Molecular Therapeutic Cancer Peptides: A Closer Look at Bovine Lactoferricin

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Authors' contributions

This work was carried out in collaboration between all authors. Author IZS generate the idea, authors LIA, DD, IZS, ARK and KB wrote the first draft of the manuscript. Author KB managed the literature searches and write the final paper. All authors read and approved the final manuscript.

Mini-review Article

ABSTRACT

Background: In spite of the progress that was recorded in the development of anticancer drugs, challenges continue to rises particularly due to resistance of the cancer chemotherapies and low sensitivity of the commercially available anticancer drugs.
Methodology: collection and review of available literatures.
Results: Bovine lactoferricin (LfcinB) is a cationic peptide with broad spectrum antimicrobial and anticancer activity. It also shows promising activity against many varieties of cancer cells including colon carcinoma cells, melanoma, fibrosarcoma, monocytic leukemic cells and neuroblastoma. It is unaffected by multidrug resistance mechanisms seen with conventional chemotherapeutic drugs,

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and displays a higher specificity for cancer cells versus normal cells in comparison to conventional chemotherapy.

Conclusion: LfcinB exhibit strong antitumor activity effectively penetrating cell membrane, activating caspases and induce apoptosis to cancer cells.

Keywords: Bovine Lactoferricin; Disulphide Bridge; apoptosis; angiogenesis; cytotoxic activity.

ABBREVIATIONS

AA : *Amino acids*
Apaf-1 : *Apoptotic protease activating factor 1*
bFGF : *Basic fibroblast growth factor*
bLF : *Bovine Latoferrin*
GSH : *Reduced glutathione*
LfcinB : *Bovine lactoferricin*
NAC : *N-acetyl cysteine*
PC 3 : *Human prostate cancer cell line-3*
ROS : *Reactive oxygen species*
THP-1 : *Human acute monocytic leukemia cell line-1*
*VEGF* : *Vascular endothelial growth factor*

1. INTRODUCTION

Bovine lactoferricin (LfcinB) is a peptide with cationic and amphipathic propriety which was reported to have cytotoxic activity to cancer cells of both human and rodent [1,2]. It is a peptide fragment generated by acid-pepsin hydrolysis of lactoferrin, a peptide obtained from cow's milk [1-3]. It consists of 25 amino acids peptide with a disulfide bridge between two cysteines (Fig. 1), thus having a cyclic twisted anti-parallel β-sheet structure [3,4]. Considerable amount of LfcinB are detected in the human stomach after consumption of bovine lactoferrin [1,4], proving the natural hydrolysis of LfcinB from the digestion of cow's milk. Due to its well-documented antimicrobial activity, many scientists have generated interest in LfcinB and it is now appearing to have antitumor activity. It broad spectrum anti-microbial activity comprises of activity against bacteria, [5] fungi [6] and viruses [7]. Evidence shows that LfcinB also possesses potent *in vivo and in vitro* activity against cancer cells [8]. Studies have indicated that subcutaneous administration of LfcinB inhibit lung and liver metastasis of both murine melanomas and lymphomas [8] and it has also been proven to induce apoptosis in human leukemic and carcinoma cell lines, [1,8] which in fact point to the evidences that LfcinB has a broad anticancer activity against many different type of cancers. In addition to its antitumor activities, it is reported to have variety of biological activities, including cells transcriptional activation [9] and regulation of immune response [10,11]. The peptide may play important role in the protective mechanism against carcinogenic effects due to its cationic property, which may oppose the negative charge of potentially carcinogenic molecules as well as the cancer cell. Research have shown that injections of human lactoferrin reduce the growth of transplanted solid tumors in mice, confirming the inhibitory effect of LfcinB to cancer cells [12]. The aim of this paper is to review the anti-cancer activity of bovine lactoferricin with it Biochemical and physiological activities within cancer cells.

