

#### Short Research Paper



# Cathelicidin LL-37 Promotes or Inhibits Cancer Cell Stemness Depending on the Tumor Origin

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#### Abstract

Antimicrobial peptides play critical protective roles in a range of human diseases, including cancer. Multiple studies have demonstrated functions—such as proliferation, angiogenesis, apoptosis and immunomodulation—of these peptides in crucial cancer pathways. We investigated the role of the antimicrobial peptide LL-37 on stemness in breast cancer (SKBR3) and melanoma cells (A375). PCR array analysis of differential gene expression in SKBR3 and A375 cancer cell lines downregulated for LL-37 expression by siRNA revealed downregulation of genes related to stemness, including telomerase reverse transcriptase, forkhead box D3 and undifferentiated embryonic cell transcription factor 1, remarkably in breast cancer cells. Furthermore, SKBR3 cells knocked down for LL-37 expression showed a decreased production of oncospheres in comparison with negative controls, while A375 cells exhibited increased production. Taken collectively, our findings indicate a role for LL-37 in cancer cell stemness depending on the cell type.

Key words: LL-37, cancer, stemness, pluripotency, self-renewal

# Introduction

Antimicrobial peptides play crucial roles in critical molecular pathways in cancer, such as cell proliferation, epithelial cell migration, angiogenesis promotion, induction of apoptosis and immunomodulation (1).

The effects of the antimicrobial peptide LL-37 in cancer remain unclear. While LL-37 acts as a positive regulator of ovarian, breast, melanoma and lung cancer progression, it also suppresses colorectal and gastric cancer cell growth (2), indicating that its effects are tumor-specific.

Recently, the concept of clonal tumor evolution has been challenged by the observation of cancer stem

cells (CSCs) in a variety of tumors. CSCs possess increased invasive and metastatic capabilities and render tumors more resistant to several microenvironmental stresses, including the action of several anti-cancer drugs (3).

Here we investigated the effects of LL-37 on stemness in both breast cancer and melanoma cells. We performed array analysis to examine the expression of 84 genes related to DNA damage in wild-type and LL-37-knockdown cancer cells.

## Material and Methods

The study protocol was approved by the

Hospital das Clinicas Ethical Committee, protocol 034/14.

## Cell culture.

Immortalized human breast cancer cells (SKBR-3) and skin malignant melanoma cells (A375) were used in this study. Cells were maintained according to the guidelines of the ATCC (American Type Culture Collection).

## Real-time PCR.

RNA was extracted from cultured cells using TRIZOL® protocol. RNA was quantified using NanoVeu (GE Heathcare) systems and RT-PCR was performed using the StepOne SuperScript® III (Applied Biosystems) protocol as provided by the manufacturer. Beta-2 microglobulin (B2M) gene was used as an internal control; primers were as follows: GAT GAG TAT GCC TGC CGT TGC, and CAA TCC AAA TGC GGC ATC T. The reactions were performed in a StepOne<sup>TM</sup> system (Applied Biosystems) at 50°C for 10 min, 95°C for 5 min and then 95°C for 15 s followed by 60°C for 30 s, and 72°C for 30 s for 40 cycles. Quantification was performed by  $2^{-\Delta\Delta CT}$  method.

## LL-37 gene silencing.

Cells were plated at  $2.5 \times 10^5$  cells per well in a 6-well plate overnight. LL-37 Silencer Selected Pre-designed short interfering RNA (siRNA) or negative scramble siRNA (Ambion<sup>®</sup>) (10 nM each) was combined with 5  $\mu$ L of Lipofectamine<sup>TM</sup> RNAiMAX reagent for 20 min. Opti-MEM<sup>®</sup> I Reduced Serum Medium (Invitrogen) was added to a final volume of 2.5 mL per well after cells were rinsed with PBS. After 24 h (SKBR3) or 48 h (A375), experimental assays were performed.

# PCR array.

Total RNA was converted into cDNA using the RT<sup>2</sup> First Strand Kit (SABiosciences, Frederick, USA) and cDNA was then combined with the RT<sup>2</sup> SYBR Green qPCR Master Mix (SABiosciences). Each sample was added to 24 Human DNA Damage PCR arrays (Qiagen, USA) according to the StepOne equipment protocol (Applied Biosystems). PCR-array data analysis was performed at the manufacturer's website (http://www.sabiosciences.com/pcrarraydat aanalysis.php).

## Sphere-forming assays.

The assay was performed as previously reported (4, 5). Briefly, after siRNA treatment, cells were detached and a single cell suspension was obtained after passing cells through 25 G needles. Cells

 $(1.0 \times 10^5)$  were plated in their respective cell culture media containing B27 supplement and rEGF (100 ng/ml; Sigma Aldrich, Poole, UK; E-9644). After 5 days, the number of spheres that were greater than 50  $\mu$ m in diameter were counted and sphere forming efficiency (%) was determined.

