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Review

MUC1: A multifunctional cell surface component of reproductive tissue epithelia

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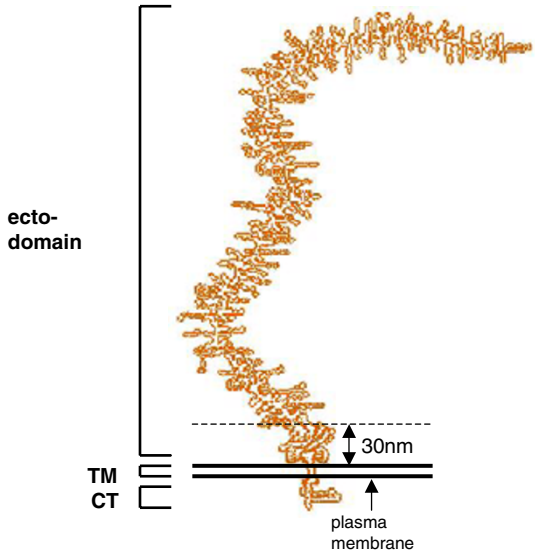
Abstract

MUC1 is a large, transmembrane mucin glycoprotein expressed at the apical surface of a variety of reproductive tract epithelia. Functions attributed to MUC1 include those generally associated with mucins such as lubrication and hydration of cell surfaces as well as protection from microorganisms and degradative enzymes. In addition, MUC1 is an effective inhibitor of both cell-cell and cell-extracellular matrix interactions in both normal and malignant contexts. Moreover, a series of recent studies has shown that the highly conserved cytoplasmic tail of MUC1 interacts specifically with a series of important signal transducing molecules including β-catenin, Grb2 and erbB family members. MUC1 expression in normal epithelia can be quite dynamic, varying in response to steroid hormone or cytokine influences. Following malignant transformation, MUC1 often becomes highly overexpressed, loses its apical restriction, and displays aberrant glycosylation and altered mRNA splice variants. Regulation of MUC1 expression can occur at the transcriptional level. In addition, post-translational regulation of cell surface expression occurs via the activity of cell surface proteases or "sheddases" that release soluble forms of the large ectodomains. This review will briefly summarize studies of MUC1 expression and function in reproductive tissues with particular emphasis on the uterus. In addition, current knowledge of the mechanisms of MUC1 gene regulation, metabolic processing and potential signal transducing functions will be presented.

MUC1 structure and expression

The MUC1 gene encodes a type-I transmembrane glycoprotein that is expressed on the apical surface of most simple epithelia, including mammary gland, female reproductive tract, lung, kidney, stomach, gall bladder, and pancreas as well as some non-epithelial cell types [reviewed in [1]]. The human MUC1 gene spans 4 to 7 kb and is comprised of 7 exons that can be alternatively spliced to form transcripts from 3.7 to 6.4 kb. The full-length protein contains three domains: short cytoplasmic and transmembrane domains that are highly conserved among species, and a large extracellular domain (Fig. 1). The extracellular domain in humans contains 20–125 tandem repeats of 20 amino acids enriched in serine, threonine, and proline residues. Due to these features, the tandem repeat domain has the potential for extensive O-glycosylation. The core protein has an estimated weight of 120–225 kDa, though the mature glycosylated form ranges from 250–500 kDa [reviewed in [1,2]]. The proline residues and glycosylation give rise to a rigid, extended structure that protrudes 200–500 nm above the cell surface, much farther than the distance spanned by most cell-surface proteins, including syndecans and integrins (fig. 2). In addition, in most simple epithelia, including those of the uterus, mucins not only are abundant, but also are concentrated at the apical surface. Collectively, these



Epithelial tissues that normally express MUC1

- mammary gland
- female reproductive tract (except ovary)
- prostate
- testis
- salivary gland
- digestive tract (except large intestine and colon)
- pancreas
- liver (bile ducts)
- lung
- kidney (distal tubules and collecting ducts)
- bladder
- eye

Non-epithelial cells that express MUC1:

