

# The human cathelicidin antimicrobial peptide LL-37 and mimics are potential anticancer drugs

Kengo Kuroda<sup>1\*</sup>, Kazuhiko Okumura<sup>2</sup>, Hiroshi Isogai<sup>3</sup> and Emiko Isogai<sup>1</sup>

<sup>1</sup>Laboratory of Animal Microbiology, Graduate School of Agricultural Science, Tohoku University, Sendai, Japan,

<sup>2</sup>Department of Oral and Maxillofacial Surgery, School of Dentistry, Health Sciences University of Hokkaido, Hokkaido, Japan,

<sup>3</sup>Animal Research Center, Sapporo Medical University, Sapporo, Japan

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Medicine, Japan

### \*Correspondence:

Kengo Kuroda,  
Laboratory of Animal Microbiology,  
Graduate School of Agricultural  
Science, Tohoku University, 1-1  
Amamiya-machi, Tsutsumodori,  
Aoba-ku, Sendai 981-8555, Japan  
[kengodoubi@gmail.com](mailto:kengodoubi@gmail.com)

### Specialty section:

This article was submitted to  
Molecular and Cellular Oncology,  
a section of the journal  
Frontiers in Oncology

**Received:** 13 April 2015

**Accepted:** 15 June 2015

**Published:** 30 June 2015

### Citation:

Kuroda K, Okumura K, Isogai H and  
Isogai E (2015) The human  
cathelicidin antimicrobial peptide  
LL-37 and mimics are  
potential anticancer drugs.  
*Front. Oncol.* 5:144.  
doi: 10.3389/fonc.2015.00144

Antimicrobial peptides (AMPs) play a critical role in innate host defense against microbial pathogens in many organisms. The human cathelicidin, LL-37, has a net positive charge and is amphiphilic, and can eliminate pathogenic microbes directly via electrostatic attraction toward negatively charged bacterial membranes. A number of studies have shown that LL-37 participates in various host immune systems, such as inflammatory responses and tissue repair, in addition to its antibacterial properties. Moreover, recent evidence suggests that it is also involved in the regulation of cancer. Indeed, previous studies have suggested that human LL-37 is involved in carcinogenesis via multiple reporters, such as FPR2 (FPRL1), epidermal growth factor receptor, and ERBB2, although LL-37 and its fragments and analogs also show anticancer effects in various cancer cell lines. This discrepancy can be attributed to peptide-based factors, host membrane-based factors, and signal regulation. Here, we describe the association between AMPs and cancer with a focus on anticancer peptide functions and selectivity in an effort to understand potential therapeutic implications.

**Keywords:** antimicrobial peptides, anticancer, carcinogenesis, LL-37, cathelicidin

## Introduction

Antimicrobial peptides (AMPs) are host defense molecules of the innate immune system of all life forms (1, 2). According to the AMP database, there are over 2,000 such peptides (3). They can be divided into seven groups: (I) linear peptides; (II) cyclic peptides; (III) glycopeptides; (IV) lipoglycopeptides; (V) lipopeptides; and (VII) thiopeptides and chromopeptides. AMPs typically contain fewer than 100 amino acids and occur in many cell types. They are generally cationic and amphipathic, and homologous peptides exist in vertebrates, invertebrates, and plants.

Mammalian AMPs belong to the defensin and cathelicidin families. Defensins contain six conserved cysteine residues in their sequence and exhibit characteristic  $\beta$ -sheet structures stabilized by intramolecular disulfide bonds (4). Cathelicidins are characterized by a highly conserved cathelin-like prosequence and variable carboxyl-terminal sequences that correspond to the mature AMPs (5, 6). Human cationic antibacterial protein of 18 kDa (hCAP18, also called LL-37 or FALL39) is the only cathelicidin in humans, and is primarily found in the secondary granules of neutrophils (6, 7); LL-37 is released as an active domain from macrophages/monocytes and various epithelial cells (8, 9).

Antimicrobial peptides were initially identified as functional antimicrobial molecules. Recently, they have been characterized as multifunctional peptides that serve a variety of biological roles,