#### Statistical analysis.

Results were analyzed suing Mann-Whitney test and are shown as mean  $\pm$  standard deviation. A p-value < 0.05 was considered significant.

#### Results

# LL-37 production upregulates several pathways related to stemness.

We next analyzed differential gene expression in cancer cell lines downregulated for LL-37 expression by siRNA. Knockdown efficiency of LL-37 expression was more than 90%, as evaluated by qPCR (data not shown). PCR arrays showed a downregulation of several genes related to stemness, especially in SKBR3, for LL-37 compared with control cells (**Tables 1 and 2**).

# LL-37 knockdown cells show decreased production of oncospheres in breast cancer cells and increased production in melanoma cells.

We next analyzed the production of cancer-derived extracellular vesicles, oncospheres, as a hallmark of stemness in cancer cell lines depleted for LL-37 expression. Our results showed that SKBR3 cells knocked down for LL-37 expression produced a decreased number of oncospheres compared with negative controls (**Figure 1A**), while A375 produced an increased number of oncospheres compared with negative controls (**Figure 1B**).

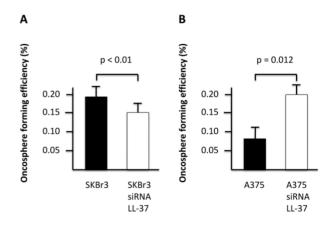


Figure 1. Oncosphere-forming efficiency in SKBR3 (A) and A375 (B) cell lines depleted for LL-37 expression by siRNA.

 Table 1. Genes upregulated in SKBR3 breast cancer cells

 compared with SKBR3 cells transfected with LL-37 siRNA

 treatment. Genes related to stemness are in bold.

Symbol	Gene Name	Fold Change	p-value
KAT2A	K(lysine) acetyltransferase 2A	1.6337	0.008484
COL2A1	Collagen, type II, alpha 1	1.8903	0.012853
GDF3	Growth differentiation factor 3	1.8402	0.014975
HNF4A	Hepatocyte nuclear factor 4, alpha	1.9064	0.016682
TERT	Telomerase reverse transcriptase	2.2468	0.018056
HAND1	Heart and neural crest derivatives expressed 1	2.1076	0.02039
HSPA9	Heat shock 70kDa protein 9 (mortalin)	3.7997	0.020459
SOX15	SRY (sex determining region Y)-box 15	2.1014	0.02309
NAT1	N-acetyltransferase 1 (arylamine N-acetyltransferase)	26.925	0.026202
ALPL	Alkaline phosphatase, liver/bone/kidney	1.838	0.026346
PARD6A	Par-6 partitioning defective 6 homolog alpha (C. elegans)	14.2326	0.026805
OLIG2	Oligodendrocyte lineage transcription factor 2	1.7598	0.031648
FOXD3	Forkhead box D3	2.6502	0.03305
MYBL2	V-myb myeloblastosis viral oncogene homolog (avian)-like 2	1.6863	0.035958
LIN28A	Lin-28 homolog A (C. elegans)	1.8108	0.036787
TP53	Tumor protein p53	1.9757	0.040021
FGF2	Fibroblast growth factor 2 (basic)	2.0355	0.044985
NES	Nestin	1.8498	0.047692
UTF1	Undifferentiated embryonic cell transcription factor 1	5.2139	0.048209

# Discussion

Self-renewal and pluripotency are the key characteristics of stem cells. Here, our findings suggest that LL-37 regulates stemness. Cancer stem cells (CSCs) are a considerable clinical problem, since they are highly resistant to radiation and chemotherapy (6). The mechanism of resistance is not fully understood, but enhanced DNA repair capacities and low intracellular reactive oxidative species concentrations are implicated (7). CSCs also proliferate more slowly than non-stem carcinoma cells, circulate in the bloodstream (8) and can lead to tumor metastasis and relapse. Definitive markers of CSCs do not exist, but our results demonstrated many interesting results in SKBR3 and A375 wild-type cells, when compared with LL-37 knockdown cells. Nestin, for example, is an important marker of CSCs, regulates proliferation, migration and invasion of cancer cells (9) correlating to a worse prognosis (10).

Telomerase reverse transcriptase, forkhead box D3 (FOXD3) and undifferentiated embryonic cell transcription factor 1 (UTF1) are other genes related to stemness, so we hypothesized that LL-37 should be important to maintain stem cell identity in breast cancer cells (**Table 1**). Upregulation of telomerase is a prerequisite for cellular immortalization and has been associated with stemness in various human cancers (11). FOXD3 induces cancer progression by epithelial-mesenchymal transition (12) and UTF1 increases stem cells reprogramming to pluripotency (13).

