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Review Article

The overview on anti-cancer effects of milk lactoferrin

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ABSTRACT

Cancer has emerged as a global health issue and become a one of major cause of deaths in 21st century. The existing therapeutic approaches to prevent cancer are indeed beneficial but pose different risks to human health and now, tumor heterogeneity, non-specificity and untargeted delivery of anti-cancer drugs arise the problem of chemoresistance. Lactoferrin especially derived from bovine milk has caught attraction to be utilized in cancer treatment because of its biological activities, immuno-compatibility and low cost effectiveness. This chapter outlined the importance of milk lactoferrin in prevention of cancer by covering different aspects of its biology and mechanism of action. Lactoferrin from bovine milk has been evaluated in different types of cancer and known to exert its anti-cancerous potential through regulation of cell cycle, apoptosis induction, inhibition of metastasis and immunomodulation. One of the major advantage of milk lactoferrin is that it crosses the blood-brain barrier which make it suitable for treatment of brain tumors especially glioblastoma. Moreover, lactoferrin has also act as a potential carrier for targeted delivery of anti-cancerous drugs to eliminate multi-drug resistance in cancer thus signifying its pharmacological importance.

Keywords: Cancer, chemoresistance, lactoferrin, immunomodulation, apoptosis

INTRODUCTION

Cancer is the leading cause of mortality all over the world. Currently, the treatment strategies to fight cancer are chemotherapy, surgery and radiation therapy. Unfortunately, all of these therapeutic regimens undergo the problem of potential risks and side effects. Therefore, the development of new treatments is very important and highly desirable. To fight cancer, multiple aspects of

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therapy are being considered involving suppression of side effects, adjunct and complementary treatments. Many epidemiological studies have revealed the importance of appropriate life style to prevent cancer [1]. A diet containing the anti-cancer agents is proposed to be a suitable strategy to control the risk of cancer.

Dairy products especially milk contain many nutritional supplements including proteins, vitamins and bioactive peptides which are not only beneficial for human health but also reported for their anti-cancer potential [2]. Lactoferrin (LF) is non-toxic globular and multipurpose protein involved in human innate immunity. Lactoferrin has been derived from various sources but here the main focus in on lactoferrin derived from milk especially bovine milk. In bovine milk, lactoferrin is one of the major component and is widely known for its biological activities including anti-bacterial, anti-viral, anti-cancer and anti-oxidant activity [3, 4]. Some of the studies have revealed that the iron-withholding ability of lactoferrin and its capacity to bind to multiple cellular receptors is responsible for its diverse biological functions [3]. Additionally, the peptides derived from lactoferrin especially bovine lactoferricin B and hololactoferrin (iron-binding form of lactoferrin) have been reported as an anti-cancer agent [5]. Multiple studies have reported the role of lactoferrin to stop cancer progression via various mechanisms. The silencing or downregulation of lactoferrin genes has been shown to be associated with cancer metastasis [6, 7], while restoration of the expression of lactoferrin gene has inhibited the proliferation of cancer cells. Moreover, several other studies have also revealed that milk lactoferrin and its derived peptides can block the progression of cancer *in vivo* and *in vitro* [8-11]. Bovine lactoferrin is proved to be stable proteins that remain active even in the form of degraded fragments. Lactoferrin as an oral supplement with concentration of 0.2 % to 2% showed the inhibition of carcinogenesis in animal models by 32.5 to 42.5% respectively. Moreover, administration of bovine or milk lactoferrin showed preventive activity against multiple types of cancer. So, the current chapter focuses on the possible mechanism of action of lactoferrin for cancer inhibition and therapeutic potential of lactoferrin in multiple cancer types. Finally, lactoferrin as a drug delivery system has been discussed for the targeted delivery of chemotherapeutic drugs.

Lactoferrin anti-cancerous activity

Mechanism of action: Although the exact mechanism of action of lactoferrin against cancer is not cleared yet but it has been suggested that the anti-cancer potential of lactoferrin is due to its multiple functions including immunostimulation, extracellular and intracellular effects (Figure 1). The extracellular effects are related to ability of lactoferrin to interact with cell membrane and bind to multiple membrane receptors [12] while intracellular effects are related to cell cycle arrest and apoptosis [11, 13].

