Host Immune Defense Peptide LL-37 Activates Caspase-Independent Apoptosis and Suppresses Colon Cancer

Shun X. Ren1, Alfred S.L. Cheng2, Ka F. To2,3, Joanna H.M. Tong3, May S. Lü, Jin Shen1, Clove C.M. Wong1, Lin Zhang1, Ruby L.Y. Chan1, Xiao J. Wang4, Simon S.M. Ng5, Lawrence C.M. Chiu6, Victor E. Marquez6, Richard L. Gallo5, Francis K.L. Chan2, Jun Yu5, Joseph J.Y. Sung2, William K.K. Wu6, and Chi H. Cho1,2

Abstract
Cathelicidins are a family of bacteriocidal polypeptides secreted by macrophages and polymorphonuclear leukocytes (PMN). LL-37, the only human cathelicidin, has been implicated in tumorigenesis, but there has been limited investigation of its expression and function in cancer. Here, we report that LL-37 activates a p53-mediated, caspase-independent apoptotic cascade that contributes to suppression of colon cancer. LL-37 was expressed strongly in normal colon mucosa but downregulated in colon cancer tissues, where in both settings its expression correlated with terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling-positive apoptotic cells. Exposure of colon cancer cells to LL-37 induced phosphatidylserine externalization and DNA fragmentation in a manner independent of caspase activation. Apoptogenic function was mediated by nuclear translocation of the proapoptotic factors, apoptosis-inducing factor (AIF) and endonuclease G (EndoG), through p53-dependent upregulation of Bax and Bak and downregulation of Bcl-2 via a pertussis toxin–sensitive G-protein–coupled receptor (GPCR) pathway. Correspondingly, colonic mucosa of cathelicidin-deficient mice exhibited reduced expression of p53, Bax, and Bak and increased expression of Bcl-2 together with a lower basal level of apoptosis. Cathelicidin-deficient mice exhibited an increased susceptibility to azoxymethane-induced colon tumorigenesis, establishing pathophysiologic relevance in colon cancer. Collectively, our findings show that LL-37 activates a GPCR-p53-Bax/Bak/Bcl-2 signaling cascade that triggers AIF/EndoG–mediated apoptosis in colon cancer cells.

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Introduction
Cathelicidin is a host defense peptide secreted by bone marrow cells, circulating leukocytes, and numerous types of epithelial tissues. It plays an active role in the maintenance of innate immunity. Not only does this peptide eliminate pathogenic microbes directly by serving as a natural antibiotic, but it also orchestrates a complex integration of host defense responses, including chemotaxis, cytokine production, and tissue repair (1). In human, cathelicidin is expressed as a 18-kDa preproprotein known as human cationic antimicrobial protein 18 (hCAP18). Proteolytic cleavage of hCAP18 is required for the release of the mature peptide LL-37. In the gastrointestinal tract, cathelicidin protects against Helicobacter pylori infection, promotes gastric ulcer healing, and alleviates inflammation in ulcerative colitis (2–4). LL-37 is highly expressed in colonic epithelial cells of the surface and upper crypts. Deeper crypt regions also have low-to-moderate level expression of LL-37 (5).

Emerging evidence supports that cathelicidin is implicated in malignant diseases. The expression of LL-37 is dysregulated in gastric, ovarian, lung, and breast cancers as well as melanoma and leukemia (6–11). The role of cathelicidin in tumorigenesis is complex and believed to be context-dependent (12). Acting as a tumor-suppressor, the expression of hCAP18/LL-37 is downregulated in gastric adenocarcinomas and acute myeloid and lymphocytic leukemia (6, 11, 13). In this regard, LL-37 induces cell-cycle arrest and apoptosis in gastric cancer cells and T lymphocytes, respectively (6, 14). The C-terminal fragment of LL-37 also exerts cytotoxic effects on both drug-resistant and drug-sensitive oral epitheloid carcinoma cells (15). Cathelicidin also enhances the antitumor activity of natural killer cells (16). The expression and function of cathelicidin in colon cancer, however, remain unclear.

