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, re-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-AAVs) 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 difference 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 microbiocidal 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 GRK-SAKKIGKRAKRI, 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 (KRSKRKRRIHQRVRIS−NH2) and P7 (TLSKEK-ERIVQRVRTS−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-

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

AMP	Sequence	AA numbers
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	37
LL-27	FRKSKEKIGKEFKRIVQRIKDFLRNLV	27
RK-31	RKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	31
KS-30	KSKEKIGKEFKRIVQRIKDFLRNLVPRTES	30
KR20	KRIVQRIKDFLRNLVPRTES	20
FK16	FKRIVQRIKDFLRNLV	16
KR12	KRIVQRIKDFLR	12
GF17	GFKRIVQRIKDFLRNLV	17
17BI	G*X*KR*L*VQR*L*KD*XL*RNLV	17
GF18	GEFKRIVQRIKDFLRNLV	18
GI-20	GIKEFKRIVQRIKDFLRNLV	20
GI-20d	*GIKEFKRIVQRIKDFLRNLV*	20

Red italic: Engineered short peptides or amino acid residues.

37 known to possess antimicrobial activities. KR-12 displayed a selective toxic effect on bacteria but not human cells [38, 39]. KE-18 (KEFKRIVQRIKDFLRNLV) 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) (KRIVKLLLKWLR-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 (MDRPA; 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 develop-

ment 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 (FKRIVQRIKDFLR-NH2) analogs based on the sequence of the 13-meric short FK13 peptide (residues 17–29 of LL-37), including FK-13-a1 (WKRIVRRIKRWLR-NH2), FK13-a2 (WKRIVRWIKRWLR-NH2), FK13-a3 (WKRIVRRIWRWLR-NH2), FK13-a4 (FKRWVQRWKRFLR-NH2), FK13-a5 (WKRWVQRWKRFLR-NH2), FK13-a6 (WKRWVQRWKRWLR-NH2) and FK-13-a7 (WKRWVRRWKRWLR-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 Ile residues at positions 4 and 8 (Ile4,8 → 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 Trp11 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

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 ESKAPE 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-IGKEFKRIVERIKRFLRELVRPLR-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 1×10^6 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 week 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 Amtimicrobial 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
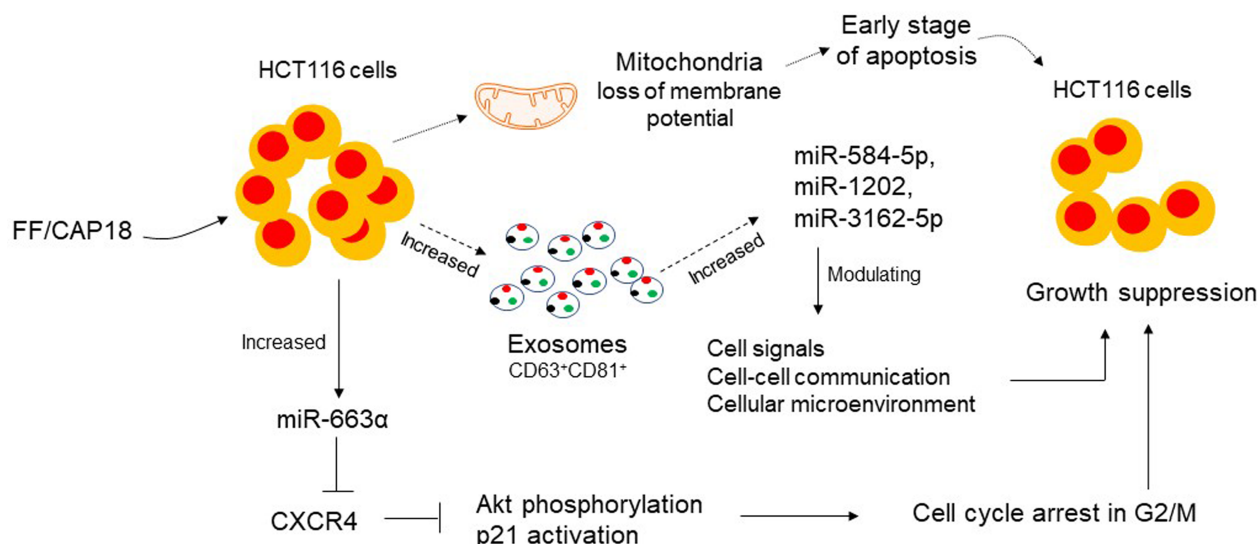
Table 2. Anti-viral mechanisms of LL-37 and its short peptides.

