroxidasin catalyzes the formation of sulfilimine crosslinks that reinforce collagen IV networks of BMs. The lack of these reinforcements led to early developmental disorders and dysfunction in several tissues and organisms [26–30]. However, observations of the dynamic distribution of peroxidasin in BMs are limited to development of *C. elegan* tissues [31] and cell lines [32], and have been implied in embryonic mouse tissues [29]. Extracellular GPBP was discovered through its binding to kidney BM [33] and has since been shown to bind major BM components laminin and collagen IV [34,35]. Overexpression of GPBP in renal tissues is associated with collagen IV rearrangement and ultrastructure expansion of glomerular BM in immune complex-mediated pathogenesis in mice and humans [36,37]. Though peroxidasin and GPBP have been identified in several BMs, whether they undergo dynamic regulation in healthy adult tissues is unknown.

In the present study, we sought to determine whether peroxidasin and GPBP undergo dynamic regulation during distributional alterations of BM in an adapted physiological state. As a model system, we explored the BM dynamics that are known to occur in uterine tissue in early pregnancy. Embryo implantation triggers the endometrium to undergo rapid and extensive changes in cell populations and extracellular matrix (ECM) for development of the decidua, a cocoon-like tissue barrier between mother-embryo throughout pregnancy [38–42]. These changes include apoptosis of endometrial epithelial and endothelial cells, restriction of immune cell infiltration, and proliferation of mesenchymal stromal cells that differentiate into cells with select epithelial-like features, thus they are often referred to as epithelioid decidua cells (See Figs. 1 and 2 in [43]). Studies of endometrial and decidual ECM show collagen IV and laminin change from limited localization underlying epithelial and endothelial cells to broad localization surrounding epithelioid cells throughout the mature decidua [12–15]. However, co-localization of these two proteins has not been examined upon the initial switch to decidua tissue during the periimplantation period. Here, our findings further reveal that the uterus reacts to implantation by triggering the dynamic regulation of peroxidasin and GPBP, as well as collagen IV and laminin, with distinct spatiotemporal expression and localization patterns in uterine tissues, suggesting both individual and collective functions of these BM toolkit proteins.

Results

Ultrastructure of BM during decidua development

Previous studies of decidualization have established the mouse as a reliable species to model and examine changes to the uterus that occur in human pregnancy, even though some aspects of anatomy and timing differ between mice and humans [44–46]. In mice, endometrium epithelial and mesenchymal cell behaviors are altered upon the initiation of pregnancy through ovarian-derived hormones and cellular compartment cross-talk [47–50]. By day 4 of the 21-day term of pregnancy in mice, the uterus is conducive to blastocyst implantation and decidualization is triggered upon embryo attachment [51–53]. The majority of decidual tissue develops by day 7 of pregnancy and persists as the embryo grows and develops placenta that merges with the decidua for nutrient and waste exchange [38,54]. Although BMs have been observed in both pre- and post-implantation uterine tissues [12–15], little is known about the BM ultrastructure properties. In the present study, we investigated these properties by comparing the BM of the luminal epithelium of day 4 uteri (Fig. 1A) and decidual zone of day 7 implantation sites (Fig. 1B) by transmission electron microscopy (TEM) that displays electron-dense layers of BMs called lamina densa.

In the endometrium on day 4 of pregnancy, a smooth and narrow layer of lamina densa was observed basal to cells of the luminal epithelium, but lamina densa was not within the stroma (Fig. 1A) (See also Fig. 3 in [43]). The location and structure of uterine epithelial lamina densa displayed characteristics of basement membrane oriented basal to polarized cells with apical-lateral localized cell–cell junctions [3,55]. In the implantation site on day 7 of pregnancy, lamina densa was positioned within the pericellular space of cells throughout the decidua. However, lamina densa of the decidua had intermittent disruptions where cell–cell contacts and electron dense junctions were observed between cells