LL37, a human antimicrobial peptide with immunomodulatory properties

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Cationic antimicrobial peptides (AMPs) represent the first line of defense against many invading pathogens. These small amphipathic peptides are part of the innate immune system and have a broad-spectrum activity against bacteria, fungi and viruses. In mammals, at least two distinct groups of AMPs are found. Defensins are the more representatives and cathelicidins form the second group. The hCAP18/LL37 is the only known human cathelicidin. The antimicrobial peptide is referred to as LL37, since it has a 37 amino acids sequence starting with two leucines. It is a 4.5 kDa, cationic (+6), amphipathic α-helical peptide, with a broad spectrum of antimicrobial activity. Besides its antimicrobial properties LL37 plays a central role in innate immune responses and inflammation. It has been identified as a potent chemoattractant for mast cells, monocytes, T lymphocytes and neutrophils using formyl-peptide receptor–like 1 (FPRL1). LL37 also promotes wound healing, angiogenesis and arteriogenesis and acts as immune adjuvant.

Keywords: Antimicrobial peptide, cathelicidin, LL37, immunomodulator

1. Antimicrobial Peptides

The increasing bacterial resistance against common antibiotics is a growing concern for the public health. Resistance began to emerge as early as the 1950s with multiresistant microorganisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* or *Enterococcus sp.,* largely as the result of antibiotic overuse [1].

Much research has been dedicated to the development of new classes of antibiotics to overcome bacterial resistance. The focus on the structure and function of antimicrobial peptides (AMPs) has greatly increased in recent years. AMPs are an important part of the innate immune system of most living organisms against invading pathogens, playing a direct antimicrobial and mediator function and providing the initial host defense mechanism. They are generally defined as peptides of less than 50 amino acid residues, bearing positive charge due to multiple lysine and arginine residues and with 50% or more of hydrophobic residues [2]. AMPs have aroused great interest due to their broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria [3], fungi [4] and protozoa [5] with minimum inhibitory concentrations (MIC) as low as 0.25-0.4 μg/ml [6]. Certain cationic peptides have been shown to inhibit the replication of enveloped viruses such as influenza A virus [7], vesicular stomatitis virus (VSV) and human immunodeficiency virus (HIV-1) [8]. Moreover, these peptides have potential to overcome bacterial resistance [9]. Therefore, AMPs or their derivatives can potentially represent a new class of antimicrobial drugs.

AMPs can be grouped according to their size, conformational structure or predominant amino acid structure; nevertheless, the diversity of these molecules is so great that it is difficult to categorize them in a generally accepted classification. On the basis of their 3D structure, peptide antibiotics can be classified in four major groups, with the first two being the most common in nature [6]:

- Group I: β-sheet structures stabilized by disulfide bridges.
- Group II: linear peptides with a α-helical structure.
- Group III: extended peptides.
- Group IV: peptides with loop structures.

The exact mechanism by which AMPs exert their antimicrobial properties is yet unclear, but it is generally accepted that cationic AMPs interact by electrostatic forces with the negatively charged phospholipids on the bacterial membrane. The interaction is complex and results in the creation of pores and membrane disruption. There are several mechanisms proposed, the most widely accepted being the “barrel-stave”, “carpet” and “toroidal” models [10].

The “barrel-stave” model describes the formation of transmembrane channel/pores by groups of amphipathic α-helical peptides, where the hydrophobic surfaces interact with the aliphatic chain of the membrane phospholipids and the hydrophilic parts point inside producing an aqueous pore [11].

In the “carpet model”, peptides accumulate on the bilayer surface. Peptides are electrostatically attracted to the anionic phospholipid head groups at numerous sites covering the surface of the membrane in a carpet-like manner. At high peptide concentrations, surface-oriented peptides are thought to disrupt the bilayer by acting like a detergent, eventually leading to the formation of micelles [12].

In the “toroidal-pore model”, antimicrobial peptide helices insert into the membrane and induce the lipid monolayers to bend continuously through the pore so that the water core is lined by both the inserted peptides and the lipid head groups. This type of transmembrane pore is induced by magainins, protegrins and melittin[13, 14]. The “toroidal”

model differs from the "barrel-stave model" as the peptides are always associated with the lipid head groups even when they are perpendicularly inserted into the lipid bilayer.

2. The human cathelicidin LL37

There are two major groups of antimicrobial peptides in humans: defensins and cathelidicins. All cathelicidins share a similar structure characterized by a highly conserved N-terminal domain of about 100 amino acid residues. This cathelin-like domain is flanked by a signal peptide (approximately 30 residues long) on its N-terminus, and by an antimicrobial peptide region on its C-terminus.

