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Review

A potential role for tissue kallikrein-related peptidases in human cervico-vaginal physiology

Julie L.V. Shaw[1,2] and Eleftherios P. Diamandis[1,2,*]

[1] Department of Pathology and Laboratory Medicine, and Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 60 Murray Street, Toronto M5T 3L9, ON, Canada

[2] Department of Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, Banting Institute, 100 College Street, Toronto M5G 1L5, ON, Canada

* Corresponding author
e-mail: ediamandis@mtsinai.on.ca

Abstract

Human tissue kallikrein-related peptidases (*KLK*) are a family of 15 genes located on chromosome 19q13.4 that encode secreted serine proteases with trypsin- and/or chymotrypsin-like activity. Relatively large levels of many KLKs are present in human cervico-vaginal fluid (CVF) and in the supernatant of cultured human vaginal epithelial cells. Many KLKs are also hormonally regulated in vaginal epithelial cells, particularly by glucocorticoids and estrogens. The physiological role of KLK in the vagina is currently unknown; however, analysis of the CVF proteome has revealed clues for potential KLK functions in this environment. Here, we detail potential roles for KLKs in cervico-vaginal physiology. First, we suggest that KLKs play a role in the vagina similar to their role in skin physiology: (1) in the desquamation of vaginal epithelial cells, similar to their activity in the desquamation of skin corneocytes; and (2) in their ability to activate antimicrobial proteins in CVF as they do in sweat. Consequently, we hypothesize that dysregulated KLK expression in the vagina could lead to the development of pathological conditions such as desquamative inflammatory vaginitis. Second, we propose that KLKs may play a role in premature rupture of membranes and pre-term birth through their cleavage of fetal membrane extracellular matrix proteins.

Keywords: cervico-vaginal fluid; cervix; kallikrein-related peptidase; menstrual cycle; pre-term birth; vagina.

Introduction

The human tissue kallikrein-related peptidase (*KLK*) family consists of 15 genes located in tandem on chromosome 19q13.4 (Yousef and Diamandis, 2001). These genes encode secreted serine proteases with either trypsin-like or chymotrypsin-like activity (Yousef and Diamandis, 2001). KLKs are known to be regulated by steroid hormones (Borgono et al., 2004; Kulasingam and Diamandis, 2007; Paliouras and Diamandis, 2007) and their expression is dysregulated in hormone-dependent malignancies (Borgono et al., 2004). Many KLKs are known biomarkers and others are candidates for many types of cancer (Paliouras et al., 2007), including prostate cancer, for which KLK3 (widely known as prostate-specific antigen or PSA) is used for diagnosis and monitoring (Barry, 2001).

The physiological functions of KLK1, KLK2 and KLK3, the so-called 'classical' kallikrein-related peptidases, have been relatively well defined; however, the functions of the remaining 12 members of this family remain largely unknown (Borgono et al., 2004). A role for KLK6 in the central nervous system (CNS) has been proposed (Scarisbrick et al., 2006) and many KLKs have been shown to cleave extracellular matrix (ECM) proteins (Ghosh et al., 2004; Kapadia et al., 2004; Michael et al., 2005; Rajapakse et al., 2005; Obiezu et al., 2006). KLKs 5, 7 and 14 are known to participate in a proteolytic cascade within the skin layers and play an important role in skin physiology, mainly through the desquamation of corneocytes (Caubet et al., 2004; Brattsand et al., 2005).

In recent studies by our group, the global expression patterns of all 15 kallikrein-related peptidases were analyzed in human tissues, biological fluids and human cell lines. We found co-expression of many KLKs in the cervix and vagina. We also reported relatively high levels of many KLKs in cervico-vaginal fluid (CVF) and in the supernatant of cultured human cervical and vaginal cell lines (unpublished data and Shaw and Diamandis, 2007).

We hypothesize that KLKs play an important role in cervico-vaginal physiology. Vaginal epithelial-cell physiology closely resembles that of skin corneocytes with respect to cell desquamation. This suggests that KLKs may play a similar role in the vagina as in skin, where their physiological and pathological functions have been well characterized. We also suggest that KLKs may be involved in the remodeling of fetal ECM proteins during pregnancy. We further propose that KLKs may be implicated in premature rupture of fetal membranes (PROM) and pre-term birth.

