Mini-Review

The potentials of short fragments of human anti-microbial peptide LL-37 as a novel therapeutic modality for diseases

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1. Abstract

Human cathelicidin antimicrobial peptide LL-37 (LL-37) is an antimicrobial peptide derived from its precursor protein hCAP18, which is an only cathelicidin in human. LL-37 not only serves as a mediator of innate immune defense against invading microorganisms, but it also plays an essential role in tissue homeostasis, regenerative processes, regulation of proinflammatory responses, and inhibition of cancer progression. Therefore, LL-37 has been considered as a drug lead for diseases. However, high levels of LL-37 may reduce cell viability and promote apoptosis of osteoblasts, vascular smooth muscle cells, periodontal ligament cells, neutrophils, airway epithelial cells and T cells. Recent evidence reveals that LL-37-derived short peptides possess similar biological activities as the whole LL-37 with reduced cytotoxicity. Thus, such small molecules constitute a pool of potential therapeutic agents for diseases.

2. Introduction

LL-37 is constitutively expressed or produced in almost all tissues and organs [1, 2]. These cells expressing LL-37 are either in direct contact with the exterior environment or operating at sites of infection and immune responses, indicating the importance of LL-37 to host defense. LL-37 plays multifunctional roles in host defense. LL-37 has a broad spectrum of microbicidal activities and is effective against Gram-positive and negative bacteria, fungi, and some viruses [3–6]. LL-37 also acts as a potent chemoattractant to guide immune cells. Upon infection, LL-37 acts as a danger signal and bridges the innate and adaptive immune system by guiding immune cells such as monocytes, neutrophils, T cells to the site of infection. Moreover, LL-37 modulates the levels of inflammatory cytokines, serving to control the delicate balance between pro-
and anti-inflammatory responses [7–9]. In addition, LL-37 is involved in many key biological processes implicating non-immune cells such as apoptosis, angiogenesis, epithelialization, wound closure and the maintenance of the intestinal epithelial barrier integrity [1, 9–13].

Accumulating knowledge indicates the possibility of LL-37 as a potential therapeutic agent for infection and cancer. Synthetic LL-37 was used for the topical treatment of hard-to-heal venous leg ulcers (VLUs) with safety and marked therapeutic effects [14]. A preliminary evaluation of orally administered recombinant L. lactis containing LL-37 peptide was performed in SARS-CoV-2-infected patients with mild symptoms. The recombinant L. lactis containing LL-37 peptide improved symptoms of CoV-2-infected patients in fever, and fatigue, myalgia, as well as gastrointestinal (abdominal discomfort, nausea, poor appetite, and diarrhea) and respiratory symptoms (pharyngalgia, cough, chest distress, shortness of breath) without adverse reactions [15]. In experimental colon cancer metastasis, mice injected intravenously with LL-37-overexpressing adeno-associated viruses (CAMP-HA-AVs) reduced human-specific cytokeratin 18 positive tumor colonies in the lung, indicating that LL-37 produced in vivo inhibited colon cancer metastasis. An in vitro study showed that LL-37 inhibited the migration of human colon carcinoma cells by disrupting the tubulin structure inside the cells [16].

However, the therapeutic application of LL-37 is limited due to its low cell selectivity and high production cost due to its large size [17]. Cytotoxic effects of LL-37 were also detected in vitro on erythrocytes, lymphocytes, and fibroblasts. LL-37 damages the plasma membrane of human cells at concentrations similar to those required for its antimicrobial activity [2, 18], constituting a potentially detrimental side effect. High levels of LL-37 (≥100 µM) are detectable in the lesions of patients with ulcerative colitis, psoriasis, rosacea, and chronic periodontitis. Such high levels of LL-37 may reduce the viability of normal cells and promote apoptosis of different cell types at different locations [19]. However, the cytotoxicity of LL-37 may be reduced by removal of its N-terminal hydrophobic amino acids [20]. Thus, LL-37-derived short peptides are proposed to be better candidates to replace LL-37 as potential novel therapeutic agents for infection and cancer. This review describes advances in the development of LL-37-derived short peptides for future therapeutics.