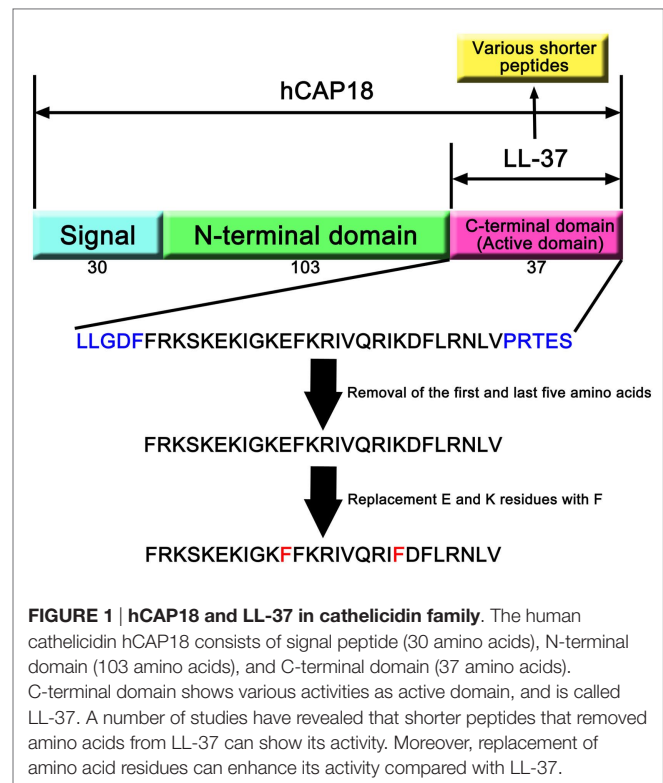
such as immune regulation, wound healing, angiogenesis, and anticancer functions. Their anticancer activity depends on cancer types. The interactions between AMPs and cancer cells influence apoptotic or other pathways and can result in cell death. Based on their multifunctional activities, there is a growing interest in the development of AMPs as anticancer agents. Magainins, cecropins, and defensins all have anticancer effects (10). An updated list of anticancer AMPs is available in the Antimicrobial Peptide Database (APD)<sup>1</sup>. The anticancer activities of human AMPs have not been widely evaluated; only six members (HNP-1, HNP-2, HNP-3, hBD-1, LL-37, and granulysin) with anticancer effects are annotated in the APD. LL-37 is overexpressed in breast, ovarian, and lung cancers, but it occasionally suppresses tumorigenesis in gastric cancer (11). Considering these reports, LL-37 can be associated with dual aspects of cancer progression via various receptors, such as epidermal growth factor receptor (EGFR), FRP2, ERBb2, P2X<sub>7</sub>, and GAPDH, or suppression via interaction with peptide-based factors and cancer membrane components. This review is described for discussion about these functional features of AMPs including LL-37.

Our research group previously found that the modified human-derived cathelicidin-related peptide FF/CAP18 has an anti-proliferative effect on the squamous cell carcinoma-derived cell line SAS-H1 (12) and the colon cancer-derived cell line HCT-116 (13), although the detailed mechanism underlying this effect is not clear. We showed that FF/CAP18 treatment inhibits the proliferation of these cancer cell lines, and results in apoptosis and cell death. The complex involvement of LL-37 and its analogs in various cancer types requires additional studies.

## LL-37

Only one cathelicidin (hCAP18/LL-37) has been found in myeloid bone marrow cDNA and isolated from neutrophils (7, 14, 15). In humans, cathelicidin exons 1–4 are located on chromosome 3p21. These are transcribed as a single gene encoding CAMP (cathelicidin antimicrobial peptide), a cationic, 18-kDa pre-pro-protein, which is also referred to as hCAP18 (14, 15). As shown **Figure 1**, hCAP18 is characterized by an N-terminal signal peptide (30 amino acid residues), a highly conserved pro-sequence (103 amino acid residues) called the cathelin-like domain, and a mature antimicrobial peptide referred to as LL-37 (37 amino acid residues with Leu–Leu at the N-terminus) at the C-terminal domain. LL-37 is expressed in almost all tissues and organs, such as neutrophils (15), myelocytes (16), testes (7), keratinocytes (17), and saliva (18). LL-37 is the accepted family name for mature AMPs from the C-terminal region rather than the full-length protein. FALL-39 (which differs from LL-37 by two amino acids) is analogous to PR-39 discovered in cattle (7).

LL-37 has a net positive charge of +6 at a physiological pH, a hydrophobic N-terminal domain, and an  $\alpha$ -helical conformation that is most pronounced in the presence of negatively charged lipids (6). LL-37 is produced from the C-terminal domain of



the hCAP18/LL-37 precursor protein by proteolytic cleavage. hCAP18/LL-37 from specific neutrophil granules is processed to the active peptide LL-37 following exposure to serine proteases, and particularly proteinase 3 from azurophil granules after exocytosis. Proteinase 3 cleaves hCAP18/LL-37 between the alanyl and leucyl residues (6). However, proteinase 3 is only expressed in myeloid cells and not in epithelial cells. The serine proteases, stratum corneum tryptic enzyme (SCTE, kallikrein 5) and stratum corneum chymotryptic protease (SCCE, kallikrein 7), control the activation of the precursor protein hCAP18/LL-37 on the skin surface and influence further processing to smaller peptides with alternate biological activity (5). Thus, the activity of cathelicidin is controlled by enzymatic processing of the proform to a mature peptide (LL-37) and/or various short forms, such as KR20 in humans (**Table 1**). In addition, the prostate-derived proteinase gastricsin (pepsin C) in the presence of vaginal fluid at low pH can also process epididymal-derived hCAP18/LL-37 in seminal plasma to functionally active ALL-38 (4). The antimicrobial activity of ALL-38 against a variety of microorganisms is equal to that of LL-37.