Lactoferrin and modulation of cell cycle: The cell cycle activity is mediated by many growth factors and hormones and it should be tightly regulated because dysregulation in cell cycle modulation can lead to development of cancer. Different proteins are involved in regulation of cell cycle like cyclin dependent kinases (CdKs) and their inhibitors are the key players to control the progression of cell cycle. Many anti-cancer agents are reported for their potential to arrest cell cycle and to induce cytotoxicity in cancer cells. But many of these chemotherapeutic agents are not able to discriminate between normal and cancer cells and thus can lead to many complications and adverse effects. Conversely, the lactoferrin has been reported as a selective agent regarding to cancerous tissues because of exerting the inhibitory effect to only tumor cells [14] while for the growth of normal cells, lactoferrin has shown to be its positive regulator [15]. The molecular mechanism of both bovine and human lactoferrin to enhance the growth of normal cells is due to shorten the cell cycle by upregulation the expression of mRNA of proliferative cell nuclear antigen thus increase the number of cells in G2 and S phase of cycle [15]. Moreover, bovine lactoferrin has also shown to increase the cell growth through phosphorylation of MAPK (mitogen-activated protein kinase) and ERK (extracellular signal regulated kinase) [16].

Concerning tumor cells, both bovine and human lactoferrin has been reported to arrest the cell growth in different phases of the cell cycle. Zhang et al. reported the selectivity of bovine lactoferrin in which bLF blocked the growth of tumor in four breast cancer cell lines but did not inhibited in normal cell lines. The authors revealed that the molecular mechanism of bovine lactoferrin for cell cycle arrest was associated with upregulation of phosphorylated AMPK and downregulation of mTOR, which is crucial for cell survival [17]. In another study, bovine lactoferrin has been reported to induce cell cycle arrest in oral carcinoma cells by mechanism in which it upregulates the expression of CDK inhibitor p21 and inhibited the cyclin D1 which is involved in the progression of cells growth through G1 phase [18].

Induction of apoptosis by lactoferrin: Apoptosis, known as programmed cell death, has been considered as the pivotal regular of physiological and pathological conditions. The basic principle for the induction of apoptosis include the activation of many extrinsic and intrinsic pathways in which the

extrinsic pathway is activated by ligand binding activation of death receptor and intrinsic pathway is initiated in response to oxidative stress, hypoxia and DNA damage which recruits many pro-apoptotic proteins regulating cell fate [19, 20]. Moreover, these regulators inducing apoptosis are modulated and controlled by various other cellular pathways including insulin signaling, JNK and ERK signaling. In cancer, beside higher proliferation rate and invasion characteristics, genetic changes accumulating in cells lead to dysregulation in extrinsic and intrinsic pathways and disrupt the balance between pro-apoptotic and anti-apoptotic proteins which confer the cell to evade apoptotic signaling [21]. Therefore, the cell resistance to undergo programmed cell death is one of the main hallmark of carcinogenesis. In this case the chemical compounds or biomolecules that can rebalance the apoptotic response can be used as chemotherapeutic agent.

Lactoferrin has been reported to activate the apoptotic signaling in various types of cancers. Bovine lactoferrin (bLf), when evaluated for its anti-cancer effect in stomach cancer cell line (SGC-7901) showed apoptosis induction through down regulation of AKT pathway [22]. Treatment with bLf cause dephosphorylation of AKT on Ser473 and Thr308 residues and further block the downstream regulators of apoptosis. In another study, bLf exerts its pro-apoptotic effects by apoptosis induction in animal model of colon cancer via upregulating the Fas signaling [23]. With the treatment of bLf, Fas protein along with caspase 3 and caspase 8 showed upregulation in their levels and immunohistochemistry confirms the presence of apoptotic and Fas-positive cells within the colon region. The same group of authors showed in another study on rat that upon treatment with bLf the two pro-apoptotic proteins Bax and Bid showed increased levels at tumor site [24]. Moreover, bLf in its both isoforms including apo and holo, was found to inhibit the survivin which is overexpressed in multiple types of cancer and bind with pro-apoptotic proteins to block apoptosis. Gibbson et al, also reported that both isoforms of bLF induced apoptosis in breast cancer cell line while no pro-apoptotic effect was observed in breast normal cell line [25]. Finally, lactoferrin B, derivative of lactoferrin, has also shown to induce ROS-dependent apoptosis induction in human leukemia cell line and in different cancer models [26].