2. STRUCTURE OF BOVINE LACTOFERRICIN

LfcinB possesses a cyclic structure formed by the disulfide bond that links its two-cysteine residues (Fig. 1). In a watery solution, LfcinB assumes an amphipathic beta-sheet conformation in which the residues that are hydrophobic line up on one face of the peptide and the basic residues on the opposing face [13]. The primary structure of LfcinB has been described as peptide consisting of 25-residue peptide with an amphipathic, antiparallel β-sheet structure [13]. The 25 AA residue peptide forms looped structure through an intramolecular disulfide bond (Fig. 1) [14]. It has a single disulfide bond and no iron-binding capacity, and is active against a broad spectrum of microorganisms, including bacteria [13]. Studies conducted using nuclear magnetic resonance (NMR) spectroscopy reveal that LfcinB exhibit a conformation that is quite different in aqueous solution from that found in the intact protein [13]. The peptide, in low salt solution, loses α-helix structure observed in intact bovine lactoferrin and changes into β-sheet. Adopting the change in structure, the released peptide becomes markedly amphipathic as nearly all the hydrophobic residues lie on one face while the positively charged residues lie on the other face [15] The amphipathic property is accounted by β twisted structure that the peptide adopts when it is in aqueous solution. The hydrophobic residues are found in one face while the opposing face

consists of the basic positively charged amino acid residues [15]. Molecular dynamic simulation can be used to observe this change in conformation in which LfcinB undergo a transition from an α-helix to a β-sheet hairpin structure in aqueous solution, but the interconversion does not proceed readily in the presence of 250 mM salt [16]. LfcinB contains five arginine and three lysine residues that confer a net charge of +8 on the peptide, as well as two tryptophan and two phenylalanine residues that are both aromatic and hydrophobic [14].
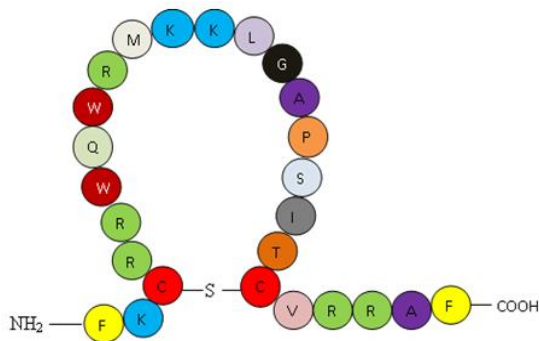
Fig. 1. Structure of Bovine lactoferricin, all amino acids in the peptide is represented by one letter code

3. STRONG CATIONIC CHARGE OF BOVINE LACTOFERRICIN LINKED TO ITS ANTICANCER ACTIVITY

The anticancer activity of LfcinB is attributed to its strong cationic charge +8 which confers the peptide the ability to selectively target the negative charge (-) of the most cancer cells, while healthy normal cells with nil charge (0) remains unaffected due to the high content of zwitterionic phosphatidylcholine located outside the leaflet of the membrane [17]. The net negative charge of most cancer cells of the leaflet of the membrane results due to the difference that exist in the branch and N-linked glycans of sialic acid which are connected with glycoprotein [18] that crosses the membrane and Over-expression of anionic molecules [19,20-22] such as phosphatidylcholine O-glycosylated mucins [17,21].The inability of the LfcinB to bind to PC3 carcinoma is probably due to the fact that some cancer cells refract the cytotoxic action of LfcinB due to insufficient negative charge through which electrostatic interaction took place between LfcinB and cancer cells [3]. Derivatives of LfcinB Containing cationic and hydrophobic moieties were reported to have enhanced activity

Owing to its high positive charge, structure and amphipathic properties whereas cancer cells cytotoxicity has not been observed with homologue of murine lactoferrricin containing glutamic acid [22-24].

