SLPI and inflammatory lung disease in females.

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Citation  
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Abbreviations: CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; E₂, estrogen; LPS, lipopolysaccharide; miRISC, miRNA induced silencing complex; miRNA, microRNA; MRE, miRNA recognition element; NE, neutrophil elastase; NFκB, nuclear factor-κB; nt, nucleotide; oxLDL, oxidised low density lipoprotein; RNAi, RNA interference; SLPI, secretory leucoprotease inhibitor; TLR, toll-like receptor; UTR, untranslated region; WAP, whey acid protein; WFDC, WAP four disulfide core.

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Abstract

During the course of certain inflammatory lung diseases secretory leukoprotease inhibitor (SLPI) plays a number of important roles. As a serine antiprotease it functions to protect the airways from proteolytic damage due to neutrophil and other immune cell-derived serine proteases. With respect to infection it has known antimicrobial and anti-viral properties that are likely to contribute to host defense. Another of its properties is the ability to control inflammation within the lung, where it can interfere with the transcriptional induction of proinflammatory gene expression induced by nuclear factor-κB (NFκB). Thus factors that regulate the expression of SLPI in the airways can impact on disease severity and outcome. Gender represents once such idiosyncratic factor. In females with cystic fibrosis (CF) it is now thought that circulating estrogen contributes, in part, to the observed gender gap whereby females have worse disease and poorer prognosis than males. Conversely in asthma sufferers females have more frequent exacerbations at times of low circulating estrogen. Here we discuss how SLPI participates in these events and speculate on whether regulatory mechanisms such as post-transcriptional modulation by microRNAs are important in the control of SLPI expression in inflammatory lung disease.
1. Secretory Leucoprotease Inhibitor (SLPI)

Secretory Leucoprotease Inhibitor (SLPI) is a member of the Whey-Acidic Protein (WAP) family of proteins. These proteins share a conserved cysteine-rich four-disulphide core (FDC) domain and as the name suggests, these domains contain eight cysteine residues forming 4 disulphide bonds. SLPI contains two of FDC domains (1), and like many other FDC-containing proteins, is encoded on chromosome 20q12-13.2. Spanning approximately 2.6 kb, the SLPI gene consists of three introns and four exons (2, 3). SLPI is expressed in many locations but expression appears to be highest in lung and cervical mucosa, body fluids and skin (4).

The primary role of SLPI is as a serine protease inhibitor. It can protect tissue from degradation by a number of proteases such as Cathepsin G, elastase, trypsin, chymotrypsin, chymase and tryptase. However, Neutrophil Elastase (NE) is considered the principal protease target of SLPI (5); NE is produced by neutrophils and the excessive tissue damage evident in certain neutrophil-dominated diseases such as cystic fibrosis (CF) may be in part attributable to the action of this protease. The antiprotease activity of SLPI lies in the C-terminal domain, and in particular, the active site is located at the Leu$^{72}$ – Met$^{73}$ residues of domain 2 (6).

The high local concentration of SLPI in the upper airways and the fact that it has been shown to associate with elastin fibres in the lung (and skin) illustrate a key role for SLPI in protecting the local lung tissue from neutrophil-derived serine proteases (7). In a high protease milieu SLPI is itself cleaved by endogenous proteases, such as the cysteinyI cathepsins (8), NE itself (9), but also by proteases derived from pathogens such as *Pseudomonas aeruginosa* (10). Furthermore oxidation of the Met$^{73}$ residue has been shown reduce the inhibitory potential of SLPI, and the increased intrapulmonary oxidative stress and pathogenic bioburden in patients with neutrophil-dominated lung diseases such as chronic obstructive pulmonary disease (COPD) or CF may help explain the sometimes lower levels of active SLPI found in these patients (7).

Although originally identified as an antiprotease, more recent data has shed light on alternative functions of SLPI. In particular, SLPI has been shown to exhibit anti-
inflammatory/immune-modulatory functions that are not directly related to its anti-protease role. Indeed the protein has been found to antagonise TNFα, lipopolysaccharide (LPS)- and oxidized low-density lipoprotein (oxLDL)-induced activation of NFκB, a major mediator of innate immune responses (11, 12). SLPI exerts its effects on NFκB signalling in a number of ways. By inhibiting the interaction between CD14 and LPS, SLPI interferes with the uptake of the latter (13). Exogenously applied SLPI has been shown, at least in peripheral blood monocytes, to be taken into the cell and distributed around both the cytoplasm and the nucleus. In monocytes, SLPI can block NFκB activation by inhibiting the degradation of IκBα and IκBβ, inhibitors of NFκB (14). Interestingly, binding of DNA by SLPI has been demonstrated, and in particular, SLPI has been shown to compete with the NFκB subunit p65 for binding to sites in the promoter regions of NFκB-regulated genes (15). This ultimately results in the down-regulation of production pro-inflammatory cytokines.

Recombinant SLPI, when administered in an aerosol form to CF patients, can not only suppress airway NE levels but can also reduce interleukin (IL)-8 levels in bronchoalveolar lavage fluid obtained from these patients (16).