Lactoferrin and inhibition of cell migration, invasion, and metastasis: Other than apoptotic resistance and proliferation, cancer cells got the ability to migrate and invade other tissues when they detach from primary site and enter into circulation thus acquired metastatic behavior. Lactoferrin has been reported to inhibit the cell invasion and migration in various models of cancer but exact molecular mechanism of its anti-invasive and anti-migratory activities is not cleared yet. Epithelial to mesenchymal transition (EMT) is one of the main process that is involved in metastasis of cancer and here cells get invasive property and they become able to infiltrate in blood vessels and surrounding tissues thus confer them metastatic behavior. Bovine lactoferrin (bLF) showed reversal of EMT process in recent investigations on oral cancer cells [27] and glioblastoma [28]. Many of the proteins and transcription factors are shown to be overexpressed in malignant cancer cells like snail, twist, STAT3 and vimentin. The overexpression of these factors are also responsible for repression of cadherins (molecules involve in cell to cell adhesion) and associated with aggressiveness of cancer. Bovine lactoferrin showed to tackle the invasiveness efficiently in oral cancer cells and HOC313 cell line by reverting the process of EMT [27]. When its mechanism of action was analyzed, it revealed the inhibition of twist by dephosphorylation of ERK1/2 upon its binding to LRP1 receptor. bLF, when administered orally to xenografts, it showed higher levels of cadherins and less infiltration of cancer cells.

Besides anti-migration and anti-invasiveness effects, lactoferrin has been also reported to suppress metastasis of cancer. Particularly, when apo form of bovine lactoferrin was injected subcutaneously in mice with lymphoma and melanoma cells, it inhibits the liver, lung and spleen cancer metastasis along with inhibition of tumor induced angiogenesis [29]. Moreover, the oral administration of bovine lactoferrin and lactoferricin B to mice having highly metastatic colon cancer, suppress the metastasis in lung and inhibit colony formation [30]. A recent study demonstrated that deficiency of lactoferrin increased the cancer metastasis to lungs through recruiting myeloid suppressor cells in lactoferrin knockout mice [31]. Hence, lactoferrin is an important agent to control the metastatic behavior of cancer.

Immunomodulation by lactoferrin

Inflammatory cells make up the tumor microenvironment which is a very important factor in tumor metastasis and inhibition. These inflammatory cells that participate in tumor microenvironment are mainly leukocytes including macrophages, dendritic cells, lymphocytes and neutrophils. These cells secrete various inflammatory mediators, cytotoxic molecules and cell killing soluble mediators to regulate cancer progression [32]. Tumor fate is usually decided by interplay between immunity and cancer regulation. Many studies reported the carcinogenic activity of

leukocytes through release of IL-1β and IL-6 which are founded to be present in high levels in different cancer models [33, 34] while some subtypes of leukocytes including cytotoxic T cells and natural killer cells showed anti-tumor activity [34]. So, the immune response is involved in both pro-cancerous and anti-cancerous activities depending on the balance between innate and adaptive immunity. Therefore, immunomodulation has a great impact in cancer biology and in this case the molecules that boost cytotoxic components of immunity can be good candidates as an adjuvant to chemotherapeutic agents.