4. CYTOTOXIC ACTIVITY OF LFCINB AGAINST CANCER CELLS

Scientists have elucidated the invitro cytotoxic activity of bovine lactoferricin using both human and mice cancer cells such as colon carcinoma cells, melanoma, fibro sarcoma, [25] monocytic leukemic cells [1,26] and neuroblastma, [2] and studies noted that treatment of these cells with LfcinB did not significantly affect the viability of normal human lymphocytes, erythrocytes, endothelial cells and fibroblasts [1,27]. The mechanism of action of LfcinB against neuroblastoma and fibro sarcoma rat cells but not human cells can be attributed to the formation of transmembrane in the plasma membrane through which the peptide is delivered to the component of cytoplasm of cancer cell. Following the entrance into the cell, the peptide interacts with the natively charged of the mitochondria, inducing apoptosis mainly via cell injury (necrosis) through cell membrane lytic. When inserted into the membrane, the peptide causes changes in the membrane mimetic systems by fostering the assembling of bicontinues cubic or inverted hexagonal phases [28-34]. Studies have reveal that LfcinB destroy THP-1 human monocytic leukemia cells through the apoptotic pathways activation, generation of ROS within the cells and activation of Ca2+/Mg2+-dependent endonucleases [25]. Eliassen et al., noted that THP-1 cells Treated with 100 μg/mL LfcinB undergo apoptosis after about 10 h, while those cells Treated with bLF did not induce cell death even at a high dose of 500 μg/mL. Fragmented DNA was exhibited by THP-1 cells treated with LfcinB in a dose-dependent manner; a time- and dose-dependent gradually decrease in integrity of the cell membrane which permitted LfcinB to go through the compartment of the cytoplasm. The disruption of cell membrane by LfcinB outside the cell does not depend on penetration of the peptide, but itself may be responsible for the cytotoxic activity of the peptide. LfcinB induce-apoptosis was found to be inhibited following the addition of Zinc Ion (Zn^{2+}), an inhibitor of endonucleases. Also, antioxidants such as N-acetyl-cysteine (NAC) and glutathione (GSH), were found to effectively abolished the capacity of LfcinB to induced cell death [25].