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, 17BI, 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 (RGGRLCYCRRRFCVCVGR); SMAP-29, Sheep Myeloid Antimicrobial Peptide 29 (*RGLRRL-GRKIAHGVKKYGPTVLRIIRIAG*); BMAP-18, Bovine Myeloid Antimicrobial Peptide-18 (*GRFKRFRKKFKKLFKKLS*).

DASamP2: DASamP2 was discovered by screening a library of representative peptides selected from the Antimicrobial Peptide Database (IKWKKLLRAAKRIL-NH$_2$).

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]. Anti-virus 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.

cal sequence to GI-20, another short peptide derived from LL-37. Ebola virus (EV) is a single-stranded, negative-sense RNA virus, which causes Ebola virus disease (EVD) characterized by fever, gastrointestinal signs, and multiple organ dysfunction syndrome [54]. HeLa cells and human macrophages, including primary and blood CD4+ monocyte-derived macrophages, were infected with VSV-eGP (a system with a recombinant vesicular stomatitis virus (rVSV) expressing EBOV-GP and green fluorescence protein) together with AMPs (LL-37, GF-17, 17BI, GI-20, GI-20d and RI-10). 17BI and GI-20d showed the highest level of inhibition of VSV-eGP infection. 17BI and GI-20d also blocked the infection of wild-type (WT) EBOV at a much higher level than the peptides containing only L-amino acids, such as GF-17 and GI-20. Mechanistic studies revealed that 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 [34] (Fig. 1).

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
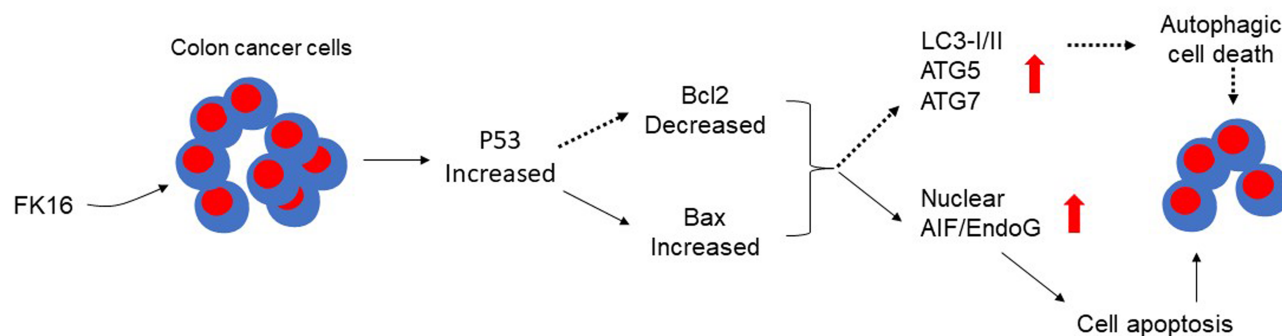
Fig. 3. FK16 induces the apoptosis and autophagic cell death in human colon cancer cells. FK16 treatment induced up-regulation of p53 expression, then increased the expression of BAX, AIF/EndoG to induce HCT116 cell apoptosis. FK16 induced Bcl2 down-regulation, resulting in up-regulation of LC3-I/II, ATG5, ATG7 expression, then activating the autophagic death of HCT116 cells. AIF, Apoptosis-inducing factor; EndoG, Endonuclease G.