- hematopoietic cells
- activated T cells

Figure 1
Protein structure and normal expression pattern of MUC1. Schematic diagram of the size and structure of the full-length MUC1 protein relative to the plasma membrane. The horizontal bar indicates the distance that most cell surface proteins extend into the pericellular space. The three major domains of MUC1 are indicated to the left: (1) an N-terminal, extracellular domain (ECTO); (2) a single, membrane spanning domain (TM); and (3) a C-terminal cytoplasmic domain (CT). A list of epithelial and non-epithelial human tissues that normally express MUC1 is indicated to the right.

observations are consistent with mucins functioning as a protective barrier with adhesion-modulating properties.

While probably the best studied, MUC1 is just one example of a class of molecules collectively referred to as mucins. Mucins may be grouped as transmembrane or soluble (see Table 1). As noted above for MUC1, many of these mucins are physically very large molecules with extended structures due to the abundance of proline residues and high degree of glycosylation (see Fig. 2). Nonetheless, some are of comparable size to other cell surface proteins, e.g., MUC13 [3]. MUC1 and MUC4 have many similar features and proposed functions. Other recently described transmembrane mucins such as MUC16 and MUC17 also may share properties with MUC1 and MUC4, but not enough information is available to suggest their physiological function. This review will focus on studies of MUC1 as a prototype of large, transmembrane mucins in the context of uterine biology. As noted in Table 1, expression of many of the known mucins has not yet been examined in uterine tissues; only MUC1 and MUC4 have been carefully examined through the menstrual cycle and during early pregnancy.

MUC1 Functions

Mucins have numerous functions in the glycocalyx. Their high degree of glycosylation provides lubrication, prevents dehydration, and offers protection from proteolysis. Microbial challenge is frequent in most mucous membranes, and mucins protect against attack by sterically inhibiting microbial access to the cell surface. Bacterial adhesins bind mucin carbohydrates at the cell surface [4,5], a process that normally protects against infection. In addition, extended transmembrane mucins, such as MUC1 and MUC4, modulate cell-cell and cell-extracellular matrix (ECM) interactions by steric hindrance

