CATHELICIDIN: A VERSATILE POLYPEPTIDE

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ABSTRACT

The human cathelicidin is an antimicrobial, immunomodulatory and tissue repair peptide found in mammalian species. Humans, mice, and rats have only one known cathelicidin gene, which has been shown to be multifunctional. The active form of vitamin D, 1,25(OH)₂D₃ has been shown to directly induce cathelicidin production in keratinocytes and myeloid cells via a vitamin D response element in the promoter of the CAMP (Cathelicidin Antimicrobial Peptide) gene. Pulmonary tuberculosis patients with vitamin D deficiency have significantly reduced local levels of the vitamin D-inducible antimicrobial peptide LL-37 in granulomatous lesions compared to distal parenchyma from the infected lung. This paper presents a concise overview of the role of cathelicidin in antimicrobial activity, immunity, wound healing, cancer etc. This understanding would help to direct future research efforts to identify therapeutic approaches that use cathelicidin as a novel drug itself, or aim to modify its expression and regulation.

KEYWORDS: Cathelicidin, Immunity, Cancer, Wound healing, Vitamin D.

INTRODUCTION

Cathelicidin is one of the most important host defense peptides known today.¹ Antimicrobial peptides are an important component of the innate immune system, providing a non-specific, rapid response to foreign pathogens.² The activity against resistant pathogens, the potential of epithelial healing after microbial injury and the neutralization of bacterial endotoxin

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underlie the most important benefits of this peptide.\[3\] Cathelicidins disrupt membranes of microorganisms and kill them. They also neutralize microbe-derived pathogens, such as lipopolysaccharide (LPS) and flagellin.\[4\] In addition to cathelicidin's antibiotic effect, it has been shown to play a role in communication between the innate and adaptive immune responses, acting as chemotactic factor for CD4 T cells, neutrophils, monocytes, and mast cells.\[5\] In this review, we will focus on the significance of the human cathelicidin.

HISTORY

The cathelicidin family of peptides was first discovered while analyzing porcine and bovine leukocytes. The cathelicidins in general have both myeloid and epithelial sources. They are produced in the bone marrow and stored in the secondary (specific) granules of neutrophils (Figure 1). The discovery of cathelicidins in many other mammalian species followed, and includes the rabbit, sheep, mouse, cow, guinea pig, goat, horse, rhesus monkey, rat, deer, and most recently, the dog. Cathelicidins have also been found in non-mammalian species, such as the shore crab, hagfish, chicken, rainbow trout and Atlantic salmon. It is interesting to note that most domesticated species have multiple cathelicidin genes, many of which have different functions, such as antimicrobial selectivity for Gram-positive or Gram-negative bacteria, viruses, or fungi (Table 1).\[6\]

![Figure 1: Schematic of cathelicidin primary structure.](image)

Table 1: Key functions of cathelicidins.

- LPS binding and neutralization.
- Blood cell chemotaxis.
• Activation of epithelial cell.
• Immunomodulation.
• Neovascularization.
• Wound re-epithelization.
• Histamine release.
• Modulation of gene expression.
• Synergistic potential.

FUNCTIONS
Cathelicidin is touted as a antimicrobial peptide with a number of health benefits as discussed below:

Antimicrobial activity
The cathelicidins, in general, display broad spectrum activity and rapid killing against Gram-positive and Gram-negative bacteria, fungi, and viruses, including HIV and HSV. Cathelicidin is produced and stored intracellularly as a prepropeptide, requiring proteolytic processing for activity. When enzymatically cleaved by serine proteinases, it releases a cationic, amphipathic C-terminal fragment termed LL-37 that has been shown to have rapid antimicrobial activity against a wide range of gram-positive and gram-negative pathogenic microbes, as well as viruses and fungi. Cathelicidins disrupt the integrity of the bacterial membrane, which accounts for their ability to kill microorganisms. The specifics of how this occurs, however, are not well understood. Several theories detailing their killing activity have been proposed, including the “barrel-stave,” “carpet,” and “toroidal pore” methods. The barrel-stave theory suggests that peptide helices are initially parallel to the membrane and then insert into the membrane in a perpendicular manner creating a “barrel” with the peptides being the “staves.” This has been shown for alamethicin, a 20-residue peptide made by a fungus. The carpet theory suggests that peptide alpha-helices bind in a parallel orientation and cover the surface of the outer membrane like a “carpet,” eventually forming micelles or toroidal pores in the bacterial membrane when they reach sufficient concentration. The carpet theory has been shown for a congener of the sheep cathelicidin, SMAP29. Finally, the toroidal pore theory describes the peptides as inserting perpendicularly to the outer membrane and causing the membrane to bend in on itself, so that the pore is made up of lipid heads and peptides. The toroidal model has been proposed as the mechanism used by magainins, which are helical peptides produced by amphibians. Interestingly, recent findings indicate that
physical disruption of the outer cellular membrane is not the only mechanism of antimicrobial activity. A recent review states that there are intracellular activities targeted as well. Briefly, these consist of flocculation of intracellular contents, alteration of cytoplasmic membrane septum formation, inhibition of cell wall synthesis, binding of nucleic acids, inhibition of nucleic acid synthesis, inhibition of protein synthesis, and the inhibition of various enzymatic activities.\[6\]

