Journal of
Cancer Science & Therapy

Kanwar et al., J Cancer Sci Ther 2012, 4.3
DOI: 10.4172/1948-5956.1000107

Review Article Open Access

Nano-Lactoferrin in Diagnostic, Imaging and Targeted Delivery for Cancer and Infectious Diseases

Jagat R. Kanwar[1]*, Rasika M. Samarasinghe[1], Rakesh Sehgal[2] and Rupinder K. Kanwar[1]

[1]Nanomedicine-Laboratory of Immunology and Molecular Biomedical Research (LIMBR), Centre for Biotechnology and Interdisciplinary Biosciences (BioDeakin), Institute for Technology & Research Innovation (ITRI), Deakin University, Geelong, Technology Precinct (GTP), Pigdons Road, Waurn Ponds, Geelong, Victoria 3217, Australia
[2]Professor, Department of Parasitology, Postgraduate Institute of Medical Education & Research, Chandigarh, India-160012

Abstract

Lactoferrin (Lf) is a natural occurring iron binding protein present in many mammalian excretions and involved in various physiological processes. Lf is used in the transport of iron along with other molecules and ions from the digestive system. However its the modulatory functions exhibited by Lf in connection to immune response, disease regression and diagnosis that has made this protein an attractive therapeutic against chronic diseases. Further, the exciting potentials of employing nanotechnology in advancing drug delivery systems, active disease targeting and prognosis have also shown some encouraging outcomes. This review focuses on the role of Lf in diagnosing infection, cancer, neurological and inflammatory diseases and the recent nanotechnology based strategies.

Keywords: Lactoferrin; Nanodelivery; Cancer; Inflammation; Infection; Oral administration

Introduction

Diagnostics play an important role in uncovering the symptoms and signs shown by patients. It can implicate the onset of a disease and also predict the different stages of that disease. In order to establish accurate prognosis, patients need to undergo a wide array of tests ranging from blood and urinary analysis to X-ray and magnetic resonance imaging (MRI). These tests often depend on immune inflammatory activated factors such as leukocyte counts, red blood cell sedimentation rate, cytokines and protein accumulation. In addition, imaging systems can only detect changes in the structure and morphology of organs but fail to stipulate a definite diagnosis.

One of the major setbacks in developing effective treatments against chronic diseases is the multi-drug resistant (MDR) phenomenon. Examples of such occurrences include the resistance of Enterococci to Vancomycin (VRE) [1] and Methicillin-Resistant *Staphylococcus aureus* [2] or the enhanced efflux of drugs due to increased transport pumps like P-glycoproteins (P-gp) [3]. Therefore, for a treatment to be optimum it needs to satisfy key requirements. These include the ability to diagnose diseases at an early stage, image the accurate site of infections, be specific enough to discriminate between the diseased and healthy cells and more importantly be effective and safe even with long term usage. This review discusses the recent therapeutic advances of the natural bioactive protein, lactoferrin (Lf) with regards to infection and cancer, its role as a diagnostic and the current nano-based findings on the use of Lf as a potential treatment agent.

Lactoferrin

Since its first isolation from bovine milk more than 70 years ago [4] and its purification and sequencing from both bovine and human milk almost 40 years later [5], Lf, also known as lactotransferrin, has caused a significant uproar in the field of cancer research. Primarily, it is an iron binding glycoprotein with a structure and size that closely resembles (60% sequence homology) to another iron-transporting family, the transferrins (Tf) [6]. The regulation of Lf is not only restricted to milk, in fact its production occurs in a wide range of secretory fluids including saliva, tears, the reproductive system and in renal organs [7]. Lower concentrations are found in plasma, bile fluids, mucosal secretions, pancreatic fluids and in neutrophils cells [8]. Structurally, Lf weighs approximately 80kDa and the polypeptide folds into two globular lobes. Each globe contains two major domains. These domains serve as the binding and glycosylation sites for iron molecules and carbonate ions. Apart from iron, Lf binds to an array of metal ions (to a much lower affinity). These include gallium, aluminium, zinc, manganese, cobalt, copper and lanthanides. Further, depending on the amount of binding, Lf can be classified as apo-Lf (iron depleted), monoferric Lf (one ferric ion) and holo-Lf (two ferric ions) [9].

Roles played by lactoferrin

In addition to its role in iron transporting, various other functions, ranging from anti-viral to anti-carcinogenic, has intrigued the curiosity of many researchers. Recent progress in our understanding of the mechanisms of Lf has led us to believe that this protein activates intracellular signalling, specifically through the modulation of cytokines, chemokines, growth factors and interleukins. Consequently, inflammatory diseases and tumour progression depends primarily on the dysfunction of these factors and the activation of immune cells such as T lymphocytes, monocytes and natural killer (NK) cells. Therefore, studying the possible influences Lf may exert on regulating these mediators is beneficial.

Metabolism and absorption of iron

Iron is a crucial element required for most biological and cellular

***Corresponding author:** Dr. Jagat R. Kanwar, MSc, PhD, Associate Professor of Immunology & Cell Biology, Head, Nanomedicine-Laboratory of Immunology and Molecular Biomedical Research (LIMBR), Centre for Biotechnology and Interdisciplinary Biosciences (BioDeakin), Institute for Technology & Research Innovation (ITRI), Geelong Technology Precinct (GTP), Deakin University, Pigdons Road, Waurn Ponds, Geelong, Victoria 3217, Australia; Tel: 0061-3 -5227 1148; Fax: 0061-3-5227 3402; E-mail: jagat.kanwar@deakin.edu.au

Received January 03, 2012; **Accepted** March 05, 2012; **Published** March 07, 2012

Citation: Kanwar JR, Samarasinghe RM, Sehgal R, Kanwar RK (2012) Nano-Lactoferrin in Diagnostic, Imaging and Targeted Delivery for Cancer and Infectious Diseases. J Cancer Sci Ther 4: 031-042. doi:10.4172/1948-5956.1000107

processes. These functions include oxygen transport by red blood cells, mitochondrial transport, nucleic acid synthesis and electron transfer during cell cycle processes. On the other hand however, excess of iron can be detrimental to cells and the dysregulation due to immune deficiency or from external agents like microbial infections can lead to severe chronic diseases like cancer, sepsis and anaemia [10]. Therefore the regulation of iron and its metabolism is essential in conserving cellular functions, treating diseases and in the elimination of infections. A controversial study by Ward and co-workers [11] demonstrated the inability of Lf to regulate iron metabolism in mice. It was reported that Lf deficient mice showed normal iron homeostasis in the serum as compared to wild type mice. However, since the presence of Lf is only increased during inflammation and sepsis, the iron metabolism activity of Lf may only come into play during these incidences.

In a study with six months old infants, radiolabelled breast milk showed an increased absorption of iron (49%) as compared to the formulated milk (10%). It was suggested that the increased level was due to the high concentration of Lf found in human milk [12]. Recently, with healthy women, absorption of iron from recombinant human Lf (rhLf) produced in rice or from ferrous sulphate showed very similar results between the two treatment groups. It was reported that no significant difference in absorption was found between heat treated and untreated rhLf and also with ferrous sulphate. The equal absorption rates proved that iron metabolism using Lf is well utilized in adults and the adverse effects such as gastrointestinal discomfort, diarrhoea and oxidative damage usually seen with ferrous iron can be avoided with the use of Lf [13]. Similarly, a clinical study on pregnant women diagnosed with iron deficiency or iron deficiency anaemia was treated with 30% iron saturated Lf. It was shown that Lf exhibited significant effects on restoring iron and haemoglobin levels in serum. In addition with Lf treated women, total iron, ferritin, haemoglobin and red blood cell levels were significantly high, while ferrous sulphate and untreated women did not shown any change. Further, Lf and ferrous sulphate were able to decrease IL-6 activation but this level increased again only with the ferrous sulphate, 30 days post-treatment [14]. Taken together these results show the benefits of Lf as a modulator of iron metabolism and absorption and that the presence of iron could possibly influence its multifunctional activities.

Role of lactoferrin receptors

Different cells such as gastrointestinal, monocytes/macrophages, lymphocytes, platelets, breast epithelial cells, brain cells, osteoblasts, chondrocytes and hepatocytes express receptors specific to Lf [8,15]. The indication that Lf interacts with cells through receptors was first suggested in 1979 by Snick and Masson [16]. An *in vitro* study with mouse peritoneal macrophages reported the distinguished internalization of human Lf (hLf) in cells as compared to the uptake of Tf. They reported the enhanced iron absorptive activity of Lf, in which the Fe present in hLf was efficiently transferred to ferritin molecules in the human duodenal mucosa. A possible mechanism for Lf's improved binding affinity was suggested to be due to the nature of Lf to be primarily cationic with a high binding affinity to anionic ligands such as receptors, glycosaminoglycans and heparin [6, 17-19]. Therefore this property enables Lf to bind to a wide array of "Lf putative receptors" expressed in different cells and organs and aid with the internalisation and absorption of Lf. The low density lipoprotein (LDL) receptor related protein-1 (LRP1) and LRP2 (megalin) are two such receptors reported to have direct interactions with Lf [20,21].

