

## Cathelicidin LL-37: A Multitask Antimicrobial Peptide

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**Abstract** The antimicrobial peptide LL-37 is the only known member of the cathelicidin family of peptides expressed in humans. LL-37 is a multifunctional host defense molecule essential for normal immune responses to infection and tissue injury. LL-37 peptide is a potent killer of different microorganisms with the ability to prevent immunostimulatory effects of bacterial wall molecules such as lipopolysaccharide and can therefore protect against lethal endotoxemia. Additional reported activities of LL-37 include chemoattractant function, inhibition of neutrophil apoptosis, and stimulation of angiogenesis, tissue regeneration, and cytokine release (e.g. IL-8). Cellular production of LL-37 is affected by multiple factors, including bacterial products, host cytokines, availability of oxygen, and sun exposure through the activation of CAP-18 gene expression by vitamin D<sub>3</sub>. At infection sites, the function of LL-37 can be inhibited by charge-driven interactions with DNA and F-actin released from dead neutrophils and other cells lysed as the result of inflammation. A better understanding of LL-37's biological properties is necessary for its possible therapeutic application for immunomodulatory purposes as well as in treating bacterial infection.

**Keywords** Antibacterial agents · Bacteria · Infection · hCAP-18

### Antimicrobial Activity of LL-37

The ability of all animals to survive in their environment relies on continuous defense against microorganisms; when this defense is overcome, infection and death can result. Before the availability of antibiotics, infectious diseases were the dominant cause of human death. Vertebrate defense against invasive microbes is provided by a well-developed immunological system. However, in invertebrates this defense is mostly assured by the function of small antibacterial molecules present on the external surface of the animal. Infections of the human eye are rarely observed although the access of immunological competent cells circulating in blood to this compartment is limited. It is believed that protection of the eyes is mostly ensured by the epithelial production of different antibacterial agents. These facts indicate that evolutionarily ancient antibacterial peptides present on the external surface of potentially all living animals possess a bactericidal mechanism that can be used against most microbes and that this mechanism is general to the extent that for millions of years, pathogens were unable to develop effective resistance.

Up to now, more than 30 different antimicrobial peptides (AMPs) have been described in different compartments of the human body, including cathelicidin (LL-37) peptide. LL-37 (NH<sub>2</sub>-LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES-COOH) is a positively charged molecule (+6 at pH ~7.4) with a high content of basic and hydrophobic amino acids. The structure of LL-37 peptide

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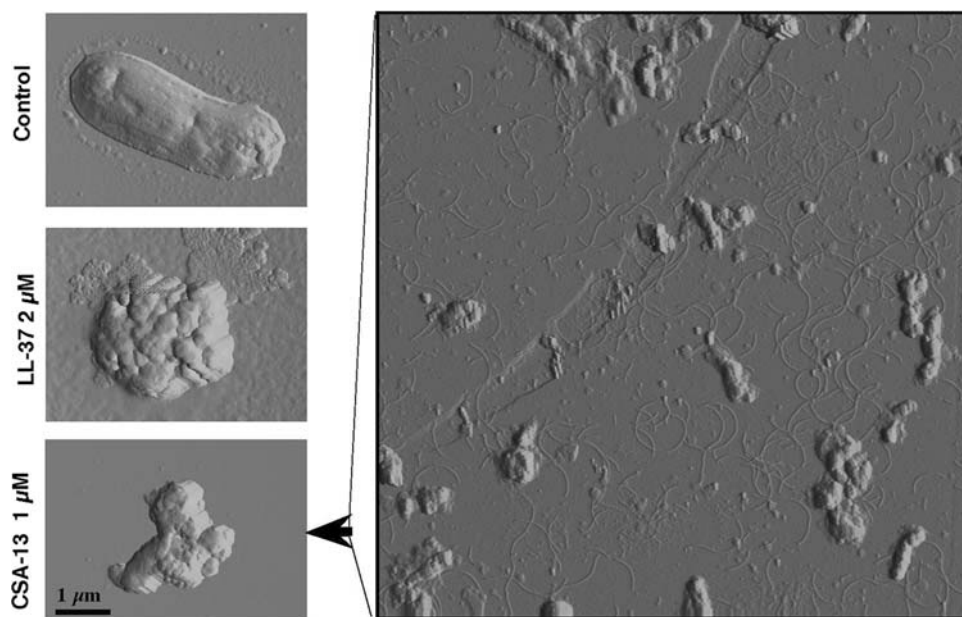
in aqueous solution is relatively disordered, but can switch to an  $\alpha$ -helix upon contact with the bacterial wall (Nijnik and Hancock 2009). LL-37 has shown a broad spectrum of activity against both Gram-negative and Gram-positive bacteria, various viruses, and fungi. In healthy individuals, the antimicrobial function of LL-37 is effective at sites of its epithelial expression at a physiological concentration of approximately 2  $\mu\text{g}/\text{ml}$  (Bals et al. 1999; Schaller-Bals et al. 2002), which may increase two- to threefold during infection. As the antibacterial activity of LL-37 is inhibited in the presence of blood plasma (Bucki et al. 2004), it is unlikely that this antibacterial molecule will kill bacteria in settings other than epithelial surfaces or the phagolysosomes of neutrophils and macrophages (Brogden 2005). In humans, LL-37, in cooperation with other cationic antibacterial peptides (CAPs), prevents infection in pulmonary and digestive systems (Bals et al. 1998, 1999; Chung et al. 2007; Wah et al. 2006), the genitourinary system (Andersson et al. 2002; Smeianov et al. 2000), esophageal tissue (Hosaka et al. 2008), salivary glands (Murakami et al. 2002a; Woo et al. 2003), skin (Frohm et al. 1997; Kim et al. 2005; Murakami et al. 2002b), and ocular surfaces (Kumar et al. 2007).

LL-37 exhibits potent activity against different bacterial strains, including *Escherichia coli* (Smeianov et al. 2000), *Pseudomonas aeruginosa* (Travis et al. 2000), *Klebsiella pneumoniae* (Smeianov et al. 2000), *Staphylococcus aureus*, methicillin-resistant *S. aureus* (Travis et al. 2000), and *Neisseria gonorrhoeae* (Bergman et al. 2005). LL-37's bactericidal activity involves membrane disruption (pore formation, change of lipid packing and organization) following interaction with negatively charged bacterial molecules and insertion into the membrane (Fig. 1).

Additionally, LL-37 inhibits the formation of *P. aeruginosa* bacterial biofilms (Overhage et al. 2008), a pattern of bacterial growth associated with antibiotic resistance. This occurs at a very low LL-37 concentration (0.5  $\mu\text{g}/\text{ml}$ ), far below that required to kill or inhibit *P. aeruginosa* growth (MIC = 64  $\mu\text{g}/\text{ml}$ ). LL-37 affects biofilm formation by decreasing the attachment of bacterial cells, stimulating twitching motility, and influencing two major quorum-sensing systems (Las and Rhl), leading to the down-regulation of genes essential for biofilm development (Overhage et al. 2008). LL-37 may also have an important role in the innate immune response against *Mycobacterium tuberculosis*, as concluded from the observation that mycobacterial infection induced the expression and production of LL-37 in A549 epithelial cells, alveolar macrophages, neutrophils, and monocyte-derived macrophages following stimulation of the Toll-like receptors TLR-2, TLR-4, and TLR-9. This last TLR was strongly activated by *M. tuberculosis* DNA (Liu et al. 2006; Rivas-Santiago et al. 2008). Additionally, it was found that the MEK1/2 and p38 MAPK signaling pathways play a critical role in the regulation of inducible LL-37 gene expression in A549 cells infected with *Mycobacterium bovis* bacillus Calmette-Guerin, the world's most widely used tuberculosis vaccine (Mendez-Samperio et al. 2008).