Ligation of the innate immune pattern recognition receptors, Toll-like receptors (TLRs) on human macrophages, causes upregulation of the intracellular vitamin D receptor (VDR) and vitamin D hydroxylase genes, resulting in induction of cathelicidin and/or β-defensin, both of which are potent antimicrobial peptides. Cathelicidin induces fusion of the phagolysosome, which is essential for the containment, degradation and subsequent killing of mycobacteria.\[7\] Control of tuberculosis (TB) requires induction and maintenance of both macrophage and T cell effector functions. Researchers have demonstrated that pulmonary TB patients with a vitamin D deficiency have significantly reduced local levels of the vitamin D-inducible antimicrobial peptide LL-37 in granulomatous lesions compared to distal parenchyma from the infected lung. Instead, TB lesions were abundant in CD3(+) T cells and FoxP3(+) regulatory T cells as well as IgG-secreting CD20(+) B cells, particularly in sputum-smear positive patients with cavitary TB. Mycobacteria-specific serum IgG titers were also elevated in patients with active TB. An upregulation of the B cell stimulatory cytokine IL-21 correlated with m-RNA expression of CD20, total IgG and also IL-10 in the TB lesions. Altogether, vitamin D-deficient TB Patients expressed a weak antimicrobial response but an IL-21 associated expansion of IgG-secreting B cells combined with a rise in FoxP3(+) regulatory T cells at the local site of infection.\[8\]

**Immunomodulatory**

Cathelicidins link the innate immune response to adaptive immune response. The human cathelicidin, LL37, by use of the formyl peptide receptor-like 1 (FPRL1), activated chemotaxis of neutrophils, monocytes, and CD4 T cells. LL37 caused monocyte migration and calcium mobilization in a dose-dependent manner. Human embryonic kidney (HEK) 293 cells were transfected, allowing them to express the FPRL1 receptor and the cells were shown to migrate in a dose-dependent response in the presence of LL37. Non-transfected HEK293 cells showed no migration. Also, when the FPRL1 receptor was blocked with an
agonist, no cell migration occurred. The fact that LL37 attracts leukocytes which are involved in both innate and adaptive immune responses supports a role in linking the two systems.\cite{6}

**Wound healing**

Keratinocytes, the predominant cell type found in the epidermis, form barriers against microbial pathogens during wound closure, and keratinocyte migration is an important step in skin wound healing. Growth factors important to wound healing induced the expression of hCAP-18/LL-37 in human keratinocytes and the P2 \times 7–SFK–Akt–CREB/ATF1 signaling pathway activated by LL-37 in keratinocytes was established. LL-37 (1 μg/ml) induced the maximum level of keratinocyte migration, and this was shown to progress via EGFR transactivation. LL-37 was also found to protect human keratinocytes from apoptosis via the activation of the COX-2 pathway. hCAP-18 is strongly expressed in healing skin epithelium, and treatment with antibodies raised and affinity purified against LL-37 inhibited re-epithelialization (wound closure) in a concentration-dependent manner.\cite{9}

**Anticancer**

In human airway epithelial cells, LL-37 has been shown to result in apoptotic TUNEL positive cells in a caspases-dependent manner. Analogue of LL-37 could induce the caspase-independent apoptosis in an oral squamous cell line SAS-H1 but not normal cells. The anti-tumorigenic effect of LL-37 is dependent on its ability to induce DNA break and mitochondrial damage in Jurkat T leukemia and A549 cells which are independent of caspase activation. It is likely that low tissue expression of LL-37 could promote tumor formation. Indeed downregulation of LL-37 in cancer tissues has also been reported in the GI tract. In normal gastric mucosa, LL-37 is expressed in surface epithelial cells and chief cells as well as parietal cells in the fundic glands. Immunochemical staining of LL-37 has revealed that the expression of LL-37 is down-regulated in gastric hyperplastic polyps, tubular adenomas, and adenocarcinomas. After *H. pylori* infection, LL-37 is markedly up-regulated in the epithelium and gastric secretions. Such upregulation could not be detected in patients with *H. pylori*-independent gastric inflammation. Moreover, a higher level of LL-37 expression has been demonstrated in wild-type *H. pylori* infection of cultured gastric epithelial cells and this higher production of *H. pylori* infection requires an intact type IV secretion system. Therefore, it is indicated that expression of LL-37 may be in a tissue- and disease-specific manner.\cite{1}
CONCLUSION
Therapeutic strategies that target cathelicidin's induction or inhibition through the vitamin D signaling pathway, as well as targeting important cytokines and proteases involved in the regulation of cathelicidin stand to be important avenues for future investigations.

REFERENCES