Lactoferrin and LRP

Lf binds to both LRP1 and LRP2 receptors with high affinity [22]. Initial studies looking at the different ligands interacting with these receptors showed Lf to inhibit the clearance of chylomicron remnants known to be efficiently cleared by LRP present in the liver or kidney, thus confirming the binding of Lf [21]. In the liver, rapid removal of circulatory Lf is mediated by the activity of LRP uptake of Lf in hepatocytes via the modulation of the G-protein dependent pathways that regulate endocytosis mechanisms [23,24]. Further, blocking with receptor-associated protein (RAP), known to inhibit ligands binding to LRP, hindered the internalization of Lf by 70% [25]. With osteoblasts and chondrocytes, LRP1 was expressed in all bone and cartilage cells tested however LRP2 was only expressed in some of the osteoblast cell lines. Further cell survival, proliferation and mitogenesis observed with Lf treatment was mediated by LRP1 by activating the mitogen activated protein kinase (MAPK) pathways and blocking this receptor with RAP hindered the endocytosis and signalling transduction activity in cells [26,27].

Intestinal lactoferrin receptors (LfR)

Another receptor suggested to be specific to Lf, involved with Lf internalization and is highly expressed in the small intestine was first suggested by Cox et al. [28] in 1979. They reported that incubation with hLf, hTf or ovo-Tf on sections of adult small intestines mediated the transfer of iron from Lf but not hTf or ovo-Tf. Extensive studies examining the tissue localization of LfR has shown that this receptor is highly expressed in the intestine, brain cells and the reproductive system [15]. Lf internalization studies on a cancerous intestinal epithelial cell line, Caco-2, revealed different modes of endocytosis of Lf and Tf proteins. It was shown that Lf entered through the apical side and was able to localize to the nucleus of cells whereas Tf internalized via the basolateral side and remained in the cytoplasm of cells [29]. Similarly, more recent studies with Caoc-2 and mouse crypt cells demonstrated the distinguished activities of these proteins. LfR expressed in the plasma membrane of both type of cells assisted the endocytosis of apo- and holo-Lf via the clathrin process. Interestingly, functional studies with apo-Lf and holo-Lf showed that apo-Lf induced significant growth of these cancerous cells activating the extracellular signal regulated kinase (ERK) and the phosphoinositide 3-kinase (PI3K) signalling pathways which was down-regulated by blocking LfR. Similar activity however was not observed with holo-Lf in which proliferation of Caco-2 and crypt cells were inhibited with holo-Lf treatment and activation of ERK and P13K cascades were not observed [30,31]. Hence, these unique characteristics of LfRs to induce specific and differential mechanisms may possibly explain the multiple biological functions exerted by Lf.

Regulation of infections

Lf is a member of the innate immune system and therefore along with its iron storage and transportation properties, it also possesses antibacterial, antiviral and antiparasitic functions, to name a few. This extensive functionality enables Lf to recognise and eradicate a wide array of microbial and parasitic strains making it an important part of the host defence system.

Anti-microbial regulation

The antimicrobial activity of Lf is well documented. With microbial agents like *Escherichia coli, Listeria monocytogenes*, *Bacillus subtilis*, *Haemophilus influenzae, Klebsiella pneumoniae* and *Salmonella typhimurium,* Lf interferes with their metabolism by sequestering

the iron required for its growth and proliferation [32,33]. Another mechanism, described originally by Arnold et al. [34], reported an iron-independent bacteriostatic effect of Lf in which direct binding to the surface of infectious agents lead to the destabilization of the cell membrane causing its destruction [34]. This bacteriostatic effect of Lf is however often masked by some microbes such as *Bifidobacterium sp*., *Lactobacillus sp* and some forms of viruses. They have the capacity to release small sized iron chelators known as siderophores that compete and remove iron from Lf thus utilizing it for its survival. Other forms have also evolved to express certain specific receptors that bind to Lf causing the dissociation of iron by altering the tertiary structure of the protein [35]. *In vivo* studies on mice have demonstrated Lf induced rapid eradication of *E. coli* in kidneys, lungs, liver and spleen, with the lungs and kidneys showing the highest level of bacterial killing [36]. Moreover, Lf exerted protective effects on mice infected with *S. aureus, Shigella flexneri* and *Salmonella spp* [37,38]. A recent study by Mosquito and colleagues [39] on the effect of bovine Lf (bLf) on *S. Typhimurium* reported Lf to be significantly active in reducing the mortality, signs of inflammation and organ necrosis in treated mice groups as compared to the untreated. Subsequently, orally administered bLf and rhLf was an effective treatment against *Helicobacter spp.* known to be the cause of gastritis, ulcers and stomach cancers [40-42].

Bovine Lf (1mg/ml) used in formulas fed to premature infants or normal infants were shown to induce the growth of the beneficial bacteria *Bifidobacterium* and inhibited proliferation of *Clostridium* and *E. coli* [43]. BLf given to patients infected with *H. pylori* in combination with a triple therapy regime completely eradicated the infection from 78% to 100% [44]. However contradictory to these studies a clinical trial with rhLf administered orally over a period of 24 hours to *H. pylori* infected subjects showed no decrease in infection [45]. Furthermore a longer treatment regime (5 to 14 days) gave similar outcomes with no significant benefits against microbial infections. The combination of these results suggests Lf alone may not be an effective therapeutic against microbial agents but it may be a significant adjunctive agent to be used with other anti-microbial agents.

Anti-viral regulation

Lf is widely reported to possess anti-viral capabilities. Lf inhibits the growth of fatal viruses such as human immunodeficiency virus (HIV), rotaviruses, Japanese encephalitis virus (JEV), influenza viruses, poliovirus, hepatitis B and C (HBV and HCV) viruses [46,47]. Possible mechanisms by which Lf may act is through direct interaction via membrane bound protein, iron sequestering or competitive binding to host cells, thus preventing viruses or parasites from entering. In viruses such as HIV, the transmission to host cells is mediated by binding its envelope protein gp120 with dendritic cells which then transmits the virus to $CD4^+$ T cells. BLf binds to the adhesion molecule, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and prevents HIV from attaching and transmitting to T cells [48]. A recent study further confirmed this interaction and linked the inhibitory activity of Lf to a Lf-33 peptide expressed in the external domain of the protein [49]. Inhibition of HCV was found to be effective in cultured hepatocyte cells in which Lf imposed a direct interaction with the HCV envelope proteins E1 and E2. Unlike with HBV, pre-treatment of the virus with Lf inhibited infection but on the other hand was ineffective when hepatocytes were pre-treated with Lf [50]. In a clinical trial performed on patients suffering from chronic hepatitis, bLf treatment (1.8g daily for 12 weeks) showed no difference in virus inhibition as compared to the control group, but given at a higher concentration (3.6g) followed by a ribavirin and interferon combined therapy a significant reduction of HCV was observed [51]. Interaction of Lf with heparin sulphate and chondroitin sulphates expressed on the cell surface has been demonstrated to inhibit herpes simplex viruses [52]. Adenovirus inhibition was reported to be induced by the binding of bLf to the viral polypeptides III and IIIa used by viruses to internalize into host cells [53] and apoptosis on host cells induced by influenza A virus was inhibited by bLf via direct interference of caspase 3 activity [54].

Anti-parasitic regulation

As an anti-parasitic agent Lf has limited the activity of various species like *Plasmodium falciparum*, *Toxoplasma gondii*, *Entamoeba histolytica* and *Eimeria stiedai*. Depletion of iron from *Pneumocystis carinii* and *E. histolytica* is one of the reported inhibitory mechanisms exerted by Lf [55-57]. In addition, apo-Lf has been found to interact with the membrane lipids and cholesterol proteins expressed in parasites to induce cell death. This is interesting as parasites are known to utilize cholesterol/lipids for growth [58]. A recent study further demonstrated the capability of Lf peptides, lactoferricin 17–30 and lactoferrampin 265–284 to completely eradicate *E. histolytica* within 24 hours of treatment [59]. This is further intensified by the ability of Lf to induce phagocytosis and destruction of *T. cruzi* by macrophages [60] and to inhibit growth of *P. falciparum* in erythrocyte [61]. In the case of another intestinal parasite, *Giardia lamblia,* it was observed that Lf and N-terminal peptides could effectively kill the parasite [62]. Recently, in Peru, a trial for supplementing children diet with bLf to decrease the prevalence of diarrhoea showed no significant reduction in diarrhoea as compared to control groups but a lower prevalence of giardiasis with bLf treatment was observed [63].

