Review Article

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The role of synthetic peptides derived from bovine lactoferricin against breast cancer cell lines: a mini-review

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Abstract: Breast cancer represents the most commonly diagnosed cancer worldwide, accounting for approximately one in eight cancers diagnosed. Despite significant advances in the diagnosis and detection of this disease, there is still a great need for more effective therapies to combat the invasive forms, especially those with a high incidence of metastasis. For that reason, bioactive molecules as peptides, including bovine lactoferricin (LfcinB), have been investigated. In this sense, there are reports that ^{20}RRWQWR^{25} motif derivate from the LfcinB has shown activity against different cancer cell lines. Thus, current studies are being carried out with synthetic derivatives (linear, palindromic, dimer and tetrameric structures) that contain the ^{20}RRWQWR^{25} motif in order to increase its activity against cancer cell lines by altering its hydrophobicity and net positive charge. In this regard, studies have focused on the use of LfcinB derivatives to combat breast cancer cell lines, with encouraging results. Therefore, in this mini-review, we present the state of the art regarding the activity of LfcinB and its analogs against breast cancer cell lines.

Keywords: breast cancer; lactoferricin bovine; peptide; breast cancer therapy

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Introduction

Breast cancer has emerged as a burgeoning global health concern, being categorized as the prevalent form of cancer among women and the leading cause of cancer-related fatalities. Epidemiological statistics reveal a significant escalation, with approximately 2.26 million new cases recorded in 2020, accounting for 11.7 % of all cancer diagnoses across genders [1].

Systemic treatments such as chemotherapy, hormone therapy, and radiotherapy still face challenges, including drug resistance, adverse effects, toxicity, high costs, and the disease's heterogeneity, which is categorized into different subtypes known as Luminal A, Luminal B, HER2 positive, and Triple-negative [1–4].

Several strategies are being explored to discover more selective and less invasive therapeutic agents, including the use of anticancer peptides. One such peptide is bovine lactoferricin (LfcinB: ^{17}FKCRRWQWRMKKLGAPSITCVRRAF^{41}), which comprises 25 amino acids derived from the N-terminal region of a protein known as bovine lactoferrin (BLF) [5]. LfcinB exhibits both antimicrobial and anticancer activities against various cancer cell lines including colon, lung, liver, leukemia, and breast cancer [6, 7].

It is hypothesized that LfcinB is attracted to the cell membrane through electrostatic interactions involving cationic amino acids. This interaction leads to membrane disruption and cellular lysis, as illustrated in Figure 1 [6–8]. In addition, studies in immunodeficient NOD-SCID-gamma (NSG) mice have shown that intratumoral injections of the LfcinB peptide result in reduced tumor size and density, as observed by live imaging with IVIS. This treatment induces apoptosis and inhibits invasion without affecting normal cells [9].

Previous studies have suggested that the anticancer activity of LfcinB is due to the ^{20}RRWQWR^{25} fragment. Therefore, current *in vitro* studies are underway to evaluate synthetic variants (linear, dimeric, tetrameric, cyclic, and palindromic peptide structures) containing this sequence