A loss of balance between cell proliferation and cell death is central to tumorigenesis. Apoptosis or type I–programmed cell death is frequently dysregulated in human cancers. In the colon, reduced rates of apoptosis are associated with an
increased risk of colon adenomas (17). In mammalian cells, apoptotic signals are generally mediated through 2 caspase-dependent molecular pathways, namely, intrinsic and extrinsic cascades. Accumulating evidence supports that the third molecular pathway of apoptosis does exist. This pathway is characterized by the nuclear translocation of apoptosis-inducing factor (AIF) and endonuclease G (EndoG) that trigger chromatin condensation and DNA fragmentation independent of caspase activation (18). In the present study, we show that LL-37 was substantially downregulated in human colon cancer tissues and induced apoptosis in cultured colon cancer cells through the third molecular pathway of apoptosis.

Materials and Methods

Reagents

All primary antibodies for Western blot analysis were purchased from Cell Signaling Technology except formyl peptide receptor 2 (FPFR2) antibody (Abcam). All other chemicals and reagents were purchased from Sigma unless otherwise specified.

Patient samples and immunohistochemistry

Paraffin-embedded tissue microarrays (TMA) consisting of a total of 102 formalin-fixed colon adenocarcinoma tissues were used. The corresponding noncancerous colon mucosal tissues available from 60 matched cases were also included. The median age of the patients was 68 years (32–92 years) and the male to female ratio was 1.12:1. Immunohistochemical staining of mature LL-37 and Ki-67 was conducted using the corresponding noncancerous colon mucosal tissues. The CCD-18Co were obtained from the American Type Culture Collection and authenticated with short-tandem repeat profiling by the vendor. Bax-knocked out (Bax+/−) and Bax-hemizygously deleted (Baxx+/−) HCT116 cells were generated as previously described (19). The mice were maintained on normal diet and under standard laboratory condition. The present study was approved by the Institutional Laboratory Animals Ethics Committee.

Cell culture and assays for cell viability, cell cycle, and necrotic cell death

The human colon cancer cell lines HT-29, HCT116, SW1116, SW620, SW480, and LoVo and the normal colon fibroblasts CCD-18Co were obtained from the American Type Culture Collection and authenticated with short-tandem repeat profiling by the vendor. Bax-knocked out (Bax−/−) and Bax-hemizygously deleted (Baxx−/−) HCT116 cells were generated as previously described (20). Cells were maintained in their respective recommended culture media, supplemented with 10% FBS (1% during treatment), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified atmosphere of 5% CO2 and 95% air. Cell viability, cell-cycle distribution, and necrotic cell death were determined by MTT, flow cytometry, and lactate dehydrogenase release assays, respectively, as previously described (6).

Methylated-DNA capture (MethylCap)-qPCR

Genomic DNA was extracted using QIAamp DNA Mini Kit (Qiagen) and bisulfite-modified using EZ DNA Methylation Kit (Zymo Research). Methylated DNA was isolated from sonicated DNA (200–600 base-pair fragments) with affinity purification using MethylMiner Methylated DNA Enrichment Kit (Invitrogen). Equal amounts of amplified MethylCap and input DNAs were quantified using PCR primers targeting the hypermethylated regions by SYBR Green-base detection (Applied Biosystems) as previously described (21).

Nuclear protein extraction and Western immunoblotting

The isolation of nuclear and cytosolic protein was conducted using NucBuster Protein Extraction Kit (EMD Biosciences). For isolation of whole-cell protein, cells were harvested in radioimmunoprecipitation buffer containing protease and phosphatase inhibitors. Equal amount of proteins were resolved by SDS–PAGE followed by a standard immunoblotting procedure.

Immunofluorescence

Cells grown on coverslips were fixed with 4% (v/v) paraformaldehyde for 30 minutes. The cells were then covered with 10% (v/v) goat serum for 60 minutes at room temperature followed by incubation with diluted primary antibody at 4°C overnight. Cells were then probed with Alexa Fluor 488 secondary antibodies (Invitrogen). Fluorescent signals were detected using a confocal fluorescence microscope (Nikon ECLIPSE C1, Nikon).

RNA interference and Bcl-2 overexpression

The expression of AIF, EndoG, Bak, and p53 were lowered using predesigned target-specific siRNAs purchased from Qiagen. The Flag-Bcl-2 expression vector (18003) was obtained from Addgene, deposited by Dr. Clark W. Distelhorst (Case Western Reserve University, Cleveland, OH), siRNAs or purified plasmids were transfected into cells using Lipofectamine 2000 reagent (Invitrogen).