Another mechanism involves the competitive binding of Lf to the membrane molecules heparan sulphates or to LRP receptors expressed on host cells. These ligands are usually utilised by *Plasmodium spp.* parasites for cell invasion [64]. Both iron free and iron saturated hLf was found to be effective in inhibiting *P. falciparum* and it was observed that a three day pre-incubation of host red blood cells (RBC) prior to infection enhances this inhibitory effect [61]. The probable mechanism was due to the release of free oxygen radicals leading to the death of the parasites. Further studies have shown that Lf inhibits the binding of *P. falciparum* erythrocytes to immobilized chondroitin sulphate receptors and CD36 receptors, which are important in the pathogenicity of infections [65]. With infections caused by toxoplamosis, intraperitoneal and oral administration of Lf was effective in reducing mortality in experimental mice [66]. In the case of *Pneumocystis carinii*, both bovine and human Lf could inhibit the cyst and trophozoite stages *in vitro* [55] and could also inhibit infections by microsporidia, another intestinal or disseminated parasite common in HIV patients [67].

Diagnosis of infections

The strategy of diagnosing the early onset of diseases and manifesting quick, non-invasive and precises prognostics on fatal diseases like sepsis, can lead to a significant reduction in mortality and morbidity. During normal physiological conditions a relatively low concentration of Lf is present in plasma varying from 0.4-2mg/ml. However, during inflammation or microbial infections the level of Lf is increased to around 200mg/ml [68], which is then significantly reduced with the eradication of infection. Lf present in plasma is primarily derived from neutrophils. It was found that the levels in healthy patients directly correlate with the number of blood neutrophils present. In a study

with leukemic and infection prone children it was found that levels of Lf in these children were significantly lower than healthy children or adults in spite of a higher blood count or unmodified intracellular instances [69]. This was similar to results obtained with HIV-1 infected patients that had decreased levels of 2.79+1.2 as compared to healthy subjects having plasma Lf levels of 4.37 + 0.83 [70]. Hence this protein could potentially be used as a biological marker to analyse activity and turnover rate of neutrophils sub-sequentially diagnosing the oncome of infections and in evaluating subjects that are highly prone to infections. Further the shortage of this molecule during complex diseases like HIV and cancer could possibly be the reason for disease progression thus further confirming the importance of Lf.

Regulation of cancer

Immense developments made over the years on understanding the pathophysiology and signalling mechanisms involved in cancer has led to the development of potential alternative treatments. Standard therapies like radiotherapy, chemotherapy and/or surgery not only destroy cancerous cells but have a high tendency to damage healthy cells and organs leading to detrimental side effects. Hence to overcome these downfalls, the use of natural bioactives as alternative therapeutics or as an adjuvant for conventional treatments is rising as a possible new avenue in the fight against cancer. Since its first *in vivo* demonstration with solid and metastatic tumours [71] the use of Lf as a potential therapeutic for cancer has been highly considered. Remarkably, the protective activities of Lf against tumorigenesis and metastatic cancers are reported in a wide range of specific organs including colon, lung, pancreas, bladder, esophagus and liver, however its use as a chemotherapeutic remains to be elucidated. Lf enhances the activation of apoptosis in colon, lung and leukemia cancers through the up-regulation of caspase 3, caspase 8, Fas receptors, the pro-apoptotic Bcl-2 members Bax and Bid [72,73]. It inhibit angiogenesis via vascular endothelial growth factor (VEGF) blocking [74] and hinders the growth of carcinoma breast, cervical, head and neck cancers at the G1 to S transition phase of the cell cycle [75,76]. Furthermore, it increases the activation of natural killer (NK) cells and lymphokine-activated killer (LAK) cells, up regulates neutrophil activity and enhances macrophage cytotoxicity by increasing the production of cytokines and reactive oxygen species (ROS) [71]. However, the actual mechanism on how Lf promotes signalling in cells remains unclear with theories of nuclear factor-kappa B (NF-κB) and MAPK signalling pathways reported to be major players [77].

Immunotherapy

The application of immunotherapy as a therapeutic strategy relies on the concepts of eliminating cancer and its metastatic counterparts primarily through the immune system. It involves grooming the immune system to not only specifically target and destroy the affected tumour cells but to also sensitize it against metastatic tumour cells and against future recurrence of cancer. Other than some of the initial immune-modulatory functions of Lf reported almost 30 years ago, it also induces the proliferation and differentiation of a range of immune cells including lymphocytes, activate polymorphonuclear leukocytes (PMN) and improve antibody dependent cytotoxic processes in cancers [71,78].

In tumour bearing mice cancer models, bLf administered orally revealed its protective effects by enhancing the activation of local intestinal mucosa and systemic immune responses. Lf was able to stimulate the activation of $CD4^+$ and $CD8^+$ T-lymphocytes and NK ((asialoGM1+) cells which in turn led to marked inhibition of tumour growth and experimental metastasis [46,79,80]. Further Lf increased production of caspase-1 protein, interferon IFN-γ and mature interleukin (IL)-18 cytokines along with other cytokines including type I IFNs, IL-6, IL-7, IL-12 and tumour necrosis factor (TNF)-α [33,78,81-83].

Chemotherapy

As a chemopreventive agent, bLf and its splice variants have shown some interesting results. With colon carcinoma 26, a highly metastatic cancer implanted in mice and examined for cell colonization in lungs revealed bLf fed for 21 days significantly inhibited colonization [79]. Similarly, rhLf orally administered to mice implanted with head and neck squamous tumours suppressed growth by 75% along with the activation of cytotoxic lymphocytes. It was also reported that with $CD3^+$ deficient mice, the inhibitory property of rhLf was abrogated, further supporting the immunomodulatory function of Lf [84].

As an adjuvant agent, rhLf (200 mg/kg) administered orally to immunocompetent BALB/c mice in combination with a conventional chemotherapeutic drug cis-platimun (5mg/kg) showed significant inhibition of head and neck squamous tumour growth. It was shown that monotherapy caused an inhibition of 6% and 66% with rhLf and cis-platinum respectively, however in combination the inhibition rate increased to a significant 79%. It was further shown that rhLf exerted its activity through the production of IL-18 and via activation of NK and $CD8^+$ T cells [80]. Another significant and interesting study examined the activity of iron saturated bLf in augmenting chemotherapy to eradicate tumours that are otherwise resistant. Kanwar et al. [33] demonstrated 100% iron saturated bLf (Lf^+) given to mice infected with EL-4 lymphoma completely hindered tumour growth, whereas this was only 13-33% with native bLf (~15% iron saturated). Lf^+ reduced the blood flow to the tumour by 71% and induced an increase in tumour apoptosis by 4.2, 5.4 and 4.9 folds when treated with paclitaxel, doxorubicin or a combination of both drugs respectively. In addition, their study demonstrated that the time Lf^+ is administered plays a critical role in its inhibitory functions. When given less than two weeks before chemotherapy or any time after therapy Lf^+ had little or no effect on tumour regression, however when given more than two weeks before chemotherapy, complete eradication of tumour was reported with no recurrence of cancer observed even after mice were returned to normal diets. This study further supported the immunomodulatory property of Lf in which an enhanced production of Th1 and Th2 cytokines, IL-4, IL-5, IL-6, IL-10, IL-18, TNF-α and IFN-γ was shown along with restored quantities of lymphocytes. It was proposed that Lf^+ could be used in between routine chemotherapy regimens as it aids in destroying resistance tumours and restoring natural defence systems [33].