## Induction of LL-37

Various stimuli can induce LL-37 (**Table 2**). Bacterial infection is a particularly strong inducer because AMPs are functional peptides against pathogens. *Mycobacterium tuberculosis* infection induces the expression and production of LL-37 in a variety of cells, such as epithelial cells, alveolar macrophages, neutrophils, and monocyte-derived macrophages (9). Furthermore, LPS induces strong production of LL-37. However, some studies have reported

<sup>1</sup><http://aps.unmc.edu/AP/main.php>

**TABLE 1 | LL-37, short peptides, and analogs.**

AMP	Sequence (type)	Net charge <sup>a</sup>	Antimicrobial activity	LPS-binding activity	Anticancer activity	Reference
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES (original)	6	+	+	+	(7, 11, 14, 15)
LL-27 (hCAP18 <sub>109-135</sub> )	FRKSKEKIGKEFKRIVQRIKDFLRNLV (short)	7	+	+	+	(12, 19)
LL-CAP18	FRKSKEKIGKLFKRIVQRILDFLRNLV (analog designed)	7	+ (high)	+ (high)	+ (high)	(12, 19)
FF-CAP18	FRKSKEKIGKFFKRIVQRIFDFLRNLV (analog designed)	7	+ (high)	+ (high)	+ (high)	(12, 13, 19, 20)
RK-31	RKSKEKIGKEFKRIVQRIKDFLRNLPRTES (short)	7	+	ND	ND	(21)
KS-30	KSKEKIGKEFKRIVQRIKDFLRNLPRTES (short)	6	+	ND	ND	(21)
KR20	KRIVQRIKDFLRNLPRTES (short)	4	+	ND	ND	(21)
FK16	FKRIVQRIKDFLRNLV (short)	4	+	ND	+	(22)
KR12	KRIVQRIKDFLR (short)	4	+	ND	ND	(23)

Net charge: (K + R) – (D + E).

**TABLE 2 | Known factors that induce LL-37.**

Factor	Cell types	Reference
Bacterial infection, LPS, TNF- $\alpha$	A549 epithelial cells, alveolar macrophages, neutrophils, monocyte-derived macrophages, and keratinocytes	(9, 36)
Vitamin D3 (via VDR)	Neutrophil progenitors and EBV-transformed B cells, and cervical epithelial cells	(25–27)
Vitamin D3 and analogs	Myeloid leukemia, immortalized keratinocyte, and colon cancer cell lines	(37)
Short-chain fatty acids	HT-29 (colon epithelial cells) and U937 (monocytic cells)	(29, 30)
Zn <sup>2+</sup>	Caco-2 and intestinal epithelial cells	(31)
Butyrate	Colon, gastric, and hepatocellular cells	(32)
Curcumin	U937, HL29	(35)

that LPS has a minimal capacity to stimulate cathelicidin production after blood mononuclear cell activation (24). This could reflect differences among cell types. It was found to be upregulated by both 1,25-hydroxyvitamin D3 and 25-hydroxyvitamin D3, and the cathelicidin gene is regulated by the vitamin D pathway in humans (25–27). Exposure to sunlight, especially ultraviolet B photons, initiates the conversion of the provitamin D3 to previtamin D3 in the skin. The second step in vitamin activation is the formation of 1,25-dihydroxyvitamin D (active vitamin D3). LL-37 can be induced by ultraviolet B irradiation and is upregulated in infected and injured skin. Gant et al. found that ultraviolet B and vitamin D may reduce the risk of several autoimmune diseases and some cancers (28). Recently, it has been reported that LL-37 is induced by various stimuli, such as short-chain fatty acids (29, 30), Zn<sup>2+</sup> (31), and butyrate, which is a major metabolite produced by intestinal bacteria (32), and curcumin. Curcumin has been found to have clinical therapeutic and prevention potential for various cancers (33). Karunagaran et al. showed that curcumin-induced apoptosis mainly involves the mitochondria-mediated pathway in various cancer cells and that it inhibits proliferation of cancer cells by arresting them at various phases of the cell cycle. These effects are similar to those of LL-37 and the analogs (34). Guo et al. reported that curcumin upregulated CAMP mRNA and protein levels in U937 and HT29 cells through a vitamin D receptor-independent manner. The anticancer effect of curcumin can mediate not only direct signaling pathway but also upregulation of CAMP mRNA/the protein level and vitamin D receptor expression (35).

