

# MOLECULAR THERAPEUTIC CANCER PEPTIDES: A CLOSER LOOK AT BOVINE LACTOFERRICIN

## ABSTRACT

Bovine lactoferricin (LfcinB) is a cationic peptide with broad spectrum antimicrobial and anticancer activity. It is made up of twenty-five (25) amino acids with a disulphide bridge between two cysteines and thus gives a cyclic structure. The peptide possesses strong cationic charge (+8) which confers the peptide the ability to selectively target the negative charge of cancer cells (-) while sparing healthy cells. It also shows promising activity against many varieties of cancer including colon carcinoma cells, melanoma, fibrosarcoma, monocytic leukemic cells and neuroblastoma. The peptide was reported to be unaffected by multidrug resistance mechanisms seen with conventional chemotherapeutic drugs, and displays a higher specificity for cancer cells versus normal cells in comparison to conventional chemotherapy. Although the exact mechanism of action of LfcinB is not fully understood, subcutaneous administration of LfcinB inhibit lung and liver metastasis of both murine melanomas and lymphomas, confirming the ability of LfcinB to hinder the development and spread of cancer to nearby tissues. The aim of this paper is to review the anti-cancer activity of bovine lactoferricin with its Biochemical and physiological activities within cancer cells.

**Keyword:** Bovine Lactoferricin; Disulphide Bridge; Apoptosis; Angiogenesis; Cytotoxic Activity

## 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 (Figure 1), thus having a cyclic twisted anti-parallel  $\beta$ -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. The broad spectrum against microorganisms 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] all this shows 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].

## 2. STRUCTURE OF BOVINE LACTOFERRICIN

LfcinB possesses a cyclic structure formed by the disulfide bond that links its two-cysteine residues (Figure 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 comprises the opposing face [13]. The primary structure of LfcinB has been described as peptide consisting of 25-residue peptide with an amphipathic, antiparallel  $\beta$ -sheet structure [13]. The 25 AA residue peptide forms looped structure through an intramolecular disulfide bond (Figure 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  $\alpha$ -helix structure observed in intact bovine lactoferrin and changes into  $\beta$ -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 [15]. The amphipathic property is accounted by  $\beta$  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  $\alpha$ -helix to a  $\beta$ -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 (Table 1) on the peptide, as well as two tryptophan and two phenylalanine residues that are both aromatic and hydrophobic [14].

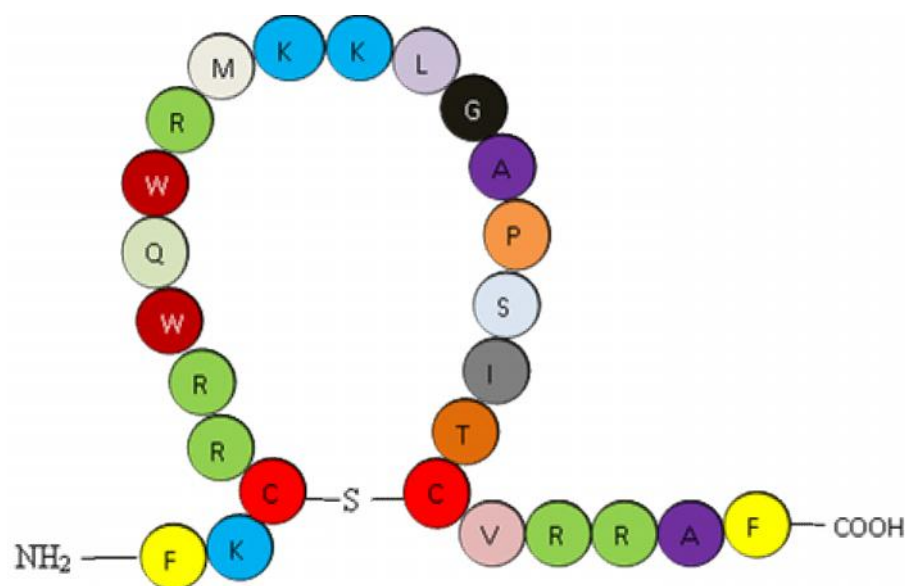


Figure 1: structure of Bovine lactoferricin, all amino acids in the peptide is represented by one letter coded as given below.