Diagnosis of cancer

Lf is famously known as a multifunctional protein primarily acting against infections and cancer. However the concept of utilising this protein as a tool in diagnosis and detection of cancer remains controversial. One of the very first studies suggesting the activity of Lf as a marker for cancers was reported by Rossiello and co-workers [85]. Their study analysed the presence of three proteins, ferritin, Tf and Lf in patients diagnosed with breast carcinomas and found Lf was highly expressed in normal breast and benign tissue but was absent in cancerous tissue [85]. Since then Lf has been studied as a biological marker to detect several forms of cancers including leukemia, colon,

breast and cervical carcinomas. In M2 and M4 forms of leukemia, Lf was expressed in low concentrations in 12 out of 35 patients suffering from acute myeloid leukaemia. Decreased production of Lf was present in serum but was absent in the control healthy group. It was suggested that the presence of Lf may manifest the dedifferentiated form of the normal cell to its cancerous state, as its production is only seen in the malignant cells [86]. Similarly, it was shown that the detection of Lf concentrations in faecal matter was an effective prognostic for confirming colorectal cancer, Crohn's diseases, colon polyps or ulcerative colitis diseases [87]. In a phase II clinical study involving 872 patients planned to undergo colorectal endoscopy, the expression of faecal Lf and haemoglobin was analysed. It was shown that patients with colorectal cancer and Crohn's disease showed a significant increase with more than 60% positive for Lf and haemoglobin expression [88]. Furthermore, examination of human endometrium revealed an overexpression of Lf protein and messenger RNA in malignant endometrial adenocarcinomas as compared to the normal epithelial cells [89]. Interestingly, in contrast to the above studies, it was shown that with certain types of tumours like breast and endocervix carcinomas, expression of Lf and its isoforms are significantly down regulated as compared to its normal tissue counterparts. It was further suggested that this difference in expression among tissues may be linked to the activation of steroidal hormones involved in the differentiation and growth of cells [90,91]. Although, the strategy of using Lf as a definite marker for diagnosing cancer is still undefined, the favourable studies reported thus far is opening pathways for it potential use as a tool for disease prognosis.

Nanoparticles as therapeutics

Since its first proposal in the 1970s as a system to effectively delivery drugs to specific locations in the body [92,93], treatment of various chronic diseases employing nanotechnology has evolved tremendously. Various nanomaterials having the capability of providing platforms with different chemical properties and biocompatible activities has allowed efficient delivery of bioactive agents to target organs. These materials present several benefits over conventional approaches, such as the ability to efficiently encapsulate several therapeutic modalities like proteins, drugs, nucleic acids and peptides. Furthermore, efficient conjugation of functionalized agents like ligands, aptamers, antibodies or peptides that can target, localize and improve binding affinity of nanoparticles (NPs) to specific areas of the body is now feasible. Therefore, these properties can further ensure the protection of bioactive molecules from degradation via proteases and DNAses and enable its prolonged sustainability and long term use without causing any severe systemic side effects [94].

Lactoferrin nanoparticles

A major problem with currently used cancer treatments like chemotherapy is the inability of drugs to differentiate between malignant and healthy cells leading to severe systemic toxicity. The distinguishing characteristics of NPs such as the small size (10-200nm), large surface-to-volume ratio allowing increased drug encapsulation and ease of functionalizing surface properties to accommodate multiple ligands that can target tumour specific markers is opening new pathways in the search for alternatives to chemotherapy. Since its first historical breakthrough in the 1980s with approved clinical trials based on polymeric protein conjugates and liposomes, research on nanocarrier cancer targeting systems have risen incredibly (Table 1). Thus utilising Lf as NPs can be a remarkable therapeutic agent, combining Lf's multifunctional properties with the added benefits brought by nanotechnology.

One of the very first studies reported with Lf NPs involved its conjugation to DNA encapsulated liposomes targeted to cervical cancer Hela cells. It was shown that Lf-liposomes enhanced uptake and transfection of genes even at a low concentration of 3µg/ml as compared to Tf conjugated liposomes [95]. Another study examined the effects of enteric coating on bLf in order to enhance its absorption from the intestine of adult rats. The enteric formulation increased protection of Lf from gastric enzymes and was transported to the lymphatic fluid within 3hours post intragastric administration [96]. The possibility of using Lf as a NP to target and treat chronic diseases was also proposed by Gupta and Curtis [97]. They demonstrated that surface functionalizing superparamagnetic iron oxide NPs with Lf and ceruloplasmin interacts with high binding affinity to cell surface receptors leading to cell specific particle internalization. Recently, we prepared a nanoformulation of iron saturated bovine lactoferrin (Fe-bLf) for cancer therapy. These nanoformulations were given orally in nanodiets prepared using combination of polymers and ceramics. Alginate enclosed chitosan coated Fe-bLf or paclitaxel (taxol) NP adsorbed onto nanocores of calcium phosphate nanocarriers (AEC-CP-Fe-bLf NCs or AEC-CP-taxol NCs) were made by combination of ionic gelation and nanoprecipitation techniques [98]. These AEC-CP-Fe-bLf NCs obtained were spherical in morphology and showed enhanced anti-cancer efficacy *in vitro*. Further, these nanocarriers were efficiently taken up by the colon cancer (Caco-2) and didn't affect the mucosal integrity during transcytosis. AEC-CP-Fe-bLf NCs were supplemented in AIN 93G diet with 1.2% (w/w) of Fe-bLf, by replacing casein and fed to mice, in both prevention and treatment xenograft colon cancer models. When nanoformulated Fe-bLf diet was given orally, as a pre-treatment, one week before Caco-2 cell injections, it was found to be highly effective and none of the mice fed with nanodiet developed tumours or showed any signs of toxicity. However the mice fed with the control AIN-93G diet, showed normal tumour growth. Fe-bLf loaded nanodiets were also found to help with absorption of iron and calcium [98].

Brain cancers

The brain is an important organ that needs to be protected from unstable conditions caused by hormones, neurotransmitters or from foreign organisms such as bacterial or viral infections. The protection and seclusion of the brain from these factors is made possible by the presence of cells that separates the circulating blood and extracellular fluid from the cerebrospinal fluid in the central nervous system. This layer, known as the blood brain barrier (BBB) consists of endothelial cells that form tightly sealed junctions allowing no direct transendothelial passageways of infections originated from bacteria. It also prevents the diffusion of macroparticles such as large drug molecules, therapeutic nucleic acids and imaging probes [99-101]. As there are so few types of drugs that can cross the BBB, delivering drugs to the brain involves a number of highly invasive measures. One such technique involves the administration of a sugar solution (mannitol) into blood vessels in the neck which induces an osmotic reaction causing the endothelial cells to shrink [102]. This technique along with several other methods such as the use of vasoactive molecules, bradkynin and cereport may force the brain to be susceptible to external infections by microbes and even from harmful substances in the circulation [103].

Since passive transport of drugs is therapeutically ineffective in treating brain cancers, active transport through the use of receptors

Targeting moiety	Platform	Tumour model	Drug encapsulated	Study outcome	Ref.
Folate receptor	Dendrimer	Oral	Methotrexate (MTX)	Enhance localization in tumour and liver tissue. Increased MTX antitumour effects and reduced drug toxicity	[133]
	Liposomes	Oral	Doxorubicin (DOX)	Internalization was 10 folds higher, significant reduction tumour growth and 30-50% increase in life span	[134,135]
		Oral	Oligo-nucleotides	Showed no difference between targeted and nontargeted NP	[136]
	Magnetite	Breast	No drug	Increased cellular internalization	[137]
PSMA antigen	PLGA-PEG	Prostate	Docetaxel	Effective uptake (77 fold increase) by cells, inhibited tumour growth and drug toxicity	[138,139]
Tranferrin receptor	Gold-PEG	Solid Tumour	No drug	Nps localized to the tumour tissues with decreased amounts in the liver	[140]
	Liposome	Leukemia	DOX and Verapamil (Pgp inhibitor)	Cytotoxicity was 5.2 time greater that non-targeted NPs	[141]
	Polymeric	Prostate/ Leukemia	Cyclodextrin	Efficient delivery of drugs to cells	[142]
	PLGA	Prostate	Paclitaxel	Single dose induce complete inhibition of tumor growth and increased mouse survival rate (80%)	[143]
Epidermal receptors	HER-2-Liposome	Breast	No drug	No significant difference observed between targeted and nontargeted Nps.	[144]
		Brain	Boron	Cellular increase by 8 fold was observed	[145]
	EGF-gelatin	Lung	No drug	Enhanced uptake in cell in a time and dose dependent manner	[146]
αvβ3 integrin	Liposome	Pancreas/ Renal	Doxorubicin	NP targeted tumour vessel, induced selective apoptosis in tumour, caused a 15 fold increase in anti-metastasis activity and reduced systemic side effects	[147]
	PLGA	Liver	Paclitaxel/ Doxorubicin	Hindered tumour growth and prolonged survival of mice	[148]
		Melanoma/Colon	ATP$^{\mu}$-Raf mutant gene	Sustained tumour regression through cell apoptosis activation	[149]
	Micelles	Connective tissue	Doxorubicin	30 fold increase of cellular uptake was observed	[150]
Vascular cell adhesion molecule (VCAM-1)	Liposomes	Lung	No drug	73.1 % localization seen within 30 min with only 30.3% seen with nontargeted NPs	[151]
Luteinizing hormone releasing hormone	Iron oxide	Breast	No drug	NPs were detected specifically in the cytosol of primary and metastatic tumours, whereas unconjugated NPs gathered in the liver	[152]
Sigma receptor	Liposomes	Lung	Survivin siRNA	Targeted NP enhanced delivery, downregulated survivn expression, induced cell cytotoxicity, apoptosis and chemosensitized cells to cisplatin. *In vivo* targeted NPs localized to specific tumour sites	[137]
	Liposomes	Prostate	Doxorubucin	Increased accumulation, inhibited tumour growth and reduced systemic toxicity usually caused by the free drug	[153]

Table 1: Targeted nanoparticles for cancer therapy.

seems to be the most sought out way. Several receptors reported to play a part in the transport of drugs included Tf, epidermal growth factor receptors (EGFR), insulin, low density lipoprotein related receptor 1 (LRP1), LRP 2 and Diphtheria toxin receptors. Furthermore several proteins successful in interacting with these receptors include Lf, Tf, melanotransferrin and OX-26 antibody [104]. A study performed by Zhang and co-workers [105] showed the conjugation of pegylated immunoliposomes with insulin or Tf antibodies was able to effectively transport EGFR antisense gene across the BBB and suppress its function by 95% [105]. In addition it was also reported that poly (ethylene glycol) and polyethylenimine nanogels cross linked with Tf or insulin proteins improved the transport and stability of oligonucleotides by almost 15 folds, with lower toxicity and adverse effects as compared to the free nucleotides [106].

