Abstract
During the course of certain inflammatory lung diseases, SLPI (secretory leucoprotease inhibitor) 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 defence. Another of its properties is the ability to control inflammation within the lung where it can interfere with the transcriptional induction of pro-inflammatory gene expression induced by NF-κB (nuclear factor-κB). 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 CF (cystic fibrosis), it is now thought that circulating oestrogen contributes, in part, to the observed gender gap whereby females have worse disease and poorer prognosis than males. Conversely, in asthma, sufferers who are females have more frequent exacerbations at times of low-circulating oestrogen. In the present paper, we discuss how SLPI participates in these events and speculate on whether regulatory mechanisms such as post-transcriptional modulation by miRNAs (microRNAs) are important in the control of SLPI expression in inflammatory lung disease.

SLPI (secretory leucoprotease inhibitor)
SLPI is a member of the WAP (whey acidic protein) family of proteins. These proteins share a conserved cysteine-rich FDC (four disulfide core) domain and as the name suggests, these domains contain eight cysteine residues forming four disulfide bonds. SLPI contains two 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, NE (neutrophil elastase) 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 CF (cystic fibrosis) may in part be 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 Leu72–Met73 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 not only by endogenous proteases, such as the cysteiny1 cathepsins [8], NE itself [9], but also by proteases derived from pathogens such as Pseudomonas aeruginosa [10]. Furthermore, oxidation of Met73 has been shown to reduce the inhibitory potential of SLPI, and the increased intrapulmonary oxidative stress and pathogenic bioburden in patients with neutrophil-dominated lung diseases such as COPD (chronic obstructive pulmonary disease) 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 results have 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 antagonize TNFα (tumour necrosis factor-α), LPS (lipopolysaccharide) and oxLDL (oxidized low-density lipoprotein)-induced activation of NF-κB (nuclear factor-κB), a major mediator of innate immune responses [11,12]. SLPI exerts its effects on NF-κB 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 NF-κB activation by inhibiting the degradation of IκB (inhibitor of NF-κB) α and IκBβ [14]. Interestingly, binding of DNA by SLPI has been demonstrated, and in particular, SLPI has been shown to compete with the NF-κB subunit p65 for binding to sites in the promoter regions of NF-κB-regulated genes [15]. This ultimately results in the down-regulation of production of 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 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 Ps. 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].

**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], while in skin and endometrium EGFR (epidermal growth factor receptor) 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 than LPS [30]. In vitro TGFβ (transforming growth factor β) 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 His3-Lys4 tri-methylation 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 deacetylation or miRNA (microRNA) repression regulate SLPI has not been shown but is doubt currently under investigation.

**miRNA**

miRNAs are 21–24 nt 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 8 years earlier in the nematode Caenorhabditis 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 pri-miRNA (primary miRNA) of up to 1000 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 (DiGeorge syndrome critical region gene 8). 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 an miRNA* strand which, in general, is degraded. RNAi (RNA interference) involving mature miRNAs occurs through the miRISC (miRNA-induced silencing complex): 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 MREs (miRNA-responsive elements) in target mRNA appears to occur through this seed region. miRNAs 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′-UTR (untranslated region) 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 bp 3′-UTR of SLPI. Thus it is likely that miRNAs 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 down-regulated 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.

**SLPI, inflammatory lung disease and the 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 characterized by infection and
miRNA biogenesis in animals

Transcription factors or other environmental regulators may induce miRNA expression, resulting in the production of long 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 nt 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 an 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 miRNA.

Recent studies have shown that patients with allergic asthma express substantially higher levels of SLPI compared with 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 block tryptase-induced bronchoconstriction and hyper-responsiveness [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 E2 (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]. Oestrogen increases SLPI expression in both rat and human uterine epithelium [50,51], and in post-menopausal women its expression is significantly decreased compared with perimenopausal 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 E2 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 E2 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 [ER (oestrogen receptor)], namely ERα and ERβ. For example, E2 acts through ERβ in the CF bronchial epithelium [41]. This is likely to be different in monocytic cells where ERα predominates over ERβ. It is also possible that E2 mediates important changes in post-transcriptional regulation of SLPI via miRNA.

**Conclusions**

With respect to gender dichotomies in lung disease, relatively little is known about the molecular mechanisms involved. It is almost certain that miRNAs 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.


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