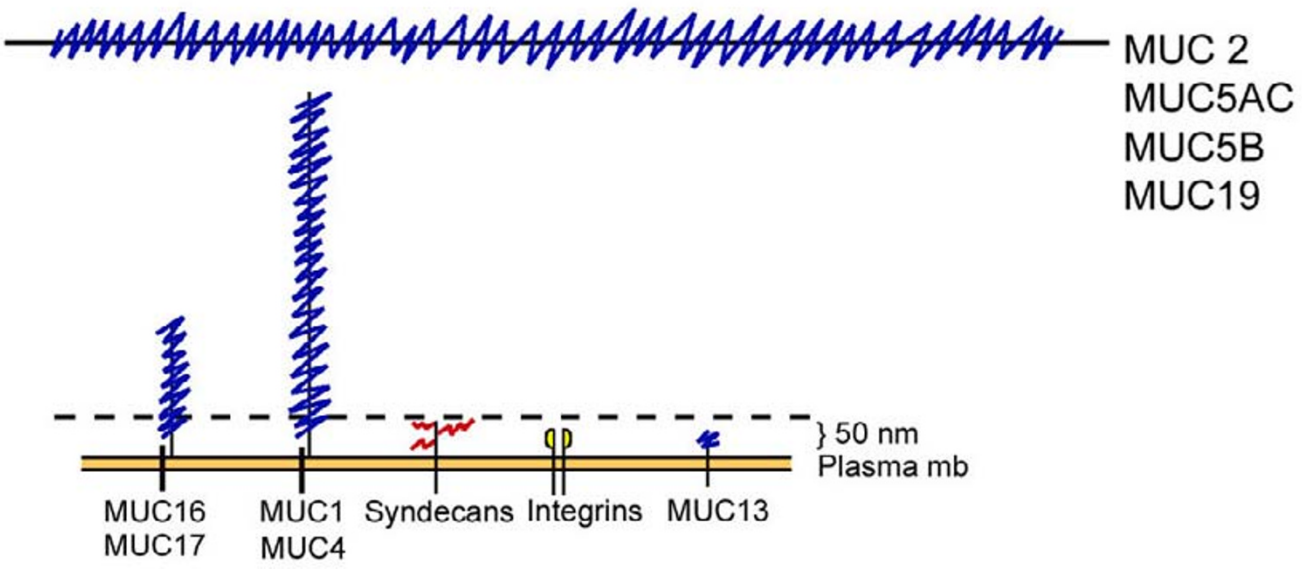


Figure 2

Model of mucins and other cell surface glycoproteins/proteoglycans. The figure schematically portrays structures of four size classes of mucins as well as consensus structures for syndecans and integrins. All extracellular portions of these molecules are roughly drawn to scale. Blue squiggles represent O-linked oligosaccharides while red squiggles represent glycosaminoglycans. Integrins, syndecans and most other all surface receptors do not extend beyond 50 nm from the cell surface; however, with the exception of MUC13, mucins extend much farther due to the extende3d structures contributed by the praline-rich heavily O-glycosylated tandem repeat domains. MUC1 and MUC4 are the largest transmembrane mucins, extending >200 nm from the cell surface. The ectodomain structures of MUC16 and MUC17 are considerably shorter than those of MUC1 and MUC4, but still much larger than other surface glycoproteins. Soluble mucins, such as MUC2, 5AC, 5B and 19 are even larger, reading 500–100 nm in length.

Table 1: Mucin Expression in the Uterus

MUCIN TYPE	UTERINE EXPRESSION	REFERENCE
Soluble or Gel-forming		
MUC2	No	Gipson *et al.*, 1997 [69]
MUC5AC	No	"
MUC5B	No	"
MUC6	Yes	"
MUC7	No	"
MUC8	Yes	D'Cruz *et al.*, 1996 [70]
MUC9	Unknown	
MUC19	Unknown	
Transmembrane		
MUC1	Yes	See text
MUC3	No	Gipson *et al.*, 1997 [69]
MUC4	Yes	See text
MUC10-18	Unknown	

[reviewed in [1,2]]. In fact, overexpression of MUC1 in tumor cells is suggested to promote metastasis through disruption of these interactions [6,7]. This activity is directly related to the number of tandem repeats in the

ectodomains of MUC1 and MUC4 since reduction of these motifs alone makes these molecules ineffective inhibitors of cell-cell and cell-ECM interactions [8,9].

In addition to mechanical functions, the MUC1 cytoplasmic tail has been shown to associate with β-catenin [10], as well as with other signaling molecules, e.g., Grb2/Sos [11], suggesting a potential role for MUC1 in cell signaling [reviewed in [12]]. In the mammary gland, MUC1 expression increases markedly during lactation along with increased MUC1:erbB1 interactions [13]. Tyrosine phosphorylation of the MUC1 cytoplasmic tail occurs in both intact MUC1 and chimeric molecules consisting of CD8 ectodomains and the MUC1 cytoplasmic tail [14,15]. It is not clear if MUC1 phosphorylation or interactions with signal transducing proteins change in response to physiological stimuli. Activation of erbB1 with EGF induces tyrosine phosphorylation of the MUC1 cytoplasmic tail [13,16] and activation of ERK 1/2 [13]. Moreover, EGF mediated activation of ERK 1/2 is drastically enhanced in the presence of high levels of MUC1 in the mouse mammary gland [13]. Thus, potential stimuli, including growth factors or cytokines directly or through activation of their receptors may affect MUC1 stability, localization at the cell surface, or phosphorylation state. Direct interactions with the MUC1 ectodomain, e.g., by microbes or selectins, also could conceivably trigger signaling events. In this regard, increased tyrosine phosphorylation of the MUC1 cytoplasmic tail is associated with cell-substratum adhesion [14]. Thus, MUC1, and perhaps other mucins, have the potential to function as receptors either alone or in cooperation with known signal transducing proteins.