Alanine = A

Arginine = R

69 Aspartic acid = D

70 Cysteine = C

71 Glutamate = E

72 Glutamine = Q

73 Glycine = G

74 Histidine = H

75 Isoleucine = I

76 Leucine = L

77 Lysine= K

78 Methionine = M

79 Phenylalanine = F

80 Proline = P

81 Serine = S

82 Threonine = T

83 Tryptophan = W

84 Tyrosine = Y

85 Valine = V

86

### 87 **3. STRONG CATIONIC CHARGE OF BOVINE LACTOFERRICIN LINKED TO ITS** 88 **ANTICANCER ACTIVITY**

89

90 The anticancer activity of LfcinB is attributed to its strong cationic charge +8 (Table1) which  
91 confers the peptide the ability to selectively target the negative charge (-) of the most cancer  
92 cells, while healthy normal cells with nil charge (0) remains unaffected due to the high  
93 content of zwitterionic phosphatidylcholine located outside the leaflet of the membrane [17].  
94 The net negative charge of most cancer cells of the leaflet of the membrane results due to

95 The difference that exist in the branch and N-linked glycans of sialic acid which are  
96 connected with glycoprotein [18] that crosses the membrane and Over-expression of anionic  
97 molecules [19,20-22] such as phosphatidylcholine O-glycosylated mucins [17,21].The  
98 inability of the LfcinB to bind to PC3 carcinoma is probably due to the fact that some cancer  
99 cells refract the cytotoxic action of LfcinB due to insufficient negative charge through which  
100 electrostatic interaction took place between LfcinB and cancer cells [3]. Derivatives of LfcinB  
101 Containing cationic and hydrophobic moieties were reported to have enhanced activity  
102 Owing to its high positive charge, structure and amphipathic properties whereas cancer cells

cytotoxicity has not been observed with homologue of murine lactoferricin containing glutamic acid [22-24].

Table 1: Amino acids component of LfcinB showing the net charge on the peptide

| Amino acid    | One letter code | Times it occurs in the peptide | Charge            | Total charge in the peptide |
|---------------|-----------------|--------------------------------|-------------------|-----------------------------|
| Phenylalanine | F               | 2                              | 0                 | 0                           |
| Lysine        | K               | 3                              | +1                | +3                          |
| Cysteine      | C               | 2                              | 0                 | 0                           |
| Arginine      | R               | 5                              | +1                | +5                          |
| Tryptophan    | W               | 2                              | 0                 | 0                           |
| Glutamine     | Q               | 1                              | 0                 | 0                           |
| Methionine    | M               | 1                              | 0                 | 0                           |
| Leucine       | L               | 1                              | 0                 | 0                           |
| Glycine       | G               | 1                              | 0                 | 0                           |
| Alanine       | A               | 2                              | 0                 | 0                           |
| Proline       | P               | 1                              | 0                 | 0                           |
| Serine        | S               | 1                              | 0                 | 0                           |
| Isoleucine    | I               | 1                              | 0                 | 0                           |
| Tyrosine      | T               | 1                              | 0                 | 0                           |
| Valine        | V               | 1                              | 0                 | 0                           |
|               |                 |                                | <b>Net charge</b> | <b>+8</b>                   |

#### 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. The peptide then interacts with the natively charged of the mitochondria, inducing apoptosis mainly via cell injury (necrosis) through cell membrane lytic. When LfcinB is inserted in the membrane, it causes changes in the membrane mimetic systems by fostering the assembling of bicontinues cubic or inverted hexagonal phases [28-32]. 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 Ca<sup>2+</sup>/Mg<sup>2+</sup>-dependent endonucleases has been linked to the apoptotic-inducing activity of LfcinB to THP-1 human monocytic leukemia cells [25]. In this same experiment, is it noted that THP-1 cells Treated with 100µg/mL LfcinB undergo apoptosis after about 10 h with maximal activity, while those cells Treated with bLF did not induce cell death even at a high dose of 500µg/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 extracellularly does not depend on internalization of the peptide, but itself may be responsible for the cytotoxic activity of the peptide. LfcinB induce-apoptosis was found to be inhibited by addition of Zn<sup>2+</sup>, an inhibitor of endonucleases which require Ca<sup>2+</sup>/Mg<sup>2+</sup> for full activity, however, LfcinB-induced cell death was effectively abolished in THP-1 cells following the addition of antioxidants, such as N-acetyl-cysteine (NAC) and glutathione (GSH), similarly to that induced by H<sub>2</sub>O<sub>2</sub>. [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 (Figure 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  $\text{Ca}^{2+}/\text{Mg}^{2+}$ -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 (Figure 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 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, and 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]