 Table 2. Genes upregulated in A375 melanoma cells compared with A375 cells transfected with LL-37 siRNA treatment. Genes related to stemness are in bold.

Symbol         Gene Name         Fold Change         p-value Change           LIN28A         Lin-28 homolog A (C. elegans)         1.5252         0           TCF3         Transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)         2.9868         0           RUNX2         Runt-related transcription factor 2         1.7998         0.000007           CCNA2         Cyclin A2         1.5997         0.000018           CD34         CD34 molecule         1.3355         0.000025           BGLAP         Bone gamma-carboxyglutamate (gla) protein         1.5147         0.000063           CD12         Cadherin 2, type 1, N-cadherin (neuronal)         1.6644         0.000046           CARPT         Fatty acid binding protein 7, brain         1.6157         0.000056           CCNE1         Cyclin E1         1.472         0.0000231           REST         RE1-silencing transcription factor         1.3996         0.00223           CDC42         Cell division cycle 42 (GTP binding protein, 2.5KDa)         1.4850         0.000278           GATA2         GATA binding protein 2         1.5476         0.000279           GDF3         Growth differentiation factor 3         1.7102         0.000278           GATA2         GATA4 binding protein 2	0 1 1		F 11	1
LIN28A         Lin-28 homolog A (C. elegans)         1.5252         0           TCF3         Transcription factor 3 (E2A immunoglobulin)         2.9868         0           enhancer binding factors E12/E47)         RUNX2         Runt-related transcription factor 2         1.7998         0.000007           CCNA2         Cyclin A2         1.3786         0.000018           CD34         CD34 molecule         1.3355         0.000025           BGLAP         Bone gamma-carboxyglutamate (gla) protein         1.5147         0.000046           CD14         Cadherin 2, type 1, N-cadherin (neuronal)         1.6644         0.000046           CCNE1         Cyclin E1         1.472         0.000063           OLG2         Oligodendrocyte lineage transcription factor         1.3996         0.000231           TUBB3         Tubulin, beta 3         1.4953         0.000248           CDC42         Cell division cycle 42 (GTP binding protein, 1.5889         0.00025           Z5KDa)         KRT15         Keratin 15         1.469         0.000278           GATA2         GATA binding protein 2         1.5476         0.000372           GDF3         Growth differentiation factor 3         1.7102         0.000242           TBA3         T-box 3         1.5775	Symbol	Gene Name		p-value
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NCAM1         Neural cell adhesion molecule 1         1.2164         0.000131           REST         RE1-silencing transcription factor         1.3996         0.000231           TUBB3         Tubulin, beta 3         1.4953         0.00025           CSC42         Cell division cycle 42 (GTP binding protein, 25kDa)         1.4953         0.000278           KRT15         Keratin 15         1.469         0.000278           GATA2         GATA binding protein 2         1.5547         0.000379           GDF3         Growth differentiation factor 3         1.7102         0.000222           TBX3         T-box 3         1.5775         0.000649           ALCDA         Activation-induced cytidine deaminase         1.3863         0.00157           RUNX1         Runt-related transcription factor 1         1.2238         0.002267           NAT1         N-acetyltransferase 1 (arylamine avactyltransferase)         1.4119         0.002267           NAT1         N-acetyltransferase 1 (arylamine avactyltransferase)         1.2117         0.003211           MYCN         V-myc myelocytomatosis viral related (avian)         1.2117         0.003221           MYCN         V-myc myelocytomatosis viral related matore         1.2172         0.003582           FGFR1         Fibrob		5	1.5713	