Cervico-vaginal fluid

The vagina contains CVF, which is composed of fluids secreted and cells shed from the vaginal epithelium, cervical epithelium, fallopian tube and endometrium. CVF plays an important role in host defense (Huggins and

Preti, 1981; Cole, 2006) and under normal physiological conditions CVF contains many different types of bacteria, mostly dominated by *Lactobacillus* (Junqueira et al., 1995; Faro, 2004). *Lactobacillus* is capable of growing in normal acidic CVF (between pH 3.8 and 4.5) and produces substances including lactic acid and hydrogen peroxide, which help to maintain a healthy state in the vagina by preventing the growth of pathogenic bacteria. Changes in the vaginal ecosystem can lead to a decrease in the number of lactobacilli present, allowing other bacteria to dominate and infections to develop (Junqueira et al., 1995).

KLK expression in CVF

Relatively high levels of many KLKs were identified in CVF from non-pregnant and pregnant women by ELISA (Shaw and Diamandis, 2007) and using proteomic approaches (Dasari et al., 2007; Shaw et al., 2007). KLKs 6, 7, 8, 10, 11, 12 and 13 were found at highest levels compared to other KLKs by ELISA (Shaw et al., 2007), shown graphically in Figure 1. KLKs 6, 7, 10, 11, 12 and 13 were identified in CVF from non-pregnant women by mass spectrometry (Shaw et al., 2007), whereas in another study KLK11 and 13 were identified in CVF from pregnant women (Dasari et al., 2007).

KLK enzyme activity in CVF and a potential proteolytic cascade

Proteolytic cascades are important in many biological processes such as coagulation and complement activation. KLKs have previously been shown to participate in proteolytic cascades in seminal plasma (Michael et al., 2006) and skin (Brattsand et al., 2005; Borgono et al., 2007). It is likely that the KLKs found in CVF may also participate in a proteolytic cascade.

Proteolytic cascades begin with an initiator enzyme that becomes active and is subsequently able to cleave and activate other zymogens to enhance the signal (Bach, 1988; Nunez et al., 1998). In the case of both skin and seminal plasma, the initiator KLK (KLK5) auto-activates and is then able to cleave and activate other (inactive) KLKs (Brattsand et al., 2005; Michael et al., 2006). In CVF the major KLKs present are KLK6, 11, 12 and 13, with lower levels of KLK5, 7, 8 and 10. There are many potential candidates for 'initiators' from this pool of enzymes. KLK5, 6 and 12, and to a lesser degree KLK11 and 14, have been shown to auto-activate (reviewed by Pampalakis and Sotiropoulou, 2007; Yoon et al., 2007) and may be responsible for initiation of a proteolytic cascade within CVF.

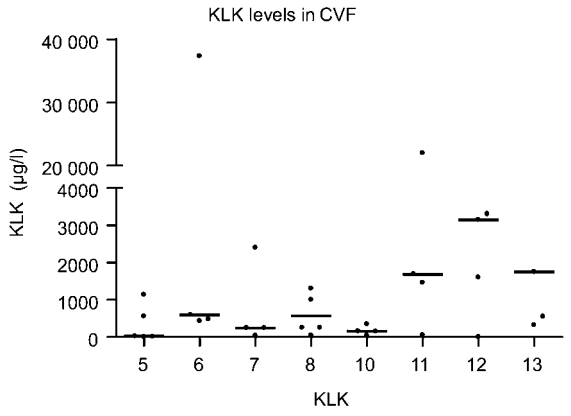

Figure 1 KLK levels in CVF measured by ELISA immunoassays specific for each KLK as described by Shaw and Diamandis (2007).
CVF samples were collected from five healthy females between 20 and 40 years old. KLK levels are expressed in µg/l and the median for each KLK level is marked with a horizontal line.