In addition to its antiprotease and immune-modulatory functions, SLPI also demonstrates antimicrobial activity in vitro. It has antimicrobial activity against many human pathogens including bacteria such as Pseudomonas aeruginosa and Staphylococcus aureus (17), fungi such as Candida albicans (18), and even HIV (19). Although the molecular mechanisms by which this antimicrobial activity is mediated are not yet well understood, it is believed that the highly cationic nature of the protein may be involved in the disruption of the cell membrane. It is also postulated that the antiprotease function of SLPI may aid in this antimicrobial effect through targeting pathogen-derived proteases (20).
2. Regulation of SLPI expression

SLPI is expressed constitutively at various mucosal surfaces however several factors including cytokines and steroid hormones can regulate its inducible expression. The SLPI promoter contains a lung specific element responsible for fine tuning its expression in lung epithelium (21). In these cells SLPI expression can be increased by LPS, IL-1β, TNFα, NE and neutrophil α-defensins (22-26). Corticosteroids can also induce SLPI expression in epithelial cells (27) whilst in skin and endometrium epidermal growth factor receptor (EGFR) signalling plays a role in its regulation (28, 29). In macrophages SLPI is also increased in response to LPS but surprisingly not IL-1β or TNFα, instead the cytokines IL-6 and IL-10 can enhance SLPI in these cells, albeit with slower kinetics that LPS (30). In vitro TGFβ decreases SLPI expression in bronchial epithelial cells (31).

Post transcriptional and epigenetic regulation of SLPI are topics that are less well explored. IL-1β-induced expression of SLPI in airway epithelial cells appears to involve histone H3-K4 trimethylation across the SLPI coding region, an effect that can be inhibited by the methylase inhibitor 5-azacytidine (32). However whether other epigenetic mechanisms such as histone deactylation or microRNA repression regulate SLPI has not been shown but is no doubt currently under investigation.

2.1 microRNA

microRNAs (miRNAs) are 21-24 nucleotide duplex RNAs involved in the translational regulation of gene expression (33). Although the term ‘microRNA’ was first coined in 2001, the first miRNA, lin-4, was discovered eight years earlier in the nematode C. elegans (34, 35). Having been initially discovered to have importance in developmental biology, interest in these small RNAs has dramatically increased since this time as they have been found to have significant roles in a range of other biological processes such as proliferation and apoptosis. Expression levels of miRNAs vary greatly between cells and tissues, and indeed aberrant levels of miRNA are associated with many diseases in humans.
Mammalian miRNAs are initially transcribed into longer primary miRNA (pri-miRNA) of up to 1000 nucleotides (nt) in length in the nucleus, where they are cleaved into resulting pre-miRNA (Figure 1). This processing involves the RNase III enzyme Drosha and the RNA binding protein DGCR8. These hairpin pre-miRNA structures, which are approximately 70-100 nt in length are actively transported into the cytoplasm, via a process involving Exportin 5. Once in the cytoplasm, the pre-miRNA is further processed into mature miRNA duplexes by Dicer. Duplexes consist of a mature miRNA strand and a miRNA* strand which, in general, is degraded. RNA interference (RNAi) involving mature miRNAs occurs through the miRNA induced silencing complex (miRISC): miRNA can bind to target mRNA and induce cleavage degradation or translational repression of the mRNA target (35, 36). An interesting aspect of miRNA regulation of mRNA translation lies in the fact that full complementarity between miRNA and target mRNA is not required. In fact, only partial complementarity is required and a 2-8 nt ‘seed region’ is thought to be crucial in the selection of targets by miRNA (37). Binding to miRNA responsive elements (MREs) in target mRNA appears to occur through this seed region. Messenger RNAs typically have many different MREs and can therefore be regulated by more than one miRNA. Although most miRNA studies have largely focused on miRNA-mRNA interactions in the 3’untranslated region (UTR) of target mRNA, these interactions can occur as efficiently in the 5’UTR (38). According to the PITA miRNA prediction algorithm the full length SLPI transcript contains 700 MREs that can potentially be bound by 408 miRNAs. By annotating this sequence and combining it with the outputs from TargetScan 5.1 and microRNA.org, there are 211 predicted MREs in the 177 base pair 3’UTR of SLPI. Thus it is likely that miRNA represent important regulators of SLPI expression.

Dysregulation of miRNA has been found to occur in many human diseases including those of the lung. These include but are not limited to cancers, COPD, CF and asthma. For example, miR-126 has been shown to be downregulated in the bronchial epithelium of patients with CF (39). The fact that this miRNA has been shown to have an involvement in allergic asthma, angiogenesis, breast cancer and other malignancies highlights the multifaceted roles of miRNA in health and disease (35).
To date there have been no studies examining whether miRNAs that regulate SLPI are altered in vivo in individuals with genetic or environmental inflammatory lung diseases.