Lactoferrin has been proved to potentiate components of adaptive immunity and has anti-inflammatory activity [35]. Both bovine and human lactoferrin are reported to enter in host cell nucleus [36] and can bind with DNA to modulate gene expression thus, controlling inflammation and regulating carcinoma. Bovine lactoferrin, when administered orally in mice, showed anti-tumor activity by enhancing the levels of mucosal IL-18 mRNA in mice small intestine [37]. Bovine lactoferrin has been also reported to inhibit growth of tumor in IFN-γ knockout mice by upregulation the IFN- α/IL-7 pathway activation [37]. In another study, bovine lactoferrin inhibited the tumor growth in human lung cancer cells and in murine models by regulating the levels of vascular endothelial growth factor. Results showed that bLF reduced the levels of VEGF in cancer cells and also reduce the expression of pro-inflammatory cytokines [38]. Along with direct modulation of immune function, bovine lactoferrin also showed to manage levels of reactive oxygen species by binding with the free iron which is the one of main component of ROS production [39]. Finally, bLF is shown to provide shield against iron disorders that lead to cancer by immunomodulation and by decreasing levels of pro-inflammatory cytokines such as Tumor necrosis factor and Interleukins [40].

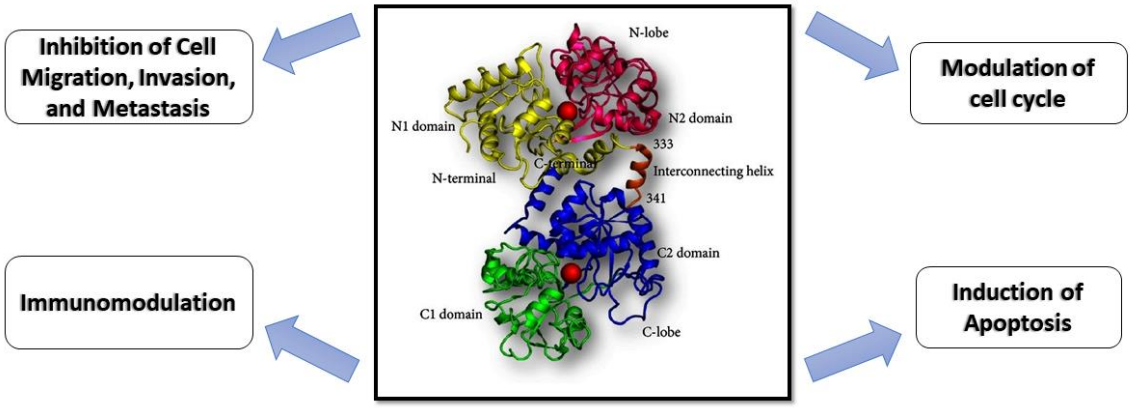

Figure 1: Crystal structure and anticancer mechanism of bovine lactoferrin

Potential of lactoferrin against different cancer types: Lactoferrin, especially bLF are found to be tested both *in vitro* and *in vivo* for its anti-cancer potential in different cancer types but majorly on breast cancer, colorectal cancer and leukemia. Interestingly, the clinical trials have been also conducted to evaluate the effectiveness of lactoferrin in prevention and treatment against metastatic cancers. Besides, the basic mechanism of action, various other functions of lactoferrin are also known to be associated with anti-cancer role like the ability of lactoferrin to bind to iron. Due to effectiveness of lactoferrin against cancer metastasis, it is considered as chemotherapeutic agent. So, it will be important to shed light on the potential of lactoferrin against different cancers.

Effects of lactoferrin on breast cancer treatment: Breast cancer is the leading cause of mortality all over the world. The potential of bovine lactoferrin has been assessed in various models of breast cancer. Duarte et al., studied the effect of bovine milk lactoferrin on two HS578T and T47D cancer cell lines of human breast cancer [41]. The cells were given exposure or treated with different concentrations of lactoferrin ranging from 0.125 to 125μM. Results showed that bLF was found to decrease the viability of T47D and HS578T cells by 54% and 47% respectively. Proliferation rate of both T47D and HS578T cells was decreased by 63.9% and 40.3% respectively. When the effect of bLF was measured on cancer cells migration, results showed that the migration was decreased only in T47D but enhanced the apoptosis in both cancer cell lines. In another study, bovine lactoferrin in its iron-binding and iron-free form was evaluated for cytotoxicity and invasion in MCF-7 and MDA-MB-231 human breast cancer

cell lines [25]. Results showed that upon treatment with both forms of bLF, the iron free form confer greater cytotoxicity in MCF-7 and MDA-MB-231 cancer cells in dose dependent manner. Interestingly, no cytotoxic effect was observed in normal breast cell line. Moreover, the both forms of bLF showed marked invasion in cancer cells. The survivin protein, upregulated in breast cancer development was found to be inhibited by both iron bound and iron-free form of bLF. The other apoptotic molecules including proteins from Bcl-2 family and p53 were found to be modulated by bovine milk lactoferrin.