3. **LL-37-derived short peptides**

The precursor protein hCAP18 of LL-37 is composed of three parts: A N-terminal signal peptide, a highly conserved cathelin like domain, and an antimicrobial peptide domain at the C-terminus [21–23]. The full peptide was first isolated from the bone marrow and subsequently from the secretions of neutrophils [24, 25]. The full-length hCAP-18 (Inactive LL-37 precursor) is cleaved by proteinase 3 [26], elastase [24], or skin-derived kallikreins [27] to release a 37-amino acid residue to form of the mature LL-37. However, hCAP-18 in seminal plasma is cleaved by prostate-derived gastricsin to release a 38-amino acid antimicrobial peptide, ALL-38. The antimicrobial spectrum and potency of ALL-38 are similar to those of the prototype LL-37 [28]. LL-37 may be further processed to release shorter fragments with biological activities. In human sweat, the mature LL-37 has been shown to be degraded to shorter peptic fragments, including RK-31, KS-30 and KR-20 [29] by two distinct kallikreins: kallikrein 5 and kallikrein 7 [27, 30].

These natural and recently developed synthetic LL-37-derived short peptides have contributed to a better understanding of the full LL-37 peptide and the replacement for LL-37 to apply in treatment of diseases [31]. For example, KS-30 (LL-8-37), KR-20 (LL-18-37), KR-12 (LL-18-29), and FK-16 (LL-17-32) released from LL-37 all exhibit microbicidal capacity [32, 33]. Moreover, replacement of amino acid residues can enhance the activity as compared with the prototype LL-37 [33]. Engineered LL-37-derived peptides inhibited Ebola [34] and Zika virus [35] infection. Interestingly, a synthetic peptide, called LL37-analogous peptide (LLAP) and with the sequence of GRKSAKKIGKRKRAKRI, possesses more potent antibacterial activity by targeting ATPases in bacterial cell membrane [36]. The sequences of some natural LL-37-derived short peptides and the engineered short peptides are listed in Table 1.

4. **Development of LL-37-derived short peptides for clinical applications**

4.1 Against multidrug resistant (MDR) clinical isolates of Escherichia coli

Aghazadeh and colleagues [37] investigated the effects of LL-37-derived short peptides; P38 (TSVRQRWRWRQRVRTS–NH2), P22 (KRSKRKKRIHQVRIS–NH2) and P7 (TLSKEKERIVQQRVRTS–NH2), against MDR clinical isolates of bacteria including five E. coli and five S. aureus strains. They found that the antibacterial activity of P38 against MDR clinical isolates of E. coli was higher than that of P7 and P22. The P38 as well as P22 and P7 have no antimicrobial activity against all S. aureus isolates. Mechanistic studies revealed that P38 had a much higher affinity for the outer membrane of Gram-negative bacteria compared with both P22 and P7, and P38 killed E. coli by disrupting the bacterial membrane. Thus, P38 has a better potential for clinical applications against infectious diseases, especially MDR clinical isolates of E. coli.

4.2 As anti-biofilm agents

KR-12 (KRIVQRIKDFLR), corresponding to the residues 18–29 of LL-37, is the smallest peptide of LL-
37 known to possess antimicrobial activities. KR-12 displayed a selective toxic effect on bacteria but not human cells [38, 39]. KE-18 (KEFKRIVQIKDFLRLNV) is another peptide corresponding to the residues 15–32 of LL-37. Luo and colleagues [40] compared the effects of KR-12 and KE-18 against Candida albicans, Staphylococcus aureus, and E. coli. All three microorganisms have been demonstrated to be involved in ventilator-acquired pneumonia (VAP) [41]. KE-18 showed a significant inhibitory activity against C. albicans and S. aureus for the biofilm-development. In contrast, KR-12 did not display any antibiofilm properties against C. albicans, S. aureus, or E. coli. Thus, KE-18 appears a good therapeutic agent for prevention of multi-species biofilm-related infections such as VAP.