5. THE CASCADES THAT TRIGGERED CANCER CELLS TO APOPTOSIS

The mechanism by which LfcinB destroy cancer cell involved binding of the cancer cell and causes the formation of transmembrane pores allowing the peptide to enter cytoplasmic compartment of the affected cancer cells and co-localized with negatively charged mitochondria [1,2,27]. Mouse fibrosarcoma cells and human neuroblastoma cells subjected to LfcinB die primarily via necrosis caused by a cell membrane lytic effect, [2]. LfcinB has been shown to kills human leukemia and breast carcinoma cells by a process that involves the sequential generation of ROS, loss of mitochondrial transmembrane potential, and activation of the caspase cascade resulting in cell death by apoptosis (Fig. 2) [1,8]. Research [1] have further confirmed the ability of LfcinB to induced apoptosis in cancer cells through the generation of ROS and activation of Ca2+/Mg2+-dependent endonucleases. Further, LfcinB-induced apoptosis in human T-leukemia cells was established to be triggered by cascades of events. The events lead to permeabilization of the cell membrane mediated by LfcinB, subsequent crossing of the LfcinB that disrupted cell membrane, colocalization of the LfcinB with mitochondria, release of cytochrome C as a result of depolarization of mitochondria, Interaction of cytochrome c with Apaf-1 and subsequent recruitment and activation of procaspase-9 forming apoptosome which triggered activation of caspases that ultimately lead to cell death through apoptosis (Fig. 2). Some studies have suggested that cytotoxicity of the peptide to cancer cells accounted by necrotic or apoptosis depend on the peptide concentration [2,24]. Even though the action of
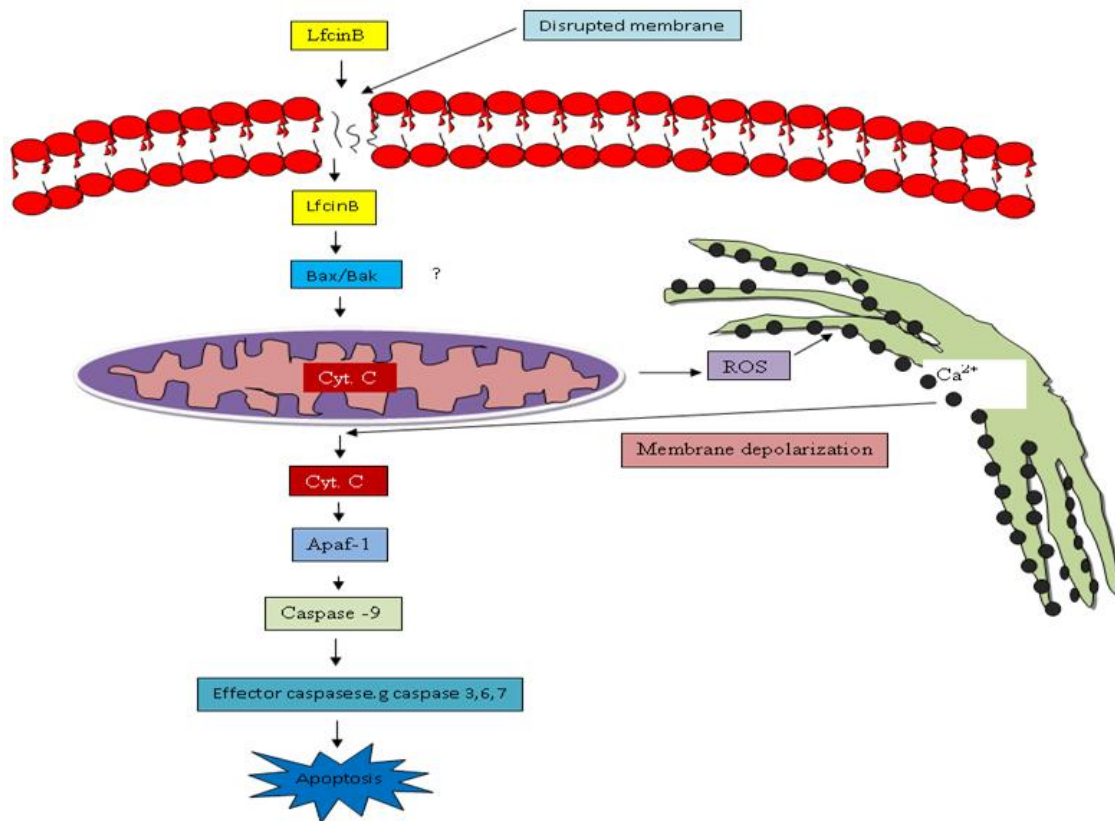
Fig. 2. Proposed mechanism of action of LfcinB. "In the diagram LfcinB causes membrane lysis and gains entry into the cytoplasm. It co-localizes with the mitochondria and causes the release of cytochrome C. The cytochrome C interacts with Apaf-1 and caspase-9 to form the apotosome. Caspase-9 then activates caspase 3 which ultimately leads to apoptosis."

the peptide decreases with increased serum [8] concentration, intratumoral or systemic administration has been reported to inhibit carcinogenesis and metastasis of a number of tumors in mice [2, 25, 26]. This effect results due to anionic serum components neutralization rather than proteolytic degradation. In recent times, LfcinB-induced apoptosis in B-lymphoma cells was found to occur via activation of cathepsin B rather than involvement of caspases activation [35].