Studies in tumor cells indicate that the amount and type of MUC1 expressed modulate immune responses to these cells. MUC1 is differentially glycosylated in many cancerous cells, exposing tumor-specific epitopes that may trigger an immune response [17,18]; however, MUC1 also has been shown to protect cancer cells from immune cell attack [19,20], indicating both immunostimulatory and immunosuppressive functions for MUC1. MUC1 and other mucins are abundantly expressed at the apical surface of luminal and glandular uterine epithelia [21-24]. Muc1 (mouse nomenclature) null mice exhibit chronic infection and inflammation of the uterus as a result of increased infection by normal bacteria of the reproductive tract [25]. *In vitro* studies have shown that cells expressing a high level of MUC1 at the cell surface are refractory to blastocyst attachment, while removing MUC1 from the cell surface allows attachment [25,26]. Taken together, these studies suggest that MUC1 plays a role in protecting the endometrium from microbial attack, but must be lost in order for embryo implantation to occur. Indeed, Muc1 expression in mice is lost throughout the uterine epithelia by day 4 of pregnancy when the uterus is receptive to blastocyst attachment [22,27]. In contrast, rabbits display generally high Muc1 expression during the receptive phase although local loss of Muc1 is observed at implantation sites [23]. Similarly, MUC1 expression in humans is maximal during the implantation phase [21]. *In vitro* studies indicate that human blastocysts produce factors that lead to local MUC1 loss on monolayers of human uterine epithelia [28]. In contrast, recent studies indicate that human blastocysts display selectins on their cell surface and that complementary selectin ligands are found on the uterine surface during the receptive phase [29]. Uterine epithelial MUC1 carries selectin ligands throughout the cycle ([30], J. Julian, S. Fisher and D.D. Carson, unpublished observations); however, it is not clear if selectin ligands are found on most, or a minor subset of, MUC1 molecules. Nonetheless, it must be considered that MUC1 participates in selectin-dependent interactions in the uterus and, thus, may promote cell-cell interactions in some contexts.

Regulation of MUC1 expression

Appropriate tissue-specific expression and regulation of MUC1 was demonstrated in a MUC1 transgenic mouse created using 1.6 kb of 5' and 2.3 kb of 3' flanking sequence [31]. *In vitro* deletion analysis of the 1.6 kb proximal promoter region demonstrated that elements in the region from 400 bp to 600 bp upstream of the transcriptional start site are required for maximal promoter activity, and elements between -150 bp and -60 bp are required for tissue specificity [32,33]. Several of the regulatory elements in the MUC1 5' flanking sequence have been functionally characterized (Fig. 3). The E box (E-*MUC1*) at -84/-72 is required for transcriptional repression in cells that do not normally express MUC1 [33]. The transcription factor *Snail* has been shown to bind this region to repress MUC1 transcription [34]. Two Sp1 sites (GC boxes) have been shown to be important for transcriptional activation in epithelial cells and repression in non-epithelial cells [33,35,36]. The Sp1 site at -99/-90 is required for cErbB2/Ras-mediated transcriptional repression [37], and this element overlaps the binding site of *SpA*, a transcriptional repressor that competes with Sp1 for binding [35]. A region from -531 to -517 was shown to be required for MUC1 transcriptional activation in human colon cancer cells in response to treatment with normal colon conditioned medium [38]. The interferon (IFN)-γ-mediated up-regulation of MUC1 expression in human mammary epithelial cells was shown to act through signal transducer and activator of transcription (STAT)-1α binding to the STAT binding site at -503/-495 [39,40]. STAT3 is able to bind the STAT binding element in human mammary epithelial cells in response to interleukin (IL)-6 treatment [40]. The κB binding element at -589/-580 in the MUC1 promoter region is required for up-regulation of MUC1 expression in response to TNF-α treatment, and TNF-α and IFN-γ can synergize to