Brain Targeting with Lactoferrin

Brain cells including oligodendrocytes, neurons and astrocytes express Lf and its up-regulation in parts of the brain is implicated in the development of brain disorders including aging, cerebral lesions, Alzheimer, Parkinson and Down syndrome diseases [107-109]. Although local synthesis of Lf is evident, the large increase observed during neurological disorders may imply that Lf might also enter the brain through the circulatory system [110]. Hence the uptake and transport of Lf from blood through the BBB is mediated specifically via dynamic receptor mediated mechanisms and is implied that the presence of LRP, expressed in brain endothelial cells is responsible for Lf binding and transcytosis [25,111].

The active process of Lf transportation can therefore be utilized beneficially to generate nano-based drug delivering and brain targeting systems that can carry these nanocarriers via similar intracellular mechanisms without causing any damage to the BBB. Studies done by Huang et al. [112] revealed for the first time the use of Lf as a ligand for actively targeting brain capillary endothelial cells and successfully delivery nucleotides to the brain. Their study utilized polyamidoamine (PAMAM) pegylated dendrimer complexes conjugated to Lf. It was shown that the uptake of the Lf/DNA dendrimer NPs were primarily mediated through clathrin-dependent and caveolae-mediated endocytosis and that the Lf coated NPs were able to localize to the central areas of the brain as compared to its uncoated counterparts

[100,112]. The Lf conjugated complexes passed through the BBB in a time, temperature and concentration dependent manner within the first two hours, successfully penetrating to the central areas of the brain and distributing within the neuronal cells. Further, a higher accumulation was reported with the conjugated NP as compared to the uncoated complexes with lower concentrations seen in the liver and other organs [112,113].

Glioma treatment

Glioma is an aggressive brain tumour with a low mortality of 5% in diagnosed patients. The main method of eradicating the cancerous growth is through surgery, however the complete removal is not always absolute hence the chance of relapse is high [114]. Recently, two very similar studies looking at the use of drug loaded, Lf conjugated liposomes as a treatment option for glioma was reported. One study incorporated the use of a cationic polymersome encapsulated with two drugs doxorubicin (DOX) and tetrandrine (TEX) which were further conjugated to Lf. DOX is a well-known chemotherapeutic drug and TEX blocks calcium associated transporter channels. It was demonstrated that Lf conjugation improved the uptake and cytotoxicity in glioma cells with an increased rate of 2.3 times more than the unconjugated TEX and DOX loaded polymersomes. The conjugated NP effectively destroyed glioma cells and significantly impeded the metastatic feature of these active cells prolonging the survival time to upto 82% [115]. This activity was confirmed in the study performed by Chen et al. [116] that demonstrated the enhanced efficiency of Lf conjugated procationic liposomes in targeting and treating glioma. Their study showed Lf conjugation increased NP uptake in endothelial and glioma cells and induced anti-glioma activity in tumour treated mice. It was suggested that these NPs internalized through a receptor-mediated pathway and caused a significant extension in animal survival rate [116].

Melanoma treatment

One of the main obstructions faced with the treatment of melanoma is its active resistance to chemotherapeutic drugs and radiotherapy. Possible mechanisms for this resistance is due to the irregulation of apoptosis and DNA, mutations of Ras genes and increased activity of transporters such as the ATP binding cassette transporters [117]. Recently, a study on murine melanoma cells reported the enhanced activity of Lf encapsulated liposomal NPs as a possible chemotherapeutic drug. It was shown that the positively charged iron free-Lf liposomes were able to protect degradation of the protein and the nanoformulation significantly enhanced the internalization into cells and cell cytotoxicity and inhibited cell proliferation via cell cycle arrest [118].

Edema treatment

The potential use of Lf NPs to enhance its anti-inflammatory effects was recently shown in a study performed at the Hoshi University by Onishi and co-workers [119,120]. It was considered that a chitosan, alginate and calcium NP loaded with Lf would have a high enough concentration of Lf to produce an immune response in the body and help in the treatment of carrageenan – induced edema. They found that the technique used to make the conjugated NPs yielded a concentration of 20 – 30% of Lf with an average of 22%. This was three times higher than what was produced with other techniques suggesting that the NPs contained high concentrations of Lf but remained at an average particle size of 1.65μm. They found that when administered to rats suffering from carrageenan – induced edema these NPs significantly exhibited better suppression and showed a faster recovery from edema as compared to the control groups, without Lf or with Lf alone [119,120].

Imaging with lactoferrin

The concept of using radiolabeled hLf as a tool for providing reliable diagnostic results during infections through scintigraphy imaging was primarily proposed by Welling and his colleagues [121]. Radiolabelling hLf peptides with Technetium-99m revealed a significantly high binding rate in animals (rabbit and mice) infected with MDR *S. aureus, Klebsiella pneumoniae* or *C. albicans* as compared to the low accumulations seen at sterile inflammatory sites. It was suggested that hLf labelled peptides as well as ubiquicidin labelled peptides have the potential of being favourable candidates to image site of infections and discriminate between microbial induced inflammatory process and non-microbial inflamed sites [121-123]. However, due to the known limitations of radiolabelled drugs such as toxicity and production cost, much work is still required with these peptides before it can be effectively used in patients.

Recently, a study demonstrated the encapsulation and delivery of fluorescein labelled Lf (FITC-Lf) conjugated to chitosan coated alginate/calcium microparticles as an effective intestinal delivery system. It was reported that the particles maintained a constant size of 1-2μm and showed a gradual release of protein over a period of 7 hours. Hence it was suggested that since these particles were able to withstand and protect the protein from gastric conditions it can be further used as a therapeutic or imaging tool to target various diseased sites [124].

Magnetic resonance imaging (MRI)

If a cell or a group of cells need to be detected through MRI they are usually labelled with magnetic particles either by surface attachment or through internalization in cells. Current diagnostic agents such as gadolinium cause severe side effects. These include renal dysfunction and increased risk of developing nephrogenic systemic fibrosis which causes fibrosis of skin, joints, eyes and internal organs. It also has poor target-specific biodistribution and rapid exertion [125]. In the 1980s, use of super paramagnetic iron oxide NPs (SPIONs) were suggested as an alternative to the currently used gadolinium based contrast imaging agents [126].

SPIONs exhibit several unique properties. Through *in vivo* testing these particles were shown to be highly sensitive, have enhanced biocompatibility and lack toxicity [127,128]. However, a key disadvantage of this imaging system is the inability to distinguish between malignant and non-malignant tumours. Therefore combining these particles to target specific ligands can possibly overcome these dilemmas. The use of Lf conjugated to iron oxide particles as a ligand for targeting specific cell receptors was first suggested by Gupta and Curtis [97] in which Lf conjugated iron oxide NPs were efficiently delivered and imaged in human fibroblast cells. Recently, Xie and colleagues [129] from the Huazhong University of Science and Technology studied the imaging and targeting activity of Lf conjugate iron oxide NPs on glioma cancers. They found that these particles were able to produce a clearer image as compared to the gadolinium based contrast agents which suggests an increased sensitivity and accumulation of NPs in glioma cells. It was shown that the Lf conjugated SPIONs had no effect on the metabolic activity in glioma infected rats and had a higher cellular uptake than that of unconjugated SPIONs. The conjugated NPs were effective in differentiating between normal and tumourous brain cells and had no observed chronic cytotoxicity. However the MR signal

degraded within 100 minutes with the Lf-SPIONs as compared to the iron oxide particles alone which gave a high intensity even after 140 minutes. Therefore, these NPs were sensitive to tumour cells and its use could be advantageous in the diagnosis of brain disorders as well as other carcinogenic tumours.