It was also recently proposed that cathelicidins produced by glia and other cells may play an important role in the innate immune response against pathogens in the central nervous system during bacterial infections (Brandenburg et al. 2008). A potential antiviral effect of LL-37 is suggested by the finding that treatment of human leukocytes with neutralizing antibodies directed against LL-37 significantly reduces the anti-cytomegalovirus (CMV) effect

**Fig. 1** Morphology of *Helicobacter pylori* visualized using AFM after LL-37 and ceragenin CSA-13 treatment (cholic-acid mimic of CAPs). The bacteria are a clinical isolate from human gastric biopsy. AFM micrographs indicate that both LL-37 and CSA-13 cause membrane disruption and blebbing, which potentially compromises its permeability, original lipid packing, and their arrangement



of LTB<sub>4</sub>, a potent lipid mediator of inflammation that possesses antiviral activities. This finding suggests that LTB<sub>4</sub>-mediated defense against CMV infection involves the activation of the high-affinity LTB<sub>4</sub> receptor (BLT1) and neutrophil degranulation (Gaudreault and Gosselin 2007). HIV-1 replication in peripheral blood mononuclear cells (PBMCs), including primary CD4<sup>+</sup> T cells, was also compromised in the presence of LL-37. This inhibition was subsequently reproduced using various HIV-1 isolates without detectable changes in the target cell expression of HIV-1 chemokine receptors. Accordingly, the HIV-1 inhibitory effect was shown to be independent of FPRL-1 signaling. Given the epithelial expression of LL-37, this peptide may contribute to the local protection against HIV-1 infection (Bergman et al. 2007). Evaluation of human specimens from patients infected with papillomavirus (condyloma acuminatum or verruca vulgaris) indicates that the immunological response to those infections involves a change in LL-37 peptide expression, which may represent a significant step in the pathogenesis of those disorders (Conner et al. 2002). All together, these findings indicate a possible application of LL-37 peptides in the treatment of CMV, HIV, and HPV infections.

The antimicrobial spectrum of the human cathelicidin LL-37 also includes fungicidal activity against *Candida albicans*, but such an action is dependent on culture conditions indicating that LL-37 may be most effective against *C. albicans* as a superficial barrier to its invasion (den Hertog et al. 2006; Lopez-Garcia et al. 2005).

### **LL-37 Inhibits The Immunostimulatory Activities of Bacteria-Derived Molecules**

The mechanism of the antibacterial action of CAPs has not been fully explored, but it involves a direct charge-driven interaction between the positive and negative charges of CAPs and bacterial wall molecules, respectively. The existence of such interaction may have a more important significance besides its involvement in the bacteria-killing mechanism, as bacterial wall molecules, upon their release from dead bacteria, create a strong immunostimulatory activity that can cause life-threatening inflammatory disorders. Accordingly, CAPs' ability to kill bacteria is as important as their ability to prevent toxicity of the bacterial wall molecules released upon bacterial death. Lipopolysaccharide (LPS)- and LTA-neutralizing activities of cathelicidin peptides were observed in a study with different cells, including a CD14<sup>+</sup> murine macrophage cell line (RAW264.7) and the murine endotoxin-shock model. More precisely, flow cytometric analysis revealed that the cathelicidin LL-37 inhibited the binding of FITC-conjugated LPS to RAW264.7 cells in addition to suppressing

LPS-induced tumor necrosis factor (TNF)- $\alpha$  mRNA and protein expression (Nagaoka et al. 2001). A strong binding of LL-37 peptide to LPS accompanied by aggregate formation prevents LPS from binding to the carrier protein, LPS-binding protein, or, alternatively, to its receptor and hence inhibits cytokine secretion (Rosenfeld et al. 2006). Microarray studies established a temporal transcriptional profile and identified differentially expressed genes in LPS-stimulated monocytes in the presence or absence of LL-37 (Mookherjee et al. 2006a). Human monocyte stimulation with 10 ng/ml LPS led to the up-regulation of 125 genes, 106 of which were suppressed in the presence of 5  $\mu$ g/ml LL-37. Consistent with these observations, LL-37 suppresses the LPS-induced translocation of the NF- $\kappa$ B subunits p50 and p65 into the nucleus of monocytes and epithelial cells and inhibits the activation of dendritic cells (DCs) with bacterial TLR ligands (Mookherjee et al. 2006b). Monocyte-derived DCs stimulated by TLR ligands such as LPS, lipoteichoic acid, and flagellin in the presence of LL-37 released lower amounts of interleukin (IL)-6, IL-12, and TNF- $\alpha$  and their surface expressions of HLA-DR, CD80, CD83, CD86, and the chemokine receptor CCR7 decreased (Kandler et al. 2006). Additionally, LL-37 inhibited LPS-induced vascular nitric oxide production (Ciornei et al. 2005) and the antibacterial activity of LL-37 peptide was compromised after addition of exogenous LTA and LPS (Bucki and Janmey 2006), indicating that this interaction results in the loss of activity of both components. All the above data support the idea that in vitro LL-37 interaction with LPS or LTA alters the aggregation state of amphipathic endotoxins, and this effect may provide an explanation for the observed inhibition of their immunostimulatory ability.

These data also motivated several studies to evaluate whether LL-37's ability to inactivate bacteria wall membrane toxicity in vitro can be reproduced in animals challenged with treatments that induce sepsis. The most intriguing hypothesis of those studies is that LL-37 can prevent LPS packing into the most toxic cubic or hexagonal phases (Brandenburg et al. 2003) or may interfere with inflammatory cell signaling pathways, acting as an agonist of formyl peptide receptors (fMLP) (Tjabringa et al. 2006) or a modulator of purinergic P2X7 (Pochet et al. 2006) and epidermal growth factor receptors (EGFR) (Tokumaru et al. 2005). Indeed, LL-37 therapy improved outcome in a rat model of peritoneal contamination and infection with human stool (to simulate clinical conditions of peritonitis in humans) (Torossian et al. 2007) as well as in rats given an intraperitoneal injection of *E. coli* 0111:B4 LPS, injection of live stock of *E. coli* ATCC 25922, or intra-abdominal sepsis induced via cecal ligation and puncture (Cirioni et al. 2006). The combination of LL-37 and granulocyte colony-stimulating factors (G-CSF) was also

very effective in protecting neutropenic mice from *P. aeruginosa* (ATCC27853)-induced sepsis (Cirioni et al. 2008). All these studies suggest that LL-37 may become an important consideration for the future treatment of septic shock, otherwise associated with a high rate of human death, for which there is yet no effective treatment once the complex inflammatory pathways stimulated by bacterial wall molecules are activated (Bucki et al. 2005, 2008a; Cinel and Opal 2009).