2.1 Structure and cellular expression of LL37

The only member of the cathelicidins family found to date in humans is LL37/hCAP18, an 18 kDa peptide encoded by the gene *CAMP* (GeneBank ID 820). It was first described in 1995 in bone marrow cells [15]. The name hCAP18 was chosen because of its close relationship to the cationic antimicrobial peptide found in rabbit, which has a molecular weight of 18 kDa and is therefore called Cationic Antimicrobial Peptide CAP18. The gene for the human cathelicidin hCAP18 is a compact gene of 1963 bp located in chromosome 3, and is composed of four exons. Exons 1 to 3 code for the signal sequence and the cathelin domain, while exon 4 codes for the antimicrobial peptide [16] (figure 1).

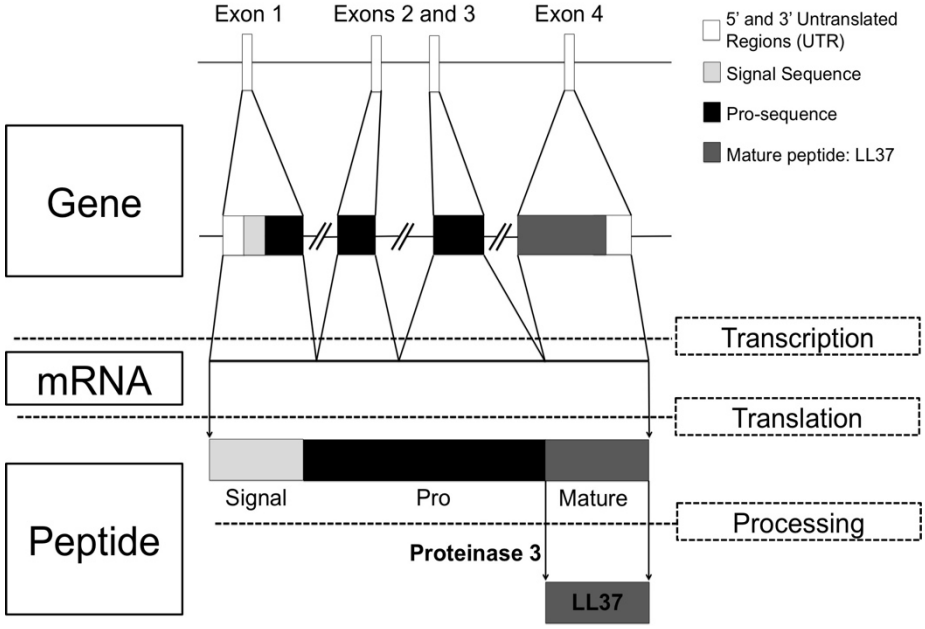

Fig.1 Schematic structure of the LL37/hCAP18 gene and its processing into LL37 peptide.

The mature antimicrobial peptide has 37 amino acid residues starting with two leucines (NH_2-LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES-COOH), hence being named LL37. It does not contain any cysteine or tryptophan residue and has a molecular weight of ~4.5 kDa. The peptide is derived by extracellular proteolysis of proteinase 3 from the C-terminal end of hCAP18 [17], differing from most cathelicidins that are cleaved by elastase [18, 19]. Initially, the active mature peptide was considered to be the 39-residue FALL39, based on the presence of a typical KR cleavage site [20]. However, Gudmundsson *et al.*[16] used anti-FALL39 imunoglobulin to locate the mature peptide and isolated it from granulocytes after degranulation. The structural analysis determined the mature peptide to be LL37. Nevertheless, two research groups have described other processed peptides. Sorensen *et al.* reported that hCAP-18 in seminal plasma is processed to generate the 38-amino acid antimicrobial peptide ALL38 by the prostate-derived protease gastricsin, when incubated at the vaginal pH. Indeed, ALL38 was found in the vagina following sexual intercourse and the antimicrobial activity of ALL38 against a variety of microorganisms tested was equivalent to that of LL37 [21]. Murakami *et al.* [22] and Yamasaki *et al.* [23] showed that after secretion onto the skin surface or sweat, the *CAMP* gene product is processed by a serine protease-dependent mechanism into multiple novel antimicrobial peptides distinct from the cathelicidin LL37. These peptides (RK31, KS30, LL29, KS22 and KR20) showed enhanced antimicrobial action, acquiring the ability to kill skin pathogens such as *Staphylococcus aureus* and *Candida albicans*.