Quantitation of DNA fragmentation

DNA fragmentation in human colon cancer cell lines and mouse tissue samples was measured by in situ terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) kit from Roche Molecular Biochemicals. The mouse mucosal TUNEL-positive cells were evaluated in 5 randomized fields at ×400 magnification, and the average was taken and expressed as the number of apoptotic cells of each samples. For human TMAs, apoptotic cells were measured using the TUNEL stain with the Dead-End Kit (Promega). Apoptosis was assessed by a proportion score (0, none; 1, <5%; 2, 5%–10%; 3, >10%).

Quantitation of phosphatidylserine externalization

Treated cells were resuspended in staining buffer containing propidium iodide (PI) and Annexin V–fluorescein isothiocyanate (FITC; Invitrogen). After incubation for 15 minutes in dark, double-labeled cells were analyzed by the FACSCalibur System and CellQuest program.
Statistical analysis

Results were expressed as means ± SD of multiple experiments, unless otherwise specified. Statistical analysis was conducted with Student’s t test or an ANOVA followed by the Tukey t test where appropriate. P values less than 0.05 were considered statistically significant.

Results

Tissue microarray revealed the downregulation of LL-37 and its correlation with apoptosis in human colon cancer tissues

In noncancerous colonic mucosa, intermediate-to-high levels of cytoplasmic stain of LL-37 was observed (Fig. 1A). In colon cancers, 68 of 102 (66.7%) cases showed complete loss of LL-37 expression. Weak, intermediate, and strong immunoreactivities were noted in 18.6% (19/102), 5.9% (6/102), and 8.8% (9/102) of the cases, respectively (Fig. 1A). In 48 corresponding adjacent noncancerous tissues in which LL-37 immune scores were available, downregulation of LL-37 was seen in 46 of 48 (95.8%) cases (Fig. 1B; P < 0.0001). The overall expression of LL-37 in 102 cases of colon cancer was also significantly lowered than that of noncancerous-colonic tissues (Fig. 1C; P < 0.0001). Colonic epithelial cells were the major cell type that expressed LL-37, although immunoreactivity to LL-37 was also detected in submucosal leukocytes. The immune score of LL-37 was also positively correlated with TUNEL- but not Ki-67–positive (Fig. 1D and E) cells. The downregulation of LL-37 was not associated with the differentiation status or stage of the tumors.

DNA demethylation restored LL-37 expression in colon cancer cells

Cultured human colon cancer cells were treated with different epigenetic modifying agents, namely, 5-aza-2’deoxycytidine (5AZA; a DNA demethylating agent), trichostatin A (TSA; a histone deacetylase inhibitor), and 3-deazaneplanocin A (DZNep; a histone methylation inhibitor; ref. 22). 5AZA but not TSA or DZNep restored LL-37 expression in cultured colon...
cancer cells (Fig. 2A–C). Concordantly, methylated-DNA capture coupled with quantitative PCR (qPCR) revealed that DNA methylation in the promoter of CAMP (the gene encoding hCAP18/LL-37) was significantly higher in primary colon cancer tissues and human colon cancer cell lines HCT116 and LoVo as compared with normal colonic mucosa (Fig. 2D). In contrast, 5AZA treatment reduced the DNA methylation level (Fig. 2E). These findings would suggest that promoter DNA methylation was responsible for the downregulation of LL-37 in human colon cancer.

LL-37 induced DNA fragmentation and phosphatidylserine externalization without caspase activation in colon cancer cells