3. SLPI, inflammatory lung disease and female gender

SLPI appears to play an important part in the progression of certain inflammatory lung diseases. For example, SLPI concentrations are higher in lung secretions of patients with chronic bronchitis, a disease characterised by infection and neutrophilic inflammation, as opposed to healthy controls. Results from studies with these patients suggest an inverse correlation exists between SLPI levels and the risk of developing an exacerbation (40). Interestingly, our group has recently shown that 17β-estradiol (E₂), the primary estrogen in non-pregnant females, is capable of abrogating Toll-like Receptor (TLR) responsiveness by upregulating SLPI in CF bronchial epithelial cells (Figure 2). This in turn inhibits the secretion of the neutrophil chemokine IL-8, by inhibiting NFκB signalling via mechanisms described earlier (41). One may wonder how this anti-inflammatory effect of E₂ could have negative consequences in the chronically inflamed airways of CF patients. The current paradigm proposes that during acute exacerbations, surges in inflammation may be protective and that E₂/SLPI-mediated inhibition of inflammation, coupled with E₂’s effect on decreasing airway surface liquid height in CF females (42), is overall disadvantageous to CF females. This role of E₂ helps explain the clinical observations that females with CF have increased mortality and tend to have more aggressive disease than their male counterparts (43).

Recent studies have shown that patients with allergic asthma express substantially higher levels of SLPI compared to healthy controls, and that SLPI may have a role in protecting the airways from asthma-induced inflammation (Figure 2) (44, 45). Expression of SLPI actually protects against allergic asthma in a mouse ovalbumin model of asthma, and this may be related to the earlier observations that SLPI can blocking tryptase-induced bronchoconstriction and hyperresponsiveness (46). E₂ may have a role in asthma severity as it has been observed that exacerbations are less common in females at times of high circulating E₂ (mid-menstrual cycle) when SLPI expression should
be highest and more common in times of low E\textsubscript{2} (menstruation). Decreased exacerbation rates and improved pulmonary function have been observed in some female asthma sufferers while undergoing hormone-replacement therapy and in those taking oral contraceptives (47).

Hormonal regulation of SLPI is not a new concept (48, 49). Estrogen increases SLPI expression in both rat and human uterine epithelium (50, 51), and in post-menopausal women its expression is significantly decreased compared to peri-menopausal women or post-menopausal women treated with hormone replacement (52). Tissue-specific expression of SLPI has also been linked to progesterone, with SLPI expression lowest in endometrium and highest in breast epithelium at times of low and high circulating progesterone, respectively (49, 53). Interestingly intrauterine expression of SLPI is decreased in women with recurring lower reproductive tract infections (54). The observation that hormonal regulation of SLPI by E\textsubscript{2} occurs in tissues other than those in the reproductive tract and breast, expands its potential role as a gender-specific factor in a variety of diseases.

It is widely accepted that E\textsubscript{2} plays a complex role in inflammatory processes and can exert both pro- and anti-inflammatory effects. These differences are due in part to the differential tissue expression of the two main forms of its receptor (Estrogen Receptor, ER), namely ER\textalpha and ER\textbeta. For example, E\textsubscript{2} acts through ER\textbeta in the CF bronchial epithelium (41). This is likely to be different in monocytic cells where ER\textalpha predominates over ER\textbeta. It is also possible that E\textsubscript{2} mediates important changes in post-transcriptional regulation of SLPI via miRNA.

4. Conclusions

With respect to gender dichotomies in lung disease, relatively little is known about the molecular mechanisms involved. It is almost certain that microRNAs play some role in mediating these gender differences in inflammatory lung diseases, but to date these remain largely unexplored. Unravelling these mechanisms will lead to a better understanding, and may open up new avenues for therapy of these diseases.
5. References.


**Figure 1. microRNA biogenesis in animals.** Transcription factors or other environmental regulators may induce miRNA expression, resulting in the production of long primary (pri) –miRNA transcripts in the nucleus. These transcripts are then generally processed by Drosha and DGCR8 into shorter hairpin structures termed pre-miRNA. After transport out of the nucleus into the cytoplasm via Exportin 5, processing by Dicer results in an approximately 21-24 nucleotide mature duplex miRNA. This results in a ‘guide strand’ and a ‘passenger strand’, the latter of which is generally degraded, and the former usually integrated into a miRNA induced silencing complex (miRISC) via the aid of Ago. The miRNA is then transported to target mRNAs, where interactions between the two RNAs lead to the cleavage degradation or translational repression of target mRNA.

**Figure 2. Effects of estrogen on the airways of patients with asthma and cystic fibrosis.** Both diseases are characterised by chronic inflammation within the lungs. High circulating estrogen levels can have a beneficial effect in asthma by inducing local SLPI expression and inhibiting tryptase-induced bronchoconstriction and hyper-responsiveness. Conversely in females with CF estrogen-induced SLPI expression leads to immune hyporesponsiveness and downregulation of pro-inflammatory cytokines via inhibition of NFκB which, combined with an estrogen-mediated reduction airway surface liquid (ASL) height, further facilitates microbial colonisation.