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Review

Molecular mechanisms of LL-37-induced receptor activation: An overview

Eddy-Tim Verjans*, Sven Zels, Walter Luyten, Bart Landuyt, Liliane Schoofs

KU Leuven, Department of Biology, Division of Neurobiology and Animal Physiology, Naamsestraat 59, 3000 Leuven, Belgium

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ABSTRACT

The human cathelicidin peptide LL-37 plays a crucial role in the immune system on many levels, from the first line of defense in epithelial cells to restoring the tissue after infection. On host cells, the majority of the LL-37-induced effects are mediated *via* the direct or indirect activation of several structurally unrelated cell surface receptors or intracellular targets. How LL-37 is able to affect multiple receptors is currently not well understood. So far, the mechanistic details underlying receptor activation are poorly investigated and evidence for a conventional ligand/receptor interaction is scarce. Over the past few decades, a large number of studies have reported on the activation of a receptor and/or components of the downstream signal transduction pathway induced by LL-37. This review summarizes the current knowledge on molecular mechanisms underlying LL-37-induced receptor activation.

Contents

Abbreviations: AMP, antimicrobial peptide; APD, antimicrobial peptide database; IFN, interferon; DC, dendritic cell; NK-cells, natural killer cells; GPCR, G protein-coupled receptor; RTK, receptor tyrosine kinase; LGIC, ligand-gated ion channel; TLR, toll-like receptor; EGFR, epidermal growth factor receptor; DNA, deoxyribonucleic acid; LPS, lipopolysaccharide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FPR2, *N*-formyl peptide receptor 2; LTB4, leukotriene B4; ROS, reactive oxygen species; NET, neutrophil extracellular trap; IAV, influenza A virus; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinases; M-CSF, macrophage colony-stimulating factor; MCP1, monocyte chemotactic protein-1; CXCR2, CXC chemokine receptor type 2; MrgX2, mas-related gene X2; P2Y11, purinoceptor 11; PTX, pertussis toxin; hβD, human beta-defensin; NMR, nuclear magnetic resonance; CXCR4, CXC chemokine receptor type 4; HSPC, human hematopoietic stem/progenitor cells; SDF-1, stromal-derived factor-1; IGF1R, insulin-like growth factor 1 receptor; ADAM, a disintegrin and metalloproteinase; HB-EGF, heparin-binding EGF-like growth factor; TGF-α, transforming growth factor-alpha; ELISA, enzyme-linked immunosorbent assay; LRC, ligand-receptor-capture; $P2X_7$, purinergic receptor 7; IL, interleukin; HEK, human embryonic kidney; TRPV2, transient receptor potential cation; BKCa, Ca^{2+}-activated K^+-channel; PRP, pattern-recognition receptor; PAMP, pathogen-associated molecular pattern; TNF-α, tumor necrosis factor-alpha; PLA_2, phospholipase A_2; PLD, phospholipase D; SPR, surface plasmon resonance; SFG, sum frequency generation.

* Corresponding author.
E-mail address: Eddytim.Verjans@bio.kuleuven.be (E.-T. Verjans).

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1. Introduction

Antimicrobial peptides and proteins (AMPs) are key components of the innate immune system, warding off invading pathogens such as bacteria, fungi, viruses and parasites [1,2]. More than hundred human AMPs have been identified and characterized from various host tissues and epithelial surfaces (for an overview see the Antimicrobial Peptide Database (APD)) [1]. AMPs are customarily positively charged and amphipathic low molecular weight proteins with a broad-spectrum antibiotic activity [1,3]. A wide variety of cell types express AMPs in a constitutive fashion or after exposure to a specific stimulus, such as vitamin D or interferon (IFN) γ [2,3]. In vertebrates, a small number of AMPs can also modulate and/or stimulate adaptive immunity *via* the specific activation of cell surface receptors or intracellular targets. Because of their prominent role in the human immune system and low inherent toxicity, AMPs are considered attractive templates to engineer new antimicrobials or therapeutics [1,4].

Cathelicidins are among the most studied classes of AMPs. In humans, there is only one representative, called LL-37 that exerts its function in concert with defensins [5]. The cathelicidin peptide plays a prominent role in innate host defense mechanisms against bacteria and some specific viruses, fungi and parasites [3,6–9]. LL-37 is expressed by cell types that are likely to encounter pathogens, such as epithelial cells of the skin, intestine, airway, ocular surface or reproductive tract, but are also localised in innate immune cells like neutrophils, dendritic cells (DC), mast cells, B-cells, natural killer (NK)-cells, γδ-T-cells, monocytes and macrophages [10,11]. The direct microbicidal activity of LL-37 is predominantly mediated by disrupting the integrity of microbial membranes, due to its inherent cationic and amphipathic nature [12]. Additionally, a multi-hit mechanism in which the peptide also interacts with several cytoplasmic targets appears to enhance microbial extirpation [13,14].