A panel of human colon cancer cell lines that consisted of HCT116 (p53 wild-type), LoVo (p53 wild-type), SW1116 (p53 mutant), SW480 (p53 mutant), and SW620 (p53 mutant), were treated with increasing concentrations of synthetic LL-37 peptide (Invitrogen) for 24 hours. The effect of LL-37 on normal colon fibroblasts CCD-18Co was also determined. MTT assay showed that LL-37 strongly reduced cell viability at doses ranging from 20 to 60 μmol/L in p53 wild-type HCT116 and LoVo. LL-37 did not show any effect on the viability of these 2 cell lines at low doses (500 ng/mL to 10 μg/mL; equivalent to 111 nmol/L to 2.2 μmol/L) that have been shown to stimulate cell proliferation in other cancer types (7–9). LL-37 also exerted significant but lesser inhibitory effects on p53-mutant SW1116, SW480, and SW620 but exhibited minimal cytotoxicity in CCD-18Co (Fig. 3A). In addition, mCRAMP (the murine cathelicidin) was used to treat the mouse cancer cell line Colon-26 (p53 wild-type) and the mouse immortalized colonocytes YAMC. Similar to the effect of LL-37, mCRAMP reduced cell viability of Colon-26 but had minimal effect on YAMC (Fig. 3A). These findings suggest that cathelicidin may have differential cytotoxicity toward p53 wild-type colon cancer cells over normal cells and p53-mutant colon cancer cells. Lactate dehydrogenase release assay indicated that LL-37 at the tested concentrations did not induce necrotic cell death (data not shown). Cell-cycle analysis showed that LL-37 treatment resulted in the accumulation of the sub-G1 phase, which was suggestive of DNA fragmentation associated with apoptosis (Fig. 3B and Supplementary Fig. S1A). The apoptogenic effect of LL-37 in colon cancer cells was subsequently confirmed by TUNEL assay (Fig. 3C and Supplementary Fig. S1B) and Annexin V staining (Fig. 3D), which detects DNA fragmentation and phosphatidylserine externalization, respectively. Activation of caspases and the
subsequent cleavage of PARP are classic molecular markers of apoptosis. Unexpectedly, after 24 and 48 hours of LL-37 treatment, no increase in PARP cleavage nor activation of caspase-3, -7, and -9 could be observed. On the contrary, both activation of caspases and cleavage of PARP were reduced by LL-37 (Fig. 3E), hinting at the possibility that LL-37 induced apoptotic cell death in a caspase-independent manner. This postulation was corroborated by the finding that the pan-caspase inhibitor z-VAD-fmk failed to reverse the loss of cell viability caused by LL-37 in HCT116 and LoVo (Fig. 3F).

**LL-37 triggered nuclear translocation of AIF and EndoG to mediate apoptosis**

AIF and EndoG are known mediators of cell death in caspase-independent apoptosis. These mediators, originally localized in the mitochondria, translocate into the nucleus to initiate DNA degradation upon activation by apoptotic signals (18). As shown in Fig. 4A, LL-37 increased the nuclear protein levels of AIF and EndoG and inversely reduced their cytosolic levels. The increases in the nuclear expression of AIF and EndoG were peaked at 6 hours after LL-37 treatment. The
Figure. 4. AIF and EndoG as mediators of LL-37–induced apoptosis. A, cytosolic and nuclear levels of AIF and EndoG were determined by Western blot analysis of fractionated proteins. GAPDH and Lamin A/C were used as loading controls for cytosolic and nuclear proteins, respectively. B and C, nuclear translocation of AIF (B) and EndoG (C) in HCT116 cells treated with or without LL-37 for 6 hours was determined by immunofluorescence. D and E, AIF- and EndoG-siRNA were transfected into HCT116 cells followed by LL-37 treatment for another 6 hours. D and E, nuclear expression (D) and subcellular localization (E) of AIF and EndoG were determined by Western blot analysis and immunofluorescence, respectively. Reciprocal regulation between AIF and EndoG in LL-37–induced apoptosis was noted. F, TUNEL staining showed that AIF- and EndoG-siRNA, in combination or alone, significantly reversed the apoptogenic action of LL-37 (40 μmol/L; 24 hours) in HCT116. Results were representative of 3 independent experiments.
change of subcellular localization of AIF and EndoG in response to LL-37 treatment was confirmed by immunofluorescence. After exposure to LL-37 for 6 hours, AIF and EndoG redistributed from the cytosol to the nucleus (Fig. 4B and C and Supplementary Fig. S1C). To determine whether AIF and EndoG were functionally involved in LL-37–induced apoptosis, RNA interference (RNAi) was used to knock down their expression. Transfection with AIF- or EndoG-siRNA reduced nuclear protein levels (Fig. 4D) and nuclear localization (Fig. 4E) of respective target induced by LL-37. RNAi targeting AIF and EndoG, alone or in combination, significantly reduced the DNA fragmentation caused by LL-37 as revealed by TUNEL assay (Fig. 4F and Supplementary Fig. S1D).