Antimicrobial role for KLKs in CVF

Currently, the physiological function of KLKs within CVF remains unknown; however, we speculate that KLKs may play a role in vaginal host defense. CVF is known to play an important role in host defense (Cole, 2006) and has been shown to contain antimicrobial substances such as cationic peptides (Venkataraman et al., 2005), lysozyme (Bard et al., 2003), lactoferrin (Harmsen et al., 1995; Groot et al., 2005), secretory leukocyte protease inhibitor (SLPI) (Turpin et al., 1996; McNeely et al., 1997; Shaw et al., 2007), human neutrophil peptides (Shaw et al., 2007), human B-defensins (Quinones-Mateu et al., 2003), hornerin (Shaw et al., 2007) and other members of the S-100 family of proteins (Shaw et al., 2007).

KLKs have recently been shown to play a role in host defense in skin and sweat through cleavage of the antimicrobial human cathelicidin protein hCAP-18 (Yamasaki et al., 2006). hCAP18 is the only human member of the cathelicidin family (Durr et al., 2006) and is expressed in the epithelium of many tissues, including those in the cervix and vagina (Frohm et al., 1999). hCAP18 is composed of an N-terminal cathelin domain and C-terminal LL-37 antimicrobial domain; proteolytic cleavage from the cathelin domain liberates the active LL-37 antimicrobial peptide.

KLK5 has been shown to cleave hCAP18 and liberate active LL-37 (Yamasaki et al., 2006), and both KLK5 and 7 are capable of further digesting LL-37 into smaller antimicrobial peptides in sweat (Yamasaki et al., 2006). We hypothesize that KLKs may also contribute to antimicrobial activity within CVF and that perhaps other KLKs, in addition to KLK5 and 7, may be responsible for cleavage of hCAP18 and LL-37. We also speculate that KLKs may be capable of producing other antimicrobial peptides through cleavage of additional precursor proteins and peptides within CVF such as dermcidin, hornerin, defensins and S-100 proteins.

Vaginal epithelium

The vagina is a tube that connects the vestibule with the cervix, the entrance to the uterus. The vaginal wall consists of three layers: a fibrous outer layer, a muscular middle layer and an inner mucosal layer. The inner mucosa is composed of stratified squamous epithelial cells with multiple layers of basal and parabasal cells. The vaginal mucosa thickens upon estrogen stimulation at puberty and from then until menopause responds cyclically to hormonal changes during the menstrual cycle (Junqueira et al., 1995; Faro, 2004). Estrogen production peaks prior to ovulation and causes the vaginal epithelial cells to mature and become cornified, thinner and flatter

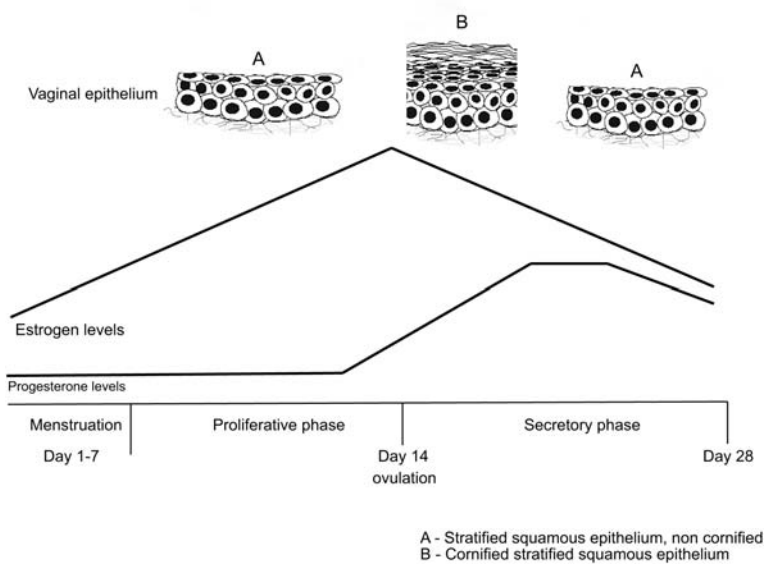

Figure 2 Schematic representation of the changes in vaginal epithelium under hormonal stimulation throughout the menstrual cycle. (A) During the follicular phase the epithelium consists of non-cornified, stratified squamous cells. (B) Estrogen levels increase during the follicular phase and peak at ovulation, causing the epithelium to mature and become increasingly cornified. Estrogen levels decrease after ovulation, causing the epithelial cells to shed. Progesterone levels peak and plateau following ovulation, further inhibiting the maturation of the epithelium, resulting in the return of the epithelium to its stratified squamous appearance (A).