Kim et al. studied the anti-cancerous effects of lactoferrin derived from caprine colostrum on different human cancer cell lines [42]. The lactoferrin from caprine was purified by ultrafiltration and chromatographic techniques. After purification, it was tested on 5 different cancer cell lines including breast cancer cells, colon, stomach, lung and cervix cancer cells. Results showed that caprine lactoferrin reduced the proliferation of cancer cells in dose dependent manner with only 20-30% survival as compared to normal cancer cell lines. Surprisingly, the greater inhibition of cell growth was observed in ZR-75-1 breast cancer cells with 27.5 µg/mL IC50 value. Thus, milk lactoferrin is proved to be a suitable anti-cancerous agent against breast cancer.

Lactoferrin and colorectal cancer: Colorectal cancer is the second most common cause of death in developing countries. Bovine lactoferrin and its peptide derivative lactoferricin B (LFcinB) have been evaluated for their anti-cancer activity against colorectal cancer cells. It has been believed that bLF and LFcinB applied its anti-cancerous activity by regulation of multiple signaling pathways. When their anti-cancer potential was analyzed in HT-29 colorectal cancer cells, they found to induce the apoptosis in HT-29 but not in normal intestinal cells showing the specificity for their target [43]. The viability of colorectal cancer cells was evaluated by cell viability assay in which HT-29 cancer cells showed reduced viability. Moreover, bLF and LFcinB also showed to regulate p53 signaling and angiopoietin signaling. Protein expression analysis represents the increased expression of p53, p21 and caspase 8 in HT-29 cells after giving treatment with bLF and LFcinB.

Colorectal polyp is abnormal tissue or cell growth on lining of colon. Colon polyps often lead to colon cancer at later stages when left untreated. The oral administration of lactoferrin from bovine milk was known to have anti-cancer effects on colorectal cancer so, a randomized controlled trial study was directed by Kozue y al. to assess the effect of bovine lactoferrin on the growth of colorectal polyps when administered orally [44]. The 104 participants in the study received 3g bLF daily for 12 months and the dose of placebo was 1.5g for same duration. After the treatment, the polyps were examined by colonoscopy. Results showed that oral administration of 3g bLF significantly reduced the growth of colorectal polyps and thus prevent the development of colorectal cancer. Generally, polypectomy is done to remove the polyps from colon but this procedure is not much efficient to eradicate 100% polyps so this study showed that oral administration of bovine lactoferrin could be employed for adjunct treatment to eradicate colorectal polyps and colorectal cancer.

Freiburghaus et al. also evaluated the potential of bovine lactoferrin and its derivative lactoferricin on human colorectal CaCo-2 cancer cells [45]. Upon the treatment with different concentrations of bLF and its derivative lactoferricin reduced the cell proliferation. When the cell cycle kinetics was assessed, it showed that bLF prolonged the S phase of cell cycle and lowered the cyclin E1 levels thus ultimately reduced the cell proliferation. Moreover, Habib et al. evaluated the therapeutic potential of lactoferrin derived from camel milk on HCT-116 colorectal cancer cells. Camel's milk lactoferrin significantly reduced the proliferation of colorectal cancer cells and prevent DNA damage.