Altered expression of Bcl-2 family members was required for AIF- and EndoG-mediated apoptosis induced by LL-37

The mitochondrial release of AIF and EndoG is mediated by members of the Bcl-2 family (23). In this connection, LL-37 upregulated the proapoptotic Bak and Bak and reduced the antiapoptotic Bcl-2 (Fig. 5A and Supplementary Fig. S1E). The upregulation of Bak and Bak and the downregulation of Bcl-2 were most prominent at 6 hours after LL-37 treatment. To this end, genetic ablation of Bak, knockdown of Bak, or restoration of Bcl-2 expression reduced the nuclear protein levels of AIF and EndoG (Fig. 5B) and apoptosis (Fig. 5C) that were induced by LL-37. The reversal effect was most prominent in Bak-downregulated Bak-ablated cells (siBak + Bax−/−) and Bcl-2–overexpressing cells. C, TUNEL-positive staining indicated that knockdown of Bak, genetic ablation of Bak, or restoration of Bcl-2 reduced LL-37 (40 μmol/L; 24 hours)–induced apoptosis in HCT116. Results were representative of 3 independent experiments.

p53 Activation was required for LL-37–induced apoptosis

The tumor-suppressor protein p53 has been shown to mediate both caspase-dependent and -independent apoptosis in a wide variety of biologic contexts (24–26). As shown in Fig. 6A, LL-37–treated HCT116 cells (p53 wild-type) showed increased total and nuclear levels of p53. LL-37 also increased p53 expression in LoVo cells (Supplementary Fig. S1E).
protein expression of p53 up-regulated modulator of apoptosis (PUMA), a direct target gene of p53, was increased upon treatment with LL-37 in HCT116. The increased nuclear levels of p53 were peaked at 3 hours after LL-37 treatment, which preceded the altered expression of Bax and Bak and downregulation of Bcl-2 by LL-37 (40 μmol/L; 6 hours). C, nuclear expression of AIF and EndoG induced by LL-37 (40 μmol/L; 24 hours) was abolished by p53 knockdown. D, Annexin V staining revealed p53 was required for LL-37 (24 hours)–induced apoptosis in HCT116. E, the p53-mutant SW1116 colon cancer cells were resistant to the apoptogenic effect of LL-37 (40 μmol/L; 24 hours) as measured by Annexin V staining. F, G-coupled GPCR was inactivated by preincubation with PTX (40 ng/mL) for 2 hours before addition of LL-37 for 6 hours in HCT116. Inactivation of G-coupled GPCR reversed the upregulation of p53, Bax, and Bak and downregulation of Bcl-2 induced by LL-37. The apoptogenic effect of LL-37 (20 μmol/L; 24 hours) was also partially blocked by PTX but not by the FPR2 antagonist WRW4 (5 μmol/L; 24 hours pretreatment) as measured by Annexin V staining in HCT116. Results were representative of 3 independent experiments.

The apoptogenic action of LL-37 was mediated by G-protein–coupled receptor

The biologic effect of LL-37 has been reported to be mediated through G-coupled G-protein–coupled receptor (GPCR) in several cell types (27, 28). Here, we have shown that pertussis toxin (PTX) as an inhibitor of G-coupled GPCR reversed the upregulation of p53, Bax, and Bak and the downregulation of Bcl-2 by LL-37. More importantly, PTX was also observed...
to reduce LL-37–induced phosphatidylserine externalization in HCT116 (Fig. 6F). We found that FPR2 (formerly known as formyl peptide receptor like-1), a G<sub>i</sub>-coupled GPCR that has been shown to mediate the biologic action of LL-37 in other cell types, was expressed in colon cancer cells (data not shown). Nevertheless, the specific antagonist WRW4 failed to reverse the action of LL-37 (Fig. 6F), suggesting that a non-FPR2 G<sub>i</sub>-coupled GPCR was responsible for the activation of p53-Bcl-2/Bax/Bak axis by LL-37 to induce caspase-independent apoptosis.