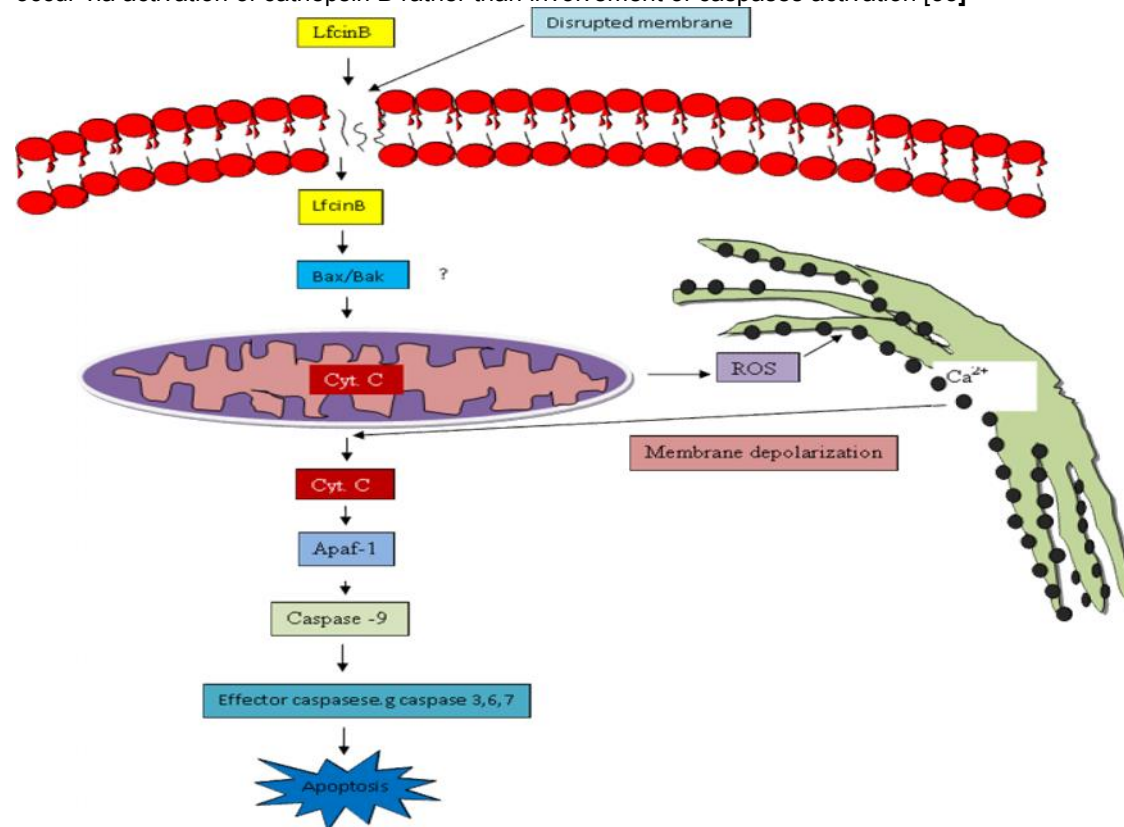


Figure 2: proposed mechanism of action of LfcinB. "In the diagram LfcinB causes membrane lysis and gains entry into the cytoplasm. It co-localizes with the mitochondria and causes the release of cytochrome C. The cytochrome C interacts with Apaf-1 and caspase-9 to form the apoptosome. Caspase-9 then activates caspase 3 which ultimately leads to apoptosis."

## 6. BOVINE LACTOFERRICIN AND ANGIOGENESIS

Angiogenesis is the process that encompasses the growth, differentiation 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. DISCUSSION

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].

Ontologically, 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 and trials

for anticancer therapeutics [53]. Hence, these peptides may emerge as diagnostic tools and as agents of choice for anti-cancer investigation through attachment to chemotherapeutic moieties including cytotoxic agents or radionuclide. The peptides can conveniently be linked directly to halogens (iodine and fluorine) or radionuclide of metals using suitable chelating agents. In view of the fact that these small peptides can be synthesized relatively easy, synthesizing new peptides 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. CONCLUSIONS

In spite of the progress that was recorded in the development of anticancer drugs, challenges continue to rise 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 approach 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.