6. BOVINE LACTOFERRICIN AND ANGIOGENESIS

Angiogenesis is the process that encompasses the growth, diffentiation and migration of endothelial cells within the wall of blood vessels. Research has been directed upon the discovery of peptide-based angiogenic inhibitor as they are least toxic and safe for therapy against abnormal angiogenic associated diseases [36]. Binding of signal molecule are required to their receptors for angiogenesis to occur on endothelial cells. Endothelial growth factors and Signal molecules such like VEGF binds to their receptors and initiate process that lead to growth and differentiation of new blood vessels. Inhibitors of angiogenesis hinder this various process. LfcinB has been shown to repress both basic fibroblast growth factor (bFGF) - and VEGF-driven proliferation and migration of human endothelial cells in vitro, and as well inhibiting with bFGF- and VEGF-induced angiogenesis in mice, it does this by competing with bFGF and VEGF for growth factor receptor-associated heparin sulfate proteoglycans on the endothelial cell surface [37,38]. LfcinB was thought to Hinder with the interaction of signal molecule such as VEGF and bFGF with their receptors on the surface of endothelial cell [37], which result in diminished angiogenesis and decreased endothelial cell proliferation [37]. Even though the precise mechanism of interaction of LfcinB with heparin-like molecules has not been fully understood, it was suggested that the LfcinB demonstrated attraction for heparin-like structures resulting in electrostatic attraction between the positive charge of LfcinB and negative charge of heparin sulfate and heparin (-).The whole anti-angiogenic activity depends on the amino acids sequence (primary structure) of the peptide, in view of the fact that a scrambled peptide composed of the same amino acid residues compete inefficiently with bFGF or VEGF for binding sites on heparin-like endothelial cells. The major drawback of systemic administration of LfcinB as therapy for anti-angiogenesis is the sensitivity of the peptide to enzymatic degradation and inactivation through anionic serum components interactions [37,38].

7. THERAPAUTICS ASPECT OF BOVINE LACTOFERRICIN

The use of peptides has gain popularity as therapeutic agents against cancer, cardiovascular diseases and diabetes and it is now growing in a rapid manner as a means of chemotherapeutics to other areas [39]. LfcinB have been proven to be effective anti-carcinogenesis molecule with a unique way of exerting it pharmacological actions. The peptide was reported to bypasses the mechanisms of multidrug resistance frequently encounter with many conventional chemotherapeutic agents [40-45] and shows high specificity to cancer cells than the normal cells in comparison to other chemotherapeutic agents [46,47]. In addition, invivo studies have established its potential as anticancer agents following primary tumor regression [2,25] and hindering of metastasis [48,49]. Intratumoral injection of LTX302, a short peptide obtained from LfcinB which occurs naturally has been shown to cause complete regression of tumors [46-49].

Active immunization has also emerged as a means of treating cancer and studies uses the component of the immune system - molecules or cells to boost the immune system [50, 51]. The concept of fighting cancer through vaccination has also been translated into clinical research and clinical trials in the last decades. Clinical oncologist specifically designed vaccines based on known antigens in order to induced immunity against cancer [50]. Vaccines made up of peptides which are obtained from sequences of protein of specific antigens or tumor concerned is used in treating neoplastic cells. In an interesting way, a small peptide derived from LfcinB, LTX302, was also found to evoke immunity hindering carcinogenesis and metastasis [50-52].

Oncologically, peptides with high affinity for receptors that are over-expressed on tumor cells are used as diagnostic tools and also have broad applicability in clinical research for anticancer therapeutics [53]. LfcinB may emerge as new anticancer molecule and as agents of choice for anti-cancer investigation through attachment to chemotherapeutic moieties including cytotoxic agents or radionuclide. peptides can conveniently be linked directly to halogens

(iodine and fluorine) or radionuclide of metals using suitable chelating agents, thus acting as cancer diagnosing agents. In view of the fact that small peptides can be synthesized relatively easy, synthesizing new peptides with better antitumor activity and enhancing the existing ones will likely be beneficial for clinical investigations [52,53]. In this scenario, many properties of these small peptides have to be taken into account such as stability of the peptide in body fluids, non- toxic to normal cells, affinity of the peptide to their receptors and improving its overall pharmacological effects [53].