**Cathelicidin-knockout mice showed reduced basal apoptotic rate in colonic mucosa and increased susceptibility to azoxymethane-induced gross colonic tumors**

The protein expression of p53, Bax, Bak, and Bcl-2 together with the basal level of apoptotic cells were determined in wild-type and cathelicidin-knockout (Cnlp<sup>−/−</sup>) mice. As compared with the wild-type mice, Cnlp<sup>−/−</sup> mice exhibited lower basal expression of p53, Bax, and Bak and higher basal expression of Bcl-2 in the colonic mucosa (Fig. 7A). Phenotypically, Cnlp<sup>−/−</sup> mice had significantly reduced number of TUNEL-positive cells in the colonic mucosa as compared with the wild-type mice (Fig. 7B). However, genetic ablation of Cnlp in mice did not have any substantial effect on crypt length or cellularity of the colon under normal conditions. To study the in vivo function of cathelicidin in the context of colon tumorigenesis, wild-type and Cnlp<sup>−/−</sup> mice (<em>n</em> = 7 in each group) were injected intraperitoneally with azoxymethane (Fig. 7C), a colon-specific carcinogen capable of inducing only microscopic premalignant lesions (i.e., aberrant crypt foci) but not normally gross tumors in mice. At 16 weeks after the last injection of azoxymethane, all 7 Cnlp<sup>−/−</sup> mice developed gross colonic tumors. In contrast, only 1 of 7 wild-type mice developed a minor colonic tumor, whereas the remaining mice were free from macroscopic lesion. The multiplicity and total tumor mass of...
azoxymethane-injected Cnlp<sup>−/−</sup> mice were significantly higher than those of wild-type mice (Fig. 7D).

### Discussion

LL-37 is the only cathelicidin found in humans. The context-dependent function of LL-37 poses some difficulties to define and understand its exact role in tumorigenesis. For example, while LL-37 has been shown to promote the growth of ovarian (7), lung (8), and breast cancers (9), the same peptide exerts tumor-suppressing effects in gastric cancer (6), acute myeloid (13), and lymphocytic leukemia (11). Consistent with its role as a tumor suppressor, LL-37 expression is downregulated in colon cancer. The correlation between LL-37 and apoptosis but not cell proliferation has also been shown by clinical sample analysis. Subsequent experiments revealed that promoter DNA hypermethylation might account for the downregulation of LL-37. Cathelicidin-deficient mice also exhibited increased susceptibility to carcinogen-induced colon tumors. The prominent downregulation of LL-37 and its association with apoptosis in human colon cancer prompt us to investigate whether LL-37 has apoptogenic function in colon tumorigenesis. In this study, our data revealed that, in p53 wild-type colon cancer cells, LL-37 induced phosphatidylserine externalization and DNA fragmentation without caspase activation, suggesting the occurrence of caspase-independent apoptosis. Such an apoptogenic role of cathelicidin has been reported in other cell types, such as periodontal ligament cells and infected airway epithelium (29, 30). But for the first time through our investigations, it is shown that cathelicidin acts through the caspase-independent pathway to mediate apoptosis in colon cancer cells.

The tumor-suppressor protein p53 accumulates in cells in response to DNA damage, oncogene activation, and other stresses. Depending on the cellular context, activation of p53 could lead to cell-cycle arrest, apoptosis, cellular senescence, differentiation, and autophagy (31). Restoring p53 activity is an attractive approach for cancer therapy and has led to the identification of a number of potential anticancer therapeutics, such as CP-31398 and Nutlin (32). Here, we show that LL-37 increased whole-cell p53 expression and p53 nuclear accumulation accompanied by induction of PUMA, Bax, and Bak and reduction of Bcl-2. In agreement, p53 has been shown to transcriptionally activate BAX and repress BCL2 (33, 34).
regulation of p53 and the Bcl-2 family members (i.e., Bax, Bak, and Bcl-2) by cathelicidin was also recapitulated in Cnp−/− mice. Our findings indicated that p53 plays a central role in cathelicidin-induced caspase-independent apoptosis.