## REFERENCES

1. Mader JS, Salsman J, Conrad DM, et al. Bovine lactoferricin selectively induces apoptosis in human leukemia and carcinoma cell lines. *Mol Cancer Ther.* 2005; 4:612-24
2. Eliasse LT, Berge G, Leknessund A, et al. The antimicrobial peptide, lactoferricin B, is cytotoxic to neuroblastoma cells in vitro and inhibits xenograft growth in vivo. *Int J Cancer.* 2006; 119:493-500.
3. Pepe G, Tenore GC, Mastrocinque R, et al. Potential anticarcinogenic peptides from bovine milk. *J Amino Acids.* 2013; 2013:1-7.
4. Kuwata H, Yip TT, Tomita M, et al. Direct evidence of the generation in human stomach of an antimicrobial peptide domain (lactoferricin) from ingested lactoferrin. *Biochem Biophys Acta.* 1998; 1429:129-41.

5. Bellamy W, Wakabayashi H, Takase M, et al. Killing of *Candida albicans* by lactoferricin B, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrin. *Med. Microbiol Immunol.* 1993; 82:97–05.
6. Cascinu S, Scartozzi M, Labianca R, et al. High curative resection rate with weekly cisplatin, 5-fluorouracil, epirubicin, 6S-leucovorin, glutathione, and filgrastim in patients with locally advanced, unresectable gastric cancer: a report from the Italian Group for the Study of Digestive Tract Cancer (GISCAD). *Br J Cancer* 2004; 90 :1521–25.
7. Andersen JH, Osbakk S A, Vorland LH, et al. Lactoferrin and cyclic lactoferricin inhibit the entry of human cytomegalovirus into human fibroblasts. *Antiviral Res* 2001; 51:141–49.
8. Yoo Y, Watanabe S, Watanabe R, et al. Bovine lactoferrin and lactoferricin, a peptide derived from bovine lactoferrin, inhibit tumor metastasis in mice. *Jpn J Cancer Res* 1997; 88:184–90.
9. He J, Furmanski P. Sequence specificity and transcriptional activation in the binding of lactoferrin to DNA. *Nature* 1995; 373:721–24.
10. Hauer J, Voetsh W, Anderer FA. Identification of a mannose-acetate-specific 87-kDa receptor responsible for human NK and LAK activity. *Immunol Lett* 1994; 42: 7–12.
11. Zagulski T, Lipinski P, Zagulska A, et al. Lactoferrin can protect mice against a lethal dose of *Escherichia coli* in experimental infection in vivo. *Brit J Exp Pathol* 1989; 70: 697–04.
12. Bezault J, Bhimani R, Wiprovnick J, et al. Human lactoferrin inhibits growth of solid tumors and development of experimental metastases in mice. *Cancer Res* 1994; 54: 2310-12.
13. Hwang P M, Zhou N, Shan X, et al. Three-dimensional solution structure of lactoferricin B, an antimicrobial peptide derived from bovine lactoferrin. *Biochemistry* 1998; 37:4288–98.
14. Bellamy W, Takase M, Yamauchi K, et al. Identification of the bactericidal domain of lactoferrin. *Biochim Biophys Acta* 1992; 1121:130-36.
15. Gifford J L, Hunter H N, Vogel H J. Lactoferricin: a lactoferrin-derived peptide with antimicrobial, antiviral, antitumor and immunological properties. *Cell Mol Life Sci* 2005; 62:2588–98.
16. Zhou N, Tieleman D P, Vogel H J. Molecular dynamics simulations of bovine lactoferricin: turning a helix into a sheet. *Biometals* 2004;17: 217–23.
17. Burdick M D, Harris A, Reid C J, et al. Oligosaccharides expressed on MUC1 by pancreatic and colon tumor cell lines. *J Biol Chem* 1997; 272:20202–4198.
18. Dennis J W. N-linked oligosaccharide processing and tumor cell biology. *Semin Cancer Biol.* 1991; 2:411–20.
19. Utsugi T, Schroit J, Connor C, et al. Elevated expression of phosphatidylserine in the outer membrane leaflet of human tumor cells and recognition by activated human blood monocytes. *Cancer Res.* 1991; 51:3062–66.
20. Dobrzyńska I, Szachowicz-Petelska B, Sulkowski S, et al. Changes in electric charge and phospholipids composition in human colorectal cancer cells. *Mol Cell Biochem.* 2005; 276:113–19.
21. Yoon W H, Park H D, Lim K, et al. Effect of O-glycosylated mucin on invasion and metastasis of HM7 human colon cancer cells. *Biochem Biophys Res Commun.* 1996; 222: 694–99.