In one recent report, endothelium-dependent relaxation mediated by the release of nitric oxide and endothelium-derived hyperpolarizing factor after LL-37 activation of the lipoxin A4 receptor (ALX, FPRL1) was observed in human omental veins (Berkestedt et al. 2008). This observation, according to the authors of that study, indicates that LL-37 released from white blood cells is a factor that contributes to blood vessel dilatation during sepsis. Therefore treatment with ALX antagonists might be proposed to interfere with the vasodilatation that occurs in patients with bacterial sepsis. It might be expected that this “anti-LL-37 therapy” will not interfere with LL-37’s ability to inactivate LPS or LTA in the extracellular space, but may interfere with some LL-37 immunomodulatory activity based on activation of cell membrane receptors.

### LL-37 Regulates Some Functions of Immunocompetent Cells

In addition to its bacteria-killing and LPS/LTA-scavenging ability, LL-37 peptide can orchestrate the function of various white blood cells, whose functions are tightly associated with host immune defense. LL-37 peptide interferes with the lifespan and inflammatory functions of the most common granulocyte subtype present in blood, the neutrophil. LL-37 inhibits human neutrophil apoptosis, signaling through P2X7 receptors and G-protein-coupled receptors. Such a process involves modulation of anti-apoptotic protein Mcl-1 expression, inhibition of the proapoptotic Bcl-2 protein BID that regulates mitochondrial membrane potential, and procaspase-3 cleavage, in addition to activation of the phosphatidylinositol-3 kinase pathway (Barlow et al. 2006). Suppression of neutrophil apoptosis may be advantageous for host defense against bacterial invasion (Nagaoka et al. 2006). On the other hand, LL-37 may decrease neutrophil lifespan by interfering with the pathway of secondary necrosis. Rapid conversion of apoptotic neutrophils (annexin V-positive/propidium iodide-negative cells with exposed negatively charge phosphatidylserine molecules in the external plasma membranes) into necrotic neutrophils (propidium iodide-positive cells with the release of IL-8, IL-1R antagonist, ATP, and intact granules) was observed in the presence of

LL-37. The effects of LL-37 on apoptotic neutrophils are neither energy dependent nor affected by pretreatment with the principal cytokines involved in granulopoiesis and differentiation of granulocytic precursors such as G-CSF, granulocyte-macrophage CSF (GM-CSF), and TNF- $\alpha$ . This effect was, however, decreased in the presence of blood plasma (Zhang et al. 2008). Taken together, this suggests that apoptotic neutrophils with negatively charged PS molecules in the external leaflet of their plasma membrane may be targets for LL-37 and membrane insertion, resulting in dysfunction of the membrane, leading to neutrophil necrosis.

LL-37 is also a potent modulator of monocyte differentiation into DCs, bridging innate and adaptive immune responses. LL-37-derived DCs displayed significantly up-regulated endocytic capacity, modified phagocytic receptor expression and function, enhanced secretion of T helper (Th)-1-inducing cytokines [T cell responses toward Th-1 differentiation by inducing IL-12 secretion from CD8<sup>+</sup> DCs (Skokos and Nussenzweig 2007)], and promoted Th-1 responses in vitro (Davidson et al. 2004). Exposure of DCs to LL-37 during LPS stimulation induced co-cultured native T cells to produce less IL-2 and interferon (IFN)- $\gamma$  and decreased their proliferation. The response of memory T cells to recall antigen was also decreased. In conclusion, these data demonstrate that the AMP LL-37 suppresses the maturation and activation of DCs by TLR ligands. (Kandler et al. 2006). LL-37 may also affect immature DCs derived from human peripheral blood monocytes following its internalization and subsequent localization in the cytoplasmic and nuclear compartments, causing phenotypic changes characterized by an increased expression of the antigen-presenting molecule HLA-DR and the co-stimulatory molecule CD86 (Bandholtz et al. 2006). Overall, LL-37 may be an attractive therapeutic candidate for manipulating T cell polarization by DCs.

Cathelicidins were also found to activate cell chemotaxis, which may contribute to innate and adaptive immunity by recruiting neutrophils, monocytes, eosinophils, and T cells to sites of microbial infection (De et al. 2000; Khine et al. 2006; Koczulla and Bals 2003). The mechanism of actin-binding protein-induced chemotaxis is potentially important in vivo because the chemotactic activity of LL-37, unlike its antibacterial action (Johansson et al. 1998), was not significantly inhibited by the presence of human serum (De et al. 2000). Another example of LL-37’s ability to affect cell function involved in immunological response is provided by a study in which LL-37 coexisting with bacterial components in the skin was able to switch mast cell function and direct human mast cells toward innate immunity (Yoshioka et al. 2008) and enhanced the proliferation and activation of NK cells after treatment in combination with a TLR9 activator, CpG

oligodeoxynucleotides (Chuang et al. 2009). All these observations indicate that LL-37 peptide is a factor that controls the activation of innate immunity. Indeed, deficiency in salivary LL-37 antimicrobial peptide has been implicated in periodontitis in patients with morbus Kostmann syndrome (infantile genetic agranulocytosis) (Koczulla and Bals 2003).

### LL-37 Regulation of Angiogenesis and Cell Growth

An inflammatory response at infection sites usually results in tissue damage that eventually switches on the mechanisms necessary to restore tissue homeostasis. LL-37 is an important molecule involved in tissue healing processes, especially revascularization and cell growth. LL37 induces angiogenesis mediated by formyl peptide receptor-like 1 expressed on endothelial cells. Application of LL-37 resulted in neovascularization in the chorioallantoic membrane assay and in a rabbit model of hind-limb ischemia (Koczulla et al. 2003). LL-37 directly activates endothelial cells, resulting in increased proliferation and the formation of vessel-like structures. Decreased vascularization during wound repair in mice deficient in the murine homologue of LL-37/hCAP-18 (CRAMP), shows that cathelicidin-mediated angiogenesis is important for cutaneous wound neovascularization in vivo (Koczulla et al. 2003). LL-37 induces wound healing, proliferation, and migration of airway epithelial cells, indicating that the peptide is likely involved in the regulation of tissue homeostasis in the airways (Shaykhiev et al. 2005). Analysis of expression profiles of human epithelial host defense peptides in burned and unburned skin tissue samples showed higher mRNA levels in partial-thickness burns than in unburned tissue (Kaus et al. 2008). In situ hybridization revealed the expression of hCAP-18/LL-37, hBD2, and hBD3 at the surface of burns that was independent of burn depth. However, the finding of higher host defense peptide gene expression rates does not correlate with the incidence of wound infection in burns (Kaus et al. 2008), indicating that the modified LL-37 expression contributes to the healing response rather than effective prevention of bacterial infection. In vitro, human cathelicidin stimulates the migration of the human keratinocyte cell line HaCaT that involves changes in actin dynamics and is associated with augmented tyrosine phosphorylation of proteins involved in focal adhesion complexes, such as focal adhesion kinase and paxillin. Other events involved in the LL-37 response were the induction of the Snail and Slug transcription factors, activation of matrix metalloproteinases, and activation of the mitogen-activated protein (MAP) kinase and phosphoinositide 3-kinase/Akt signaling pathways

(Carretero et al. 2008; Chamorro et al. 2009; Tokumaru et al. 2005). These signaling events could be mediated not only through the transactivation of EGFR, but also through the induction of G-protein-coupled receptor FPRL-1 expression in these cells. After in vivo adenoviral transfer of the AMP to excisional wounds in ob/ob mice, LL-37 significantly improved re-epithelialization and granulation tissue formation.