Conclusion and Future Directions

There is still without a doubt an unmet necessity to discover a better treatment and diagnostic system that can fight cancerous and infection diseases. In addition to the currently used chemotherapeutic drugs, several promising pharmaceutical agents have entered the market, but sadly, like with other conventional drugs they cause adverse side effects and exert detrimental effects on normal organs. Hence, with the recent advances in technology, NPs may provide opportunities as an effective agent that can be used for diagnostic applications, monitoring disease progression and eradicating cancerous occurrences.

Due to the unique physiochemical characteristics shared by NPs, these systems can be modified and tuned to encompass cell targeting, drug trafficking and imaging systems within a single existence. Hence the ideal nanocarrier will specifically target and deliver drug to specific sites, minimising systemic toxicity, deliver adequate doses of drugs and monitor disease progress efficiently while avoiding invasive imaging techniques. Indeed, there are improved therapeutic nanocarriers currently established in over 100 clinical trials and various animal models that involve the use of a wide array of targeting ligands and antibody conjugates. However, in an ideal situation not only is targeting, delivering and diagnosis of the cancer important, but it also needs to be biocompatible and safe in humans, as this is a common problem faced with nonviral therapeutics. Further, NPs need to be effective as well as exhibit minimum cytotoxicity when used in the long run, as this is a major difficulty faced with most metal based NPs [130].

Toxicity that maybe caused by NPs is a crucial factor frequently scrutinized in cancer research. The physiochemical characteristics like morphology, size, crystalline structure, surface charge and concentration of NPs administered during treatments are generally some of the main factors leading to particle cytotoxicity [131]. Manipulating size allows NPs to reach sites through the leaky vasculature in tumours and gain access to areas that are usually unreachable by microparticles. Further modifying surface charge can enhance biodistribution and specificity of particles. A method that is suggested in several studies is modifying these NPs by the attachment of PEG complexes to reduce its toxicity. This process reduces toxicity of materials, improve circulation time and enhance linking of bioactive compounds however, it can also impede with cellular uptake due to the size increase of particles [130].

Functionalizing NPs so that they can have longer circulatory half-lives and entail several targeting ligands such as aptamers, peptides, antibodies and imaging probes in one system may also increases the efficacy of nanocarriers. These systems will not only improve the targeting specificity of diseases, it can show the location of the tumours with the use of imaging probes and destroy tumour cells by releasing the encapsulated drugs. However, a major setback with this system is linking NPs efficiently with all these parameters. The modification with these entities may decrease its targeting capability due to a change in it physical properties during conjugation and significantly affect its binding affinity to the specific biomarkers [132]. Therefore research on understanding and optimizing these conditions thus enabling NPs to carry these components can result in an intelligent system that can not only diagnose diseased cells from normal cells but can be personalized

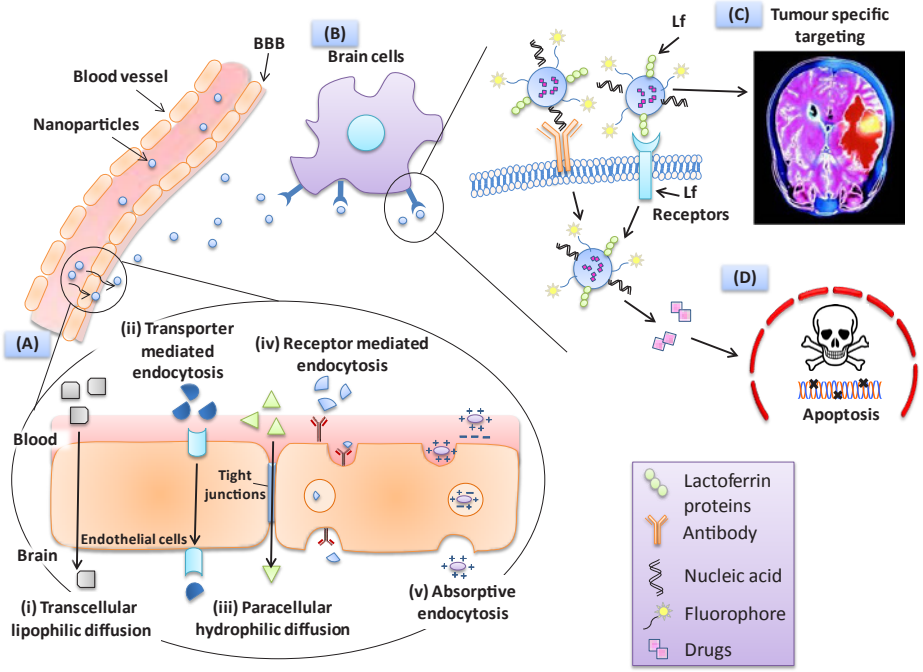

Figure 1: Drug encapsulated transport of multi-ligand targeted nanoparticles. The transport of molecules through the bood brain barrier (BBB) is mediated through different mechanism (A). These include (i) transcellular diffusion which depends on the lipophilicity and size of molecules, (ii) transporter protein mediated endocytosis, primarily used in the delivery of nutrients, (iii) paracellular diffusion of hydrophilic and small moleculaes through tight juctions of epithelial cells, (iv) receptor mediated endocytosis, enabling the transport of various molecules such a genes, proteins and targeted nanoparticles and (v) absorptive transport depending on the surface charge of molecules. (B) Nanoparticels trasported through the BBB, targets specific neuronal cells due to the presence of cell specific targeting ligands. Multi-functional nanoparticles can be used to detect tumours via MRI [154] (C) and simultaneously induce cell death by releasing chemotherapeutic drugs (D).

according to the diseases and its severity. Figure 1 shows a schematic representation of multi-ligand conjugated NPs targeted at cancerous neuronal cells.

Although the idea of combating all complex and diverse obstacles in cancer therapy with a single treatment regime may still be a few years away, many prominent nanotechnologist believe that functional nano-based systems is the most definite approach in facing these issues. Developing and designing NP formulations that address multifunctional strategies, have higher specificity, is effective and safe can therefore be effectively used in diagnostic and personalized therapies.

Acknowledgement

Authors would like to thank the Australia-India Strategic Research Fund (AISRF, BF 030016) for financial support. Authors would like to thank DBT, Govt. India for funding (BT-PR-12672 ICD55102009).