[61]. Autophagy in colon cancer cells is activated by the miRNA (or LncRNA)-induced PI3K/Akt/mTOR signaling pathway or the Reactive oxygen species (ROS)-induced AMPK/mTOR signaling pathway [61]. For example, LncRNA is upregulated in clinical CRC tissues. Blockage of LncRNA in HCT116 cells induces apoptosis and inhibits autophagy by regulating the mir-100/ATG5 axis and the PI3K/AKT/mTOR pathway [62]. Oridonin, a compound isolated from medicinal herb *Rabdosia rubescens*, initiates colon cancer cell apoptosis through upregulation of caspase-3 cleavage and activation of autophagy by increasing the expression of Becin1 and LC3-II via AMPK/mTOR/ULK1 [63].

FK-16 is a short peptide corresponding to the residues 17–32 of human LL-37. Both FK-16 and LL-37 significantly reduced the viability of the human colon cancer LoVo and HCT116 cells [32]. Notably, FK-16 displayed a more potent activity against colon cancer cells than LL-37. Moreover, FK-16 had a minimal effect on the viability of normal human colon mucosal epithelial NCM460 cells. FK-16 upregulated nuclear p53 expression, thereby upregulating expression of Bax and downregulating Bcl-2 expression. The Bax signal was increased followed by increased nuclear levels of AIF and EndoG, which originally localized in mitochondria and translocated into the nuclei upon activation to trigger chromatin condensation and DNA fragmentation in cancer cells to induce programmed cell death. As FK-16 induced downregulated Bcl-2 expression, HCT116 cells increased autophagy-related proteins LC3-I and LC3-II as well as Atg5 and Atg7, which is required for the formation of autophagosomes at an early stage in cancer cells, to trigger cancer cell death. This fact was supported by the evidence that increased formation of LC3+ autophagic vacuoles and knockdown of Atg5 or Atg7 in HCT116 cells significantly reduced the cytotoxic effect of FK-16 for cancer cells. However, the full-length LL-37 had a minimal effect on LC3-I/II expression, indicating the importance of the short peptide FK-16 to induce autophagic death of colon cancer [32] (Fig. 3).

5. Future challenges

LL-37-drived 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 II$^{\alpha}$ study [64, 65].

As discussed earlier, a high number of LL-37-drived 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-drived 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.

12. References

[1] Zhang M, Liang W, Gong W, Yoshimura T, Chen K, Wang JM. The Critical Role of the Antimicrobial Peptide LL-37/CRAMP in Protection of Colon Microbiota Balance, Mucosal Homeostasis, Anti-Inflammatory Responses, and Resistance to Carcinogenesis. Critical Reviews in Immunology. 2019; 39: 83–92.

[2] Xhindoli D, Pacor S, Benincasa M, Scocchi M, Gennaro R, Tossi A. The human cathelicidin LL-37–a pore-forming antibacterial peptide and host-cell modulator. Biochimica et Biophysica Acta - Biomembranes. 2016; 1858: 546–566.

[3] Travis SM, Anderson NN, Forsyth WR, Espiritu C, Conway BD, Greenberg EP, *et al*. Bactericidal Activity of Mammalian Cathelicidin-Derived Peptides. Infection and Immunity. 2000; 68: 2748–2755.

[4] Gordon YJ, Huang LC, Romanowski EG, Yates KA, Proske RJ, McDermott AM. Human Cathelicidin (LL-37), a Multifunctional Peptide, is Expressed by Ocular Surface Epithelia and has Potent Antibacterial and Antiviral Activity. Current Eye Research. 2005; 30: 385–394.

[5] Tanaka D, Miyasaki KT, Lehrer RI. Sensitivity of Actinobacillus actinomycetemcomitans and Capnocytophaga spp. to the bactericidal action of LL-37: a cathelicidin found in human leukocytes and epithelium. Oral Microbiology and Immunology. 2000; 15: 226–231.