The protective and regenerative activities of LL-37 support its therapeutic potential to promote wound healing (Carretero et al. 2008; Tokumaru et al. 2005). In addition, LL-37 induced phosphorylation of EGFR, signal transducer and activator of transcription STAT1, and STAT3, which are intracellular signaling molecules involved in keratinocyte migration and proliferation (Niyonsaba et al. 2007). LL-37 can directly act on dermal fibroblasts and may have antifibrotic action during the wound repair process. In human dermal keloids, fibrosis was inversely related to the expression of cathelicidin, which was found to affect the expression of types I and III collagen genes in dermal fibroblasts (Park et al. 2009).

LL-37 peptide protects primary human keratinocytes from apoptosis induced by the topoisomerase I inhibitor camptothecin, which activates caspase-3. Expression profiling of keratinocytes treated with LL-37 identified the upregulation of cyclooxygenase-2 (COX-2) expression, which might act as an inhibitor of apoptosis (IAP) by increasing prostaglandin E-2 production. LL-37 also induced the expression of IAP-2, implicated in the COX-2/prostaglandin E-2 anti-apoptotic pathway. Pretreatment with a selective COX-2 inhibitor abolished the anti-apoptotic effect of LL-37 and reduced IAP-2 expression. This finding suggests that the anti-apoptotic effect of LL-37 in keratinocytes is mediated by a COX-2-dependent mechanism involving IAP-2 (Chamorro et al. 2009).

Considering that LL-37's bacterial-killing primarily occurs by depolarizing and permeabilizing bacterial plasma membranes and/or induced autolysis (Ginsburg 2004), it was suggested that similar mechanisms of action can be used to target the disruption of cancer cells (Li et al. 2006). Unexpectedly, in a series of breast carcinomas it was found that hCAP18/LL-37 was strongly expressed in the tumor cells and not in the adjacent stroma, suggesting that hCAP18/LL-37 may provide a growth advantage for the tumor cells. Indeed, human epithelial cell lines treated with synthetic biologically active LL-37 peptide show a significant increase in cell proliferation. Transgenic expression of hCAP18 in two different human epithelial cell lines increased proliferation of both cell types (Heilborn et al. 2005). hCAP-18/LL-37 is significantly over-expressed in ovarian tumors, suggesting that LL-37 might contribute to ovarian tumorigenesis through direct stimulation of tumor cells, initiation of angiogenesis, and/or recruitment of



immune cells (Coffelt et al. 2008). Similarly, LL-37/hCAP-18 acts as a growth factor for human lung cancer cells (von Haussen et al. 2008). These findings do not support the hypothesis that LL-37 has an antitumor effect, but rather suggest that hCAP18/LL-37 may promote tumor cell growth (Coffelt and Scandurro 2008; Coffelt et al. 2008; Heilborn et al. 2005; von Haussen et al. 2008).

### LL-37-Mediated Cytokine Release

LL-37 modulates cellular immune responses by stimulation of chemokine production, particularly IL-8. Such an effect is observed in myeloid and epithelial cells (Khine et al. 2006) and it increases neutrophil host defense functions at inflamed and infected sites. LL-37-enhanced production of IL-8 is under the control of MAPK p38 and extracellular signal-regulated kinase (ERK), as evidenced by the effects of p38 and ERK1/2 inhibitors on LL-37-mediated IL-8 production (Khine et al. 2006; Zheng et al. 2007). In human PBMCs, LL-37 synergistically enhanced the IL-1 $\beta$ -induced production of IL-6, IL-10, and the macrophage chemoattractant proteins MCP-1/MCP-3, indicating a role in enhancing certain innate immune responses. Similarly, LL-37 synergistically enhanced chemokine production in the presence of GM-CSF, but IFN- $\gamma$ , IL-4, or IL-12 addition led to antagonism, indicating that the role of LL-37 in reinforcing specific immune responses is selective and restricted to particular endogenous immune mediators. LL-37 increased the level of TLR4 mRNA and TLR4 protein and induced the release of IL-4, IL-5, and IL-1 $\beta$  from skin mast cells which are involved in the innate immune system response against microbial infections via Toll-like receptors (Yoshioka et al. 2008). Human airway smooth muscle cells also respond to the AMP LL-37 by releasing IL-8, suggesting that LL-37 is a regulator of the inflammatory process in various inflammatory lung diseases by enhancing IL-8 production (Zuyderduyn et al. 2006). Together, those results indicate that the LL-37 acts as a human host defense peptide that can work in synergy with the endogenous inflammatory mediator to enhance the induction of specific inflammatory effectors by a complex mechanism involving multiple pathways, thus reinforcing certain innate immune responses (Yu et al. 2007).

### Regulation of LL-37 Cellular Expression

As a baseline defense mechanism, some cells constitutively produce a range of CAPs, including LL-37. However, the expression of this peptide is up-regulated by a variety of stimuli, such as pro-inflammatory cytokines, growth factors, nutrients, and bacterial products, especially at inflammation

and repair sites. The active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH) $_2$ D), is a potent inducer of the antimicrobial protein cathelicidin (Adams et al. 2007; Liu et al. 2006; Nijnik and Hancock 2009; Zasloff 2006). In macrophages and trophoblastic cells, this response is dependent on the intracrine synthesis of 1,25(OH) $_2$ D from the precursor 25-hydroxyvitamin D (25OHD) (this precursor originates from sunlight-mediated conversion of 7-dehydrocholesterol in the skin), catalyzed by the enzyme 25-hydroxyvitamin D-1- $\alpha$ -hydroxylase (CYP27B1) (Liu et al. 2006). Potentially, certain drugs such as ritonavir (HIV-protease inhibitor) and ketokonazole (antifungal), which causes CYP27B1 inhibition, may result in a decreased antimicrobial capacity (Cozzolino et al. 2003; Zasloff 2006). Macrophage differentiation triggered by TLR2/1-induced IL-15 was required for the induction of CYP27B1, vitamin D receptor, and downstream AMP LL-37 production [in agreement with an earlier study showing that specific inhibition of Cyp27B1 blocked TLR2/1L activation of cathelicidin mRNA in monocytes (Liu et al. 2006). 1,25(OH) $_2$ D boosts levels of LL-37 in keratinocytes in tissue culture and after topical administration onto the skin of human subjects (Mallbris et al. 2005). As sunlight, especially within the UVB spectrum, induces immunosuppression (a UVB-mediated increase in  $\alpha$ -MSH synthesis results in inhibited activation of NF- $\kappa$ B in a wide number of tissues) and makes us more vulnerable to infection, 1,25(OH) $_2$ D, potentially to balance this effect, stimulates the synthesis of LL-37 in skin and circulating phagocytic cells (Zasloff 2005). This physiological mechanism may provide a more accurate explanation for the effective treatment of skin tuberculosis (lupus vulgaris) with the use of high-intensity light produced by an electric arc lamp, as first described by Niels Finsen of Denmark in 1895, who was awarded a Nobel Prize in 1903 for this finding (Zasloff 2006). However, it might be concluded that optimal function of our innate immune system requires some necessary amount of vitamin D.