## Function of LL-37 in Cancer

Cancer is a major world health problem, and it is predicted that there will be approximately 26 million new cancer cases and 17 million cancer-related deaths annually by 2030 (38). The management of cancer currently suffers from several issues. Cancer treatment strategies include radiation therapy, chemotherapy, and a combination of these, chemoradiotherapy, all of which exert cytotoxicity on cancer cells (39, 40). In addition, specific inhibitors are available, which are used for cancer therapy, such as RTK or kinase inhibitors, in the form of monoclonal antibodies or small organic molecules (41–43). Although these treatments lead to improvements in many tumor types, they can cause severe side effects and delayed neurotoxicity owing to their non-specific mechanisms, which is the first crucial matter. The second issue is the development of resistance, which is caused by a number of factors. Many conventional anticancer reagents target factors related to cancer cell growth and show poor tumor penetration, resulting in reduced sensitivity of hypoxic cells in tumors that are in a growth-arrested state (44). Furthermore, the *ABCB1* (*MDR-1*) gene can confer multidrug resistance in cancer cells via P-glycoprotein (P-gp), which belongs to the ATP-binding cassette family of transporters (45–47). P-gp expression may be low before chemotherapy; however, it is induced by chemotherapy, resulting in the transport of anticancer reagents from the cell before they interact with their intracellular targets (48). Therefore, to combat cancer, it is necessary to develop an innovative and unique therapeutic strategy. Several studies have indicated possible new targets of cancer treatment, such as the mitochondria (49), hybrid tubulin-targeting compounds (50), and anti-angiogenesis (51). However, it is generally accepted that the accumulation of oncogenes and tumor suppressor gene mutations promotes cancer development and cellular heterogeneity. High-throughput DNA sequencing data suggest that thousands of point mutations, translocations, amplifications, and deletions contribute to cancer development, and that the mutational range differs, even among tumors with identical histopathology (52). Therefore, any therapeutic strategy designed to target a single biological event or individual signaling molecules is limited with respect to its ability to improve current survival rates, and novel strategies are needed.

The identification and development of peptides with therapeutically useful anticancer potential can be an innovative strategy (53, 54). AMPs function in first-line defense against infections and

exhibit potent cancer cell toxicity (55, 56). According to the APD<sup>1</sup>, more than 170 peptides have anticancer effects. Accumulating evidence supports the role of the human cathelicidin antimicrobial peptide LL-37 in carcinogenesis. LL-37 and its fragments and analogs show anticancer effects for various cancer cell lines. In this review, we introduce the role of AMPs, with a focus on LL-37 in human cancer in the next section.

## LL-37 as a Therapeutic Target

LL-37 is actively involved in physiological responses in eukaryotic cells, such as tissue repair and wound healing, although it was originally identified as an antimicrobial peptide. Previous studies have suggested that the possible molecular targets are involved in these effects (Table 3). LL-37 induces cell migration and downstream innate immunity via transactivation of EGFR (57, 58), and stimulates chemotaxis and angiogenesis via G-protein-coupled formyl-peptide receptor 2 (FRP2), also known as formyl-peptide receptor-like 1 (FPRL1) (8, 59, 60) (Figure 2). Based on these findings, it is not surprising that LL-37 is linked to cancer progression and metastasis. Indeed, hCAP18/LL-37 is expressed in breast cancer cells, and its production is most markedly higher in the breast epithelium of high-grade tumors than in normal mammary epithelia or low-grade tumors (>5 ng/mg total protein) (61); furthermore, FPRL1 is expressed in breast cancer (8, 62). Heilborn et al. also revealed that transgenic expression of LL-37 significantly increases proliferation in the human keratinocyte cell line (HaCaT) and HEK293. Furthermore, Weber et al. showed that mRNA expression of hCAP18/LL-37 is strongly correlated with that of ERBB2 and with the presence of lymph node metastasis in estrogen receptor-positive tumors from clinical samples, and LL-37 synergistically increases ErBb2 signaling (63) (Figure 2). These effects can be inhibited, suggesting the possibility of therapeutic strategies targeting LL-37. A truncated N-terminal peptide of LL-37, LL-25, inhibits LL-37 signaling and induces migration and changes in cancer cell colony morphology. Therefore, LL-37 is a putative therapeutic target to prevent progression to metastatic disease, although the detailed molecular mechanisms remain to be clarified.

Interestingly, these reports indicate the involvement of a receptor; AMPs generally exert effects via electrostatic interactions with the cell membrane. Several studies have shown that AMPs other than LL-37, such as SK84, a glycine-rich AMP derived from the larvae of *Drosophila virilis*, NRC-3 and NRC-7 from Atlantic flounder species, and Temporin-1CEa isolated from skin secretions of

the Chinese brown frog, show breast carcinoma cytotoxicity via membrane destruction (67–69). Accordingly, the abovementioned investigations suggest the existence of signaling pathways via an LL-37-specific receptor, despite the lack of a detailed understanding of this mechanism.