[6] Chen K, Yoshimura T, Gong W, Tian C, Huang J, Trinchieri G, *et al*. Requirement of CRAMP for mouse macrophages to eliminate phagocytosed *E. coli* through an autophagy pathway. Journal of Cell Science. 2021; 134: jcs252148.

[7] Nijnik A, Pistolic J, Wyatt A, Tam S, Hancock RE. Human cathelicidin peptide LL-37 modulates the effects of IFN-gamma on APCs. Journal of Immunology. 2009; 183: 5788–5798.

[8] Verjans ET, Zels S, Luyten W, Landuyt B, Schoofs L. Molecular mechanisms of LL-37-induced receptor activation: an overview. Peptides. 2016; 85: 16–26.

[9] Chen K, Yoshimura T, Yao X, Gong W, Huang J, Dzutsev AK, *et al*. Distinct contributions of cathelin-related antimicrobial peptide (CRAMP) derived from epithelial cells and macrophages to colon mucosal homeostasis. Journal of Pathology. 2021; 253: 339–350.

[10] Koczulla R, von Degenfeld G, Kupatt C, Krötz F, Zahler S, Gloe T, *et al*. An angiogenic role for the human peptide antibiotic LL-37/hCAP-18. The Journal of Clinical Investigation. 2003; 111: 1665–1672.

[11] Barlow PG, Li Y, Wilkinson TS, Bowdish DME, Lau YE, Cosseau C, *et al*. The human cationic host defense peptide LL-37 mediates contrasting effects on apoptotic pathways in different primary cells of the innate immune system. Journal of Leukocyte Biology. 2006; 80: 509–520.

[12] Heilborn JD, Nilsson MF, Kratz G, Weber G, Sørensen O, Borregaard N, *et al*. The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. The Journal of Investigative Dermatology. 2003; 120: 379–389.

[13] Ramos R, Silva JP, Rodrigues AC, Costa R, Guardão L, Schmitt F, *et al*. Wound healing activity of the human antimicrobial peptide LL37. Peptides. 2011; 32: 1469–1476.

[14] Grönberg A, Mahlapuu M, Ståhle M, Whately-Smith C, Rollman O. Treatment with LL-37 is safe and effective in enhancing healing of hard-to-heal venous leg ulcers: a randomized, placebo-controlled clinical trial. Wound Repair and Regeneration. 2014; 22: 613–621.

[15] Pahar B, Madonna S, Das A, Albanesi C, Girolomoni G. Immunomodulatory Role of the Antimicrobial LL-37 Peptide in Autoimmune Diseases and Viral Infections. Vaccines. 2020; 8: 517.

[16] Wang J, Cheng M, Law IKM, Ortiz C, Sun M, Koon HW. Cathelicidin Suppresses Colon Cancer Metastasis via a P2RX7-Dependent Mechanism. Molecular Therapy Oncolytics. 2019; 12: 195–203.

[17] Rajasekaran G, Kim EY, Shin SY. LL-37-derived membrane-active FK-13 analogs possessing cell selectivity, anti-biofilm activity and synergy with chloramphenicol and anti-inflammatory activity. Biochimica et Biophysica Acta - Biomembranes. 2017; 1859: 722–733.

[18] Johansson J, Gudmundsson GH, Rottenberg ME, Berndt KD, Agerberth B. Conformation-dependent antibacterial activity of the naturally occurring human peptide LL-37. The Journal of Biological Chemistry. 1998; 273: 3718–3724.

[19] Svensson D, Wilk L, Mörgelin M, Herwald H, Nilsson B. LL-37-induced host cell cytotoxicity depends on cellular expression of the globular C1q receptor (p33). The Biochemical Journal. 2016; 473: 87–98.

[20] Ciornei CD, Sigurdardóttir T, Schmidtchen A, Bodelsson M. Antimicrobial and chemoattractant activity, lipopolysaccharide neutralization, cytotoxicity, and inhibition by serum of analogs of human cathelicidin LL-37. Antimicrobial Agents and Chemotherapy. 2005; 49: 2845–2850.