RESTRE1-silencing transcription factor1.39960.000211TUBB3Tubulin, beta 31.49530.000248CDC42Call division cycle 42 (GTP binding protein, 25kDa1.58890.000278KRT15Keratin 151.4690.000278GATA2GATA binding protein 21.55470.000372ALPLAlkaline phosphatase, liver/bone/kidney1.48160.000379GDF3Growth differentiation factor 31.71020.000422TBX3T-box 31.57050.000679ALCAActivation-induced cytidine deaminase1.38630.00157RUNX1Runt-related transcription factor 11.22380.002267KLF4Kruppel-like factor 4 (gut)1.11390.00216FGF2Fibroblast growth factor 2 (basic)1.42190.002267NATN-acetyltransferase 1 (arylamine N-acetyltransferase)1.38830.00311MYCNV-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)1.003220.00321FMADHeart and neural crest derivatives expressed 11.1160.003263FGF4Fibroblast growth factor 4 ceptor 11.36260.00583FGF4Fibroblast growth factor 4 ceptor 11.3620.00583FGF4Fibroblast growth factor 4 ceptor 11.36260.00583FGF4Fibroblast growth factor 4 ceptor 11.36260.00583FGF4Fibroblast growth factor 4 alphan1.4120.003263FGF4Fibroblast growth factor 4 alpha1.2090.00585<	NCAM1	0 1 0 1		
TUBB3Tubulin, beta 31.49530.000248CDC42Cell division cycle 42 (GTP binding protein, 25kDa)1.58890.00025KRT15Keratin 151.4690.000278GATA2GATA binding protein 21.55470.000379GDF3Growth differentiation factor 31.71020.000422TBX3T-box 31.57750.000669AICDAActivation-induced cytidine deaminase1.38630.00157RUNX1Run-related transcription factor 11.22380.0002267NAT1N-acetyltransferase 1 (arylamine n-acetyltransferase)1.38880.002578VAT1N-acetyltransferase)1.42190.002167NAT1N-acetyltransferase)1.42190.003271MYCNV-myc myelocytomatosis viral related n-acetyltransferase)1.6720.003271LEFTY2Left-right determination factor 21.34890.003161MYCNV-myc myelocytomatosis viral related n-acetyltransferase)1.21170.003824ZFP42Zinc finger protein 42 homolog (mouse)1.24110.003271HSP39Heat shock 70kDa protein 9 (mortalin)1.40290.005582FGF41Fibroblast growth factor 41.17890.003671FGF4Fibroblast growth factor 41.17890.003671PAX6Paired box 61.5540.005687FGF4Fibroblast growth factor 41.17890.005682FGF41Fibroblast growth factor 41.17890.005682FGF4Fibroblast growth factor 4<				
CDC42Cell division cycle 42 (GTP binding protein, 25kDa)1.58990.000278KRT15Keratin 151.4690.000278GATAGATA binding protein 21.5120.000372ALPLAlkaline phosphatase, liver/bone/kidney1.8100.000372GDF3Growth differentiation factor 31.71020.000422TBX0T-box 31.57050.000691AlCDAActivation-induced cytidine deaminase1.38630.001057RUNX1Runt-related transcription factor 11.2280.00216FGF2Fibroblast growth factor 2 (basic)1.4190.002267NAT1N-acetyltransferase 1 (arylamine n-acetyltransferase)1.38890.002516LEFTY2Left-right determination factor 21.34890.003161MYCNY-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)1.40290.003282FGF4Fibroblast growth factor receptor 11.40290.00582FGF4Fibroblast growth factor receptor 11.40290.00582FGF4Fibroblast growth factor 41.11790.00634Aldryde derlydrogenase 1 family, meme Al1.20160.00582FGF4Fibroblast growth factor 41.11740.00324AlstyAldendo G(mouse)1.6040.00582FGF4Fibroblast growth factor 41.11750.00582FGF4Fibroblast growth factor 41.11750.00582FGF4Fibroblast growth factor 41.11750.00582FGF4Gotyfier, fabrid		0 1		
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GATA2GATA binding protein 25547.0.003721ALPLAlkaline phosphatase, liver/bone/kidney1.48160.00379GDF3Growth differentiation factor 371020.000422TBX3T-box 357750.000669AICDAActivation-induced cytidine deaminase1.38630.00179 <b>RUNA1Runerelated transcription factor 1</b> 1.22380.002161FGF2Fibroblast growth factor 2 (basic)1.42190.002267NAT1N-acetyltransferase 1 (arylamine n-acetyltransferase)1.38880.003161MYC1V-myc myelocytomatosis viral related (arian) moregene, neuroblastoma derived (arian)1.16720.003161MAD1Heart and neural crest derivatives expressed 11.11790.003282FGF4Fibroblast growth factor 7 ceptor 11.36260.00583FGF4Fibroblast growth factor 4 ceptor 11.40290.00582FGF4Fibroblast growth factor 4 ceptor 11.40290.00583FGF4Fibroblast growth factor 4 apha1.4020.00583FGF4Fibroblast growth factor 41.7890.00483NDALNodal homolog (mouse)1.6940.00164ALDH1Idetry 4 ceptor 4 apha1.14160.00583FGF4Fibroblast growth factor 4 apha1.14160.00583FGF4Fibroblast growth factor 4 apha1.14160.00584FGF4Fibroblast growth factor 4 apha1.14160.01587FGF4Fibroblast growth factor 4 apha1.14160.015871 <td></td> <td></td> <td></td> <td></td>				