Haussen et al. reported that LL-37 is expressed in human lung cancer cells (20–30 ng/mL) and acts as a growth factor (64). In this study, the EGFR signaling inhibitor AG1478 and MEK inhibitors, PD98059 and U1260, significantly inhibited LL-37-induced proliferation. Additionally, the activation of MAP kinases was detected. Thus, the effects of LL-37 on lung cancer depend on the EGFR pathway, and its effects on breast cancer depend on the downstream activation of MEK and MAP kinases (Figure 2). It is noteworthy that the concentration of LL-37 necessary to activate lung cancer cell proliferation was on the order of nanogram per milliliter, whereas the administration of 20 µg/mL LL-37 decreased rather than increased cell numbers. The LL-37 expression level in the lungs is increased during inflammatory and infectious lung diseases (70–73), and this could promote local cancer growth. Cigarette smoke induces chronic obstructive pulmonary disease, which is an inflammatory disease, and increases the risk of lung cancer development (74, 75). Recently, it was reported that mouse homolog cathelicidin-related antimicrobial peptide (CLAMP) expressed in myeloid cells promotes cigarette smoke-induced lung tumor growth by recruiting inflammatory cells (76). Therefore, there may be a strong association between human cathelicidin antimicrobial peptide LL-37, inflammation, and cancer development, and LL-37 may have unexpected positive effects for several types of cancer in normal conditions.

In prostate cancer, LL-37 is also overexpressed. *In vitro* and *in vivo* studies have demonstrated that proliferation and invasive potential decreased as a result of the targeted downregulation of CLAMP, indicating that the targeting of LL-37 in human prostate cancers could be the basis for new therapeutic strategies (77).

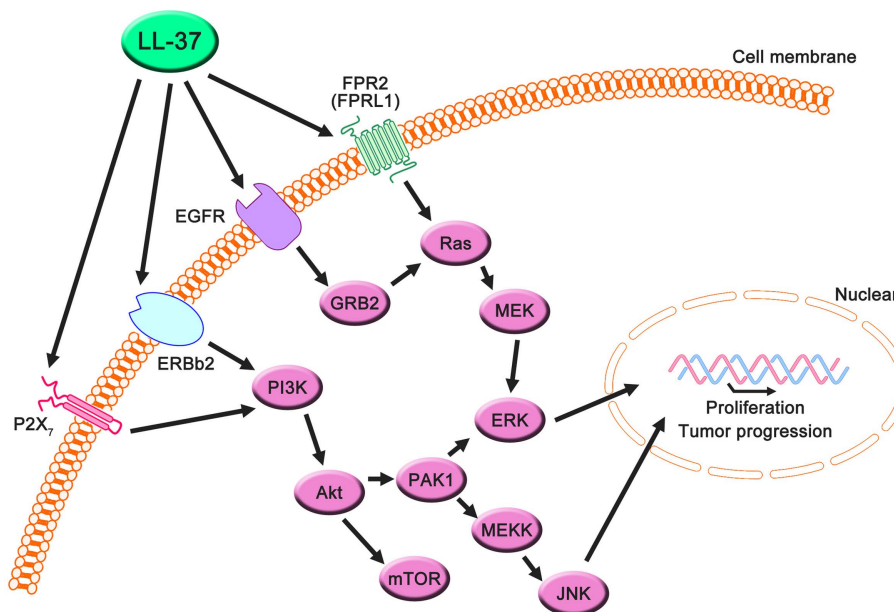
Coffelt et al. reported that LL-37 is significantly overexpressed in ovarian cancers relative to normal ovarian tissue and stimulates ovarian cancer cell proliferation, migration, invasion, and matrix metalloprotease secretion (1–25 µg/mL) (78). FPR2 (FPRL1) is not only involved in LL-37-stimulated cell growth but also promotes a more aggressive phenotype in ovarian cancer cells via a number of transcription factors in LL-37-FPRL1 signaling, such as cAMP response element binding protein (CREB), which may contribute to the invasive behavior of ovarian cancer cells (79). These findings indicate that LL-37-FPRL1 interactions in ovarian cancer cells are a potential target for a novel therapeutic strategy (Figure 2). The combination of CpG oligodeoxynucleotides (CpG-ODN) and LL-37 generates significant therapeutic antitumor effects in *in vivo* experiments (50–100 µg/mL) (19). Chuang et al. also observed that this combination enhances the proliferation and activation of peritoneal natural killer cells, resulting in antitumor effects. LL-37 promotes DNA translocation and can significantly increase interferon-α production in plasmacytoid dendritic cells (80); thus, it potentially delivers CpG-ODN to peritoneal immune effectors, causing potent tumor cytotoxicity. Based on these reports, LL-37 can be both a target and a candidate for therapeutic strategies for ovarian cancer.