[21] Larrick JW, Lee J, Ma S, Li X, Francke U, Wright SC, *et al*. Structural, functional analysis and localization of the human CAP18 gene. FEBS Letters. 1996; 398: 74–80.

[22] Cowland JB, Johnsen AH, Borregaard N. hCAP-18, a cathelin/pro-bactenecin-like protein of human neutrophil specific granules. FEBS Letters. 1995; 368: 173–176.

[23] Dürr UHN, Sudheendra US, Ramamoorthy A. LL-37, the only human member of the cathelicidin family of antimicrobial peptides. Biochimica et Biophysica Acta - Biomembranes. 2006; 1758: 1408–1425.

[24] Gudmundsson GH, Agerberth B, Odeberg J, Bergman T, Olsson B, Salcedo R. The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. European Journal of Biochemistry. 1996; 238: 325–332.

[25] Larrick JW, Hirata M, Balint RF, Lee J, Zhong J, Wright SC. Human CAP18: a novel antimicrobial lipopolysaccharide-binding protein. Infection and Immunity. 1995; 63: 1291–1297.

[26] Sørensen OE, Follin P, Johnsen AH, Calafat J, Tjabringa GS, Hiemstra PS, *et al*. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood. 2001; 97: 3951–3959.

[27] Morizane S, Yamasaki K, Kabigting FD, Gallo RL. Kallikrein expression and cathelicidin processing are independently controlled in keratinocytes by calcium, vitamin D(3), and retinoic acid. Journal of Investigative Dermatology. 2010; 130: 1297–1306.

[28] Sørensen OE, Gram L, Johnsen AH, Andersson E, Bangsbøll S, Tjabringa GS, *et al*. Processing of seminal plasma hCAP-18 to all-38 by gastricsin: a novel mechanism of generating antimicrobial peptides in vagina. The Journal of Biological Chemistry. 2003; 278: 28540–28546.

[29] Murakami M, Lopez-Garcia B, Braff M, Dorschner RA, Gallo RL. Postsecretory processing generates multiple cathelicidins for enhanced topical antimicrobial defense. Journal of Immunology. 2004; 172: 3070–3077.

[30] Yamasaki K, Schauber J, Coda A, Lin H, Dorschner RA, Schechter NM, *et al*. Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. FASEB Journal. 2006; 20: 2068–2080.

[31] Feng X, Sambanthamoorthy K, Palys T, Paranavitana C. The human antimicrobial peptide LL-37 and its fragments possess both antimicrobial and antibiofilm activities against multidrug-resistant Acinetobacter baumannii. Peptides. 2013; 49: 131–137.

[32] Ren SX, Shen J, Cheng ASL, Lu L, Chan RLY, Li ZJ, *et al*. FK-

16 derived from the anticancer peptide LL-37 induces caspase-independent apoptosis and autophagic cell death in colon cancer cells. PLoS ONE. 2013; 8: e63641.

[33] Kuroda K, Okumura K, Isogai H, Isogai E. The Human Cathelicidin Antimicrobial Peptide LL-37 and Mimics are Potential Anticancer Drugs. Frontiers in Oncology. 2015; 5: 144.

[34] Yu Y, Cooper CL, Wang G, Morwitzer MJ, Kota K, Tran JP, *et al*. Engineered Human Cathelicidin Antimicrobial Peptides Inhibit Ebola Virus Infection. iScience. 2020; 23: 100999.

[35] He M, Zhang H, Li Y, Wang G, Tang B, Zhao J, *et al*. Cathelicidin-Derived Antimicrobial Peptides Inhibit Zika Virus Through Direct Inactivation and Interferon Pathway. Frontiers in Immunology. 2018; 9: 722.