ALPLAlkaline phosphatase, liver/bone/kidney1.48160.000379GDF3Growth differentiation factor 31.71020.000422TBX3T-box 31.57750.000669AlCDAActivation-induced cytidine deaminase1.38630.001057RUNX1Runt-related transcription factor 11.22380.001949KLF4Kruppel-like factor 4 (gut)1.11390.00216FGF2Fibroblast growth factor 2 (basic)1.42190.002267NAT1N-acetyltransferase 1 (arylamine n-acetyltransferase)1.38880.003161MYCNV-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)1.16720.003221HAND1Heart and neural crest derivatives expressed 11.21170.003824ZFP42Zinc finger protein 42 homolog (mouse)1.24210.00582FGF4Fibroblast growth factor receptor 11.36260.00583NODALNodal homolog (mouse)1.69440.0043ALDH1A1Aldehyde dehydrogenase 1 family, member A11.20060.007585PAX6Paired box 61.15780.003869DNMT3BDNA (cytosine-5)-methyltransferase 3 beta1.20050.011056CDK1Cyclin-dependent kinase 11.4710.012871LEFTY1Left-right determination factor 11.2260.01597PAX6Paired box 61.5780.02679DNMT3BDNA (cytosine-5)-methyltransferase 3 beta1.20160.011256CDK1Cyclin-dependent kinase 11.14140.01275	KRT15	Keratin 15	1.469	0.000278
GDF3       Growth differentiation factor 3       1.7102       0.000422         TBX3       T-box 3       1.5775       0.000669         AICDA       Activation-induced cytidine deaminase       1.3863       0.001057         RUNX1       Runt-related transcription factor 1       1.2238       0.001949         KLF4       Kruppel-like factor 4 (gut)       1.1139       0.00216         FGF2       Fibroblast growth factor 2 (basic)       1.4219       0.002528         NAT1       N-acetyltransferase 1 (arylamine N-acetyltransferase)       1.3489       0.003161         LEFTY2       Left-right determination factor 2       1.3489       0.003221         mocogene, neuroblastoma derived (avian)       1.00226       0.003224         HAND1       Heart and neural crest derivatives expressed 1       1.2117       0.003824         ZFP42       Zinc finger protein 42 homolog (mouse)       1.2421       0.006582         FGF1       Fibroblast growth factor receptor 1       1.3626       0.005687         FGF4       Fibroblast growth factor 4       1.1789       0.006383         NODAL       Nodal homolog (mouse)       1.6944       0.007374         A1       A1       A1       0.007585         PAX6       Paired box 6       1.578 </td <td>GATA2</td> <td>GATA binding protein 2</td> <td>1.5547</td> <td>0.000372</td>	GATA2	GATA binding protein 2	1.5547	0.000372
TBX3T-box 31.57750.000699AICDAActivation-induced cytidine deaminase1.38630.001057 <b>RUNX1Runt-related transcription factor 1</b> 1.22380.00216FGF2Fibroblast growth factor 2 (basic)1.42190.002267NAT1N-acetyltransferase 1 (arylamine N-acetyltransferase)1.38880.002528LEFT2Left-right determination factor 21.34890.003161MYCNV-myc myelocytomatosis viral related (avian) oncogene, neuroblastoma derived (avian)0.003271HAND1Heart and neural crest derivatives expressed 11.21170.003282FGF4Sibroblast growth factor receptor 11.36260.00582FGF4Fibroblast growth factor receptor 11.36260.00583FGF4Fibroblast growth factor 41.7890.003631FGF4Fibroblast growth factor 41.7890.00383FGF4Fibroblast growth factor 41.7890.00583FGF4Fibroblast growth factor 41.7890.00583FDADHAldehyde dehydrogenase 1 family	ALPL	Alkaline phosphatase, liver/bone/kidney	1.4816	0.000379
AICDAActivation-induced cytidine deaminase1.38630.001057RUNX1Runt-related transcription factor 11.22380.002167KLF4Kruppel-like factor 4 (gut)1.11390.002167FGF2Fibroblast growth factor 2 (basic)1.42190.002267NAT1N-acetyltransferase 1 (arylamine N-acetyltransferase)1.38880.002528LEFTY2Left-right determination factor 21.34890.003161MYCNV-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)1.016720.003824JEP42Zinc finger protein 42 homolog (mouse)1.21170.003824JEFFY3Heat shock 70kDa protein 9 (mortalin)1.40290.00582FGF4Fibroblast growth factor receptor 11.3660.00643FGF4Fibroblast growth factor 41.7890.006383NODALNodal homolog (mouse)1.21440.007582FGF4Fibroblast growth factor 41.20050.006383NDDALNdal homolog (mouse)1.2340.007582PAX6Paired box 61.5780.008689DNM13BDNA (cytosine-5-)-methyltransferase 3 beta1.20050.011056FUNT3Left-right determination factor 11.2260.011276CDK1Cytin-dependent kinase 11.14710.012381CDK1Cytin-dependent kinase 11.31360.024156FUNT3Developmental pluripotency associated 21.31380.024156CDK1Cytin-dependent kinase 71.31470.026879	GDF3	Growth differentiation factor 3	1.7102	0.000422