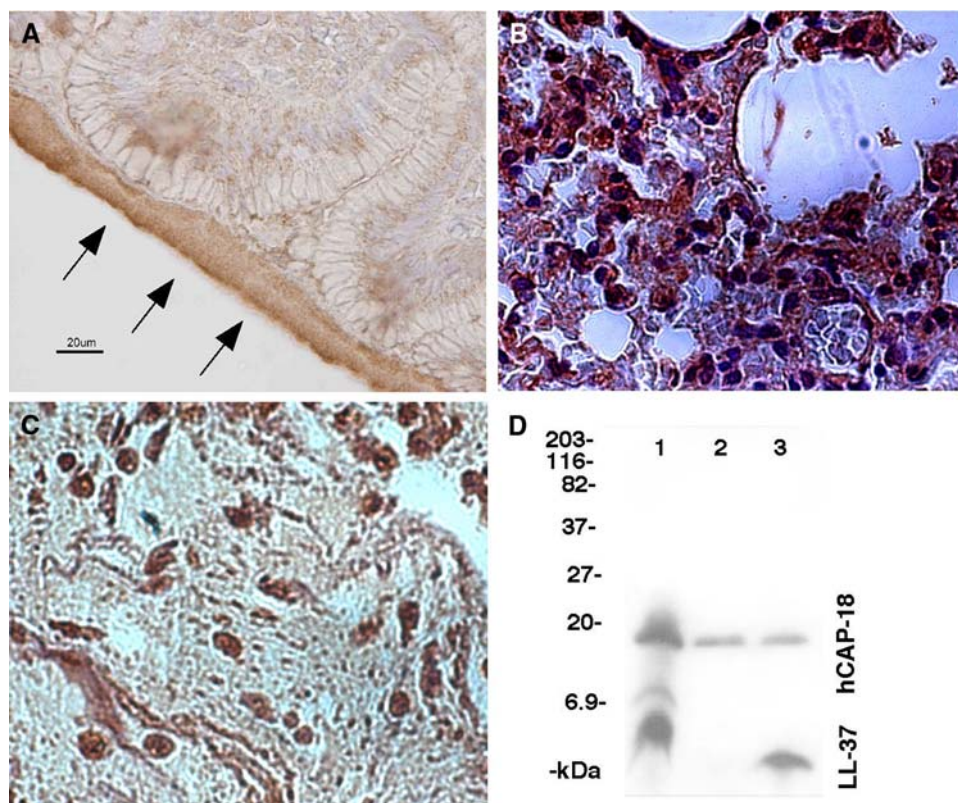
Hypoxia-inducible transcription factor 1 $\alpha$  (HIF1 $\alpha$ ), should also be included on the list of factors that govern LL-37 cellular expression. A recent study shows that selective inactivation of HIF1 $\alpha$  (siRNA knockdown) correlates with decreasing LL-37 expression in myeloid cells and keratinocytes (Peyssonnaud et al. 2008). LL-37 cellular expression was also found to be regulated by *P. aeruginosa* flagellin recognized by TLR-5 in human corneal epithelial cells (Kumar et al. 2007), fungal allergens in nasal tissue from chronic rhinosinusitis patients (Ooi et al. 2007), and *Helicobacter pylori* in gastric epithelium (Hase et al. 2003), suggesting that this cathelicidin contributes to determining the balance between the host mucosal defense and the pathogen survival mechanisms that usually govern the chronic infection process. On the

other hand, *N. gonorrhoeae*, which preferentially attaches to and invades epithelial cells of the genital tract, was found to down-regulate the expression of LL-37 (confirmed at both the peptide and transcriptional levels), indicating that pathogenic *Neisseria* may gain a survival advantage in the female genital tract by decreasing LL-37 expression (Bergman et al. 2005). Similarly, cholera toxin (CT) and labile toxin, the major virulence proteins of *Vibrio cholerae* and enterotoxigenic *E. coli*, respectively, are predominantly responsible for the suppression of LL-37 and HBD-1 expression in intestinal epithelial cells both in vitro and in vivo. CT transcriptionally down-regulates the AMPs by activating several intracellular signaling pathways involving protein kinase A, ERK MAP kinase, and COX-2 downstream of cAMP accumulation, and inducible cAMP early repressor may mediate this role of CT, at least in part (Chakraborty et al. 2008). Transcriptional repression of LL-37 peptide was also observed in biopsies from patients with bacillary dysenteries (*Shigella spp.* infections) and it was mediated by bacterial DNA involvement (Islam et al. 2001). Together, these findings indicate that

the regulation of LL-37 expression at the transcriptional level may be successfully modulated and directed to its increase or decrease.

### LL-37 Antibacterial Activity in the Presence of Charged Biopolymers

The counterion condensation around DNA and F-actin released from dead neutrophils and other cells lysed as a result of inflammation at infection sites promotes the binding of larger-valence CAPs over smaller-valence ions, and when a critical concentration of CAPs is present at sufficiently high concentrations of polyvalent counterions, the polyelectrolytes collapse into bundles that trap both polyelectrolytes and CAPs in dense, stable structures. Large bundles containing both DNA/F-actin and LL-37 peptide, as demonstrated in Fig. 2, are a very common feature of CF sputum (Bucki et al. 2007a; Sheils et al. 1996) and can be expected to form at other sites where purulent infection with large neutrophil accumulation takes



**Fig. 2** Immunohistochemical staining of **a** gastric mucosa specimen, **b** rat lungs, and **c** CF sputum with anti-hCAP-18/LL-37 antibody. The sections were incubated with primary antibody, washed with 1% PBSA, and subjected to binding with secondary antibody (biotinylated goat anti-Rabbit IgG). Amplification was performed with a Vectastain ABC kit and an horseradish peroxidase (HRP) detection system was used to colocalize peroxidase activity with a DAB substrate. The sections were then counterstained with hematoxylin.

The high intensity of the DAB signal suggests that LL-37 and hCAP18 are present in high concentration in the mucins layer covering the gastric mucosa, airway fluid of alveoli, and the bundle structure present in CF sputum. **d** Immunoblotting analysis of LL-37 peptide in CF sputum before (*lane 1*) and after centrifugation in supernatant without treatment of the original sample (*lane 2*) and the sample treated with (*lane 3*) DNase I (30 µg/ml) and poly-aspartate (50 µM)

place. CAPs with many positive charges are able to overcome the strong electrostatic repulsion between DNA/F-actin filaments that cannot be overcome by the physiological concentrations of monovalent and divalent ions (Tang and Janmey 1996). The ability of negatively charged biopolymers to inactivate CAP activity may represent an important issue for the appropriate function of naturally produced CAP as well as for the practical use of exogenous, positively charged synthetic peptides or their mimics. For this reason, addition or over-expression of CAPs at infection sites may be ineffective if compromised by DNA, F-actin, or other anionic polyelectrolytes.