Kim and colleagues [42] designed and synthesized three analogs of KR-12-a5 (KRIVKLILKWLR-NH2); KR-12-a5(5-DK) (KRIVKLILKWLR-NH2) and KR-12-a5(7-DL) (KRIVKLILKWLR-NH2) and KR-12-a5(6-DL) (KRIVKLILKWLR-NH2). D-Lys (K) and D-Leu (L) substituted the Lys5 and Ile7 in the central position of the polar and non-polar face of KR-12-a5, respectively. KR-12-a5(6-DL) was designed by substituting D-Leu for Leu6 in the polar-nonpolar interface of KR-12-a5 [42]. KR-12-a5 and its analogs show higher antimicrobial activities against all tested antibiotic-resistant bacterial strains than LL-37, including methicillin-resistant Staphylococcus aureus strains (MRSA; CCARM 3089, CCARM 3090, and CCARM 3095), multidrug-resistant Pseudomonas aeruginosa strains (MDGRA; CCARM 2095, and CCARM 2109) and vancomycin-resistant Enterococcus faecium (VREF; ATCC 51559). Antibiotics and chemicals have difficulty penetrating and killing the bacteria which have formed biofilm. Biofilm is formed by bacteria with bacteria-secreted slimy, glue-like substances, adhering to the surface of some object in a moist environment [43]. KR-12-a5 and its analogs KR-12-a5(5-DK), KR-12-a5(6-DL), and KR-12-a5(7-DL) inhibited biofilm development of MDRPA (CCARM 2095). By contrast, the parental peptide, LL-37, did not show a significant anti-biofilm activity. KR-12-a5 and its analogs also displayed a remarkable synergy with antibiotics, including chloramphenicol, ciprofloxacin, and oxacillin against MDRPA. Thus, KR-12-a5 and its analogs can be developed further as novel antimicrobial/anti-inflammatory agents to treat antibiotic-resistant infections.

Rajasekaran and colleagues [17] synthesized a series of FK13 (FKRIVQIKDFLRLNV) analogs based on the sequence of the 13-meric short FK13 peptide (residues 17–29 of LL-37), including FK-13-a1 (WKRIVRKKRWLR-NH2), FK13-a2 (WKRIVRWIKRWLR-NH2), FK13-a3 (WKRIVRWIRWLR-NH2), FK13-a4 (FKRWVQRWKFR-NH2), FK13-a5 (FKRWVQRWKFR-NH2), FK13-a6 (WKRWVQRWKFR-NH2) and FK-13-a7 (WKRWVRWRRWLR-NH2). FK13-a1 was designed by replacing Phe1 and Phe11 of FK13 with Trp, FK13-a2 and FK13-a3 were designed by Arg7 to Trp7 and Lys9 to Trp9 substitution in FK13-a1, respectively. FK13-a4 was obtained from FK13 by Trp substitution of the two consecutive Leu residues at positions 4 and 8 (Leu4,8 → Trp4,8), and an additional Asp10 to Arg10 substitution. FK13-a5 was obtained from FK13-a4 by Phe1 to Trp1 substitution. A subsequent Phe11 to Trp1 substitution resulted in FK13-a6. FK13-a7 from FK13-a6 by Gln6 to Arg6 substitution. FK13 and its analogs showed antimicrobial activities against three Gram-positive bacteria (B. subtilis, S. epidermidis, and S. aureus) and three Gram-negative bacteria (E. coli, P. aeruginosa, and S. typhimurium). Both FK13-a1 and FK13-a7 exhibited higher antimicrobial activity against all tested antibiotic-resistant bacterial strains than did LL-37. These tested antibiotic-resistant bacterial strains included MRSA strains (CCARM 3089, CCARM 3090, and CCARM 3095), two MDRPA strains (CCARM 2109, and CCARM 2095), and a VREF strain (ATCC

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**Table 1. The sequences of natural LL-37 and some short peptides.**

| AMP   | Sequence AA numbers |
|-------|---------------------|
| LL-37 | LLGDFRKSKEKIGKEFKRIVQIKDFLRLNVPTES 37 |
| LL-27 | FRKSKEKIGKEFKRIVQIKDFLRLNV          27 |
| RK-31 | RKSKEKIGKEFKRIVQIKDFLRLNVPTES      31 |
| KS-30 | KSKEKIGKEFKRIVQIKDFLRLNVPTES       30 |
| KR20  | KRIQVQIKDFLRLNPTRTES               20 |
| FK16  | FKRIQVQIKDFLRLV                   16 |
| KR12  | KRIQIVQIKDFLRI                   12 |
| GF17  | GFKRIQVQIKDFLRLV                  17 |
| I7BI  | GXRKRIQVQIKDFLRLV                 17 |
| GF18  | GEFKRIQVQIKDFLRLV                  18 |
| GI-20 | GIKEFKRIVQIKDFLRLV                 20 |
| GI-20d | GIKEFKRIVQIKDFLRLV                 20 |

Red italic: Engineered short peptides or amino acid residues.
51559). Importantly, FK13-a1 and FK13-a7 effectively inhibited the MDRPA (CCARM 2095) biofilm formation [17].