In the last decade, P2X<sub>7</sub> receptor expression and activity have been reported in several cancers (81, 82), and LL-37 is a potential

**TABLE 3 | Possible molecular targets of LL-37.**

Target	Cell Types	Reference
EGFR	Lung carcinoma cell line, bronchial epithelial cell line, keratinocyte	(57, 58, 64)
FRP2 (FPRL1)	293 cells stably transfected with FPRL1, eosinophils, neutrophils, umbilical vein endothelial cells, lung cancer cell lines	(8, 59, 60, 62)
ERBB2	Breast cancer cell lines	(63)
P2X <sub>7</sub>	Monocyte	(65)
GAPDH	Monocyte	(66)





**FIGURE 2 | Proposed LL-37 signaling pathways involved in cancer cell proliferation, migration, and tumor progression.** Many studies have suggested that the PI3K/Akt and MAPK/Erk signaling pathways are activated

via the interaction between LL-37 and several receptors, such as FPR2 (FRPL1), EGFR, ERBB2, and P2X<sub>7</sub>. These signaling molecules can promote proliferation, migration, and tumor progression in cancer cells.

ligand (65) (**Figure 2**). P2X<sub>7</sub> triggers a range of responses including cell proliferation via the PI3K/Akt pathway (83). These findings indicate that LL-37 may promote growth via the P2X<sub>7</sub> receptor in several cancers (**Figure 2**).

## Anticancer Effects of LL-37 as well as its Fragments and Analogs

Part of the LL-37 C-terminal domain (hCAP18<sub>109–135</sub>: FRKSKEKIGKEFKRIVQRIKDFLRNLV) shows anti-proliferative effects on human squamous cell carcinoma, SAS-H1, cells (20–40 µg/mL) (12). Specifically, hCAP18<sub>109–135</sub> causes apoptosis via mitochondrial depolarization and DNA fragmentation but not via caspase activation. Furthermore, analog peptides with replacements of a glutamic acid residue and a lysine residue with leucine (LL/CAP18: FRKSKEKIGKLFKRIVQRILDFLRNLV) or phenylalanine (FF/CAP18: FRKSKEKIGKFFKRIVQRIKDFLRNLV) at positions 11 and 20, respectively, induce apoptotic cell death to a greater extent than did the original peptide (10–40 µg/mL). These analog peptides were designed to increase antimicrobial effects (84), which are associated with potent hydrophobic residues. This observation was based on the interactions between peptides and cancer cell membrane. These peptides, the LL-37 fragment, and its products containing amino acid substitutions can cause apoptotic cell death in cancer cells that have a more negatively charged cell membrane than in non-cancerous cells.

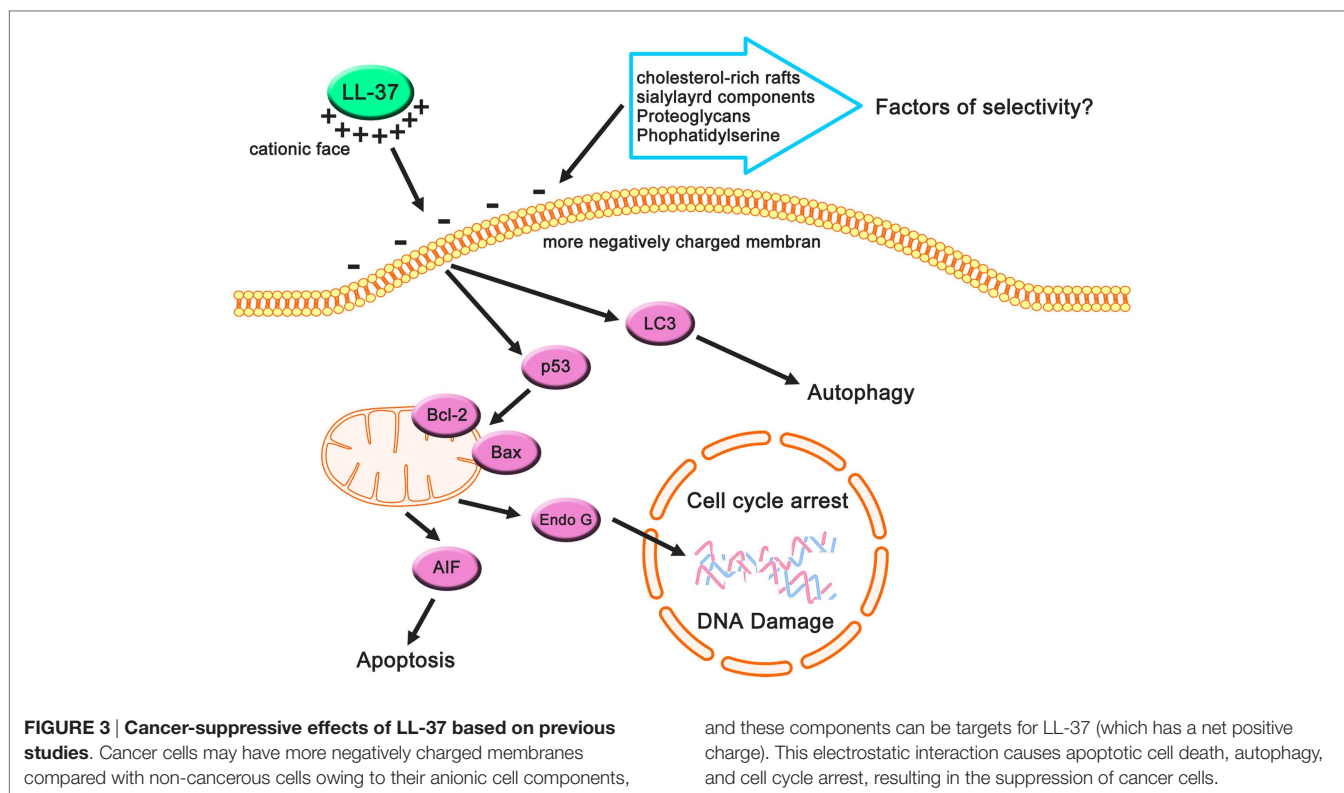
Several studies indicate that LL-37 and its fragments and analogs show cytotoxicity in other cancer cell types. LL-37 inhibits gastric cancer cell proliferation by the activation of bone morphogenetic protein (BMP) signaling via a proteasome-dependent