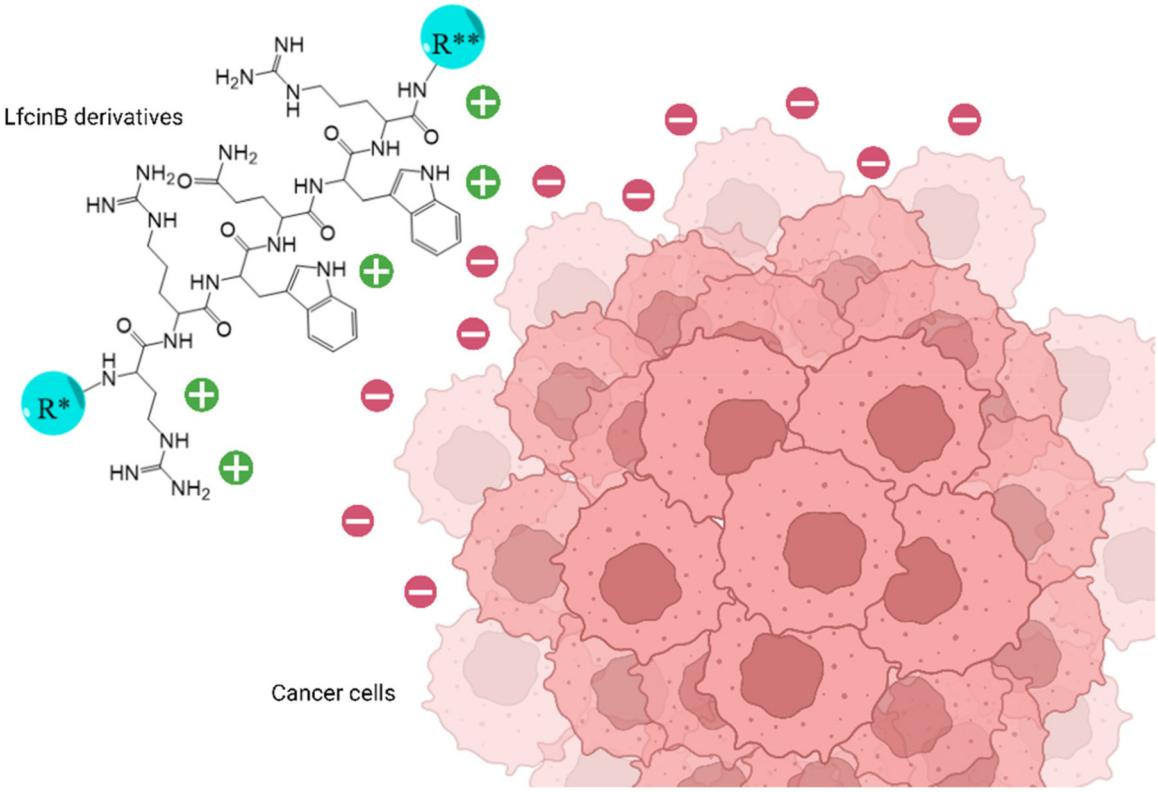

Figure 1: Electrostatic interactions within cancer cell membranes and the minimal motif and LfcinB derivatives. R* and R**=hydrogen atom or different amino acid sequences.

against cancer cell lines such as MCF-7, MDA-MB-231, and MDA-MB-468. In Table 1, we present a summary of information gathered over the last 10 years regarding these LfcinB derivatives.

The role of synthetic LfcinB derivatives against breast cancer

In vitro studies have shown that synthetic analogs containing the ^{20}RRWQWR^{25} motif in their structure increase the activity against Luminal A (MCF-7) and show low cytotoxicity against the human trophoblast cell line (CRL-3271) and the non-tumorigenic cell line MCF-12 [4, 6, 7, 10]. Among these synthetic derivatives, we would like to highlight the study by Insuasty-Cepeda et al., in which the dimeric peptide LfcinB $(20–30)_2$ was synthesized six times with changes in the 26th position amino acid [4]. This study suggested that not only electrostatic interactions of the peptide but also increased hydrophobicity contributed to the cytotoxic effect. They observed that ^{26}[F]-LfcinB $(20–30)_2$ (IC_{50}=6 µM) and ^{26}[L]-LfcinB $(20–30)_2$ (IC_{50}=20 µM) induced cell damage via the apoptosis pathway [4]. Comparable outcomes were achieved using a tetrameric structure, LfcinB $(20–25)_4$, which at a concentration of 12.2 µM (30 µg/mL), selectively damaged MCF-7 cells and led mitochondrial membrane depolarization associated with an apoptotic pathway [10]. However, it is imperative to acknowledge that *in vitro* experiments cannot fully replicate the intricacies of organ systems. Consequently, ongoing research should prioritize *in vivo* approaches.

It has been suggested that increasing the net positive charge and peptide length also increases the cytotoxic effect. To investigate this, a study utilizing a linear palindromic peptide analog, Lfcin $(21–25)_{Pal}$, six peptides were synthesized by adding and deleting arginine (Arg) at the N-terminal, C-terminal, or both ends of the structure. The results showed that adding arginine at the N-terminus increased the cytotoxic effects while maintaining some level of selectivity [7].

Cytometry experiments showed that this palindromic peptide induced apoptotic events in MCF-7 cells, making it a promising molecule with a therapeutic window of 70–140 µM. In addition, it shows a broad spectrum of activity against various cancer cell lines [6]. Notably, Lfcin $(21–25)_{Pal}$ is the only peptide derivative that has been tested *in vivo* using a *Galleria mellonella* larvae model, showing a 90 % survival rate after 10 days when administered at a dosage of 800 µg/mg of peptide [7].