RUNX1Runt-related transcription factor 11.22380.00194KLF4Kruppel-like factor 4 (gut)1.11390.00216FGF2Fibroblast growth factor 2 (basic)1.42190.002267NAT1N-acetyltransferase 1 (arylamine N-acetyltransferase)1.38880.002528LEFTY2Left-right determination factor 21.34890.003161MYCNV-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)1.06720.003271HAND1Heart and neural crest derivatives expressed 11.21170.003824ZFP42Zinc finger protein 42 homolog (mouse)1.24210.004222HSPA9Heat shock 70kDa protein 9 (mortalin)1.40290.00583FGF4Fibroblast growth factor receptor 11.36260.00587FGF4Fibroblast growth factor 41.17890.00374ALDH1A1Aldehyde dehydrogenase 1 family, member A11.69440.00422PAX6Paired box 61.5780.007585PAX6Paired box 61.5780.008689DNMT38DNA (cytosine-5)-methyltransferase 3 beta1.20050.011056HNF4AHepatocyte nuclear factor 4, alpha1.41460.012371CDK1Cyclin-dependent kinase 11.17810.012381COL1A1Collagen, type I, alpha 11.16720.015877LEFTY1Left-right determination factor 11.2640.02768PAX6Noclear receptor subfamily 5, group A, member 21.31480.024156CDF11Collagen, type I, alpha	TBX3	T-box 3	1.5775	0.000669
KLF4Kruppel-like factor 4 (gut)1.11390.00216FGF2Fibroblast growth factor 2 (basic)1.42190.002267NAT1N-acetyltransferase 1 (arylamine N-acetyltransferase)1.38880.002528LEFT2Left-right determination factor 21.34890.003161MYCNV-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)1.16720.003271HAND1Heart and neural crest derivatives expressed 11.21170.003824ZFP42Zinc finger protein 42 homolog (mouse)1.24210.004222HSPA9Heat shock 70kDa protein 9 (mortalin)1.40290.005837FGF4Fibroblast growth factor receptor 11.36260.006687FGF4Fibroblast growth factor 41.17890.00374ALDH1A1Aldehyde dehydrogenase 1 family, member A11.25440.007374FAX6Nola locetyltransferase 2A1.20060.0011056PAX6Paired box 61.15780.008689DNMT3BDNA (cytosine-5)-methyltransferase 3 beta1.20050.011056HNF4AHepatocyte nuclear factor 4, alpha1.41460.012751CDK1Cyclin-dependent kinase 11.17110.012881COL1A1Collagen, type I, alpha 11.12610.015877LEFT21Left-right determination factor 11.2260.015976DPA3Developmental pluripotency associated 21.31380.024156NR5A2Nuclear receptor subfamily 5, group A, member 21.36470.029885FARD6A<	AICDA	Activation-induced cytidine deaminase	1.3863	0.001057
FGF2Fibroblast growth factor 2 (basic)1.42190.002267NAT1N-acetyltransferase 1 (arylamine N-acetyltransferase)1.38880.002528LEFTY2Left-right determination factor 21.34890.003161MYCNV-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)1.16720.003271HAND1Heart and neural crest derivatives expressed 11.21170.003824ZFP42Zinc finger protein 42 homolog (mouse)1.24210.004222HSPA9Heat shock 70kDa protein 9 (mortalin)1.40290.00582FGF1Fibroblast growth factor receptor 11.36260.006687SNODALNodal homolog (mouse)1.69440.0064ALDH1A1Aldehyde dehydrogenase 1 family, member A10.007374KAT2AK(lysine) acetyltransferase 2A1.20060.007585PAX6Paired box 61.15780.008689DNMT3BDNA (cytosine-5-)-methyltransferase 3 beta1.20050.011056HNF4AHepatocyte nuclear factor 4, alpha1.41460.01275CDK1Cyclin-dependent kinase 11.14710.012381COL1A1Collagen, type I, alpha 11.16720.005677LEFTY1Left-right determination factor 11.2260.011976DPPA3Developmental pluripotency associated 21.31380.02218NR5A2Nuclear receptor subfamily 5, group A, member 21.14210.029885PARD6APar-6 partitioning defective 6 homolog alpha (c. elegans)1.27030.033306 <td>RUNX1</td> <td>Runt-related transcription factor 1</td> <td>1.2238</td> <td>0.001949</td>	RUNX1	Runt-related transcription factor 1	1.2238	0.001949