(Figure 2). Progesterone stimulation following ovulation inhibits this maturation. Following menopause the decrease in estrogen results in fewer mature cells and subsequent shedding of vaginal epithelial cells (Faro, 2004).

KLK expression and hormonal regulation in the vaginal epithelium

Many KLKs (1, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14) are co-expressed in vaginal tissue at both the mRNA and protein levels (Shaw and Diamandis, 2007). Cultured human vaginal epithelial (VK2) cells have also been shown to express many KLKs (5, 6, 7, 8, 10, 11, 12 and 13) (unpublished data), in some cases at relatively high levels (mg/l range), such as KLK5, 7 and 10. Hormonal studies have shown that KLK6, 11 and 13 are downregulated in these cells upon estrogen treatment (Table 1), suggesting that expression of these KLK may be influenced by hormonal changes during the menstrual cycle. Interestingly, KLK5, 6, 7 and 10 levels have been shown to be significantly downregulated by treatment with the corticosteroid dexamethasone in vaginal epithelial cells (Table 1 and unpublished data).

Table 1 Hormonal regulation of KLKs in vaginal epithelial cells (VK2).

KLK	Expression (μg/l)[a]		
	Constitutive	Dexamethasone	Estradiol
5	4324	2959[b]	No change
6	30	19[b]	24[b]
7	417	277[b]	No change
10	308	202[c]	197[b]
11	51	No change	47[c]
13	2.9	No change	1.9[b]

[a]Concentrations of KLKs in tissue culture supernatants after 7 days of incubation.
[b]$p<0.01$.
[c]$p<0.05$.

Kallikrein-related peptidases in epithelial cell desquamation

The role of KLKs in skin physiology has been well characterized. Many kallikrein-related peptidases are expressed in human skin and are known to play a central role in the desquamation of skin corneocytes (Komatsu et al., 2006b). In particular, KLK5, 7 and 14 have been shown to participate in a proteolytic cascade in skin (Caubet et al., 2004; Brattsand et al., 2005) and cleave cell-cell adhesion molecules such as cadherins (Descargues et al., 2006), corneodesmosin, desmogleins (Borgono et al., 2007) and desmocollins (Caubet et al., 2004).

The vaginal epithelium undergoes cyclical desquamation in response to hormonal changes during the menstrual cycle (Faro, 2004). Given this and the relatively high levels of KLK expression in vaginal epithelial cells, we believe that KLKs may play a role in vaginal epithelial-cell desquamation through cleavage of cell-cell adhesion molecules, similar to their role in skin. Given the especially high levels of KLK5 and 7 in vaginal epithelial cells, we also speculate that these KLKs may participate in a proteolytic cascade in the vaginal epithelium similar to the cascade in skin. In a recent proteomic analysis of human CVF, several cell-cell adhesion molecules such as desmogleins and desmocollins (Shaw et al., 2007) were identified, in further support of the above hypotheses.

Kallikrein-related peptidases and over-desquamation of epithelial cells

Kallikrein-related peptidases have been extensively studied with respect to their role in over-desquamation of corneocytes in human skin. Over-desquamation is impli-

cated in many skin disease pathologies such as Netherton syndrome (Komatsu et al., 2002; Descargues et al., 2005, 2006; Hachem et al., 2006), peeling skin syndrome (Komatsu et al., 2006a), psoriasis (Komatsu et al., 2007b) and atopic dermatitis (Komatsu et al., 2007a). The activity of KLK5, 7 and 14 has been shown to be controlled by the lympho-epithelial kazal-type-related inhibitor (LEKTI) in skin (Egelrud et al., 2005; Descargues et al., 2005; Schechter et al., 2005; Hachem et al., 2006; Borgono et al., 2007; Deraison et al., 2007). Mutations in the serine peptidase inhibitor kazal-type 5 (*SPINK5*) gene encoding LEKTI have been shown to result in increased KLK activity and over-desquamation of skin corneocytes, implicated in Netherton syndrome (Descargues et al., 2005).