Depolymerization of these polymers can overcome these environmental factors. Indeed, with use of recombinant DNase I and recombinant human plasma gelsolin (rhpGSN) it was shown that in solubilized CF sputum, the bacterial load decreases (Tang et al. 2005; Weiner et al. 2003), indicating recovery of CAP function due to their release from bundle structures. This suggestion was further supported by a study in which increasing amounts of LL-37 peptide were observed in the supernatant of CF sputum samples treated with DNase or its combination with rhpGSN (Bucki et al. 2007a, 2007b). Addition of poly-anions, such as poly-aspartate and poly-glutamate (Tang et al. 2005), represents an intriguing possibility as an alternative to this specific treatment with DNase and gelsolin. Interestingly, the presence of polyanionic polymers at the site of infection may also cause changes in pathogenic organisms that grow in such an environment. By changing the bacterial pattern of growth from planktonic to biofilm, they may become less sensitive to CAPs' killing action (Walker et al. 2005). In human colonic cells, LL-37 can directly stimulate mucus synthesis through activation of MUC1 and MUC2 expression and the MAP kinase pathway. Real-time PCR data showed that the addition of LL-37 induced more than a 50% increase in MUC1 and MUC2 mRNA levels. Treatment with MUC1 and MUC2 siRNAs normalized the stimulatory action of LL-37 on mucus synthesis. LL-37 also activated the phosphorylation of MAP kinase in the cells. A specific inhibitor of the MAP kinase pathway, U0126, completely blocked the increase in MUC1 and MUC2 expression as well as mucus synthesis by LL-37 (Tai et al. 2008). On the other hand, the antibacterial activity of LL-37 peptide was found to decrease in the presence of mucins (Bucki et al. 2008b).

## Conclusion

Our understanding of the biological importance of cationic AMPs, including the cathelicidin LL-37, has significantly increased in the past decade. It is now well established that

LL-37 can act as an antibacterial and immunomodulatory factor by altering membrane dynamics, binding to intracellular targets, and directly activating transmembrane receptors. Some natural antibacterial peptides, including LL-37, or their synthetic analogs, such as pexiganan acetate (Lamb and Wiseman 1998), which have gone to clinical trial, will likely provide a new option of treatment against infections. A complex array of responses in many immunocompetent cells upon LL-37 treatment may also provide a new option for immunomodulatory use of this molecule. As a result of its activity on keratinocytes, LL-37 may be used as a pleiotropic agent against infection and as a wound healing enhancer in situations such as severe burn wound or skin transplant (Thomas-Virnicig et al. 2009). This new path for LL-37 therapeutic intervention deserves future investigation.

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## References

- Adams JS, Liu PT, Chun R et al (2007) Vitamin D in defense of the human immune response. *Ann N Y Acad Sci* 1117:94–105
- Andersson E, Sorensen OE, Frohm B et al (2002) Isolation of human cationic antimicrobial protein-18 from seminal plasma and its association with prostasomes. *Hum Reprod* 17:2529–2534
- Bals R, Wang X, Zasloff M et al (1998) The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. *Proc Natl Acad Sci USA* 95:9541–9546
- Bals R, Weiner DJ, Meegalla RL et al (1999) Transfer of a cathelicidin peptide antibiotic gene restores bacterial killing in a cystic fibrosis xenograft model. *J Clin Invest* 103:1113–1117
- Bandholtz L, Ekman GJ, Vilhelmsson M et al (2006) Antimicrobial peptide LL-37 internalized by immature human dendritic cells alters their phenotype. *Scand J Immunol* 63:410–419
- Barlow PG, Li Y, Wilkinson TS et al (2006) The human cationic host defense peptide LL-37 mediates contrasting effects on apoptotic pathways in different primary cells of the innate immune system. *J Leukoc Biol* 80:509–520
- Bergman P, Johansson L, Asp V et al (2005) Neisseria gonorrhoeae downregulates expression of the human antimicrobial peptide LL-37. *Cell Microbiol* 7:1009–1017
- Bergman P, Walter-Jallow L, Broliden K et al (2007) The antimicrobial peptide LL-37 inhibits HIV-1 replication. *Curr HIV Res* 5:410–415
- Berkestedt I, Nelson A, Bodelsson M (2008) Endogenous antimicrobial peptide LL-37 induces human vasodilatation. *Br J Anaesth* 100:803–809
- Brandenburg K, Andra J, Muller M et al (2003) Physicochemical properties of bacterial glycopolymers in relation to bioactivity. *Carbohydr Res* 338:2477–2489
- Brandenburg LO, Varoga D, Nicolaeava N et al (2008) Role of glial cells in the functional expression of LL-37/rat cathelin-related antimicrobial peptide in meningitis. *J Neuropathol Exp Neurol* 67:1041–1054