NAT1N-acetyltransferase 1 (arylamine N-acetyltransferase)1.38880.002528LEFTY2Left-right determination factor 21.34890.003161MYCNV-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)1.16720.003271HAND1Heart and neural crest derivatives expressed 11.21170.003824ZFP42Zinc finger protein 42 homolog (mouse)1.24210.004222HSPA9Heat shock 70kDa protein 9 (mortalin)1.40290.00582FGF1Fibroblast growth factor receptor 11.36260.006687FGF4Fibroblast growth factor 41.17890.00633NODALNodal homolog (mouse)1.69440.0064ALDH1A1Aldehyde dehydrogenase 1 family, member A10.007374FA72K(lysine) acetyltransferase 2A1.20060.007585PAX6Paired box 61.15780.008689DNMT3BDNA (cytosine-5-)-methyltransferase 3 beta1.20150.011056HNF4AHepatocyte nuclear factor 4, alpha1.41460.012751CDK1Cyclin-dependent kinase 11.17610.012381COL1A1Collagen, type I, alpha 11.16720.015877LEFTY1Left-right determination factor 11.2640.026799PM30Developmental pluripotency associated 21.31380.024156NR5A2Nuclear receptor subfamily 5, group A, member 21.9670.029885PARD64Par-6 partitioning defective 6 homolog alpha (c. elegans)1.0420.033506PO	KLF4	Kruppel-like factor 4 (gut)	1.1139	0.00216
N-acetyltransferase)         N-acetyltransferase)           LEFTY2         Left-right determination factor 2         1.3489         0.003161           MYCN         V-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)         1.1672         0.003271           HAND1         Heart and neural crest derivatives expressed 1         1.2117         0.003824           ZFP42         Zinc finger protein 42 homolog (mouse)         1.2421         0.004222           HSPA9         Heat shock 70kDa protein 9 (mortalin)         1.4029         0.005882           FGF1         Fibroblast growth factor receptor 1         1.3626         0.006687           SMODAL         Nodal homolog (mouse)         1.6944         0.0064           ALDH1AI         Aldehyde dehydrogenase 1 family, member A1         0.007374           KAT2A         K(lysine) acetyltransferase 2A         1.2006         0.007585           PAX6         Paired box 6         1.1578         0.008689           DNMT3B         DNA (cytosine-5-)-methyltransferase 3 beta         1.2005         0.011056           HNF4A         Hepatocyte nuclear factor 4, alpha         1.4146         0.012371           CDL1A1         Collagen, type I, alpha 1         1.1671         0.028481           DNF32         Developmental pluripotency	FGF2	Fibroblast growth factor 2 (basic)	1.4219	0.002267
LEFTY2Left-right determination factor 21.34890.003161MYCNV-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)1.16720.003271HAND1Heart and neural crest derivatives expressed 11.21170.003824ZFP42Zinc finger protein 42 homolog (mouse)1.24210.004222HSPA9Heat shock 70kDa protein 9 (mortalin)1.40290.00582FGF1Fibroblast growth factor receptor 11.36260.006483NODALNodal homolog (mouse)1.69440.0064ALDH1A1Aldehyde dehydrogenase 1 family, member A10.007374KAT2AK(lysine) acetyltransferase 2A1.20060.007585PAX6Paired box 61.15780.008689DNMT3BDNA (cytosine-5-)-methyltransferase 3 beta1.20050.0112056HNF4AHepatocyte nuclear factor 4, alpha1.41460.0112751CDK1Cyclin-dependent kinase 11.14710.012381COL1A1Collagen, type I, alpha 11.17610.015876DPPA3Developmental pluripotency associated 21.31380.024156NR5A2Nuclear receptor subfamily 5, group A, member 21.34870.029084FAT7K(lysine) acetyltransferase 71.9670.029885PARD6APare for partitioning defective 6 homolog alpha (c. elegans)1.01230.033506POU5F1POU class 5 homeobox 11.11310.042862	NAT1	N-acetyltransferase 1 (arylamine	1.3888	0.002528
MYCNV-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)1.16720.003271HAND1Heart and neural crest derivatives expressed 11.21170.003824ZFP42Zinc finger protein 42 homolog (mouse)1.24210.004222HSPA9Heat shock 70kDa protein 9 (mortalin)1.40290.005582FGF1Fibroblast growth factor receptor 11.36260.005687FGF4Fibroblast growth factor 41.17890.006383NODALNodal homolog (mouse)1.69440.0064ALDH1A1Aldehyde dehydrogenase 1 family, member A10.007374KAT2AK(lysine) acetyltransferase 2A1.20060.007585PAX6Paired box 61.15780.008689DNMT3BDNA (cytosine-5-)-methyltransferase 3 beta1.20050.011205CDK1Cyclin-dependent kinase 11.14710.012871CDL1A1Collagen, type I, alpha 11.17610.015871LEFTY1Left-right determination factor 11.2260.015965DPPA3Developmental pluripotency associated 21.31380.024156NR5A2Nuclear receptor subfamily 5, group A, member 21.41420.029084FAT7K(lysine) acetyltransferase 71.9670.029885PARD6APar-6 partitioning defective 6 homolog alpha (c. elegans)1.27030.033506POU5F1POU class 5 homeobox 11.11310.042862		N-acetyltransferase)		