22. Eliassen L T, Haug B E, Berge G, et al. Enhanced antitumor activity of 15-residue bovine lactoferricin derivatives containing bulky aromatic amino acids and lipophilic N terminal Modifications. *J Pept Sci.* 2003; 9:510–17.
23. Yang N, Strøm M B, Mekonnen S M, et al. The effects of shortening lactoferrin derived peptides against tumor cells, bacteria and normal human cells. *J Pept Sci.* 2004; 1:37–46.
24. Gifford J L, Hunter H N, Vogel H J. Lactoferricin: a lactoferrin-derived peptide with antimicrobial, antiviral, antitumor and immunological properties. *Cell Mol Life Sci* 2005; 62:2588–98.
25. Eliassen L T, Berge G, Sveinbjørnsson B, et al. Evidence for a direct antitumor mechanism of action of bovine lactoferricin. *Anticancer Res* 2002; 22: 2703–10.
26. Yoo YC, Watanabe R, Koike Y, et al. Apoptosis in human leukemic cells induced by lactoferricin, a bovine milk protein derived peptide: involvement of reactive oxygen species. *Biochem Biophys Res Commun* 1997; 237: 624 – 28.
27. Furlong S J, Mader J S, Hoskin DW, Lactoferricin induced apoptosis in estrogen-nonresponsive MDA-MB-435 breast cancer cells is enhanced by C6 ceramide or tamoxifen,” *Oncol rep.* 2006;15:1385–90.
28. Staudegger E, Prenner E J, Kriechbaum M, et al. X-ray studies on the interaction of the antimicrobial peptide gramicidin S with microbial lipid extracts: evidence for cubic phase formation. *Biochim. Biophys. Acta* 2000; 1468: 213–30.
29. Willumeit R, Kumpugdee M, Funari SS, et al. Structural rearrangement of model membranes by the peptide antibiotic NK-2. *Biochimica Biophys Acta, Biomembr.* 2005; 1669:125–34.
30. Hickel A, Danner-Pongratz S, Amenitsch H, Degovics G, et al. Influence of antimicrobial peptides on the formation of nonlamellar lipid mesophases *Biochim Biophys Acta, Biomembr.* 2008; 1778: 2325–33
31. Zweglick D, Tumer S, Blondelle S E, Lohner K. Membrane curvature stress and antibacterial activity of lactoferricin derivatives. *Biochem. Biophys Res Commun* 2008; 369:395–00.
32. Zweglick D, Deutsch G, Andraß J, et al. Studies on Lactoferricin-derived Escherichia coli membrane-active peptides reveal differences in the mechanism of N-acylated versus nonacylated peptides. *J Biol Chem.* 2011; 286:21266–76.
33. Mader JS, Richardson A, Salsman J, et al. Bovine lactoferricin causes apoptosis in Jurkat T-leukemia cells by sequential permeabilization of the cell membrane and targeting of mitochondria. *Exp Cell Res.* 2007; 313:2634–50.
34. Onishi J, Roy M K, Juneja L R, et al. A lactoferrin-derived peptide with cationic residues concentrated in a region of its helical structure induces necrotic cell death in a leukemic cell line (HL-60). *J Pept Sci.* 2008; 14:1032–38.
35. Furlong S J, Mader J S, Hoskin D W. Bovine lactoferricin induces caspase-independent apoptosis in human B-lymphoma cells and extends the survival of immune-deficient Mice bearing B-lymphoma xenografts. *Exp Mol Pathol* 2010; 88:371–75.
36. Rosca E V, Koskimaki J E, Rivera C G, et al. Anti-angiogenic peptides for cancer therapeutics. *Curr Pharm Biotechnol* 2011; 12:1101–16.
37. Mader J S, Smyth D, Marshall J, et al. Bovine lactoferricin inhibits basic fibroblast growth factor- and vascular endothelial growth factor165-induced angiogenesis by competing for heparin-like binding sites on endothelial cells. *Am J Pathol* 2006; 169:1753–66.