mechanism (4–40 µg/mL) (22), and also induces apoptosis via the mitochondrial-associated pathway in Jurkat human T leukemia cells (25–200 µg/mL) (20). FK-16 (FKRIVQRIKDFLRNLV), which is a shorter fragment of LL-37, induces caspase-independent apoptosis and autophagy via the common p53-Bcl-2/Bax cascade in colon cancer cells (20–40 µM) (85). We have also observed that FF/CAP18 suppresses colon cancer cell proliferation via apoptotic cell death and changes metabolome levels (10–40 µg/mL) (13, 86).

In all cancer cells in which it suppressed proliferation or promoted apoptosis, autophagy, and cell cycle arrest, LL-37 expression was downregulated (87–89). In addition, cathelicidin-deficient mice exhibit increased susceptibility to azoxymethane-induced colon carcinogenesis (89). These observations suggest that LL-37 has a direct role in the suppression of tumorigenesis in several types of cancer (**Figure 3**), but other types of cancer may be affected by LL-37 through receptors related to proliferation or migration. These characteristics are more strongly affected by targeting the cancer membrane than by signaling induced by LL-37-receptor interactions due to the anionic cancer membrane. According to this view, the interaction between LL-37, which has a cationic charge, and the negatively charged membrane of cancer cells is extremely important with respect to the development of new therapeutic strategies, and we review the current understanding of these interactions in the next section.

## Interaction between AMPs and the Cancer Membrane

When discussing the anticancer effects of LL-37 and its fragments and analogs, it is important to consider both peptide-based factors



and membrane-based factors. LL-37, one of the CAMPs, has a helical structure associated with increased peptide concentration, anions, pH, detergent, and lipids (6, 90), and interacts with the membranes of eukaryotic cancer cells. Its hydrophobicity and amphiphilicity may contribute to these interactions (91). Previous studies based on designed peptides have revealed that hydrophobicity is a critical factor in interactions between peptides and target cytoplasm membranes and the associated anticancer activity (92, 93). Moreover, anticancer peptide designed using a *de novo* approach show high specificity, i.e., they differentiate between cancerous and non-cancerous cells. It is generally recognized that amphiphilicity is a major determinant of the ability of peptides to partition the membrane; many studies have shown that changes in the amino acid distribution that disrupt the amphiphilic structure decrease the activity against bacteria or bacteria-mimic vesicles of many  $\alpha$ -helical AMPs (94). A net positive charge is also critical for the anticancer action of AMPs (95, 96). Lysine has strong preference for anionic membranes; however, arginine, another basic residue, exhibits a strong binding affinity to both zwitterionic and anionic membranes (97). Both arginine and lysine residues have a +1 charge in neutral buffer, indicating that lysine residues could be important in determining the selectivity of cancer cell membranes, which are more anionic, relative to those of non-cancerous cells. However, these factors alone are not sufficient to predict anticancer activities (98); thus, cancer membrane-based factors may also be important.