At physiological pH, LL37 has a positive net charge of +6. The peptide has 6 lysines and 5 arginines that carry 11 positive charges, while 3 glutamates and 2 aspartates residues bear 5 negative charges. It is relatively disordered in aqueous solution, but folds into an amphipathic α-helix in other environments, such as in contact with lipid membranes

[24]. Guangshun Wang studied the structure of ^{13}C,^{15}N-labeled LL37 by three-dimensional triple-resonance NMR and reported that the helical region covers residues 2 to 30, while the C-terminus is mobile [25]. All antimicrobial peptides are amphipathic, meaning that they have hydrophobic and hydrophilic regions, allowing the electrostatic interaction with anionic bacterial membranes. Figure 2 illustrates the three-dimensional structure of LL37. The hydrophobic residues are shown in red and are aligned on one side of the peptide.

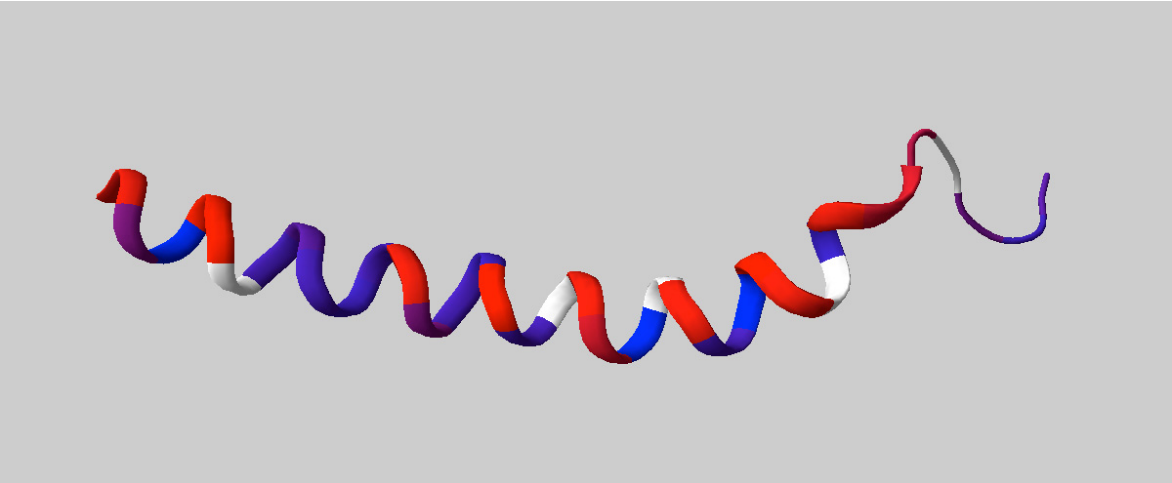

Fig.2α-helical structure of LL37. Hydrophobic residues are shown in red.

LL37 and its precursor, hCAP18, are found at different concentrations in very different cells, tissues and body fluids, predominantly in leukocytes and various epithelial linings. But the expression of hCAP18/LL37 has been reported in many other tissues or body fluids like breast milk, sweat, wound fluids, saliva, gingiva, testis, spermatozoa, seminal plasma, amniotic fluids and more, as well summarized by Durr *et al.*[20]. In a recent work, Laudien *et al.* also reported the expression of LL37 and defensins in the nasal epithelium providing a chemical defense shield [26]. AMPs are also present in ear wax preventing bacteria and fungi from causing infections in the external auditory canal [27]. The quantification of hCAP18/LL37 concentrations *in vivo* is challenging. Sorensen *et al.* developed a specific ELISA for the detection of hCAP18 in cells, plasma and urine. They determined that the amount of hCAP18 in neutrophils is 0.627 μg/10^6 cells and is barely excreted in the urine. The plasma level is 1.18 μg/ml, several fold higher than for other neutrophil specific granule proteins [28]. In the airway fluids, the levels of LL37 are estimated to be 2 μg/ml in adults and 5 μg/ml in neonates.