38. Hoskin DW, Ramamoorthy A. Studies on anticancer activities of antimicrobial peptides. *Biochimica Biophys Acta*. 2008; 1778, 357–75.
39. Thundimadathil J. Cancer treatment using peptides: current therapies and future prospects. *J. Amino Acids* 2012; 2012:1–13
40. Johnstone SA, Gelmon K, Mayer LD, et al. In vitro characterization of the anticancer activity of membrane-active cationic peptides. I. Peptide-mediated cytotoxicity and peptide-enhanced cytotoxic activity of doxorubicin against wild-type and p-glycoprotein Over-expressing tumor cell lines. *Anticancer Drug Des*. 2000; 15:151-60.
41. Cruciani RA, Barker JL, Durell SR, et al. Magainin 2, a natural antibiotic from frog skin, forms ion channels in lipid bilayer membranes. *Eur J Pharmacol*. 1992; 226:287- 96.
42. Ohsaki Y, Gazdar AF, Chen HC, et al. Antitumor activity of magainin analogues against human lung cancer cell lines. *Cancer Res*. 1992; 52:3534-38.
43. Yeaman MR, Yount NY: Mechanisms of antimicrobial peptide action and resistance. *Pharmacol Rev*. 2003; 55:27-55.
44. Dennison SR, Whittaker M, Harris F, et al. Anticancer alpha-helical peptides and structure/function relationships underpinning their interactions with tumour cell membranes. *Curr Protein Pept Sci*. 2006; 7:487- 99.
45. Mader JS, Hoskin DW: Cationic antimicrobial peptides as novel cytotoxic agents for cancer treatment. *Expert Opin Investig Drugs*. 2006; 15, 933-46.
46. Papo N, Shahar M, Eisenbach L, et al. A novel lytic peptide composed of DL-amino acids selectively kills cancer cells in culture and in mice. *J Biol Chem*. 2003; 278:21018-23.
47. Papo N, Seger D, Makovitzki A, et al. Inhibition of tumor growth and elimination of multiple metastases in human prostate and breast xenografts by systemic inoculation of a host defense-like lytic peptide. *Cancer Res*. 2006; 66:5371-78.
48. Makovitzki A, Fink A, Shai Y. Suppression of human solid tumor growth in mice by intratumor and systemic inoculation of histidine-rich and pH dependent host defense-like lytic peptides. *Cancer Res*. 2009; 69:3458-63.
49. Papo N, Braunstein A, Eshhar Z, et al. Suppression of human prostate tumor growth in mice by a cytolytic D-, L-amino Acid Peptide: membrane lysis, increased necrosis, and inhibition of prostate-specific antigen secretion. *Cancer Res* 2004; 64: 5779-86.
50. Henderson R A, Mossman S, Nairn N. Cancer vaccines and immunotherapies: emerging perspectives. *Vaccine* 2005; 23: 2359–62.
51. Berzoksky J A, Ahlers J D, Belyakov I M. Strategies for designing and optimizing new generation vaccines. *Nature* 2001; 1: 209–19.
52. Berge G, Eliassen LT, Camilio KA, et al. Therapeutic vaccination against a murine lymphoma by intratumoral injection of a cationic anticancer peptide. *Cancer Immunol Immunother*. 2010; 59:1285-94.
53. Lozza C, Navarro-Teulon I, Pèlegri A, et al. Peptides in receptor-mediated radiotherapy: from design to the clinical application in cancers. *Front Oncol*. 2013; 3: 247.
54. Riedl S, Zweytick D, Lohner K. Membrane-active host defense peptides – Challenges and perspectives for the development of novel anticancer drugs. *Chem Phys Lipids*. 2011; 164: 766–81.

- 456 55. Fadnes B, Uhlin-Hansen L, Lindin I, et al. Small lytic peptides escape the inhibitory effect of  
457 heparan sulfate on the surface of cancer cells. BMC Cancer 2011; 11:116.  
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460 **ABBREVIATIONS**

- 461 AA, amino acids  
462 Apaf-1, Apoptotic protease activating factor 1  
463 bFGF, Basic fibroblast growth factor  
464 bLF, Bovine Lactoferrin  
465 GSH, Reduced glutathione  
466 LfciB, Bovine lactoferricin  
467 NAC, N-acetyl cysteine  
468 PC 3, Human *prostate cancer* cell line-3  
469 ROS, Reactive oxygen species  
470 THP-1, Human acute monocytic leukemia *cell* line-1  
471 VEGF, Vascular endothelial growth factor