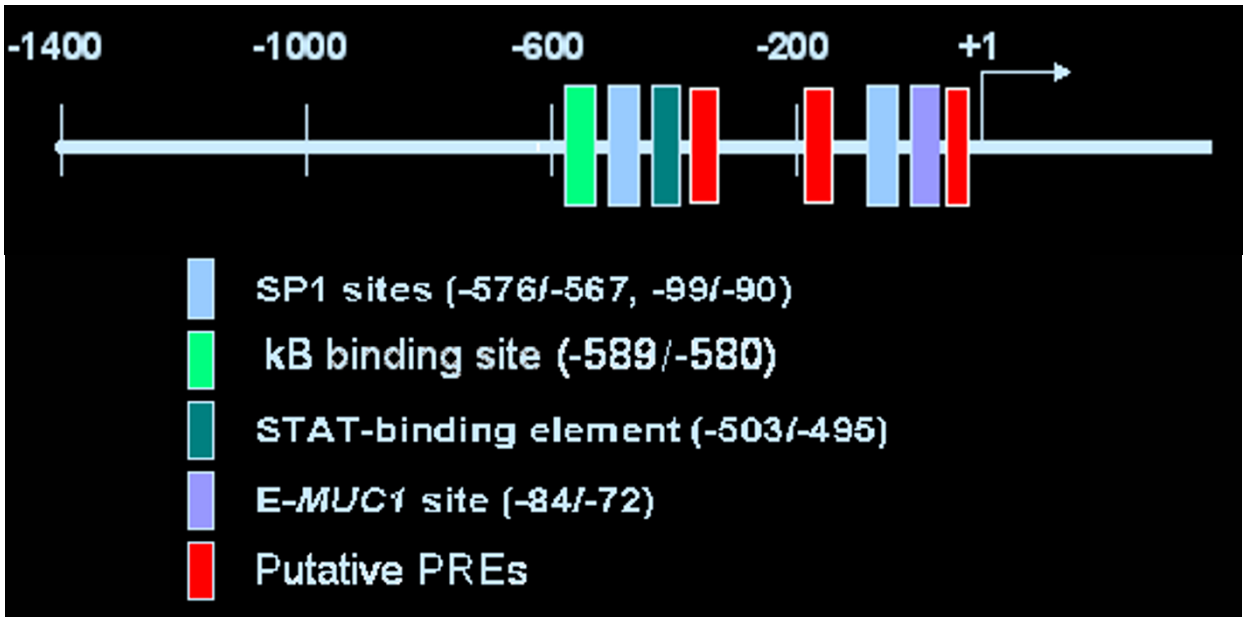


Figure 3
The MUC1 proximal promoter. The 1.4 kb human MUC1 proximal promoter is sufficient for driving proper tissue-specific expression in a transgenic mouse model. *Cis* elements that have been shown to be important for MUC1 regulation include the two Sp1 sites, the κB site, a STAT binding element, and E-MUC1 (E box). The location of several potential progesterone response elements (PRE) also are shown.

stimulate MUC1 expression in human mammary epithelial cells [39].

Similar to its actions on MUC1 gene expression, TNFα, acting through NFκB activation, greatly stimulates MUC2 and MUC5AC gene activation in human lung epithelial cell lines [41-44]. This activation proceeds via a Src-dependent, Ras-MEK1/2/ERK1/2-pp90srk pathway. MUC4 expression also is cytokine regulated, but in a different, intriguing fashion. TGFβ1 strongly inhibits MUC4 expression at the post-translational level of mucin processing [45]. Recent studies indicate that the TGFβ1 response is repressed by IFN-γ, an activator of the expression of other mucin genes. This response involves interplay between SMADs and STAT1 that appear to control activity of the MUC4 processing step [46]. It is not clear if the TGFβ1/SMAD system similarly regulates the expression of other mucins.