The down and upregulation of LL37 have been identified in many diseases. A higher concentration of the peptide is more common in the presence of infections. In fact, in pulmonary infections the levels of LL37 are augmented two or three times [29]. A low expression of LL37 is associated with skin disorders like atopic dermatitis [30] or chronic ulcers [31]. Putsep *et al.*[32] reported that the neutrophils from patients with morbus Kostmann syndrome are deficient in LL37. However, in acute inflammation, LL37 concentrations can dramatically increase, reaching 1.5 mg/ml in the skin lesions of psoriasis patients [33]. High concentrations of LL37 have also been associated to other inflammatory disorders like lupus erythematosus, contact dermatitis or inflamed synovial membranes [20, 34]. Gilliet and Lande[35] proposed that these inflammatory disorders are caused by the inappropriate recognition of self-nucleic acids with induction of interferon responses by plasmacytoid dendritic cells (pDCs). In normal conditions self-DNA released by dying cells is unable to enter pDCs and is degraded rapidly by extracellular DNases. LL37 binds to self-DNA and forms aggregated particles that are resistant to degradation by DNases and able to enter pDCs through proteoglycan-mediated attachment of LL37 to the cell membrane and lipid-raft-mediated endocytosis. Aggregated self-DNA particles are retained in early endocytic compartments to trigger induction of interferons (IFNs) by activating endosomal toll-like receptor 9 (TLR9) as if they were viruses. Type I IFNs trigger local maturation of myeloid dendritic cell and activation of autoreactive T cells leading to the formation of psoriatic skin lesions. In this process, activated Th17 cells produce IL-22 and IL-17 that stimulate psoriatic keratinocytes to sustain the expression of cationic antimicrobial peptides (AMP) including LL37, which further forms complexes with self-DNA that is abundantly released in psoriatic skin lesions by apoptotic cells. As a result, LL37/self-DNA complexes constantly promote dendritic cell maturation and activation of autoreactive T cells, providing a self-sustaining feedback mechanism that amplifies and maintains autoimmune skin inflammation in psoriasis. Leung *et al.* measured the serum concentrations of LL37 in eczema children patients and reported that the concentration of the peptide increased with the eczema severity. They considered that LL37 might be a biomarker for the severity of childhood eczema [36].

The expression of LL37 is upregulated by several stimuli, such as pro-inflammatory cytokines, growth factors, nutrients, and bacterial products, especially at inflammation and repair sites. The mechanisms regulating the LL37

production are not fully understood. Recently, the roles of the vitamin D_3 and the hypoxia response pathways in the regulation of LL37 production in leucocytes and keratinocytes have been described. Vitamin D_3 is naturally produced in the skin during exposure to sunlight, and is activated by hydroxylases CYP27A1 and CYP27B1 to generate the biologically active 1,25-dihydroxyvitamin D_3 [35], which is a potent inducer of the antimicrobial protein cathelicidin in isolated human keratinocytes, monocytes, neutrophils and gingival epithelial cells [37-39]. As sunlight induces immunosuppression increasing the vulnerability to infection, Vitamin D_3, potentially to balance this effect, stimulates the synthesis of LL37 in skin and circulating phagocytic cells [40]. Morevoer, Hata *et al.* have reported that oral administration of vitamin D for 21 days induced cathelicidin production in atopic dermatitis lesional skin [41].
Hypoxia-inducible transcription factor 1α (HIF1α) also plays an important role in LL37 cellular expression. In fact, HIF1α-null mouse neutrophils show a dramatic reduction in the levels of mouse cathelin-related antimicrobial peptide (CRAMP) [42]. Also, the siRNA knock down of HIF1α in human keratinocytes decreased the expression of hCAP18 [43]. On the other hand, Stroinigg and Srivastava [44] reported that the human beta-defensin 2 (HBD-2), present in significant amounts in human milk, greatly increased LL37 expression in colon and breast epithelial cells. Moreover, the exposure of human primary bronchial epithelial cells to IL-4 and IL-13 cytokines during mucociliary differentiation increased mRNA expression of hCAP18/LL37 and hBD-2 [45].

Finally, microorganisms can also regulate the expression of LL37. Bacterial products from both gram-positive and gram-negative bacteria were shown to increase LL37 production in cultured sinus epithelial cells [46]. Butyrate, a product of bacterial microflora in the colon, induces LL37 production [47]. LL37 cellular expression was also found to be augmented by *Pseudomonas aeruginosa* flagellin [48], fungal allergens in nasal tissue from chronic rhinosinusitis patients [49], and *Helicobacter pylori* in gastric epithelium [50]. On the other hand, other pathogens reduce the expression of the peptide: *Shigella flexneri* suppress LL37 production [51]; *Neisseria gonorrhoeae*, a human pathogen causing the sexually transmitted disease gonorrhoeae, that preferentially attach to and invade epithelial cells of the genital tract was found to down-regulate the expression of LL37, indicating that pathogenic *Neisseria* may gain a survival advantage in the female genital tract by decreasing LL37 expression [52]; Cholera toxin and labile toxin, the major virulence proteins of *Vibrio cholerae* and enterotoxigenic *Escherichia coli*, respectively, are predominantly responsible for the suppression of LL37 and human beta-defensin 1 (HBD-1) expression in intestinal epithelial cells both *in vitro* and *in vivo* [53].