4.3 As surface coated agents for implanted medical devices

FK-16 (FKRIVQRIKDFLRNLV) is a potently active peptide corresponding to the residues 17–32 of human LL-37 [44]. FK-16-coated titanium surface also demonstrated a significant anti-biofilm activity against methicillin-resistant S. aureus and E. coli [44].

Biofilm-related infections by implanted medical devices cause implant failures, increase treatment costs, and lead to higher patient morbidity and mortality [45]. In the past years, both metals (e.g., silver, zinc, copper, and zirconium) and non-metals (e.g., selenium and antibiotics) have been used for coating biomaterial surface to prevent such infections [46]. The effective use of metals, however, is complicated by leaching and cytotoxicity, whereas a prolonged use of antibiotics results in reduced efficacy due to the emergence of multi-drug resistant pathogens [47]. Titanium surface coated with LL-37-derived FK16 showed a broad-spectrum of activity against ESKEAPE pathogens, including Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter cloacae. FK-16-coated titanium surface also demonstrated a significant anti-biofilm activity against methicillin-resistant S. aureus and E. coli [44]. Antimicrobial effects of LL-37-derived peptides are achievable at the concentrations not toxic to human cells. Thus, the titanium surfaces covalently immobilized with LL-37-derived peptides with a potent antimicrobial activity may be harnessed to prevent biofilm-related infection on medical devices [44]. On the other hand, the compounds conjugated with both metal and a LL-37-derived peptide exerted more potent antimicrobial activities with improved water solubility and air stability of LL-37-derived short peptides through structural changes of peptides upon metal conjugation and redox chemistry [48].

The LL-37-derived synthetic peptide OP-145 (another name is P60.4Ac) (acetyl-IGKEFKRIVERIKDFLRNLV-amide) was more effective than LL-37 in eradicating S. aureus in wound infection models in vitro and proved to be safe and successful for treatment for patients with chronic suppurative otitis media [49, 50]. De Breij and colleagues incorporated OP-145 into a Polymer-Lipid Encapsulation Matrix (PLEX)-coating to obtain high peptide levels for prolonged periods at the implant-tissue interphase [50]. They found that OP-145 incorporated PLEX coating on Tricalcium phosphate (TCP) granules showed a burst release of approximately 55% of the peptide by the coating during the first 48 h followed by a daily release of about 1% for 30 days, and completely killed $1 \times 10^6$ CFU of S. aureus, in vitro. In a rabbit intramedullary nail-related infection model, 67% of rabbits with PLEX-OP-145-coated nails had culture-negative nails after 28 days as compared to 29% of rabbits with uncoated nails. The bone and soft tissue samples were culture-negative in 67% and 80% in PLEX-OP-145-coated nails, respectively, whereas all bone samples and 71% of the soft tissue samples of rabbits with uncoated nails were infected. Thus PLEX-OP-145 coatings can prevent implant colonization and S. aureus-induced biomaterial-associated infections (BAIs) [50].

4.4 As neutralizing agents of LPS and LTA

Nowadays, most patients with upper airway infections are treated with antibiotics because bacterial products, like lipopolysaccharide (LPS) from Gram-negative bacteria and lipoteichoic acid (LTA) from Gram-positive bacteria are involved in a large part of symptoms of these infections [51, 52]. Nell and colleagues found that P60.4, a 24 amino acid peptide they designed and synthesized, had a similar efficacy as LL-37 in terms of LPS and LTA neutralization and lower pro-inflammatory activity. Moreover, the acetylated and amidated version of this peptide showed no toxicity and displayed higher or equal antimicrobial activity compared to LL-37 [52]. FK13 nearly lost its ability to bind LPS, but all FK13 analogs (FK-13-a1, FK-13-a2, FK-13-a3, FK-13-a4, FK-13-a5, FK-13-a6 and FK-13-a7) showed much higher LPS-binding ability than FK13. Among the FK13 analogs, FK13-a4 and FK13-a5 showed the lowest LPS-binding ability [17]. Other LL-37 derived short peptides KE-18 and KR-12 retained the LPS-binding activity of LL-37. However, KE-8 showed significantly enhanced LTA-binding activity, while KR-12 retained the weak LTA-binding activity of the parent LL-37 peptide [40].