MUC1 expression is controlled by steroid hormones in the mammary gland and uterus [22,28,47]. Several putative progeterone and estrogen response elements have been identified in the human MUC1 promoter by sequence analysis [48,49]. In both mice and humans, progesterone levels are maximal, relative to estrogen, at the implantation phase, but changes in MUC1 expression in response to steroid hormones is different in the two species. In mice, uterine Muc1 expression is greatly stimulated by estrogen. While progesterone alone has no effect, it strongly antagonizes estrogen-stimulated Muc1 expression [22,27]. Experiments with antiestrogens and antiprogestins indicate that the actons of both hormones are mediated by nuclear receptors [22]. Nonetheless, direct regulation of the Muc1 promoter by ER-α or -β, or PR-A or -B, has not been demonstrable [50]. Conversely, endometrial MUC1 expression in humans is higher during the secretory phase, a progesterone-dominated portion of the cycle [21,28]. The reason for these differences in hormonal responsiveness among species is not clear. Possibilities include species-specific differences in *cis* promoter elements and transcription factor/transcriptional coregulator contexts. Further studies are necessary to understand the molecular basis of steroid hormone regulation of MUC1 gene expression.

MUC1 Metabolism and Shedding

In mouse uterine epithelial cells, Muc1 is translated, glycosylated and moves to the cell surface with a median transit time of 142 min [51]. During this process, it is proteolytically cleaved at a site between the transmembrane and tandem repeat domains. In spite of this, the heterodimer remains tightly associated in an SDS-labile, but otherwise stable, interaction [52-54]. After arrival at the cell surface, MUC1 may undergo several fates. One is recycling through the *trans* aspect of the Golgi apparatus ([55], a process that would lead to no net loss; however, Muc1's metabolic half life ranges from 12–16 hr ([51] and refs. within) demonstrating that mechanisms exist to degrade even these extremely biochemically resistant molecules. A second fate is endocytosis followed by degradation in an intracellular acidic compartment, presumably lysosomes [51]. The balance of Muc1 turnover probably is due to cell surface release or shedding (see below). Shedding results in separation of the cytoplasmic tail from the ectodomain in all cases studied, implicating a proteolytic event [51,54,56]; however, recent studies indicate that mutation of the site of intracellular metabolic cleavage inhibits MUC1 shedding, indicating that cleavage at this site is critical for subsequent cell surface release [57].

Soluble, presumably shed, MUC1 fragments are found in bodily fluids [58,59] as well as uterine flushings from women during the receptive phase of the cycle [60]. Women who suffer recurrent spontaneous miscarriages have reduced levels of MUC1 in uterine flushings, suggesting that a defect in the MUC1 release system underlies some human fertility defects [60,61]. These observations suggest that MUC1 proteolytic release occurs *in vivo* as well. Alternative mRNA splicing generates a secreted form of MUC1, MUC1/SEC, lacking the transmembrane and cytoplasmic tail [62]. Transcripts for MUC1/SEC are detectable in human endometrium indicating that at least a portion of the soluble MUC1 found in uterine flushings is contributed by MUC1/SEC, rather than shedding [63].

Although the intracellular cleavage site 65 amino acids upstream of the MUC1 transmembrane domain has been identified, the protease mediating the metabolic cleavage has not [53]. As noted above, this site appears to be critical for MUC1 shedding, at least in certain cellular contexts [57]. Nonetheless, these results do not preclude an additional cleavage occurring at a later stage of processing. Thus, due to its extreme resistance to externally added proteases [64], it is unlikely that cell surface MUC1 release is mediated by the actions of an external protease [52,64]. Various serine, cysteine and aspartic acid protease inhibitors do not inhibit Muc1 ecotodomain release [51], implicating the involvement of other protease classes. Others have suggested that release is catalyzed post-translationally by an endogenous proteolytic activity [56,65].

Muc1 expression is elevated during the peri-implantation period in rabbits [23]; however, careful examination of implantation sites *in vivo* and *in vitro* reveals that Muc1 is lost solely at the site of embryo-uterine apposition [23]. Interestingly, elevated expression of the cell surface protease, ADAM 9, accompanies Muc1 loss at implantation sites in rabbits [66], implicating ADAM 9 in the implantation process in this species. Uterine MUC1 also appears to be elevated during the receptive phase in humans [21]. Although implantation sites have not been studied in humans, *in vitro* implantation models indicate that MUC1 is lost at the site of embryo attachment in humans as well [26,28], suggesting that factors expressed on the blastocyst surface or released with limited diffusibility trigger MUC1 loss in rabbits and humans. These findings are consistent with an induced loss of MUC1 at the site of attachment, perhaps triggered by the blastocyst itself or a factor(s) produced by the blastocyst and mediated through activation of a uterine cell surface protease. Taken together, these observations led to the hypothesis that an endogenous protease(s) mediates constitutive and induced MUC1 release from the human uterine epithelial cell surface.