2.2 Functions of LL37

LL37 was initially recognized for its antimicrobial properties. Nevertheless, it has been found to have additional defensive roles such as regulating the inflammatory response and chemo-attracting cells of the adaptive immune system to wound or infection sites, binding and neutralizing LPS, promoting re-epthelialization and wound closure and more as illustrated in figure 3.

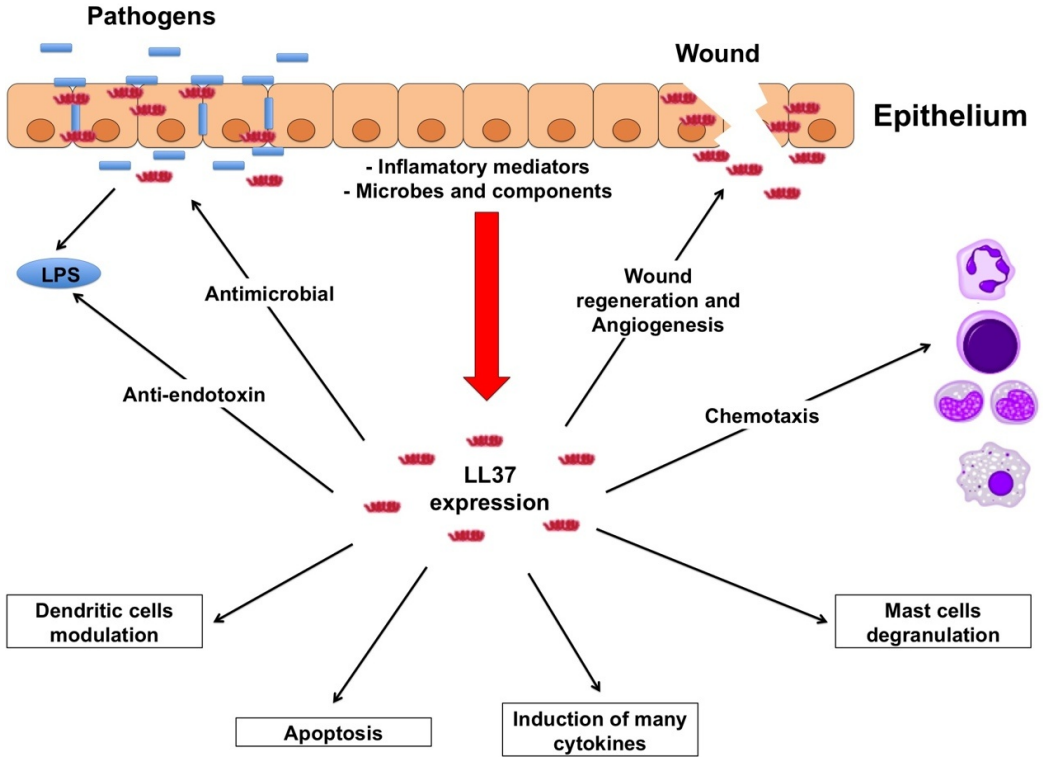

Fig.3 Biological activities of LL37.

Antimicrobial activity

The antibacterial activity of LL37 has mostly been studied *in vitro* using the synthetic peptide. The reported activities of LL37 vary in several studies, indicating that it is sensitive to differences in the experiment conditions, such as salt, pH and the phase of bacterial growth. Nevertheless, LL37 has a broad range of activity against both Gram-negative and Gram-positive bacteria. LL37 exhibits potent activity against *Escherichia coli*, *Salmonella typhimurium and Salmonella minnesota*, *Pseudomonas aeruginosa, Neisseria gonorrhoeae, Klebsiella pneumoniae* and group A *Streptococcus* with MIC values of 0.6-7.6 μg/ml (0.1-2 μM) [54], 0.2-3.6 μg/ml (0.1-0.8 μM) [54], 0.9-5.7 μg/ml (0.2-1 μM) [54], 0.9 μg/ml (0.2 μM) [52], 4.2 μg/ml (0.9 μM) [55], and 5-72 μg/ml (1-16 μM) [56] respectively, while low activity has been detected against the yeast *Candida albicans* (MIC >250 μg/ml or 56 μM) [57] and *spirochaete* (MIC>145 μg/ml or 32 μM) [58]. Besides its low activity against *C. albicans,* LL37 was still able to reduce the *C. albicans* infectivity by inhibiting its adhesion to plastic surfaces, oral epidermoid cells, and urinary bladders of female BALB/c mice. The inhibitory effects of LL-37 on cell adhesion and aggregation were mediated by its preferential binding to mannan, the main component of the *C. albicans* cell wall, and partially by its ability to bind chitin or glucan, which underlie the mannan layer [59]. LL37 has also been shown to possess some antiviral activity against herpes simplex virus [60] and vaccinia virus [61] and inhibits HIV-1 replication in peripheral blood mononuclear cells, including primary CD4(+) T cells [62]. Additionally, LL37 inhibits the formation of *P. aeruginosa* bacterial biofilms [63]. Like most other AMPs, LL37 kills microorganisms by membrane disruption, resulting in lysis. By using solid-state NMR data and differential scanning calorimetry, Wildman *et al.* proposed that LL37 covers the surface of the membrane, resulting in toroidal pore formation with subsequent leakage of the cytoplasmic content [64]. LL37 can also be cytotoxic to eukaryotic cells, including both erythrocytes and leukocytes. However, the cytotoxic concentrations (>58 μg/ml or 13 μM) are generally higher than the concentrations required for elimination of microorganisms [65].