The discovery that LL-37 acts as a potent chemoattractant to guide monocytes, neutrophils and T cells to the site of infection led to a more thorough understanding of the role of the peptide [15]. LL-37 is not only involved in the innate immune system, but also exerts immunostimulatory and immunomodulatory effects. Upon infection, LL-37 acts as a danger signal and bridges the innate and adaptive immune system by recruiting immunocompetent cells to the site of infection. Additionally, LL-37 modulates the levels of inflammatory cytokines, serving to control the delicate balance between pro- and anti-inflammatory responses [16–18]. This 'alarming' effect of the peptide complements its role as endogenous antibiotic. Moreover, LL-37 is implicated in many key biological processes involving non-immune cells such as apoptosis, angiogenesis, re-epithelialization, wound closure and the maintenance of the intestinal epithelial barrier integrity [11,19–23]. Defects in LL-37 expression or processing are therefore frequently associated with the pathogenesis of several human diseases including psoriasis, rosacea, cystic fibrosis and cancer [18,24,25]. Detailed knowledge about the exact molecular mode of action on a variety of host cells and tissues is therefore a prerequisite to understand the pathology of these diseases.

Considering its small size and low structural complexity, it is remarkable that LL-37 contains all the necessary information to perform its pleiotropic tasks. On host cells, the majority of LL-37-induced effects are mediated *via* specific activation of various putative cell surface receptors, membrane channels or intracellular targets. LL-37 has been associated with at least nine receptors belonging to different receptor classes, including four G protein-coupled receptors (GPCRs), three receptor tyrosine kinases (RTKs), a ligand-gated ion channel (LGIC) and Toll-like receptors (TLRs) [2,26]. Recently, it has been demonstrated that LL-37 also inhibits the CD36 fat receptor in adipocytes and hepatocytes [27].

It remains a conundrum how one peptide is able to directly activate multiple receptors belonging to different receptor classes, especially since most 'ligand-receptor' interactions are classically thought to occur in a very specific manner. The current general consensus hypothesis appears to be that LL-37 activates eukaryotic cells by virtue of at least five distinctly different mechanisms, including direct and indirect modes of action.

LL-37 either acts as a surrogate ligand for a specific receptor or influences the formation or stabilization of membrane microdomains containing the receptor [3,28]. Transactivation of epidermal growth factor receptors (EGFRs) *via* metalloproteinase-mediated cleavage of membrane-anchored EGFR ligands has also been reported for LL-37 (*e.g.* 'triple-membrane-passing-signaling' model) [29]. More recently, LL-37 was found to contribute to the activation of receptors by promoting their incorporation in lipid rafts [30]. Receptors that congregate in lipid rafts can be more sensitive for their cognate agonists or even show activation in the absence of agonists. Furthermore, LL-37 and LL-37 aggregates can penetrate a variety of host cells, mainly *via* receptor-mediated endocytic pathways [26]. This provides a mechanism for the selective uptake of extracellular anionic molecules such as deoxyribonucleic acid (DNA) or lipopolysaccharide (LPS) and allows access for LL-37 to several intracellular targets, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [31].

Because of the large number of publications reporting on a specific receptor and/or components of the downstream signaling transduction pathway of LL-37, as well as functional effects on host cells, this review primarily intends to provide the reader with an updated and comprehensive synopsis on the subject. Also, during the last few years, novel molecular targets of LL-37 have been identified which have not been reviewed before, as exemplified by studies from Hoang-Yen Tran et al., Zhang *et al.*, and Gambade et al. [27,32,33]. Our aim is to review these recent findings in order to acquire a more thorough understanding of the complex modes of action of LL-37 on host cells. Finally, many studies rely on a collection of broad-spectrum or receptor-selective antagonists to identify the cognate receptor of LL-37. Administration of receptor-selective antagonists to cell lines displaying a response to LL-37 suggests direct interaction between the peptide and its receptor, but true functional coupling of LL-37 to a defined receptor binding pocket has been questioned by several research groups.

Currently, it is believed that LL-37 operates in a non-canonical manner by first binding to the membrane interface and then interacting with receptor transmembrane domains, but consensus on this matter has not yet been attained [26]. In order to establish a starting point for novel drug development efforts, improved knowledge on the interaction mechanism of LL-37 with its putative receptors is imperative.

Therefore, we want to provide the reader with an overview of the receptors reported to be activated by LL-37 and investigate whether there is compelling evidence for a direct interaction between receptor and peptide. This review will help researchers to compare their data with the current scientific literature (as of July 2016), or provide a source of inspiration for future experiments on this fascinating peptide.

2. GPCRs

GPCRs, also known as seven-transmembrane domain receptors, constitute a large and diverse family of cell surface proteins that mediate a wide variety of physiological processes in multiple cell types. In 2000, De Yang and colleagues identified *N*-formyl peptide receptor 2 (FPR2; formerly known as formyl peptide receptor like-1) as the first functional receptor for LL-37 [15]. FPR2 belongs to a class of G_i protein-coupled receptors activated by a wide