Lactoferrin against prostate cancer: Prostate cancer is one of the leading malignancy and 2nd most common type of cancer affecting male population. Due to lack of symptoms on early stages, prostate cancer is diagnosed on the later stages when proper treatment and surgery is not possible. The use of natural and non-toxic products is highly attractive towards therapeutics in prostate cancer. In this scenario, lactoferrin which possess anti-cancerous and anti-metastatic activity was evaluated for it potential to manage prostate cancer. Guedes et al. evaluated the potential of bLF against PC-3 cells which are highly metastatic cancer cells of prostate cancer [46]. The cells were displaying V-ATPase which promote the cancer invasion, metastasis and produce acidic tumor microenvironment. The cells were treated with bLF and rate of cell proliferation, intracellular pH, apoptosis and extracellular acidification was analyzed. Results showed that bLF reduced the cell proliferation in PC-3 metastatic cancer cells and induced the cell death. bLF also inhibited the rate of intracellular acidification in cancer cells. The same experiments were also conducted for BJ-5ta cells which are normal cells. Upon exposure to lactoferrin, BJ-5ta cells remain insensitive to bovine lactoferrin showing the specificity of bLF for only cancerous cells. The authors speculated that mechanism behind the inhibition in cancers cells is the inhibition of V-ATPase which promote

cancer metastasis. These experiments showed that lactoferrin from milk source can be used to manage prostate cancer and its metastasis. Zadvornyi, T.V., et al. also evaluated the biological effects of lactoferrin from exogenous sources on the invasiveness of DU145 and LNCaP human prostate cancer cells [47]. Human PC cells were cultured with lactoferrin in dose dependent manner. After culturing, invasiveness of the cancer cells was evaluated and results showed that upon treatment with lactoferrin, invasiveness activity of PC cells were reduced by 40% in DU145 cancer cells and 30% in LNCaP human prostate cancer cells. In DU145 cells, treatment with exogenous lactoferrin enhanced the expression of oncosuppressive micro RNAs (miR-200b and miR-133a) when assessed with qPCR. It was concluded that lactoferrin has the potential to change the phenotypic characteristics of prostate cancer cells and can regulate major cellular processes involved in cell growth.

Lactoferrin and other cancer types: Bovine lactoferrin, a component of milk, recently appeared to be a solid chemopreventive agent of lung carcinoma advancement. A research study has been conducted on rat models by Ushida et al., in order to check the influence of bovine lactoferrin on esophagus and lung carcinogenesis [48]. This investigation revealed that in the esophagus, an inclination for decrease in developing of papillomas was obvious in the bovine lactoferrin treated rats, alongside a huge concealment of generally huge sized papillomas. Tumor multiplicity (adenomas and carcinomas) in the lung was additionally diminished in animals treated with bovine lactoferrin. In another study, potential of bLF was assessed both *in vivo* and *in vitro* against lung carcinoma [38]. The A549 human lung cancer cell line was used for *in vitro* system and it has higher levels of vascular endothelial growth factor (VEGF) which is one of the hallmark of lungs cancer. For the in vivo system, transgenic strain of mice was used as a model carrying human VEGF-A165 gene having pulmonary tumor. Both *invitro* and *in vivo* models were treated with bLF and results showed that bovine lactoferrin inhibit the proliferation of human lung cancer A549 cells in dose dependent way. The expression of vascular endothelial growth factor was found to be downregulated upon treatment with bovine lactoferrin. Moreover, when the treatment was given to *in vivo* model expressing higher levels of human VEGF-A165 gene, tumor was suppressed in mice and expression of human VEGF-A165 gene was reduced. Interestingly, upon the treatment with bLF, the expression of pro-inflammatory and anti-inflammatory cytokines including interleukins (IL-4, IL-6, IL-10) and TNF-alpha was also reduced which suggested that reduced expression of cytokines resulted in inhibition of inflammation which further inhibit the proliferation of lung cancer cells. So, it was concluded that bovine lactoferrin has considerable potential for therapeutics against lungs carcinoma by inhibiting cell inflammation and angiogenesis.

Chemopreventive impacts of bovine lactoferrin, which is found at high amount in colostrum, on rat bladder carcinogenesis were examined by Masuda et al., utilizing a rat bladder medium-term bioassay [49]. Finding indicates that 2% bovine lactoferrin diminished tumor multiplicity and would in general decrease cell proliferation in N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) induced rat bladder carcinogenesis, potentially by direct impacts through its presence in urine.