The MIC values of LL37 determined *in vitro* are similar with the physiological concentrations observed during infectious and inflammatory disorders. Several studies reported the antimicrobial activity of LL37 to be noticeably reduced in high salt concentrations, under serum conditions, in the presence of a lung surfactant preparation as well as in the presence of artificial tears. However, *in vivo*, LL37 acts together with other antimicrobial components, i.e. beta-defensin 2 [66], lysozyme and lactoferrin [67], to show optimal killing capacity. Since the reported MIC values are determined for LL37 alone, there might be an underestimation of the antimicrobial activity under *in vivo* conditions, where synergistic effect with other antimicrobial components has to be considered. In fact, the role of LL37 in disease has been demonstrated in several reports. Nizet *et al.* [68] showed that mice with disrupted *Cnlp*, the gene coding for CRAMP (cathelin-related antimicrobial peptide), bear increased susceptibility to skin infections. Since CRAMP is similar to LL37 in structure, tissue distribution and antimicrobial activity, the CRAMP knockout model is a useful one for studying the function of the human cathelicidin. The *in vivo* role of LL37 in preventing infections is also supported by an observation made in patients with morbus Kostmann syndrome. These patients lack LL37 in the saliva neutrophils, which may contribute to increased susceptibility to oral infections. A different approach to evaluate the role of LL37 in certain diseases is to overexpress the peptide. As example, an adenovirus vector was utilized to transfer the LL37-gene systemically or into trachea of mice [69]. Furthermore, the vector was inserted into human bronchial xenograft, derived from patients with cystic fibrosis [70]. The overexpression of LL37 augmented the killing of bacteria in all these cases.

Lipopolysaccharide neutralizing properties

Lipopolysaccharides (LPS) endotoxins are heteropolymeric components of the outer layer membrane of Gram-negative bacteria with strong immunotoxic properties. LPS play an important role in pathogenesis of many exogenous respiratory diseases, including organic dust toxic syndrome and chronic illnesses such as chronic obstructive pulmonary disease, asthma or allergic alveolitis (hypersensitivity pneumonitis) [71]. LPS is released upon cell death, activating mononuclear phagocytes (monocytes and macrophages) to produce and release proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and others [72]. This event is initiated by binding of LPS to LPS-binding protein (LBP), followed by a second binding of the LPS-LBP complex to CD14, the primary receptor of LPS, which is expressed mainly on macrophages [73]. The LPS-CD14 complex initiates intracellular signaling by interacting with the transmembrane protein Toll-like receptor-4 (TLR-4), which activates the NF-kB transcription factor, resulting in the production and secretion of pro-inflammatory cytokines [74]. The massive release of LPS can lead in extreme cases to endotoxic shock and therefore, death.

Some AMPs, including LL37, have the capacity to neutralize LPS by binding it with high affinity, inhibiting LPS interaction with LBP and/or CD14 [75, 76]. Another mechanism has also been suggested, where LL37 binds directly to CD14, inhibiting the association of LPS with its receptor. LL37 significantly inhibited the expression of specific proinflammatory genes upregulated by NF-kB in the presence of LPS in human monocytes [77]. It reduced TNF-α and nitric oxide levels produced by LPS and IFN-γ-polarized mouse bone marrow-derived macrophages [78]. Also, LL37 suppressed the LPS-induced apoptosis of endothelial cells [79]. Kandler *et al.* showed that LL37 also blocked the effect of flagellin and lipoteichoic acid on dendritic cells [80]. The antiendotoxic effect of LL37 has been confirmed *in vivo*

using rat as a model system, making it a promising candidate for treatment of endotoxin shock or sepsis that is associated with a high rate of human death, for which there is yet no effective treatment [81].