4.5 As anti-inflammatory agents

KR-12-a5 and its analogs KR-12-a5(5-DK), KR-12-a5(6-DL), and KR-12-a5(7-DL) inhibited the expression and production of iNOS, TNF-α, IL-6 and MCP-1 by LPS-stimulated RAW264.7 cells [42, 43]. Interestingly, FK13 and FK13 analogs displayed different inhibitory effects against inflammatory cytokine production. FK13-a1, FK13-a2, FK13-a3, FK13-a6, and FK13-a7 displayed a strong inhibition of nitrite production, FK13-a4 and FK13-a5 displayed a moderate inhibition, while FK13 showed very weak inhibition of nitrite production. For TNF-α release from LPS stimulated RAW264.7 cells, FK13-a1, FK13-a2, FK13-a3, and FK13-a7 significantly blocked its release. At the same concentration, FK13-a4, FK13-a5, and FK13-a6 displayed lower inhibition rates, while FK13 failed to inhibit TNF-α release [17].

4.6 As anti-viral agents

Several mimetics derived from LL-37 or other Antimicrobial peptides (AMPs) have been used as antiviral agents. For example, GI-20, GI-20d, GF-17, 17BI, RI-10, BMAP-18, and DAsamP2 were used for Zika virus (ZIKV) infection [35]; GF-17, 17BI, GI-20, GI-20d and RI-10 were
Table 2. Anti-viral mechanisms of LL-37 and its short peptides.

| Virus                                | Peptides         | Proposed mechanism                                                                 |
|--------------------------------------|------------------|-------------------------------------------------------------------------------------|
| SARS-CoV-2                           | LL-37            | Binding virus and inhibiting viral replication.                                      |
| Dengue virus (DENV)                  | LL-37            | Binding E-protein dimmers of the virus to prevent cell–virus interaction.            |
| Human rhinoviruses (HRVs)            | LL-37, PG-1, SMAP-29 | Direct antiviral effects on HRVs and reducing the metabolic activity of infected cells. |
| Respiratory syncytial viruses (RSV)  | LL-37            | Binding RSV to reduce the level of colocalization of the F- and N-proteins.          |
| Vaccinia virus (VV)                  | LL-37            | Inhibiting the replication of VV.                                                   |
| Venezuelan equine encephalitis virus (VEEV) | LL-37           | Inhibiting the VEEV replication.                                                    |
| Ebola virus (EV)                     | LL-37, GF-17, 17BI, GI-20, GI-20d and RI-10 | Acting as cathepsin B (Cat-B) inhibitors to block the endosomal processing of EBOV glycoprotein (GP). |
| Zika virus (ZIKV)                    | LL-37, GI-20, GI-20d, GF-17, 17BL, RI-10, BMAP-18, and DASamP2 | Interfering with ZIKV entry host cells by increasing IFN-α production by host cells. |
| Neurotropic herpes simplex virus 1 (HSV-1) | LL-37           | Suppressing HSV-1 replication.                                                      |
| Human immunodeficiency virus (HIV)   | LL-37            | Binding HIV-1 reverse transcriptase to block its activity.                          |

Notes: PG-1, Porcine Cathelicidin Protegrin-1 (RGGRLCYPYRRFFCVCVGR); SMAP-29, Sheep Myeloid Antimicrobial Peptide 29 (RGLRRLGRKIAHVGKYGPTVLRIIRIAG); BMAP-18, Bovine Myeloid Antimicrobial Peptide-18 (GRFKEFRKKFKKLFKIALS); DASamP2: DASamP2 was discovered by screening a library of representative peptides selected from the Antimicrobial Peptide Database (IKWKKLLRAAKRIL-NH₂).

Fig. 1. 17BI and GI-20d inhibit Ebola virus infection of host cells. 17BI and GI-20d act as cathepsin B (Cat-B) inhibitors to block the endosomal processing of EBOV glycoprotein (GP), thus preventing virus entry because EBOV requires the cleavage of EBOV-GP by cathepsins within host cell endosomes.

for Ebola virus (EV) infection [34] and PG-1, SMAP-29 were for Human rhinoviruses (HRVs) infection [53]. Antivirus effects of some of LL-37 and LL-37-derived short peptides were listed in Table 2.

17BI was engineered based on the sequence of GF-17, a fragment of LL-37 with three D-form amino acids and two biphenylalanines, whereas GI-20d is a novel peptide engineered with all D-form amino acids with identi-
Fig. 2. FF/CAP18 inhibits the growth of human colon cancer cells. Human colorectal carcinoma HCT116 cells cultured in the presence or absence of FF/CAP18 secreted exosomes in the supernatant. FF/CAP18 treated HCT116 and exosomes expressed miR-584-5p, miR-1202 and miR-3162-5p, which reduced the growth of HCT116. FF/CAP18 treated HCT116 cells increased miR-663α expression, which showed anti-proliferative effects on HCT116 cells by suppressing the chemokine receptor CXCR4 expression, and cell cycle arrest in G2/M.