Bovine lactoferrin is considered to be a natural defense protein containing iron present in the bodily secretions, has been accounted for to repress carcinogenesis and the development of tumors. Kanwar et al., utilized bovine lactoferrin saturated with iron and examined its capability to augment chemotherapy of cancer in lymphoma [50]. Iron saturated Bovine lactoferrin and natural bovine lactoferrin that contain less percentage of iron was supplemented in the diet of C57BL/6 mice fed subcutaneously with cells of tumor and then chemotherapy was provided. Chemotherapy annihilated huge (0.6 cm measurement) EL-4 lymphomas in mice that had been augmented by iron led bovine lactoferrin in a duration of 6 weeks preceding chemotherapy, but surprisingly not in mice that were supplemented with iron free bovine lactoferrin form. The results of the current investigation uncover unexpectedly that the capacity of bovine lactoferrin to augment chemotherapy is subject to its degree of iron binding.

Chea et al. demonstrated the anti-cancer effects of bovine lactoferrin on oral squamous cancer cells [18]. In this study, 4 cell lines including HSC2, HSC3, HSC4 and normal RT7 cells were given treatment with bLF in different concentrations of 1, 10 and 100 μg/ml. All the concentrations of bLF found to be effective in controlling proliferation of cells and in induction of apoptosis. Result from flow cytometry and western blotting showed the molecular mechanism of bLF to manage oral squamous cancer. bLF exerts its effect by activation of p53 which found to be correlated with apoptosis induction and cell cycle arrest in G1/S phase of oral squamous cancer cells. The western blot analysis also revealed that bLF downregulate the phosphorylation of Akt and cytokine signaling suppressor providing a link that bLF can attenuate various signaling cascades including JAK/STAT pathway and mTOR signaling. Hence, bovine

lactoferrin was found to be beneficial for control of oral squamous cancer cells.

Lactoferricin B is a peptide fragment of Bovine lactoferrin synthesized by acid pepsin hydrolysis of lactoferrin got from the milk of cow. Mader et al., explored in vitro therapy with Lactoferricin B quickly actuated apoptosis in a several distinctive human leukemia and carcinoma cell lines [11]. Treatment of Jurkat T leukemia cells with Lactoferricin B brought about the production of reactive oxygen species followed by caspase-2-incited dissipation of mitochondrial transmembrane potential and consecutive activation of caspase-9 and caspase-3. Besides, Jurkat T leukemia cells that overexpressed Bcl-2 were less sensitive to Lactoferricin B -instigated apoptosis, which was portrayed by swelling of mitochondria and the arrival of cytochrome c from mitochondria into the cytosolic segment. Authors infer that Lactoferricin B repress cancer cells by setting off the mitochondrial pathway of apoptosis partially through the production of reactive oxygen species.

Montezuma, S. et al., evaluated the capability of Lactoferricin B and bovine lactoferrin to forestall tentatively induced neovascularization in C57BL/6J mice utilizing two models [51]. Model 1-the laser instigated choroidal neovascularization and model 2-the cornea micro pocket examine model. Bovine lactoferrin was directed either intraperitoneally or intravitreal whereas Lactoferricin B was managed through a cornea pellet. Authors analyzed in model 1 that Bovine lactoferrin controlled at portion of 100 mg/kg intraperitoneally, consistently for 10 days or 200ug/ml single dose intravitreal, diminished the volume of choroidal neovascularization by 40% and indicated a pattern for decrease in the surface territory by 20%. In model 2: Lactoferricin B pellet at 50-mg/ml amount diminished the territory of corneal neovascularization by 45%. Bovine lactoferrin and Lactoferricin B seems to have a strong antiangiogenic impact in two animal models of neovascularization.