The levels of kallikrein-related peptidases 5, 6, 7, 8, 10, 11, 13 and 14 are elevated in peeling skin syndrome, psoriasis and atopic dermatitis, resulting in increased trypsin-like and chymotrypsin-like activity and subsequent over-desquamation of corneocytes (Komatsu et al., 2006a, 2007a,b).

Vaginitis and a potential role for kallikrein-related peptidases?

Vaginitis is inflammation of the vagina resulting in itching and pain. There are several types of vaginitis resulting from different causes, such as atrophic vaginitis, desquamative inflammatory vaginitis and bacterial vaginitis (Faro, 2004).

Atrophic vaginitis This vaginitis sub-type results from a decrease in estrogen levels during menopause (Faro, 2004), which results in thinning of the vaginal epithelium and changes in the stratified squamous epithelium (Pandit and Ouslander, 1997). Microscopic examination of vaginal smears reveals a decrease in the number of mature cells and increased numbers of intermediate and parabasal cells (Pandit and Ouslander, 1997; Faro, 2004). These cellular changes can cause irritation and painful sexual intercourse (Pandit and Ouslander, 1997). There is also a decrease in the number of *Lactobacillus* present, resulting in an increase in vaginal pH to >5.0 (Pandit and Ouslander, 1997; Faro, 2004). Atrophic vaginitis is most commonly treated by estrogen replacement either orally or intravaginally (Pandit and Ouslander, 1997).

Desquamative inflammatory vaginitis Desquamative inflammatory vaginitis (DIV) is similar to atrophic vaginitis except that patients do not have an estrogen deficiency. DIV is not caused by infection (Faro, 2004) and mostly affects pre-menopausal women, resulting in discomfort, irritation, increased discharge and painful intercourse (Newbern et al., 2002). Microscopic analysis reveals a similar pattern to atrophic vaginitis, with increased squamous cell exfoliation, an increased number of immature epithelial cells, a decrease in lactobacilli and an increase in pH from 4.5–5.5 to 7.4 (Murphy, 2004). DIV is most commonly treated with clindamycin or intravaginal corticosteroids (Newbern et al., 2002; Faro, 2004; Murphy, 2004).

Potential role for KLKs in vaginitis It is probable that KLKs play a role in the normal desquamation of vaginal epithelial cells similar to their role in the desquamation of skin corneocytes. In syndromes such as vaginitis, we hypothesize that KLK levels and/or KLK activity are elevated and contribute to over-desquamation, just as in skin pathologies.

We hypothesize that under normal conditions a basal level of KLK activity is required for normal vaginal epithelial-cell desquamation. Proteomic analysis of CVF showed the presence of many serine protease inhibitors, including LEKTI (Shaw et al., 2007), responsible for controlling KLK activity. We speculate that KLK levels and activity are increased during DIV or contribute to its development. A recent study showed that KLK activity in skin is higher at lower pH than at higher pH because of lower affinity between KLKs and their inhibitor LEKTI at low pH (Deraison et al., 2007). It is possible in this case that the increased pH associated with vaginitis may encourage increased association between KLK and LEKTI or other inhibitors and therefore reduce KLK activity. Further to this, we have shown that treatment of vaginal epithelial cells with corticosteroids and estrogen reduces KLK expression. We therefore hypothesize that treatment with corticosteroids and/or estrogen helps to reduce KLK levels associated with vaginitis, therefore reducing proteolytic activity and desquamation.

Cervical epithelium

Unlike the vagina, the cervix is lined with mucus-secreting epithelial cells and the cervical mucosa does not undergo desquamation during the menstrual cycle (Junqueira et al., 1995). However, the amount and type of mucus secreted by the cervical mucosa change throughout the menstrual cycle (Junqueira et al., 1995). During ovulation, mucus secretions are less viscous to facilitate sperm penetration of the uterus and subsequent fertilization (Junqueira et al., 1995). During the luteal phase and during pregnancy, the cervical mucosa contains mucus glands that proliferate and produce more viscous secretions to prevent microorganisms from entering the uterus.