- Brogden KA (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol* 3:238–250
- Bucki R, Janmey PA (2006) Interact ion of the Gelsolin-derived antibacterial PBP10 peptides with cell membranes and lipids bilayers. *Antimicrob Agents Chemother* 50:2932–2940
- Bucki R, Pastore JJ, Randhawa P et al (2004) Antibacterial activities of rhodamine B-conjugated gelsolin-derived peptides compared to those of the antimicrobial peptides cathelicidin LL37, magainin II, and melittin. *Antimicrob Agents Chemother* 48:1526–1533
- Bucki R, Georges PC, Espinassous Q et al (2005) Inactivation of endotoxin by human plasma gelsolin. *Biochemistry* 44:9590–9597
- Bucki R, Byfield FJ, Janmey PA (2007a) Release of the antimicrobial peptide LL-37 from DNA/F-actin bundles in cystic fibrosis sputum. *Eur Respir J* 29:624–632
- Bucki R, Sostarecz AG, Byfield FJ et al (2007b) Resistance of the antibacterial agent ceragenin CSA-13 to inactivation by DNA or F-actin and its activity in cystic fibrosis sputum. *J Antimicrob Chemother* 60:535–545
- Bucki R, Byfield FJ, Kulakowska A et al (2008a) Extracellular gelsolin binds lipoteichoic acid and modulates cellular response to proinflammatory bacterial wall components. *J Immunol* 181:4936–4944
- Bucki R, Namiot DB, Namiot Z et al (2008b) Salivary mucins inhibit antibacterial activity of the cathelicidin-derived LL-37 peptide but not the cationic steroid CSA-13. *J Antimicrob Chemother* 62:329–335
- Carretero M, Escamez MJ, Garcia M et al (2008) In vitro and in vivo wound healing-promoting activities of human cathelicidin LL-37. *J Invest Dermatol* 128:223–236
- Chakraborty K, Ghosh S, Koley H et al (2008) Bacterial exotoxins downregulate cathelicidin (hCAP-18/LL-37) and human beta-defensin 1 (HBD-1) expression in the intestinal epithelial cells. *Cell Microbiol* 10:2520–2537
- Chamorro CI, Weber G, Gronberg A et al (2009) The human antimicrobial peptide LL-37 suppresses apoptosis in keratinocytes. *J Invest Dermatol* 129:937–944
- Chuang CM, Monie A, Wu A et al (2009) Treatment with LL-37 peptide enhances the antitumor effects induced by CpG oligodeoxynucleotides against ovarian cancer. *Hum Gene Ther* 20:303–313
- Chung WO, Dommisch H, Yin L et al (2007) Expression of defensins in gingiva and their role in periodontal health and disease. *Curr Pharm Des* 13:3073–3083
- Cinel I, Opal SM (2009) Molecular biology of inflammation and sepsis: a primer. *Crit Care Med* 37:291–304
- Ciornei CD, Sigurdardottir T, Schmidtchen A et al (2005) Antimicrobial and chemoattractant activity, lipopolysaccharide neutralization, cytotoxicity, and inhibition by serum of analogs of human cathelicidin LL-37. *Antimicrob Agents Chemother* 49:2845–2850
- Cirioni O, Giacometti A, Ghiselli R et al (2006) LL-37 protects rats against lethal sepsis caused by Gram-negative bacteria. *Antimicrob Agents Chemother* 50:1672–1679
- Cirioni O, Ghiselli R, Tomasinsig L et al (2008) Efficacy of LL-37 and granulocyte colony-stimulating factor in a neutropenic murine sepsis due to *Pseudomonas aeruginosa*. *Shock* 30:443–448
- Coffelt SB, Scandurro AB (2008) Tumors sound the alarmin(s). *Cancer Res* 68:6482–6485
- Coffelt SB, Waterman RS, Florez L et al (2008) Ovarian cancers overexpress the antimicrobial protein hCAP-18 and its derivative LL-37 increases ovarian cancer cell proliferation and invasion. *Int J Cancer* 122:1030–1039
- Conner K, Nern K, Rudisill J et al (2002) The antimicrobial peptide LL-37 is expressed by keratinocytes in condyloma acuminatum and verruca vulgaris. *J Am Acad Dermatol* 47:347–350
- Cozzolino M, Vidal M, Arcidiacono MV et al (2003) HIV-protease inhibitors impair vitamin D bioactivation to 1, 25-dihydroxyvitamin D. *AIDS* 17:513–520
- Davidson DJ, Currie AJ, Reid GS et al (2004) The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. *J Immunol* 172:1146–1156
- De Y, Chen Q, Schmidt AP et al (2000) LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. *J Exp Med* 192:1069–1074
- den Hertog AL, van Marle J, Veerman EC et al (2006) The human cathelicidin peptide LL-37 and truncated variants induce segregation of lipids and proteins in the plasma membrane of *Candida albicans*. *Biol Chem* 387:1495–1502
- Frohm M, Agerberth B, Ahangari G et al (1997) The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *J Biol Chem* 272:15258–15263
- Gaudreault E, Gosselin J (2007) Leukotriene B4-mediated release of antimicrobial peptides against cytomegalovirus is BLT1 dependent. *Viral Immunol* 20:407–420
- Ginsburg I (2004) Bactericidal cationic peptides can also function as bacteriolysis-inducing agents mimicking beta-lactam antibiotics? It is enigmatic why this concept is consistently disregarded. *Med Hypotheses* 62:367–374
- Hase K, Murakami M, Iimura M et al (2003) Expression of LL-37 by human gastric epithelial cells as a potential host defense mechanism against *Helicobacter pylori*. *Gastroenterology* 125:1613–1625
- Heilborn JD, Nilsson MF, Jimenez CI et al (2005) Antimicrobial protein hCAP18/LL-37 is highly expressed in breast cancer and is a putative growth factor for epithelial cells. *Int J Cancer* 114:713–719
- Hosaka Y, Koslowski M, Nuding S et al (2008) Antimicrobial host defense in the upper gastrointestinal tract. *Eur J Gastroenterol Hepatol* 20:1151–1158
- Islam D, Bandholtz L, Nilsson J et al (2001) Downregulation of bactericidal peptides in enteric infections: a novel immune escape mechanism with bacterial DNA as a potential regulator. *Nat Med* 7:180–185
- Johansson J, Gudmundsson GH, Rottenberg ME et al (1998) Conformation-dependent antibacterial activity of the naturally occurring human peptide LL-37. *J Biol Chem* 273:3718–3724
- Kandler K, Shaykhiev R, Kleemann P et al (2006) The anti-microbial peptide LL-37 inhibits the activation of dendritic cells by TLR ligands. *Int Immunol* 18:1729–1736
- Kaus A, Jacobsen F, Sorkin M et al (2008) Host defence peptides in human burns. *Burns* 34:32–40
- Khine AA, Del Sorbo L, Vaschetto R et al (2006) Human neutrophil peptides induce interleukin-8 production through the P2Y6 signaling pathway. *Blood* 107:2936–2942
- Kim JE, Kim BJ, Jeong MS et al (2005) Expression and modulation of LL-37 in normal human keratinocytes, HaCaT cells, and inflammatory skin diseases. *J Korean Med Sci* 20:649–654
- Kocuzilla AR, Bals R (2003) Antimicrobial peptides: current status and therapeutic potential. *Drugs* 63:389–406
- Kocuzilla R, von Degenfeld G, Kupatt C et al (2003) An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. *J Clin Invest* 111:1665–1672
- Kumar A, Yin J, Zhang J et al (2007) Modulation of corneal epithelial innate immune response to pseudomonas infection by flagellin pretreatment. *Invest Ophthalmol Vis Sci* 48:4664–4670
- Lamb HM, Wiseman LR (1998) Pexiganan acetate. *Drugs* 56:1047–1052 (discussion 1053–1054)