The widely accepted model of action of AMP interactions with cancer cell membranes is that AMPs are involved in a bilayer interaction involving a membranolytic mechanism and membrane translocation for the utilization of intracellular sites. A number

of studies have revealed that cancer cell membranes have distinct features relative to non-cancerous cells, such as cholesterol and a variety of anionic components (91, 96, 99). Increased cholesterol, a major sterol in eukaryotic cell membranes (100), inhibits the lytic ability of a number of  $\alpha$ -helical peptides toward membranes of non-cancerous cells and their lipid mimics. Therefore, cholesterol plays an important role in the general lack of anticancer action on cell membranes (91, 101–103). Interestingly, some cancer cells have increased cholesterol–lipid rafts (104), indicating that the relationship between AMPs and cholesterol–lipid rafts may decrease the cancer cell toxicity (99). The formation of cholesterol-rich lipid rafts may reduce cholesterol-depleted bulk membranes harboring phosphatidylserine that are more susceptible to peptide attacks owing to increased fluidity and hence less tightly packed lipids (96). Therefore, cholesterol-rich rafts can be a key factor in the anticancer effect of AMPs (Figure 3).

The main determinant of the selectivity and toxicity of AMPs specific to cancer cells and not non-cancerous cells appears to be the overexpression of anionic membrane components, including glycoproteins, glycolipids, proteoglycans (PGs), and phospholipids on the surface of cancer cells compared with non-cancerous cells. In cancer cells, changes in the glycosylation of glycoproteins and glycolipids, including the increased expression of their terminal sialic acids (105), contribute to the selectivity of AMPs toward cancer cells (91). Several studies have shown that anticancer effects are reduced by enzymatic digestion of sialyl residues on the surface of cancer cell membranes, strongly supporting this suggestion (106, 107). Thus, sialylated components of the cancer cell membrane play an important role in the selectivity and toxicity (Figure 2). PGs, which are negatively charged, contribute to the negatively

charged glycosaminoglycan side chains (108). Several studies have suggested that the expression of PGs on cancerous cell surfaces is much higher than on the surfaces of non-cancerous cells (109–111). Zwaal et al. reported that phosphatidylserine, a negatively charged phospholipid, can be exposed on the surface of the outer membrane leaflet in cancer cells (112) (Figure 2). These factors can contribute to the anticancer effects of AMPs including LL-37.

## Conclusion and Future Challenges

Despite the accumulation of scientific knowledge from a large number of studies showing that the anticancer action of LL-37 and other AMPs has potential applications for novel cancer treatment strategies, there are a few remaining challenges. In particular, its selectivity and toxicity are complicated and it will be important to consider the effects of both peptide-based and membrane-based factors. Furthermore, as we described in this review, there is a variation in the sensitivity of LL-37 among the cancer types. In breast, lung, and prostate cancers, LL-37 promotes proliferation, migration, and tumorigenesis through receptor signaling, but in other types of cancers, such as gastric cancer, colon cancer, and T-cell leukemia, it can suppress proliferation and induce apoptotic and autophagic cell death. There is no conclusive evidence to explain the opposite effects in various cancers. To resolve this issue, we may need to examine the effects of LL-37 on cancer cells from a different perspective.

It is also conceivable that LL-37 contributes to immune systems and exerts effects in combination with additional factors. Indeed,

several antimicrobial agents, such as human beta defensin and LL-37, have synergistic antibacterial and anti-inflammatory activities (113–116). Therefore, LL-37 can interact with several factors to induce both positive and negative effects on cancer cells. Combination therapies with anticancer agents are a possible novel cancer treatment strategy.

Two receptors, FPR2 (55) and P2X<sub>7</sub> (65), are thought to be involved in mediating the effects of LL-37 in various cell types. The glycolytic enzyme GAPDH has also been identified as a novel intracellular receptor, and is a direct binding partner for LL-37 in monocytes (66). However, the functions of these receptors including the intracellular effects mediated by LL-37 are not fully understood in cancer cells. For the application of AMPs as new therapeutic agents, it is necessary to clarify their receptor interactions and cellular mechanisms.

There are many well-known barriers to drug entry. Recently, new drug-delivery systems have been proposed (117). These approaches include mucoadhesives, viscous polymer vehicles, nanoparticles, and others. Local applications or missile treatments are possible for AMPs, irrespective of their toxicity. A novel nanocarrier was used to deliver the anticancer drug 5-fluorouracil to increase antitumor efficacy against breast cancer cells *in vitro* and *in vivo* (118). Eguchi et al. explained that magnetic anticancer drugs have the potential to greatly advance cancer chemotherapy for new theranostics and drug-delivery strategies (119). Anticancer therapy with AMPs could be successful when used in conjunction with new drug-delivery systems.

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**Conflict of Interest Statement:** This research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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