KLK and the cervix

KLK expression in the cervix and cervical cancer cell lines KLK1, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 are co-expressed in cervical tissue (Shaw and Diamandis, 2007). We and others have also shown that many KLKs are expressed by human cultured cervical cancer cell lines (Cane et al., 2004; Santin et al., 2004). In particular, KLK7 and 8 have both been shown to be overexpressed in cervical tumor tissue and in cervical cancer cell lines compared to normal tissue and normal cervical keratinocytes, respectively (Cane et al., 2004; Santin et al., 2004).

KLK regulation in cervical cancer KLKs appear to be under hormonal regulation in cervical cancer cells, particularly by glucocorticoids and estrogen. We have shown that KLK5, 6, 8, 11 and 13 are consistently down-regulated by the synthetic glucocorticoid dexamethasone in several cervical cancer cell lines such as Ht-3,

Caski, Me-190 and Ms-751 (unpublished data). In contrast, KLK5, 6 and 11 were shown to be upregulated by estradiol in Caski and Me-180 cell lines.

Cervical mucus The opening to the cervix is filled with a substance referred to as cervical mucus. This mucus acts by preventing microorganisms in the vagina from moving into the uterus (Hein et al., 2002). Cervical mucus has a neutral pH (~8.0) (Owen and Katz, 1999) and is primarily composed of water. This mucus is also known to contain enzymes, antibacterial proteins and proteins, mainly mucins (Andersch-Bjorkman et al., 2007).

As mentioned, the composition of cervical mucus changes throughout the menstrual cycle in response to changes in hormone levels. In the period before ovulation, cervical mucus is viscous and composed of compact fiber structures (Brunelli et al., 2007). In response to estrogen during ovulation, the volume of mucus increases and it becomes less viscous (Bigelow et al., 2004). This highly hydrated mucus allows for migration of sperm through the cervix into the uterus (Bigelow et al., 2004). Cervical mucus is found in all women, and in non-pregnant women it is a viscous fluid (Hein et al., 2002). Following conception, the increase in progesterone levels causes the mucus to thicken and form a plug that blocks the entrance into the uterus from the vagina (Hein et al., 2001, 2002).

Cervical mucus, infection and pre-term birth

Pre-term birth remains the most significant cause of neonatal mortality (Noori et al., 2007) and in approximately 30% of cases is thought to be the result of an intra-amniotic infection (Greig, 1998). The microorganisms causing such infections originate from either the abdominal cavity or the vagina (Goldenberg et al., 2000). Microorganisms present in the vagina ascend, migrate past the cervical mucus plug and enter the intra-amniotic cavity, resulting in infection.

The cervical mucus plug acts as a blockade to prevent ascension of microorganisms from the vagina into the uterus and has been shown to have antimicrobial activity against many forms of bacteria (Hein et al., 2001). Cervical plugs have been found to contain many antimicrobial proteins and peptides such as SLPI, lysozyme, calprotectin, lactoferrin, neutrophil defensins and epithelial β-defensins (Hein et al., 2002). SLPI, a known protease inhibitor, is thought to prevent cleavage and inactivation of antimicrobial peptides. Disruption of the integrity of the mucus plug (possibly though degradation of mucin proteins making up the plug) removes the blockade and allows microorganisms to ascend (Wiggins et al., 2002). This suggests that the levels of proteolytic enzymes may be increased during infection and may be responsible for disruption of the mucus plug.

A study by Diaz-Cueto et al. (2006) showed that matrix metalloprotease 8 (MMP-8) levels are elevated in the CVF of women with bacterial vaginosis. Another study found that the risk of pre-term birth could be correlated with increased levels of granulocyte elastase (Nakai et al., 2005). It is possible that KLK levels may also be increased on infection and may be implicated in disruption of the mucus plug.

Premature rupture of membranes (PROM) and pre-term birth

Fetal membranes, which are composed of ECM proteins, undergo modifications and remodeling during the later stages of pregnancy and onset of labor (Bryant-Greenwood, 1998). Premature remodeling and subsequent PROM are responsible for one-third of pre-term births (Greig, 1998). PROM can occur pre-term or at term and is associated with latency between rupture and delivery. This latency increases the risk of perinatal infection (American Society of Obstetricians and Gynecologists, 2007).