Table 1: Bovine lactoferricin derivates, its amino acid sequence, and breast cancer cell lines used as experimental models.

Peptide	Amino acid sequence	BCCL	Observations	Ref.
^{26}[D]-LfcinB $(20–30)_2$	$(RRWQWR\mathbf{D}KKLG)_2$-K*-Ahx	MDA-MB-468 MCF-7	The cytotoxic effect increases when hydrophobicity increases: ^{26}[F]>^{26}[L]>^{26}[A] >^{26}[M]. Dimeric peptides show low cytotoxic effect against non-tumorigenic cell line MCF-12 and it is also suggested by cytometry that they generate cell death through the apoptosis pathway.	[4]
^{26}[K]-LfcinB $(20–30)_2$	$(RRWQWR\mathbf{K}KKLG)_2$-K*-Ahx			
^{26}[M]-LfcinB $(20–30)_2$	$(RRWQWR\mathbf{M}KKLG)_2$-K*-Ahx			
^{26}[A]-LfcinB $(20–30)_2$	$(RRWQWR\mathbf{A}KKLG)_2$-K*-Ahx			
^{26}[L]-LfcinB $(20–30)_2$	$(RRWQWR\mathbf{L}KKLG)_2$-K*-Ahx			
^{26}[F]-LfcinB $(20–30)_2$	$(RRWQWR\mathbf{F}KKLG)_2$-K*-Ahx			
LfcinB (20–25)	^{20}RRWQWR^{25}	MDA-MB-231 MDA-MB-468	Dimeric and tetrameric peptides show higher cytotoxic effect against triple-negative cancer cell lines when compared with linear peptides derived of LfcinB. Also, it was observed low cytotoxic effect against in fibroblast (PCS-201-012). Cyclic peptides show low cytotoxic effect against breast cancer cell lines.	[5]
LfcinB $(20–25)_2$	$(RRWQWR)_2$-K*-Ahx			
LfcinB $(20–25)_4$	$(RRWQWR)_4$-K*$_2$-Ahx$_2$-C_2			
LfcinB $(20–25)_{cyc}$	C[a]-RRWQWR-Ahx$_2$-C[a]			
LfcinB (20–30)	^{20}RRWQWRMKKLG^{30}			
LfcinB $(20–30)_2$	$(RRWQWRMKKLG)_2$-K*-Ahx			
LfcinB $(20–30)_4$	$(RRWQWRMKKLG)_4$-K*$_2$-Ahx$_2$-C_2			
LfcinB $(20–30)_{cyc}$	C[a]-RRWQWRMKKLG-Ahx-C[a]			
^{19}[A]-LfcinB (17–31)	^{17}FK**A**RRWQWRMKKLGA^{31}			
^{19}[A]-LfcinB $(17–31)_2$	$(FK\mathbf{A}RRWQWRMKKLGA)_2$-K*-Ahx			
^{19}[A]-LfcinB $(17–31)_4$	$(FK\mathbf{A}RRWQWRMKKLGA)_4$-K*$_2$-Ahx$_2$-$C_2$			
^{19}[A]-LfcinB $(17–31)_{cyc}$	C[a]-FK**A**RRWQWRMKKLGA-Ahx-C[a]			
LfcinB $(21–25)_{Pal}$	RWQWRWQWR	MDA-MB-231 MCF-7	It was observed that the palindromic derivate has great potential *in vitro* against Luminal A and triple-negative cancer cell lines which had a better result with MCF-7. It was also tested with non-cancerogenic cells showing low toxicity. It was suggested that trough flow cytometry assays LfcinB $(21–25)_{Pal}$ generates late apoptosis in MCF-7.	[6]
1	_WQWRWQW_NH_2	MDA-MB-231 MCF-7	It was suggested that it is not only the positive net charge of the peptide that increase the cytotoxic effect against cancer cell lines but also the position in which is add. In that order RR-1-R peptide increased its activity while maintaining selectivity. It was shown that RR-1-R peptide activates intrinsic apoptosis pathways and reduced the migration of MCF-7 cells.	[7]
R-1-R	**R**-WQWRWQW-**R**			
RR-1-RR	**RR**-WQWRWQW-**RR**			
R-1-RR	**R**-WQWRWQW-**RR**			
RR-1-R	**RR**-RWQWRWQW-R			
R-1	**R**-WQWRWQW_NH_2			
1-R	_WQWRWQW**R**-NH_2			
LfcinB $(21–25)_{Pal}$	RWQWRWQWR	MDA-MB-468	It is suggested that substituting each amino acid in the palindromic peptide for alanine shows a decrease in its activity against MDA-MB-468 compared with the original LfcinB $(21–25)_{Pal}$ peptide indicating that there is a correlation between net charge and hydrophobicity with the cytotoxic effect.	[8]
A1	**A**WQWRWQWR			
A2	R**A**QWRWQWR			
A3	RW**A**WRWQWR			
A4	RWQ**A**RWQWR			
A5	RWQW**A**WQWR			
A6	RWQWR**A**QWR			
A7	RWQWRW**A**WR			
A8	RWQWRWQ**A**R			
A9	RWQWRWQW**A**			