[36] Chingaté S, Delgado G, Salazar LM, Soto C. The ATPase activity of the mycobacterial plasma membrane is inhibited by the LL37-analogous peptide LLAP. Peptides. 2015; 71: 222–228.

[37] Aghazadeh H, Memariani H, Ranjbar R, Pooshang Bagheri K. The activity and action mechanism of novel short selective LL-37-derived anticancer peptides against clinical isolates of Escherichia coli. Chemical Biology and Drug Design. 2019; 93: 75–83.

[38] Jacob B, Park I, Bang J, Shin SY. Short KR-12 analogs designed from human cathelicidin LL-37 possessing both antimicrobial and antiendotoxic activities without mammalian cell toxicity. Journal of Peptide Science. 2013; 19: 700–707.

[39] Wang G. Structures of human host defense cathelicidin LL-37 and its smallest antimicrobial peptide KR-12 in lipid micelles. The Journal of Biological Chemistry. 2008; 283: 32637–32643.

[40] Luo Y, McLean DT, Linden GJ, McAuley DF, McMullan R, Lundy FT. The Naturally Occurring Host Defense Peptide, LL-37, and Its Truncated Mimetics KE-18 and KR-12 Have Selected Biocidal and Antibiofilm Activities Against *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli In vitro*. Frontiers in Microbiology. 2017; 8: 544.

[41] Hellyer TP, Morris AC, McAuley DF, Walsh TS, Anderson NH, Singh S, *et al*. Diagnostic accuracy of pulmonary host inflammatory mediators in the exclusion of ventilator-acquired pneumonia. Thorax. 2015; 70: 41–47.

[42] Kim EY, Rajasekaran G, Shin SY. LL-37-derived short antimicrobial peptide KR-12-a5 and its d-amino acid substituted analogs with cell selectivity, anti-biofilm activity, synergistic effect with conventional antibiotics, and anti-inflammatory activity. European Journal of Medicinal Chemistry. 2017; 136: 428–441.

[43] Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrobial Resistance and Infection Control. 2019; 8: 76.

[44] Mishra B, Wang G. Titanium surfaces immobilized with the major antimicrobial fragment FK-16 of human cathelicidin LL-37 are potent against multiple antibiotic-resistant bacteria. Biofouling. 2017; 33: 544–555.

[45] Migliolo L, Felício MR, Cardoso MH, Silva ON, Xavier ME, Nolasco DO, *et al*. Structural and functional evaluation of the palindromic alanine-rich antimicrobial peptide Pa -MAP2. Biochimica et Biophysica Acta - Biomembranes. 2016; 1858: 1488–1498.

[46] Gallo J, Holinka M, Moucha CS. Antibacterial surface treatment for orthopaedic implants. International Journal of Molecular Sciences. 2014; 15: 13849–13880.

[47] Turner RJ. Metal-based antimicrobial strategies. Microbial Biotechnology. 2017; 10: 1062–1065.

[48] Di Natale C, De Benedictis I, De Benedictis A, Marasco D. Metal-Peptide Complexes as Promising Antibiotics to Fight Emerging Drug Resistance: New Perspectives in Tuberculosis. Antibiotics. 2020; 9: 337.

[49] Haisma EM, de Breij A, Chan H, van Dissel JT, Drijfhout JW, Hiemstra PS, *et al*. LL-37-derived peptides eradicate multidrug-resistant Staphylococcus aureus from thermally wounded human skin equivalents. Antimicrobial Agents and Chemotherapy. 2014; 58: 4411–4419.

[50] de Breij A, Riool M, Kwakman PHS, de Boer L, Cordfunke RA, Drijfhout JW, *et al*. Prevention of Staphylococcus aureus biomaterial-associated infections using a polymer-lipid coating containing the antimicrobial peptide OP-145. Journal of Controlled Release. 2016; 222: 1–8.

[51] Kita H, Himi T. Cytokine and chemokine induction using cell wall component and toxin derived from gram-positive bacteria in the rat middle ear. Acta Oto-Laryngologica. 1999; 119: 446–452.