AND1         Heart and neural crest derivatives expressed 1         1.2117         0.003824           ZFP42         Zinc finger protein 42 homolog (mouse)         1.2421         0.004222           HSPA9         Heat shock 70kDa protein 9 (mortalin)         1.4029         0.005582           FGF1         Fibroblast growth factor receptor 1         1.3626         0.005687           FGF4         Fibroblast growth factor 4         1.1789         0.006383           NODAL         Nodal homolog (mouse)         1.6944         0.0064           ALDH1A1         Aldehyde dehydrogenase 1 family, member A1         1.2544         0.007374           KAT2A         K(lysine) acetyltransferase 2A         1.2006         0.007585           PAA6         Paired box 6         1.1578         0.008689           DNMT3B         DNA (cytosine-5-)-methyltransferase 3 beta A1.011056         1.2006         0.011275           CDL1A1         Collagen, type I, alpha 1         1.1471         0.012381           COL1A1         Collagen, type I, alpha 1         1.1761         0.015877           LEFTY1         Left-right determination factor 1         1.226         0.015976           DPPA3         Developmental pluripotency associated 2         1.3138         0.024156           NR5A2	LEFTY2	Left-right determination factor 2	1.3489	0.003161
HAND1Heart and neural crest derivatives expressed 11.21170.003824ZFP42Zinc finger protein 42 homolog (mouse)1.24210.004222HSPA9Heat shock 70kDa protein 9 (mortalin)1.40290.005582FGF1Fibroblast growth factor receptor 11.36260.006883FGF4Fibroblast growth factor 41.17890.006383NODALNodal homolog (mouse)1.69440.0064ALDH1A1Aldehyde dehydrogenase 1 family, member A10.007374KAT2AK(lysine) acetyltransferase 2A1.20060.007585PAK6Paired box 61.15780.008689DNM3BDNA (cytosine-5-)-methyltransferase 3 beta1.20160.011275CDL1A1Cyclin-dependent kinase 11.14710.012381COL1A1Collagen, type I, alpha 11.17610.015877LEFTY1Left-right determination factor 11.2260.015765DPPA3Developmental pluripotency associated 21.31380.024156NR5A2Nuclear receptor subfamily 5, group A, member 21.14210.029881FY300E1A binding protein p3001.38470.029881FARD6APar-6 partitioning defective 6 homolog alpha (c. elegans)1.02710.033506POU5F1POU class 5 homeobox 11.11310.042862	MYCN	5 5 5	1.1672	0.003271
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(C. elegans)           POU5F1         POU class 5 homeobox 1         1.1131         0.042862	PARD6A			
		(C. elegans)		
ESRRB Estrogen-related receptor beta 1.2021 0.044154	POU5F1	POU class 5 homeobox 1	1.1131	0.042862
	ESRRB	Estrogen-related receptor beta	1.2021	0.044154

The results were not so evident in A375 melanoma cells, but the presence of Runt-related transcription factors 1 and 2 (14-16), Cadherin 2 (17, 18), Nodal homolog (mouse) (19) and Aldehyde dehydrogenase 1 (family member A1) pointed to the same direction (20) (**Table 2**). However, the production of oncospheres in A375 cells put in evidence that LL-37 has opposing effects on cancer cell stemness depending on the cell type.

# Conclusion

Emerging evidence highlights the role of antimicrobial peptides in non-infectious diseases, such as cancer. The mechanisms triggered by antimicrobial peptides are broad and lead to unexpected cell responses. Here, we show that the antimicrobial peptide LL-37 is implicated in cancer stemness. Further research needs to be directed to better clarify this phenomenon and the role of other antimicrobial peptides in this scenario.

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## **Competing Interests**

The authors have declared that no competing interest exists.

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