References

1. Uttley AH, Collins CH, Naidoo J, George RC (1988) Vancomycin-resistant enterococci. Lancet 1: 57-58.
2. Benner EJ, Morthland V (1967) Methicillin-resistant Staphylococcus aureus. Antimicrobial susceptibility. N Engl J Med 277: 678-680.
3. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, et al. (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci USA 84: 7735-7738.
4. Soerensen M, Soerensen SP (1939) The proteins in whey. CR Trav Lab Carlsberg 23: 55-99.
5. Metz-Boutigue MH, Jollès J, Mazurier J, Schoentgen F, Legrand D, et al. (1984) Human lactotransferrin: amino acid sequence and structural comparisons with other transferrins. Eur J Biochem 145: 659-676.
6. Baker HM, Baker EN (2004) Lactoferrin and iron: structural and dynamic aspects of binding and release. Biometals 17: 209-216.
7. Teng CT, Beard C, Gladwell W (2002) Differential expression and estrogen response of lactoferrin gene in the female reproductive tract of mouse, rat, and hamster. Biol Reprod 67: 1439-1449.
8. Legrand D, Pierce A, Elass E, Carpentier M, Mariller C, et al. (2008) Lactoferrin structure and functions. Adv Exp Med Biol 606: 163-194.
9. Baker EN, Baker HM (2009) A structural framework for understanding the ultifunctional character of lactoferrin. Biochimie 91: 3-10.
10. Weiss G (2002) Pathogenesis and treatment of anaemia of chronic disease. Blood Rev 16: 87-96.
11. Ward PP, Paz E, Conneely OM (2005) Multifunctional roles of lactoferrin: a critical overview. Cell Mol Life Sci 62: 2540-2548.
12. Saarinen UM, Siimes MA, Dallman PR (1977) Iron absorption in infants: high bioavailability of breast milk iron as indicated by the extrinsic tag method of iron absorption and by the concentration of serum ferritin. J Pediatr 91: 36-39.
13. Lönnerdal B, Bryant A (2006) Absorption of iron from recombinant human lactoferrin in young US women. The Am J Clin Nutr 83: 305-309.
14. Paesano R, Torcia F, Berlutti F, Pacifici E, Ebano V, et al. (2006) Oral administration of lactoferrin increases hemoglobin and total serum iron in pregnant women. Biochem Cell Biol 84: 377-380.
15. Suzuki YA, Lopez V, Lönnerdal B (2005) Mammalian lactoferrin receptors: structure and function. Cell Mol Life Sci 62: 2560-2575.
16. Van Snick JL, Masson PL (1976) The binding of human lactoferrin to mouse peritoneal cells. J Exp Med 144: 1568-1580.
17. Legrand D, van Berkel PH, Salmon V, van Veen HA, Slomianny MC, et al. (1997) The N-terminal Arg2, Arg3 and Arg4 of human lactoferrin interact with sulphated molecules but not with the receptor present on Jurkat human lymphoblastic T-cells. Biochem J 327: 841-846.
18. Mann DM, Romm E, Migliorini M (1994) Delineation of the glycosaminoglycan-binding site in the human inflammatory response protein lactoferrin. J Biol Chem 269: 23661-23667.
19. van Berkel PH, Geerts ME, van Veen HA, Mericskay M, de Boer HA, et al. (1997) N-terminal stretch Arg2, Arg3, Arg4 and Arg5 of human lactoferrin is essential for binding to heparin, bacterial lipopolysaccharide, human lysozyme and DNA. Biochem J 328: 145-151.
20. Strickland DK, Gonias SL, Argraves WS (2002) Diverse roles for the LDL receptor family. Trends Endocrinol Metab 13: 66-74.
21. Willnow TE, Goldstein JL, Orth K, Brown MS, Herz J (1992) Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance. J Biol Chem 267: 26172-26180.
22. Bennatt DJ, Ling YY, McAbee DD (1997) Isolated rat hepatocytes bind lactoferrins by the RHL-1 subunit of the asialoglycoprotein receptor in a galactose-independent manner. Biochemistry 36: 8367-8376.
23. Bennett RM, Kokocinski T (1979) Lactoferrin turnover in man. Clin Sci (Lond) 57: 453-460.
24. Goretzki L, Mueller BM (1998) Low-density-lipoprotein-receptor-related protein (LRP) interacts with a GTP-binding protein. Biochemical J 336: 381-386.
25. Fillebeen C, Descamps L, Dehouck MP, Fenart L, Benaïssa M, et al. (1999) Receptor-mediated transcytosis of lactoferrin through the blood-brain barrier. J Biol Chem 274: 7011-7017.
26. Grey A, Banovic T, Zhu Q, Watson M, Callon K, et al. (2004) The low-density lipoprotein receptor-related protein 1 is a mitogenic receptor for lactoferrin in osteoblastic cells. Mol Endocrinol 18: 2268-2278.
27. Brandl N, Zemann A, Kaupe I, Marlovits S, Huettinger P, et al. (2010) Signal transduction and metabolism in chondrocytes is modulated by lactoferrin. Osteoarthritis Cartilage 18: 117-125.
28. Cox TM, Mazurier J, Spik G, Montreuil J, Peters TJ (1979) Iron binding proteins and influx of iron across the duodenal brush border. Evidence for specific lactotransferrin receptors in the human intestine. Biochim Biophys Acta 588: 120-128.
29. Ashida K, Sasaki H, Suzuki YA, Lönnerdal B (2004) Cellular internalization of lactoferrin in intestinal epithelial cells. Biometals 17: 311-315.
30. Jiang R, Lönnerdal B (2012) Apo- and holo-lactoferrin stimulate proliferation of mouse crypt cells but through different cellular signaling pathways. Int J Biochem Cell Biol 44: 91-100.
31. Jiang R, Lopez V, Kelleher SL, Lönnerdal B (2011) Apo- and holo-lactoferrin are both internalized by lactoferrin receptor via clathrin-mediated endocytosis but differentially affect ERK-signaling and cell proliferation in Caco-2 cells. J Cell Physiol 226: 3022-3031.
32. Gupta I, Sehgal R, Kanwar RK, Sehgal A, Kanwar JR (2009) Recent advances of metal binding protein lactoferrin as an anti-microbial agent. Curr Bioactive Comp 5: 226-233.
33. Kanwar JR, Palmano KP, Sun X, Kanwar RK, Gupta R, et al. (2008) 'Iron-saturated' lactoferrin is a potent natural adjuvant for augmenting cancer chemotherapy. Immun Cell Bio 86: 277-288.
34. Arnold RR, Cole MF, McGhee JR (1977) A bactericidal effect for human lactoferrin. Science 197: 263-265.
35. Brock JH (2002) The physiology of lactoferrin. Biochem Cell Biol 80: 1-6.
36. Zagulski T, Lipiński P, Zagulska A, Jarzabek Z (1998) Antibacterial system generated by lactoferrin in mice *in vivo* is primarily a killing system. Int J Exp Pathol 79: 117-123.
37. Diarra MS, Petitclerc D, Lacasse P (2002) Effect of lactoferrin in combination with penicillin on the morphology and the physiology of Staphylococcus aureus isolated from bovine mastitis. J Dairy Sci 85: 1141-1149.
38. Gomez HF, Ochoa TJ, Herrera-Insua I, Carlin LG, Cleary TG (2002) Lactoferrin protects rabbits from Shigella flexneri-induced inflammatory enteritis. Infect Immun 70: 7050-7053.
39. Mosquito S, Ochoa TJ, Cok J, Cleary TG (2010) Effect of bovine lactoferrin in Salmonella ser. Typhimurium infection in mice. Biometals 23: 515-521.
40. Wada T, Aiba Y, Shimizu K, Takagi A, Miwa T, et al. (1999) The therapeutic effect of bovine lactoferrin in the host infected with Helicobacter pylori. Scand J Gastroenterol 34: 238-243.

41. Dial EJ, Lichtenberger LM (2002) Effect of lactoferrin on Helicobacter felis induced gastritis. Biochem Cell Biol 80: 113-117.

42. Dial EJ, Romero JJ, Headon DR, Lichtenberger LM (2000) Recombinant human lactoferrin is effective in the treatment of Helicobacter felis-infected mice. J Pharm Pharmacol 52: 1541-1546.

43. Tomita M, Wakabayashi H, Shin K, Yamauchi K, Yaeshima T, et al. (2009) Twenty-five years of research on bovine lactoferrin applications. Biochimie 91: 52-57.

44. Di Mario F, Aragona G, Dal Bò N, Cavestro GM, Cavallaro L, et al. (2003) Use of bovine lactoferrin for Helicobacter pylori eradication. Dig Liver Dis 35: 706-710.

45. Guttner Y, Windsor HM, Viiala CH, Marshall BJ (2003) Human recombinant lactoferrin is ineffective in the treatment of human Helicobacter pylori infection. Aliment Pharmacol Ther 17: 125-129.

46. Kanwar JR, Kanwar RK, Sun X, Punj V, Matta H, et al. (2009) Molecular and biotechnological advances in milk proteins in relation to human health. Curr Protein Pept Sci 10: 308-338.

47. Kanwar RK, Singh N, Gurudevan S, Kanwar JR (2011) Targeting hepatitis B virus and human papillomavirus induced carcinogenesis: novel patented therapeutics. Recent Pat Antiinfect Drug Discov 6: 158-174.

48. Groot F, Geijtenbeek TB, Sanders RW, Baldwin CE, Sanchez-Hernandez M, et al. (2005) Lactoferrin prevents dendritic cell-mediated human immunodeficiency virus type 1 transmission by blocking the DC-SIGN--gp120 interaction. J Virol 79: 3009-3015.

49. Carthagena L, Becquart P, Hocini H, Kazatchkine MD, Bouhlal H, et al. (2011) Modulation of HIV Binding to Epithelial Cells and HIV Transfer from Immature Dendritic Cells to CD4 T Lymphocytes by Human Lactoferrin and its Major Exposed LF-33 Peptide. Open Virol J 5: 27-34.

50. Ikeda M, Sugiyama K, Tanaka T, Tanaka K, Sekihara H, et al. (1998) Lactoferrin markedly inhibits hepatitis C virus infection in cultured human hepatocytes. Biochem Biophys Res Commun 245: 549-553.

51. Kaito M, Iwasa M, Fujita N, Kobayashi Y, Kojima Y, et al. (2007) Effect of lactoferrin in patients with chronic hepatitis C: combination therapy with interferon and ribavirin. J Gastroenterol Hepatol 22: 1894-1897.

52. Marchetti M, Trybala E, Superti F, Johansson M, Bergström T (2004) Inhibition of herpes simplex virus infection by lactoferrin is dependent on interference with the virus binding to glycosaminoglycans. Virology 318: 405-413.

53. Pietrantoni A, Di Biase AM, Tinari A, Marchetti M, Valenti P, et al. (2003) Bovine lactoferrin inhibits adenovirus infection by interacting with viral structural polypeptides. Antimicrob Agents Chemother 47: 2688-26891.