[52] Nell MJ, Tjabringa GS, Wafelman AR, Verrijk R, Hiemstra PS, Drijfhout JW, *et al*. Development of novel LL-37 derived antimicrobial peptides with LPS and LTA neutralizing and antimicrobial activities for therapeutic application. Peptides. 2006; 27: 649–660.

[53] Casanova V, Sousa FH, Shakamuri P, Svoboda P, Buch C, D'Acremont M, *et al*. Citrullination Alters the Antiviral and Immunomodulatory Activities of the Human Cathelicidin LL-37 During Rhinovirus Infection. Frontiers in Immunology. 2020; 11: 85.

[54] Jacob ST, Crozier I, Fischer WA, Hewlett A, Kraft CS, Vega MDL, *et al*. Ebola virus disease. Nature Reviews Disease Primers. 2020; 6: 13.

[55] Hayashi M, Kuroda K, Ihara K, Iwaya T, Isogai E. Suppressive effect of an analog of the antimicrobial peptide of LL-37 on colon cancer cells via exosome-encapsulated miRNAs. International Journal of Molecular Medicine. 2018; 42: 3009–3016.

[56] Kuroda K, Fukuda T, Yoneyama H, Katayama M, Isogai H, Okumura K, *et al*. Anti-proliferative effect of an analogue of the LL-37 peptide in the colon cancer derived cell line HCT116 p53+/+ and p53-/-. Oncology Reports. 2012; 28: 829–834.

[57] Kloosterman WP, Plasterk RHA. The diverse functions of microRNAs in animal development and disease. Developmental Cell. 2006; 11: 441–450.

[58] Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nature Cell Biology. 2007; 9: 654–659.

[59] Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nature Reviews. Cancer. 2006; 6: 259–269.

[60] Kuroda K, Fukuda T, Krstic-Demonacos M, Demonacos C, Okumura K, Isogai H, *et al*. MiR-663a regulates growth of colon cancer cells, after administration of antimicrobial peptides, by targeting CXCR4-p21 pathway. BMC Cancer. 2017; 17: 33.

[61] Xie Q, Liu Y, Li X. The interaction mechanism between autophagy and apoptosis in colon cancer. Translational Oncology. 2020; 13: 100871.

[62] Zheng Y, Tan K, Huang H. Long noncoding RNA HAGLROS regulates apoptosis and autophagy in colorectal cancer cells via sponging miR-100 to target ATG5 expression. Journal of Cellular Biochemistry. 2019; 120: 3922–3933.

[63] Bu H, Liu D, Zhang G, Chen L, Song Z. AMPK/mTOR/ULK1 Axis-Mediated Pathway Participates in Apoptosis and Autophagy Induction by Oridonin in Colon Cancer DLD-1 Cells. OncoTargets and Therapy. 2020; 13: 8533–8545.

[64] Malanovic N, Leber R, Schmuck M, Kriechbaum M, Cordfunke RA, Drijfhout JW, *et al*. Phospholipid-driven differences determine the action of the synthetic antimicrobial peptide OP-145 on Gram-positive bacterial and mammalian membrane model systems. Biochimica et Biophysica Acta - Biomembranes. 2015; 1848: 2437–2447.

[65] Peek NFAW, Nell MJ, Brand R, Jansen-Werkhoven T, van Hoogdalem EJ, Verrijk R, *et al*. Ototopical drops containing a novel antibacterial synthetic peptide: Safety and efficacy in adults with chronic suppurative otitis media. PLoS ONE. 2020; 15: e0231573.

[66] Vlieghe P, Lisowski V, Martinez J, Khrestchatisky M. Synthetic therapeutic peptides: science and market. Drug Discovery Today. 2010; 15: 40–56.

[67] Parachin NS, Mulder KC, Viana AAB, Dias SC, Franco OL. Expression systems for heterologous production of antimicrobial peptides. Peptides. 2012; 38: 446–456.