Table 1: (continued)

Peptide	Amino acid sequence	BCCL	Observations	Ref.
LfcinB $(20–25)_4$	$(RRWQWR)_4–K^*_2–Ahx_2–C_2$	MCF-7	It was shown that the tetrameric structure of ^{20}RRWQWR^{25} sequence has great activity against MCF-7 and also is selective when comparing with the low activity against non-tumorogenic trophoblastic cell line. Furthermore, this peptide induces mitochondrial membrane depolarization and increase of cytoplasmic calcium concentration indicating an apoptotic pathway.	[10]

BCCL, breast cancer cell lines; R, arginine (arg); W, tryptophan (trp); Q, glutamine (glu); A, alanine (ala); L, leucine (leu); G, glycine (gly); F, phenylalanine (phe); M, methionine (met); K, lysine (lys); P, proline (pro); S, serine (ser); I, isoleucine; T, threonine (thr); V, valine (val); K*, precursor of lysine for dimeric and tetrameric peptide, Ahx, 6-aminohexanoic residue; C, precursor of tetrameric peptide. [a]Precursor of cyclic peptide.

Moreover, the ^{20}RRWQWR^{25} motif and cyclic derivatives containing that sequence showed low cytotoxicity against cancer cell lines that are more aggressive subtypes such as MDA-MB-231 and MDA-MB-468. For this reason, the synthesis of dimeric and tetrameric structures containing the ^{20}RRWQWR^{25} motif was tested against triple-negative subtype cancer cell lines, as in the case of LfcinB $(20–25)_4$, LfcinB $(20–30)_2$ and LfcinB $(20–30)_4$ in which IC_{50} is less than 40 μM [5]. Again, it is suggested that increasing hydrophobicity such as ^{26}[F]-LfcinB $(20–30)_2$ and ^{26}[L]-LfcinB $(20–30)_2$ increases the cytotoxic effect against MDA-MB-231 and maintains low cytotoxicity against MCF-12 [4]. Similar results have been shown with the palindrome analog LfcinB $(21–25)_{Pal}$, which showed promising results against both cancer cell lines [6–8]. However, there is a lack of preclinical studies and a great need for more effective treatments for triple-negative subtypes.

Finally, in an attempt to investigate the hypothesis that Trp residues within the peptide's structure play a pivotal role in its internalization into cancer cells, Barragán-Cárdenas et al. synthetized 9 Lfcin $(21–25)_{Pal}$ peptides substituting Ala in each position (Table 1) and evaluated the cytotoxicity effect against MDA-MB-468 [8]. They found that substitution of Trp for Ala decreased its cytotoxic effect suggesting an association between hydrophobicity and Trp position with the mode of its internalization.

In conclusion, there is a strong relationship between the position, hydrophobicity and net charge of LfcinB derivatives with their cytotoxic effect against cancer cells. Furthermore, it would be very interesting to study how each family of derivatives (linear, dimeric, tetrameric, palindromic or cyclic) affects preclinic models *in vivo*.

Future perspectives

Overall, the potential of LfcinB and its derivatives against specific breast cancer subtypes, such as triple-negative and Luminal A, is remarkable. Here, we particularly highlight the palindrome peptide Lfcin $(20–25)_{Pal}$. However, further studies are needed to gain a better understanding of their mechanisms of action. Additionally, preclinical studies should be conducted to establish their safety and efficacy *in vivo*. Furthermore, it is important to encourage and emphasize future research focused on the green synthesis of peptides and the development of drug delivery systems that ensure their stability in biological systems, such as nanostructured systems.

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