Immunomodulatory properties

In addition to its antimicrobial activity and ability to neutralize LPS, LL37 plays a central role in innate immunity. It is chemoattractant for mast cells [82], monocytes, T lymphocytes and neutrophils [83]. Formyl Peptide Receptor Like-1 (FPRL-1) is the only receptor found to activate direct migration of immunological cells to a site of infection. As leukocytes participate in both innate and adaptive immunity, the fact that LL37 can chemoattract human leukocytes may provide one additional mechanism by which LL37 contributes to host defense against microbial invasion, by participating in the recruitment of leukocytes to sites of infection. This mechanism is potentially important *in vivo*, because the chemotactic activity of LL37, unlike its antimicrobial action, is not significantly inhibited by the presence of human serum. The activation of FPRL-1 requires relatively high concentrations of LL37 (10^{-5} M) when compared to other classical chemoattractant agents, suggesting a low-affinity peptide-receptor interaction [84]. This implies that cellular FPRL-1 mediated recruitment by LL37 in vivo may be active only when a threshold concentration of the peptide is reached following upregulation of the LL37 gene in epithelial cells or after massive release from invading neutrophils. Therefore, LL37 can potentially reach its optimal chemotactic concentration at local inflammatory sites.
LL37 modulates cellular immune responses by stimulation of chemokine production. LL37 activates airway epithelial cells as demonstrated by activation of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and increases release of the potent chemoattractant IL-8. LL37 transactivates the epidermal growth factor receptor (EGFR) via metalloproteinase-mediated cleavage of membrane-anchored EGFR-ligands [85]. LL37 also induces IL-8 secretion through phosphorylation and activation of the MAPKs, extracellular signal-regulated kinase 1/2 (ERK1/2) in monocytes [86]. Human airway smooth muscle cells also respond to LL37 by releasing IL-8, suggesting that LL-37 is a regulator of the inflammatory process in various inflammatory lung diseases [87]. Yoshioka *et al.* [88] reported that LL37 also causes functional changes in mast cells. Mast cells in the skin are involved in the innate immune system response against microbial infections via Toll-like receptors (TLRs), such as TLR-4, which that is known to recognize LPS. They observed that LL37 increased the level of TLR-4 mRNA and TLR-4 protein, and induced the release of IL-4, IL-5 and IL-1β from mast cells. Additionally, mast cells show degranulation upon stimulation with LL37. Degranulation releases pro-inflammatory mediators, such as histamine and prostaglandins, into the surrounding tissue, thus increasing vascular permeabilization and promoting the infiltration of inflammatory cells [89]. LL37 also interacts with the purinoceptor $P2X_7$, predominantly expressed on monocytes, macrophages and DCs and induces the processing and release of the potent cytokine IL-1β [90] and IL-8 in human gingival fibroblasts [91].

LL37 shows different effects on apoptotic cell death of different cell types. LL37 promotes apoptosis in various T-cells, smooth muscle cells and epithelial cells, while it is a potent inhibitor of human neutrophil apoptosis [65, 92]. The inhibition of neutrophils apoptosis may be a survival strategy, leading to an increase of viable neutrophils at the site of infection, which is beneficial for the host during bacterial invasion. It has been proposed that the underlying mechanism for the inhibition of apoptosis by LL37 is mediated via FPRL-1 and $P2X_7$ receptors, resulting in the inhibition of caspase 3 activity [93]. Lau *et al.* [94] reported that the apoptosis of airway epithelial cells is inhibited by human serum. Finally, Davidson *et al.* [95] identified LL37 as a potent modifier of dendritic cells differentiation, bridging innate and adaptive immune responses. LL-37-derived DCs displayed significantly up-regulated endocytic capacity, modified phagocytic receptor expression and function, up-regulated costimulatory molecule expression, enhanced secretion of Th-1 inducing cytokines, and promoted Th-1 responses *in vitro*. Moreover, when internalized by immature human DCs, LL37 caused phenotypic changes, characterized by an increased expression of the antigen-presenting molecule HLA-DR, and the costimulatory molecule CD86 [96].