4.7 Suppression of colon cancer cell growth by FF/CAP18

FF/CAP18, also known as LL-27, is a 27mer peptide that lacks the first and the last 5 amino acids of LL-37. FF/CAP18 was designed by replacing a glutamic acid and a lysine residue with phenylalanine of hCAP18 to enhance its antimicrobial and anticancer activity [55, 56]. Human colorectal carcinoma HCT116 cells cultured in the presence or absence of FF/CAP18 secreted exosomes in the supernatant. The exosomes indicated by CD63 and CD81 expression were 40–100 nm in size with 3.8 × 10^{-8} and 4.9 × 10^{-8} mg/cell in untreated and FF/CAP18-treated cells, respectively [55]. Exosomes formed by FF/CAP18-treated cells reduced the growth of HCT116 cells. In contrast to exosomes formed by un-treated cells, FF/CAP18 treatment did not affect tumor cell viability, directly. Microarray analysis indicated that FF/CAP18-treatment increased the expression of three miRNAs (miR-584-5p, miR-1202 and miR-3162-5p) in both HCT116 cells and their exosomes [55]. miRNAs as small non-coding RNAs that control gene expression by binding to the 3’-untranslated region of their target mRNA [57] are present in cancer cells and in exosomes [58], and they may act both as tumor suppressors or oncogenes [59]. LL-37 and FF/CAP18 also induced upregulation of miR-663α expression in HCT116 cells. Over-expression of miR-663α in HCT116 cells showed anti-proliferative effects by suppressing the expression of the chemokine receptor CXCR4 and blocking cell cycle in G2/M [60]. Interestingly, treatment of HCT116 cells with FF/CAP18 caused a loss of mitochondrial membrane potential and increased DNA fragmentation, suggesting an early stage of apoptosis induced by LL-37-derived short peptides [56] (Fig. 2).

4.8 Induction of colon cancer cell death by FK-16

Both autophagy and apoptosis are programmed cell death processes and may occur within the same cell. Normally autophagy is followed by apoptosis [61]. DNA fragmentation, ER stress, hypoxia, and metabolic stress initiate intrinsic death pathways through activation of the proteins BAX and BAK, the release of cytochrome c from mitochondria and activation of caspase-9 which in turn cleaves Caspase-3 into the effector Caspase-3. Once caspases-3 is activated, cell apoptosis becomes irreversible...
Fig. 3. FK16 induces the apoptosis and autophagic cell death in human colon cancer cells. FK16 treatment induced up-regulation of p53 expression, then increased the expression of BAX, AIF/EndoG to induce HCT116 cell apoptosis. FK16 induced Bcl2 down-regulation, resulting in up-regulation of LC3-I/II, ATG5, ATG7 expression, then activating the autophagic death of HCT116 cells. AIF, Apoptosis-inducing factor; EndoG, Endonuclease G.

5. Future challenges

LL-37-derived short peptides have several potential advantages over the full-length LL-37 as future therapeutics. They have the capacity to neutralize endotoxins released by pathogens and enhance host immune responses to infection as well as their broad spectrum of antimicrobial activities. There are numerous AMPs in clinical development to treat infections associated with pathogenic microbes, but most of them are intended for topical applications. OP-145 (P60.4Ac) is a synthetic antimicrobial short peptide derived from LL-37. OP-145 has the capacity to bind LPS and LTA [52] and was formulated in eardrops to treat chronic bacterial middle ear infections [64]. Chronic suppurative otitis media (CSOM) is a chronic infectious disease with worldwide prevalence that causes hearing loss and decreased quality of life. Application of P60.4Ac-containing ototopical drops in the ear canal of patients suffering from CSOM was found to be safe and well-tolerated as well as treatment success in 47% of cases versus 6% in the placebo group [65]. The optimal dose of P60.4Ac was selected for the subsequent phase IIa study [64, 65].