8. PEPTIDES AS SUITABLE AGENTS FOR DESIGNING EFFECTIVE ANTICANCER AGENTS

In spite of the progress that was recorded in the development of anticancer drugs, challenges continue to rises particularly due to resistance of the cancer chemotherapies and low sensitivity of the commercially available anticancer drugs [53] Peptides such as LfcinB which are host defense peptides and effectors molecules of innate immunity as well may give rise to a unique way for designing effective anticancer agents. The peptide bypasses the mechanism of drug resistance [54,55] and provides immunological response [55] against mass of tumors, indicating its suitability for design and development of novel anticancer therapeutics. The anticancer peptide is able to make distinction between cancer and normal cells binding specifically to the negative charge of the component of the plasma membrane (sialic acid, heparan sulfate or phosphatidylserine), which differentiate between neoplastic and non-neoplastic cells. Susceptibility of cancer cells to anti-cancer peptide has been found to be enhanced by increased in the number of microvilli on the cancer cells which also result in increased surface area. Due to their selective target between neoplastic and non-neoplastic cells membrane, host defense peptides have high exceptional penetration power and are taken effectively to both distant metastasis and primary tumors. The chance of resistance occurs less likely due to the fact that this peptide attacked their target rapidly by disrupting plasma membrane integrity [55]. Designing a peptide that is extremely effective having serum stability would lead to a novel approached in cancer therapy. The present cancer situation clearly indicates that better treatment options that selectively target cancer with little or no toxicity to normal tissues are urgently necessary. Novel

and cheaper alternatives to current cancer therapeutics, including specific cytotoxic molecules and vaccines that can boost the human immune system to fight deadly forms of cancer are needed these days. The challenge lies in developing the clinical application of therapeutic peptides. Improving delivery to tumors, serum stability, minimizing non-specific toxic effects and discerning pharmacokinetic properties are high among the needs to produce a powerful therapeutic peptide for cancer treatment.

9. DISCUSSION

Bovine lactoferricin has gain a wide range of use in medical and therapeutic applications. A potent cationic anticancer peptide, LfcinB display it antitumor activity with lysine and arginine accounting for the overall charge of the peptide. It exerts it pharmacological activities against many human and rodent cancer cells including colon carcinoma cells, melanoma, fibrosarcoma, monocytic leukemic cells and neuroblastoma [1,2, 25-27]. Many of the metabolic changes as well as the biochemicals changes causes modifications in mechanism of transport, regulation of program cell death and enzymes activities contribute to drug resistance within cancer cells [56].

One peculiar quality about this peptide is that it is unaffected by multidrug resistance mechanisms frequently encountered with many conventional chemotherapeutic agents, and displays a higher specificity for cancer cells versus normal cells in comparison to conventional chemotherapy [46,47]. The peptide rapidly induced apoptosis in several different cancer cell lines indicated by DNA fragmentation assays and phosphatidylserine headgroup inversion detected by Annexin V binding to the surface of cancer cells. Although this peptide did not adversely affect the viability of untransformed human lymphocytes, fibroblasts, or endothelial cells indicating it safety on the normal cells, Production of reactive oxygen species was observing after Treatment of Jurkat T leukemia cells with the peptide which ultimately lead to activation of caspases.

A major limitation of cancer therapeutic efficacy is often systemic non-specific biodistribution which often leads to the reduced bioavailability of drug delivered to target cancer cells and therefore reduce therapeutic index. In this respect, LfcinB bioavailability can be increase to

rapidly reached the target tissue without much serum proteases degradation while increase it serum stability.

10. CONCLUSION

We come into conclusion that LfcinB possesses strong anticancer activity against many different cancer cells. It exerts its cytotoxic activity causing apoptosis to tumor cells through mitochondria pathway leading to DNA fragmentation without Affecting the Viability of normal Cells. Accordingly, increase in LfcinB activity would lead to designed of better cancer therapeutics.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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