Examination of MUC1 shedding in the human uterine epithelial cell line, HES, demonstrated that this process was unaffected by a variety of synthetic and naturally occurring protease inhibitors; however, a subset of synthetic and one endogenous metalloprotease inhibitor blocked constitutive as well as phorbol ester-stimulated MUC1 shedding [67], implicating an ADAM (a disintegrin and metalloprotease) as a MUC1 sheddase. PCR profiling identified a subset of catalytically active ADAMs including tumor necrosis-α converting enzyme (TACE)/ADAM17 present in HES cells as well as the receptive phase human endometrium. Cotransfection of MUC1 into wild-type as well as TACE/ADAM17-deficient mouse embryonic fibroblasts revealed that constitutive and phorbol ester-stimulated shedding was abolished in TACE/ADAM17-deficient cells. Furthermore, TACE/ADAM17 was identified in both lumenal and glandular epithelium in the receptive phase human endometrium implicating a role for this protease in MUC1 shedding *in vivo*. No information is yet available on whether endometrial TACE/ADAM17 expression or activation is modulated during the cycle in any species. Additional studies indicate that pervanadate-stimulated MUC1 shedding occurred even in TACE/ADAM17-deficient cells, demonstrating that additional sheddases are operative under certain circumstances (A. Thathiah and D.D. Carson, manuscript submitted). Thus, it is possible that multiple proteases contribute to MUC1 shedding. Alterations in the expression of activation of these enzymes may contribute to acute, localized MUC1 removal.

Conclusion

In many respects, MUC1 is a prototypical large, transmembrane mucin. In the uterus these glycoproteins are almost completely restricted to the uterine epithelium where they lubricate and maintain hydration of the cell surface as well as present major barriers to microbes and implanting blastocysts. In the latter case, it is critical that this barrier be removed to create embryonic access to the uterine epithelium. In many species, this appears to be accomplished by down-regulation of MUC1 gene expression. In this regard, progress has been made in understanding transcriptional regulation of MUC1 as well as other mucin genes. Nonetheless, more work needs to be done to understand the mechanisms underlying steroid hormone regulation of these genes. In other cases, MUC1 expression and function appears to be modulated by shedding. TACE/ADAM17 and MT1-MMP represent two enzymes likely to mediate MUC1 shedding in uterine epithelia. While MUC1 shedding provides a dynamic and rapid way of modulating cell surface MUC1 expression and function, many questions remain. In order to create blastocyst access to the epithelial surface, it is predicted that a large fraction of the apically-disposed MUC1 must be lost; however, so far we have found no individual factors that reduce the cellular complement of MUC1 by more than 5–10% (A. Thathiah, M. Brayman, N. Dharmaraj and D.D. Carson, unpublished observations). The receptive phase endometrial milieu is complex and influenced by factors of both maternal and embryonic origin. It is possible that a combination of factors synergize, potentially activating multiple sheddases, to clear MUC1 from the apical cell surface. Adressing this possibility will require careful examination of candidate factors alone and in combination. Sheddases also require activation to be functional and assays need to be developed to determine whether proteins not only are present, but also active during the transition to the receptive state. Since sheddases are likely to play important roles in regulating bioavailability of other important epithelial cell surface components, e.g., HB-EGF [68], developing a thorough understanding of the enzymes operative in the endometrium is likely to impact other aspects of our understanding of uterine physiology. Finally, the roles of mucins in uterine signal transduction are only starting to be appreciated. How mucins activate or function in intracellular signaling cascades and what factors modulate mucin participation in these events should be carefully studied in physiologically relevant contexts.

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