As discussed earlier, a high number of LL-37-derived short peptides have been discovered with promising and potent activities to combat infections and colon cancer, but their translation into the clinic has been difficult. One of the greatest challenges restricting the development of LL-37-derived short peptides into therapeutics is their low metabolic stability. There is a low oral bioavailability for oral administration of peptide drugs because of degradations by proteolytic enzymes of the digestive system and their poor ability for penetration across the intestinal mucosa [66]. In the same way, intravenous administration is also limited due to rapid cleavage by proteolytic enzymes in the blood plasma and rapid removal from the circulation by excretory organs (liver and kidneys) before distribution to the surrounding tissues [66].

LL-37-derived short peptides can be obtained by purification from natural sources, chemical synthesis and expression using biological systems. Isolation from natural
sources is not viable to produce in bulk quantities for clinical trials. Expression systems can also be used to produce short peptides in bacteria, yeast, insect, plants and mammalian cells but with lower yields and limitations to introduce chemical modifications into the peptide sequence [67]. Chemical synthesis is generally considered as the most mature technology available to produce linear peptides up to 50 residues with sufficient yields [68]. Moreover, the short peptides can be engineered to improve the bioavailability [17, 34]. Currently, head-to-tail cyclisation and incorporation of D-amino acids are good strategies to improve stability and antimicrobial activity and to reduce salts sensitivity of various LL-37-derived short peptides. However, to overcome some of the hurdles like high biodegradability and rapid removal from circulation, new strategies are needed to facilitate short peptide antibiotic development.

In addition, the mechanisms of action of LL-37-derived short peptides need further studies. First, synergistic effects of LL-37-derived short peptides with other factors are worth further investigations. Several antimicrobial agents, such as human β-defensin and LL-37, have been shown to have synergistic antibacterial and anti-inflammatory activities [69–72]. LL-37 is reported to promote the IFNβ signaling and to contribute to the induction of an antiviral state in susceptible cells [73]. Thus, combination of LL-37-derived short peptides with other anti-infection or anti-cancer agents may constitute novel treatment strategies. The sensitivity of cells to LL-37-derived short peptides also needs careful consideration. In breast, lung, and prostate cancers, LL-37 promotes tumor cell proliferation, migration, and tumorigenesis through the cell surface receptor signaling. However, in other types of cancers, such as gastric cancer, colon cancer, and T-cell leukemia, LL-37 suppresses the proliferation and induces apoptotic and autophagic cell death. There is no conclusive evidence to explain the opposing effects of LL-37 on various cancers [33], presumably due to the promiscuous capacity of LL-37 to interact with cellular receptors that transduce divergent signaling pathways. Finally, the mechanisms of LL-37-derived short peptides interacting with different receptors need further in-depth investigations. LL-37 acts through several receptors, including at least four G-protein-coupled receptors (GPCRs), three Receptor tyrosine kinases (RTKs), a ligand-gated ion channel (LGIC) and Toll-like receptors (TLRs) [8, 74–80]. The mechanisms of LL-37 and LL-37-derived short peptides interacting with these receptors have thus far not been completely clear. More in-depth research is warranted to better understand the activities of LL-37 and its short peptides both in vivo and in vitro to determine precise mechanistic basis for more effective clinical applications.

6. Strategies to coping with challenges

For the challenges posed by the nonspecific distribution and short half-lives of LL-37 and its short peptides, an important consideration is to develop degradable, controlled-release polymers and polymeric nanoparticles as carriers. Polymeric drug delivery system is represented by a formulation or a medical device that is able to deliver drugs to target cells or organs, while the degradation and release of therapeutic molecules are controlled [81, 82]. Since the first generation of polymeric drug delivery system passively carrying PEGylated molecules to the second-generation system which was able to selectively bind specific targets, the third-generation system has emerged [81], which has evolved into the utilization of local biochemical changes in aberrant disease states to trigger activation-driven drug release [81]. It is also important to better understand the role of LL-37 in the pathogenesis of inflammation and tumorigenesis because LL-37 may display opposing activities in different tumors [33]. In addition, it should be kept in mind that LL-37 has been reported to interact with several receptors expressed by different cells or tissues to elicit diverse biological activities [8, 74–80]. Therefore, use of LL-37 and its short peptides as future therapeutic agents must be based on the development of more efficient delivery system and understanding of the precise mechanisms of human diseases.

7. Author contributions

KC, WG, JH—writing original draft; TY, JMW—editing the manuscript. All authors have read and agreed to the published version of the manuscript.

8. Ethics approval and consent to participate

Not applicable.

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11. Conflict of interest

The authors declare no conflict of interest.

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