Angiogenesis and wound healing

The healing of wound is a complex process that involves different steps: haemostasis, inflammation, remodeling/granulation tissue formation and re-epithelialization. Angiogenesis is critical to wound repair. Newly formed blood vessels participate in provisional granulation tissue formation and provide nutrition and oxygen to growing tissues. Angiogenesis, in response to tissue injury, is a dynamic process that is highly regulated by signals from both serum and the surrounding extracellular matrix environment [97].

LL37 is importantly involved in tissue healing processes, especially revascularization and cell growth. LL37 induces angiogenesis mediated by FPRL-1 expressed on endothelial cells. Koczulla *et al.* [98] reported that the application of LL37 stimulated neovascularization in a chorioallantoic membrane assay and in a rabbit model of hind-limb ischemia. The peptide directly activates endothelial cells, resulting in increased proliferation and formation of vessel-like structures in cultivated endothelial cells. Also, decreased vascularization during wound repair was observed in mice deficient for CRAMP. LL37 induces wound healing, proliferation, and migration of airway epithelial cells. Therefore, the peptide is likely involved in the regulation of tissue homeostasis in the airways [99]. It also promotes the migration of keratinocytes via EGFR transactivation [100]. More importantly, upon injury, a large increase in the expression of

cathelicidins in human and murine skin after sterile incision was observed and returned to normal levels as the wound closed [56]. The appearance of cathelicidins in skin was due to both synthesis within epidermal keratinocytes and deposition from granulocytes that migrate to the site of injury. In human burns, higher mRNA levels of hCAP18/LL37, hBD-2 and hBD-3 were detected [101]. The importance of LL37 in re-epithelialization was clearly demonstrated by Heilborn *et al.* [102]. The authors showed that the use of antibodies against LL37 inhibited re-epithelialization in a concentration-dependent manner in a non-inflammatory *ex vivo* wound healing model and that the peptide is lacking in chronic ulcers. In a recent work, Carretero *et al.* [103] demonstrated that LL37 significantly improved re-epithelialization and granulation tissue formation by *in vivo* adenoviral transfer of the peptide to excisional wounds in ob/ob mice. According to Gronberg *et al.* the proteolytic environment of chronic wounds does not seem to prevent the therapeutic use of topical LL37. The authors showed that LL37 was not degraded by matrix metalloproteinase-9, and was fairly resistant to proteolytic cleavage *ex vivo* by incubation with wound fluid from non-healing venous leg ulcers [104]. Moreover, we have recently demonstrated that the topical application of recombinant LL37 produced in our laboratory [105] induced angiogenesis and re-epithelialization in wounds of dexamethasone-treated mice (paper submitted).

As already stated, LL37 promotes cells proliferation and angiogenesis. Unfortunately, angiogenesis is also necessary for cancerous tumors to keep growing and spreading. LL37 has emerged as a novel modulator of tumor growth and metastasis in carcinogenesis in ovarian, lung, breast and prostate cancers [106-109]. In fact, LL37 expression is upregulated in these tumors. However, in gastric cancer, the peptide inhibits cell proliferation [110]. LL37 also exhibits antitumor effects on epidermoid carcinoma cells, suggesting that the effect of LL37 on cancer phenotypes depends on the tissue origin of the tumor [111]. Although LL37 is demonstrated to promote cancer metastasis, fragments of the peptide have tumor inhibitory effects. These findings suggest that the fragments of LL37 have the potential to be developed into anticancer agents [112]. As an alternative strategy, LL37 has been shown to enhance the antitumor effects induced by CpG oligodeoxynucleotides (ODNs) through stimulating the tumor-suppressing activity of natural killer cells [113, 114]. Unmethylated CpG sites can be detected by TLR-9 on pDCs and B lymphocytes in humans to detect intracellular viral, fungal, and bacterial pathogen DNA, resulting in the secretion of proinflammatory cytokines, which may contribute to the immune response to the pathogen [115]. The potent immunostimulatory effects of TLR-9 activation by CpG ODNs have resulted in enormous interest in the potential uses of these TLR-9 agonists as adjuvants for clinical therapy [116]. Hurtado and Peh reported that LL37 promotes the rapid sensing of CpG ODNs by B lymphocytes and pDCs but not T cells. These results are relevant to contemporary studies of TLR9 agonists as adjuvants for vaccines and cancer therapy [117].

3. Conclusion

LL37 is a multifunctional host defense peptide. Besides its antimicrobial activity, it stimulates a complex group of responses in many cells, either directly or through modulation of cellular responses to microbial compounds and other immune mediators. A better understanding of the biological activity of LL37 and its interactions with other immune mediators will permit to create strategies and opportunities for therapeutic intervention in infectious and inflammatory diseases.

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