AIF/EndoG–mediated DNA fragmentation represents a major mechanism of caspase-independent apoptosis (18). Here, we clearly showed that LL-37 induced nuclear translocation of AIF and EndoG to mediate apoptosis. We also showed that knockdown of AIF partially reduced the nuclear expression and localization of EndoG and vice versa (Fig. 4D and E), suggesting a novel reciprocal regulation between these 2 cell death mediators. Although AIF has been shown to bind to EndoG (35), it is the first time to show the existence of such a cooperative interaction. The release of AIF and EndoG is regulated by mitochondrial outer membrane permeability, which is in turn controlled by the relative abundance of the pro- and antiapoptotic members of the Bcl-2 family. Consistent with its role in apoptosis, LL-37–induced nuclear translocation of AIF/EndoG was paralleled by the upregulation of Bax and Bak and the downregulation of Bcl-2. The BH3-only protein PUMA, which is required for Bax- and Bak-mediated cell death (36), was also upregulated by LL-37. Bax and Bak can form complexes at the mitochondrial outer membrane to facilitate the release of apoptogenic mediators, whereas Bcl-2 sequesters BH3-only molecules to prevent the activation of Bax and Bak (37). In particular, Bax and Bak could promote the release of AIF and EndoG in a caspase-independent manner (38). Bcl-2 could also interact with Bax directly to prevent its homooligomerization that is required for membrane permeabilization (39). Our study shows that altered expression of these Bcl-2 family members triggers the activation of AIF and EndoG in LL-37–treated colon cancer cells.

In this study, PTX as an inhibitor of G, coupled GPCR blocked the apoptogenic effect and downstream signaling of LL-37. Several receptors, including FRP2, EGF receptor (EGFR), P2×γ purinoreceptor, Toll-like receptor 9 (TLR-9), and type 1 insulin-like growth factor receptor (IGF-1R), have been reported to mediate the biologic actions of cathelicidin (27, 40–43). Among these, only FRP2 is a PTX-sensitive GPCR. The others are receptor tyrosine kinases (EGFR and IGF-1R), membrane ion channel (P2×γ), or MyD88-dependent receptor (TLR-9) that are not coupled to G-protein. Although FRP2 was expressed in colon cancer cells, the specific antagonist WRW4 failed to reverse the action of LL-37, suggesting a FRP2-independent mechanism. However, it is still unstated whether LL-37 by itself could bind to FRP2 and to initiate the downstream signaling in colon cancer cells. It is also unclear whether the binding of LL-37 to FRP2 could be blocked by WRW4. Aside from FRP2, Niyonsaba and colleagues reported that the chemotactic effect of LL-37 on mast cells is mediated by another G, coupled GPCR (28). Further investigations will be needed to identify the receptor for LL-37 in colon cancer cells. Whether the tissue-dependent expression of receptors may alter the oncogenic or tumor-suppressing function of LL-37 also warrants further study.

In cancer therapy, the rapid regrowth of tumor after initial response to chemotherapeutic agents constitute a major clinical challenge. A recent study has shown that caspase-3 activation in dying cancer cells triggered the release of prostaglandin E2, which in turn stimulated the growth of surviving tumor cells (44). This finding would suggest that novel therapeutics agents that modulate through a caspase-independent mechanism may hold specific promises for cancer drug development. Our data support that LL-37 could induce caspase-independent apoptosis in human colon cancer cells via the activation of G, coupled GPCR-p53-Bcl-2/Bax/AIF/EndoG cascade. Our study not only elucidated the tumor-suppressing mechanism of LL-37 in colon tumorigenesis, but also supports the development of synthetic LL-37 peptide as an inducer of caspase-independent apoptosis.

Disclosure of Potential Conflicts of Interest

F.K.L. Chan has honoraria from Speakers Bureau of AstraZeneca, Eisai, and Pfizer and is a consultant/advisory board member of Pfizer. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.X. Ren, K.F. To, J.H.M. Tong, M.S. Li, S.S.M. Ng, I.C.M. Chiu


Writing, review, and/or revision of the manuscript: S.X. Ren, A.S.L. Cheng, K.F. To, S.S.M. Ng, V.E. Marquez, F.K.L. Chan, W.K.K. Wu

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.F. To, J.H.M. Tong, R.L. Gallo


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Correction: Host Immune Defense Peptide LL-37 Activates Caspase-Independent Apoptosis and Suppresses Colon Cancer

In this article (Cancer Res 2012;72:6512–23), which appeared in the December 15, 2012, issue of Cancer Research (1), the name of the sixth author was misspelled. The correct name is Jing Shen. The authors regret this error.

Reference

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