- Li X, Li Y, Han H et al (2006) Solution structures of human LL-37 fragments and NMR-based identification of a minimal membrane-targeting antimicrobial and anticancer region. *J Am Chem Soc* 128:5776–5785
- Liu PT, Stenger S, Li H et al (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 311:1770–1773
- Lopez-Garcia B, Lee PH, Yamasaki K et al (2005) Anti-fungal activity of cathelicidins and their potential role in *Candida albicans* skin infection. *J Invest Dermatol* 125:108–115
- Mallbris L, Edstrom DW, Sundblad L et al (2005) UVB upregulates the antimicrobial protein hCAP18 mRNA in human skin. *J Invest Dermatol* 125:1072–1074
- Mendez-Samperio P, Miranda E, Trejo A (2008) Expression and secretion of cathelicidin LL-37 in human epithelial cells after infection by *Mycobacterium bovis* Bacillus Calmette-Guerin. *Clin Vaccine Immunol* 15:1450–1455
- Mookherjee N, Brown KL, Bowdish DM et al (2006a) Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37. *J Immunol* 176:2455–2464
- Mookherjee N, Wilson HL, Doria S et al (2006b) Bovine and human cathelicidin cationic host defense peptides similarly suppress transcriptional responses to bacterial lipopolysaccharide. *J Leukoc Biol* 80:1563–1574
- Murakami M, Ohtake T, Dorschner RA et al (2002a) Cathelicidin antimicrobial peptides are expressed in salivary glands and saliva. *J Dent Res* 81:845–850
- Murakami M, Ohtake T, Dorschner RA et al (2002b) Cathelicidin anti-microbial peptide expression in sweat, an innate defense system for the skin. *J Invest Dermatol* 119:1090–1095
- Nagaoka I, Hirota S, Niyonsaba F et al (2001) Cathelicidin family of antibacterial peptides CAP18 and CAP11 inhibit the expression of TNF-alpha by blocking the binding of LPS to CD14(+) cells. *J Immunol* 167:3329–3338
- Nagaoka I, Tamura H, Hirata M (2006) An antimicrobial cathelicidin peptide, human CAP18/LL-37, suppresses neutrophil apoptosis via the activation of formyl-peptide receptor-like 1 and P2X7. *J Immunol* 176:3044–3052
- Nijnik A, Hancock RE (2009) The roles of cathelicidin LL-37 in immune defences and novel clinical applications. *Curr Opin Hematol* 16:41–47
- Niyonsaba F, Ushio H, Nakano N et al (2007) Antimicrobial peptides human beta-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines. *J Invest Dermatol* 127:594–604
- Ooi EH, Wormald PJ, Carney AS et al (2007) Fungal allergens induce cathelicidin LL-37 expression in chronic rhinosinusitis patients in a nasal explant model. *Am J Rhinol* 21:367–372
- Overhage J, Campisano A, Bains M et al (2008) Human host defense peptide LL-37 prevents bacterial biofilm formation. *Infect Immun* 76:4176–4182
- Park HJ, Cho DH, Kim HJ et al (2009) Collagen synthesis is suppressed in dermal fibroblasts by the human antimicrobial peptide LL-37. *J Invest Dermatol* 129:843–850
- Peyssonnaud C, Boutin AT, Zinkernagel AS et al (2008) Critical role of HIF-1alpha in keratinocyte defense against bacterial infection. *J Invest Dermatol* 128:1964–1968
- Pochet S, Tandel S, Querriere S et al (2006) Modulation by LL-37 of the responses of salivary glands to purinergic agonists. *Mol Pharmacol* 69:2037–2046
- Rivas-Santiago B, Hernandez-Pando R, Carranza C et al (2008) Expression of cathelicidin LL-37 during *Mycobacterium tuberculosis* infection in human alveolar macrophages, monocytes, neutrophils, and epithelial cells. *Infect Immun* 76:935–941
- Rosenfeld Y, Papo N, Shai Y (2006) Endotoxin (lipopolysaccharide) neutralization by innate immunity host-defense peptides. Peptide properties and plausible modes of action. *J Biol Chem* 281:1636–1643
- Schaller-Bals S, Schulze A, Bals R (2002) Increased levels of antimicrobial peptides in tracheal aspirates of newborn infants during infection. *Am J Respir Crit Care Med* 165:992–995
- Shaykhiiev R, Beisswenger C, Kandler K et al (2005) Human endogenous antibiotic LL-37 stimulates airway epithelial cell proliferation and wound closure. *Am J Physiol Lung Cell Mol Physiol* 289:L842–L848
- Sheils CA, Kas J, Travassos W et al (1996) Actin filaments mediate DNA fiber formation in chronic inflammatory airway disease. *Am J Pathol* 148:919–927
- Skokos D, Nussenzweig MC (2007) CD8- DCs induce IL-12-independent Th1 differentiation through Delta 4 Notch-like ligand in response to bacterial LPS. *J Exp Med* 204:1525–1531
- Smeianov V, Scott K, Reid G (2000) Activity of cecropin P1 and FA-LL-37 against urogenital microflora. *Microbes Infect* 2:773–777
- Tai EK, Wong HP, Lam EK et al (2008) Cathelicidin stimulates colonic mucus synthesis by up-regulating MUC1 and MUC2 expression through a mitogen-activated protein kinase pathway. *J Cell Biochem* 104:251–258
- Tang JX, Janmey PA (1996) The polyelectrolyte nature of F-actin and the mechanism of actin bundle formation. *J Biol Chem* 271:8556–8563
- Tang JX, Wen Q, Bennett A et al (2005) Anionic poly(amino acid)s dissolve F-actin and DNA bundles, enhance DNase activity, and reduce the viscosity of cystic fibrosis sputum. *Am J Physiol Lung Cell Mol Physiol* 289:L599–L605
- Thomas-Virrig CL, Centanni JM, Johnston CE et al (2009) Inhibition of multidrug-resistant *Acinetobacter baumannii* by nonviral expression of hCAP-18 in a bioengineered human skin tissue. *Mol Ther* 17:562–569
- Tjabringa GS, Ninaber DK, Drijfhout JW et al (2006) Human cathelicidin LL-37 is a chemoattractant for eosinophils and neutrophils that acts via formyl-peptide receptors. *Int Arch Allergy Immunol* 140:103–112
- Tokumar S, Sayama K, Shirakata Y et al (2005) Induction of keratinocyte migration via transactivation of the epidermal growth factor receptor by the antimicrobial peptide LL-37. *J Immunol* 175:4662–4668
- Torossian A, Gurschi E, Bals R et al (2007) Effects of the antimicrobial peptide LL-37 and hyperthermic preconditioning in septic rats. *Anesthesiology* 107:437–441
- Travis SM, Anderson NN, Forsyth WR et al (2000) Bactericidal activity of mammalian cathelicidin-derived peptides. *Infect Immun* 68:2748–2755
- von Haussen J, Koczulla R, Shaykhiiev R et al (2008) The host defence peptide LL-37/hCAP-18 is a growth factor for lung cancer cells. *Lung Cancer* 59:12–23
- Wah J, Wellek A, Frankenberger M et al (2006) Antimicrobial peptides are present in immune and host defense cells of the human respiratory and gastrointestinal tracts. *Cell Tissue Res* 324:449–456
- Walker TS, Tomlin KL, Worthen GS et al (2005) Enhanced *Pseudomonas aeruginosa* biofilm development mediated by human neutrophils. *Infect Immun* 73:3693–3701
- Weiner DJ, Bucki R, Janmey PA (2003) The antimicrobial activity of the cathelicidin LL37 is inhibited by F-actin bundles and restored by gelsolin. *Am J Respir Cell Mol Biol* 28:738–745
- Woo JS, Jeong JY, Hwang YJ et al (2003) Expression of cathelicidin in human salivary glands. *Arch Otolaryngol Head Neck Surg* 129:211–214
- Yoshioka M, Fukuishi N, Kubo Y et al (2008) Human cathelicidin CAP18/LL-37 changes mast cell function toward innate immunity. *Biol Pharm Bull* 31:212–216

- Yu J, Mookherjee N, Wee K et al (2007) Host defense peptide LL-37, in synergy with inflammatory mediator IL-1beta, augments immune responses by multiple pathways. *J Immunol* 179:7684–7691
- Zasloff M (2005) Sunlight, vitamin D, and the innate immune defenses of the human skin. *J Invest Dermatol* 125:16–17
- Zasloff M (2006) Fighting infections with vitamin D. *Nat Med* 12:388–390
- Zhang Z, Cherryholmes G, Shively JE (2008) Neutrophil secondary necrosis is induced by LL-37 derived from cathelicidin. *J Leukoc Biol* 84:780–788
- Zheng Y, Niyonsaba F, Ushio H et al (2007) Cathelicidin LL-37 induces the generation of reactive oxygen species and release of human alpha-defensins from neutrophils. *Br J Dermatol* 157:1124–1131
- Zuyderduyn S, Ninaber DK, Hiemstra PS et al (2006) The antimicrobial peptide LL-37 enhances IL-8 release by human airway smooth muscle cells. *J Allergy Clin Immunol* 117:1328–1335