Lactoferrin as a carrier for drug delivery in cancer: In cancer therapeutics, different biomolecules and compounds have been evaluated for the targeted killing of cancer cells, without posing any harmful effects to normal cells of body. Most of the compounds showed great anti-cancer potential but they lack specificity for cancer cells resulting in cytotoxic behavior in normal tissues [52]. Moreover, untargeted delivery of chemotherapeutic agents is one of main contributing factor for multi-drug resistance in cancer. Therefore, specific targeting of cancer cells in cancer therapy is highly desirable. Many ligands and particles have been evaluated for specific targeting of cancer cells including antibodies, organic molecules, Nanoparticles and lactoferrin [53]. Lactoferrin has been shown to conjugate with nanoparticles loaded with anti-cancerous drugs for specific targeting of cancer cells and interestingly, Lactoferrin itself can act as a carrier for targeted delivery of anti-cancerous drugs.

Doxorubicin (Dox), an anti-cancerous drug used in chemotherapy, has been evaluated in conjugation with Bovine lactoferrin to enhance its working and internalization in prostate cancer cell line and then in mice models. Results showed that conjugation of Dox with bovine lactoferrin resulted in enhanced Dox-mediated cytotoxicity and better retention in cancer cells [54]. Furthermore, bLF-Dox complex significantly eliminate the multi-drug resistance in drug resistant cancer cells. Lastly, the potential of bLF-Dox complex was assessed in in vivo model of cancer. Oral administration of bLF-Dox complex in mice revealed better survival of animals and tumor growth was significantly reduced. Surprisingly, bLF-Dox complex was much safer than solo treatment with Doxorubicin as the complex removes the general toxicity associated with Doxorubicin and also increased the levels of TNF-alpha, CCL4 and IFN-gamma in serum.

Liposomes loaded with lactoferrin have been also evaluated as anti-cancer agents and for drug delivery purpose. Particularly, polyethylene glycol (PEG) modified liposomes loaded with bovine lactoferrin have been employed to deliver Dox in cell lines and animal models of liver cancer [55]. The complex (Dox loaded bLF-PEG-Liposomes) showed better cellular uptake of Dox and inhibited the growth of tumor cells in mice xenografts of liver cancer. Moreover, the Dox loaded bLF-PEG-Liposome complex were injected intravenously in models of breast cancer which shows the accumulation of this nanocomposite complex in tumor site leading to better suppression of tumor as compared to sole Dox loaded PEG-Liposomes [56]. The results clearly showed the influence of bovine lactoferrin both in drug delivery and tumor suppression. Likewise, micelles containing bLF are used to deliver rapamycin for targeted delivery and specific killing of breast cancer cells [57]. The excellent bio compatibility and serum stability of bLF-micelles enable them to deliver rapamycin to target tumor site and induce cytotoxicity.

Glioblastoma is very dangerous melanoma in central nervous system and remain untreated due to ineligibility of chemotherapeutic agents to reach glioma cells because most of the agents don't cross blood brain barrier and if they cross they are unable to reach to targeted site and can cause serious complications. Lactoferrin has the ability to cross blood brain barrier and possess good safety profile. Lactoferrin derived nanoparticles loaded with different anti-cancer agents are shown to be safe,

having increased permeation to blood brain barrier and efficient in delivery of chemotherapeutic agents to glioma cells [58]. Investigation of all the above evidences cleared that bovine lactoferrin is attractive molecule that can be used as cargo for specific delivery of chemotherapeutic agents and can overcome the problem of multi-drug resistance in cancer.

Conclusion

Currently, none of the cancer therapeutic approaches are optimal to ensure the life of patient. Natural biomolecules gain the interest in scientific community to evaluate them for their anti-cancer potential because of their high stability, specificity, biocompatibility and less side effects. In this scenario, the molecule or compound with both cancer prevention property and to boost existing treatments is considered to be important. From literature review and data presented here, Lactoferrin from bovine milk has been emerged as a suitable protein for cancer treatment because of its biological activities and provide more stable treatment. Bovine lactoferrin has known to exert its anti-cancerous potential in different types of cancers through its different extracellular and intracellular effects. Moreover, bLF has the ability to cross blood brain barrier which make it useable for tumors in brain. Lastly, bLF has been emerged as a potential carrier for targeted delivery of chemotherapeutic agents for specific killing of cancer cells.

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