One factor implicated in PROM is weakening of fetal membranes due to degradation of ECM proteins such as collagens (Bryant-Greenwood, 1998), laminin (Steadman et al., 1993) and fibronectin (Mercer et al., 2000). In fact, the presence of fetal fibronectin in maternal CVF is currently used as a biomarker for risk of PROM and pre-term birth (Lockwood and Dudenhausen, 1993; Krupa et al., 2006).

Proteolytic enzymes have been implicated in PROM because of their role in the degradation of ECM proteins. Elastase, a chymotryptic protease, is known to cleave elastin present in fetal membranes (Malak and Bell, 1994), as well as other ECM proteins (Gadek et al., 1980; Mainardi et al., 1980; McDonald and Kelley, 1980; Steadman et al., 1993). The activity of elastase is controlled by SLPI, an inhibitor found in cervical mucus (Helmig et al., 1995) and CVF (Shaw et al., 2007). Helmig et al. (2002) found increased levels of elastase and decreased levels of SLPI in patients with PROM, indicating that increased elastase activity may be responsible for degradation of ECM proteins resulting in PROM.

Members of the MMP family of proteases have also been shown to be expressed in fetal membranes (Weiss et al., 2007). Increased MMP-9 levels have been shown during labor, when MMP-9 is responsible for gelatinolytic activity in fetal membranes (Weiss et al., 2007). MMPs have also been implicated in PROM owing to their role in ECM protein degradation. Fetal MMP-9 has been shown to be increased in PROM (Romero et al., 2002), and TIMP-2, the inhibitor of MMP-2, has been shown to be decreased in PROM (Maymon et al., 2001), both suggesting an increase in MMP activity associated with PROM.

Role for KLKs in pre-term birth

As previously mentioned, we have shown that relatively high levels of many KLKs are present in CVF (Shaw and Diamandis, 2007) and that at least one active KLK (KLK6) exists in CVF (unpublished data). We hypothesize that, in addition to the other proteases mentioned (elastase and MMPs), KLKs may also play a role in PROM and pre-term birth.

Many KLKs have previously been shown to degrade ECM proteins *in vitro*, including KLK6 (Ghosh et al., 2004) and 13 (Kapadia et al., 2004), two major CVF KLKs. We hypothesize that under normal conditions, active KLKs

are kept in check and away from fetal membranes via inhibition by the high levels of protease inhibitors found in cervical mucus and probably the low pH of CVF. It is possible that decreased levels of protease inhibitors such as SLPI (Helmig et al., 2002) associated with PROM lead to increased KLK activity and subsequent degradation of fetal ECM proteins in fetal membranes by KLKs.

Conclusions and future directions

The relatively high levels of KLKs in CVF (Shaw and Diamandis, 2007; Shaw et al., 2007) and their expression by vaginal epithelial and cervical cancer cell lines indicate that KLKs play a role in cervico-vaginal physiology. The coordinated expression of multiple KLKs suggests that KLKs may participate in a proteolytic cascade in CVF, similar to their participation in proteolytic cascades in other systems such as seminal plasma (Michael et al., 2006) and skin (Brattsand et al., 2005; Borgono et al., 2007).

There appear to be parallels between vaginal and skin physiology, suggesting that KLKs may play a similar role in vaginal epithelium as in skin in terms of the desquamation of vaginal epithelial cells and skin corneocytes. It is also likely that KLKs may be involved in vaginal pathologies such as vaginitis, comparable to their implication in skin conditions such as dermatitis and Netherton syndrome.

CVF is known to be important in host defense (Huggins and Preti, 1981; Cole, 2006) and we and others have shown that many antimicrobial factors are present in CVF. KLKs have previously been shown to be involved in antimicrobial activity through their cleavage and activation of hCAP18, the human cathelicidin protein (Yamasaki et al., 2006). It is possible that KLKs play an antimicrobial role in CVF, possibly through cleavage of hCAP18 or other antimicrobial proteins or peptides.

KLKs are known to cleave ECM proteins (Ghosh et al., 2004; Kapadia et al., 2004; Michael et al., 2005; Rajapakse et al., 2005; Obiezu et al., 2006), and it is thus possible that they are implicated in PROM and pre-term birth through cleavage of fetal ECM proteins. Further experiments and development of both *in vitro* and *in vivo* models for vaginal physiology will help to delineate the physiological and pathological roles played by KLKs in this system.

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