[68] Münzker L, Oddo A, Hansen PR. Chemical Synthesis of Antimicrobial Peptides. Methods in Molecular Biology. 2017; 1548: 35–49.

[69] Chen X, Niyonsaba F, Ushio H, Okuda D, Nagaoka I, Ikeda S, *et al*. Synergistic effect of antibacterial agents human beta-defensins, cathelicidin LL-37 and lysozyme against Staphylococcus aureus and Escherichia coli. Journal of Dermatological Science. 2005; 40: 123–132.

[70] Nagaoka I, Hirota S, Yomogida S, Ohwada A, Hirata M. Synergistic actions of antibacterial neutrophil defensins and cathelicidins. Inflammation Research. 2000; 49: 73–79.

[71] Midorikawa K, Ouhara K, Komatsuzawa H, Kawai T, Yamada S, Fujiwara T, *et al.* Staphylococcus aureus susceptibility to innate antimicrobial peptides, beta-defensins and CAP18, expressed by human keratinocytes. Infection and Immunity. 2003; 71: 3730–3739.

[72] Maisetta G, Batoni G, Esin S, Luperini F, Pardini M, Bottai D, *et al*. Activity of human beta-defensin 3 alone or combined with other antimicrobial agents against oral bacteria. Antimicrobial Agents and Chemotherapy. 2003; 47: 3349–3351.

[73] Ahmed A, Siman-Tov G, Keck F, Kortchak S, Bakovic A, Risner K, *et al*. Human cathelicidin peptide LL-37 as a therapeutic antiviral targeting Venezuelan equine encephalitis virus infections. Antiviral Research. 2019; 164: 61–69.

[74] De Marchi E, Orioli E, Pegoraro A, Sangaletti S, Portararo P, Curti A, *et al*. The P2X7 receptor modulates immune cells infiltration, ectonucleotidases expression and extracellular ATP levels in the tumor microenvironment. Oncogene. 2019; 38: 3636–3650.

[75] Lara R, Adinolfi E, Harwood CA, Philpott M, Barden JA, Di Virgilio F, *et al*. P2X7 in Cancer: From Molecular Mechanisms to Therapeutics. Frontiers in Pharmacology. 2020; 11: 793.

[76] Scheenstra MR, van Harten RM, Veldhuizen EJA, Haagsman HP, Coorens M. Cathelicidins Modulate TLR-Activation and Inflammation. Frontiers in Immunology. 2020; 11: 1137.

[77] Herster F, Bittner Z, Archer NK, Dickhöfer S, Eisel D, Eigenbrod T, *et al*. Neutrophil extracellular trap-associated RNA and LL37 enable self-amplifying inflammation in psoriasis. Nature Communications. 2020; 11: 105.

[78] Murakami T, Suzuki K, Niyonsaba F, Tada H, Reich J, Tamura H, *et al*. MrgX2-mediated internalization of LL-37 and degranulation of human LAD2 mast cells. Molecular Medicine Reports. 2018; 18: 4951–4959.

[79] Alford MA, Baquir B, Santana FL, Haney EF, Hancock REW. Cathelicidin Host Defense Peptides and Inflammatory Signaling: Striking a Balance. Frontiers in Microbiology. 2020; 11: 1902.

[80] Zhang Z, Le K, La Placa D, Armstrong B, Miller MM, Shively JE. CXCR2 specific endocytosis of immunomodulatory peptide LL-37 in human monocytes and formation of LL-37 positive large vesicles in differentiated monoosteophils. Bone Reports. 2020; 12: 100237.

[81] Kamaly N, Yameen B, Wu J, Farokhzad OC. Degradable Controlled-Release Polymers and Polymeric Nanoparticles: Mechanisms of Controlling Drug Release. Chemical Reviews. 2016; 116: 2602–2663.

[82] Sung YK, Kim SW. Recent advances in polymeric drug delivery systems. Biomaterials Research. 2020; 24: 12.

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