54. Pietrantoni A, Dofrelli E, Tinari A, Ammendolia MG, Puzelli S, et al. (2010) Bovine lactoferrin inhibits influenza A virus induced programmed cell death *in vitro*. Biometals 23: 465-475.

55. Cirioni O, Giacometti A, Barchiesi F, Scalise G (2000) Inhibition of growth of Pneumocystis carinii by lactoferrins alone and in combination with pyrimethamine, clarithromycin and minocycline. J Antimicrob Chemother 46: 577-582.

56. León-Sicairos N, López-Soto F, Reyes-López M, Godínez-Vargas D, Ordaz-Pichardo C, et al. (2006) Amoebicidal activity of milk, apo-lactoferrin, sIgA and lysozyme. Clin Med Res 4: 106-113.

57. León-Sicairos N, Reyes-López M, Canizalez-Román A, Bermúdez-Cruz RM, Serrano-Luna J, et al. (2005) Human hololactoferrin: endocytosis and use as an iron source by the parasite Entamoeba histolytica. Microbiology 151: 3859-3871.

58. Bansal D, Bhatti HS, Sehgal R (2005) Role of cholesterol in parasitic infections. Lipids Health Dis 4: 10.

59. López-Soto F, León-Sicairos N, Nazmi K, Bolscher JG, de la Garza M (2010) Microbicidal effect of the lactoferrin peptides lactoferricin17-30, lactoferrampin265-284, and lactoferrin chimera on the parasite Entamoeba histolytica. Biometals 23: 563-568.

60. Lima MF, Kierszenbaum F (1987) Lactoferrin effects on the interaction of blood forms of Trypanosoma cruzi with mononuclear phagocytes. Int J Parasitol 17: 1205-1208.

61. Fritsch G, Sawatzki G, Treumer J, Jung A, Spira DT (1987) Plasmodium falciparum: inhibition *in vitro* with lactoferrin, desferriferrithiocin, and desferricrocin. Exp Parasitol 63: 1-9.

62. Turchany JM, Aley SB, Gillin FD (1995) Giardicidal activity of lactoferrin and N-terminal peptides. Infect Immun 63: 4550-4552.

63. Ochoa TJ, Chea-Woo E, Campos M, Pecho I, Prada A, et al. (2008) Impact of lactoferrin supplementation on growth and prevalence of Giardia colonization in children. Clin Infect Dis 46: 1881-1883.

64. Sinnis P, Willnow TE, Briones MR, Herz J, Nussenzweig V (1996) Remnant lipoproteins inhibit malaria sporozoite invasion of hepatocytes. J Exp Med 184: 945-954.

65. Eda S, Eda K, Prudhomme JG, Sherman IW (1999) Inhibitory activity of human lactoferrin and its peptide on chondroitin sulfate A-, CD36-, and thrombospondin-mediated cytoadherence of plasmodium falciparum-infected erythrocytes. Blood 94: 326-332.

66. Isamida T, Tanaka T, Omata Y, Yamauchi K, Shimazaki K, et al. (1998) Protective effect of lactoferricin against Toxoplasma gondii infection in mice. J Vet Med Sci 60: 241-244.

67. Leitch GJ, Ceballos C (2009) A role for antimicrobial peptides in intestinal microsporidiosis. Parasitology 136: 175-181.

68. Nakao K, Imoto I, Gabazza EC, Yamauchi K, Yamazaki N, et al. (1997) Gastric juice levels of lactoferrin and Helicobacter pylori infection. Scand J Gastroenterol 32: 530-534.

69. Venge P, Foucard T, Henriksen J, Håkansson L, Kreuger A (1984) Serum-levels of lactoferrin, lysozyme and myeloperoxidase in normal, infection-prone and leukemic children. Clin Chim Acta 136: 121-130.

70. Defer MC, Dugas B, Picard O, Damais C (1995) Impairment of circulating lactoferrin in HIV-1 infection. Cell Mol Biol (Noisy-le-grand) 41: 417-421.

71. Bezault J, Bhimani R, Wiprovnick J, Furmanski P (1994) Human lactoferrin inhibits growth of solid tumors and development of experimental metastases in mice. Cancer Res 54: 2310-2312.

72. Mader JS, Salsman J, Conrad DM, Hoskin DW (2005) Bovine lactoferricin selectively induces apoptosis in human leukemia and carcinoma cell lines. Mol Cancer Ther 4: 612-624.

73. Fujita K, Matsuda E, Sekine K, Iigo M, Tsuda H (2004) Lactoferrin enhances Fas expression and apoptosis in the colon mucosa of azoxymethane-treated rats. Carcinogenesis 25: 1961-1966.

74. Norrby K, Mattsby-Baltzer I, Innocenti M, Tuneberg S (2001) Orally administered bovine lactoferrin systemically inhibits VEGF(165)-mediated angiogenesis in the rat. Int J Cancer 91: 236-240.

75. Damiens E, El Yazidi I, Mazurier J, Duthille I, Spik G, et al. (1999) Lactoferrin inhibits G1 cyclin-dependent kinases during growth arrest of human breast carcinoma cells. J Cell Biochem 74: 486-498.

76. Xiao Y, Monitto CL, Minhas KM, Sidransky D (2004) Lactoferrin down-regulates G1 cyclin-dependent kinases during growth arrest of head and neck cancer cells. Clin Cancer Res 10: 8683-8686.

77. Oh SM, Pyo CW, Kim Y, Choi SY (2004) Neutrophil lactoferrin upregulates the human p53 gene through induction of NF-kappaB activation cascade. Oncogene 23: 8282-8291.

78. Kanwar RK, Kanwar R (2011) Immunomodulatory lactoferrin in the regulation of apoptosis modulatory proteins in cancer. Protein Pept Lett.

79. Iigo M, Kuhara T, Ushida Y, Sekine K, Moore MA, et al. (1999) Inhibitory effects of bovine lactoferrin on colon carcinoma 26 lung metastasis in mice. Clin Exp Metastasis 17: 35-40.

80. Varadhachary A, Wolf JS, Petrak K, O'Malley BW Jr, Spadaro M, et al. (2004) Oral lactoferrin inhibits growth of established tumors and potentiates conventional chemotherapy. Int J Cancer 111: 398-403.

81. Iigo M, Alexander DB, Long N, Xu J, Fukamachi K, et al. (2009) An Anticarcinogenesis pathways activated by bovine lactoferrin in the murine small intestine. Biochimie 91: 86-101.

82. Kanwar JR, Haggarty NW, Palmano KP, Krissansen GW (2005) Methods of immune or haematological enhancement, inhibiting tumour formation or growth, and treating or preventing cancer.

83. Kuhara T, Iigo M, Itoh T, Ushida Y, Sekine K, et al. (2000) Orally administered

lactoferrin exerts an antimetastatic effect and enhances production of IL-18 in the intestinal epithelium. Nutr Cancer 38: 192-199.

84. Wolf JS, Li G, Varadhachary A, Petrak K, Schneyer M, et al. (2007) Oral lactoferrin results in T cell-dependent tumor inhibition of head and neck squamous cell carcinoma *in vivo*. Clin Cancer Res 13: 1601-1610.

85. Rossiello R, Carriero MV, Giordano GG (1984) Distribution of ferritin, transferrin and lactoferrin in breast carcinoma tissue. J Clin Pathol 37: 51-55.

86. Panella TJ, Liu YH, Huang AT, Teng CT (1991) Polymorphism and altered methylation of the lactoferrin gene in normal leukocytes, leukemic cells, and breast cancer. Cancer Res 51: 3037-3043.

87. Uchida K, Matsuse R, Tomita S, Sugi K, Saitoh O, et al. (1994) Immunochemical detection of human lactoferrin in feces as a new marker for inflammatory gastrointestinal disorders and colon cancer. Clin Biochem 27: 259-264.

88. Hirata I, Hoshimoto M, Saito O, Kayazawa M, Nishikawa T, et al. (2007) Usefulness of fecal lactoferrin and hemoglobin in diagnosis of colorectal diseases. World J Gastroenterol 13: 1569-1574.

89. Walmer DK, Padin CJ, Wrona MA, Healy BE, Bentley RC, et al. (1995) Malignant transformation of the human endometrium is associated with overexpression of lactoferrin messenger RNA and protein. Cancer Res 55: 1168-1175.

90. Benaïssa M, Peyrat JP, Hornez L, Mariller C, Mazurier J, et al. (2005) Expression and prognostic value of lactoferrin mRNA isoforms in human breast cancer. Int J Cancer 114: 299-306.

91. Farley J, Loup D, Nelson M, Mitchell A, Esplund G, et al. (1997) Neoplastic transformation of the endocervix associated with downregulation of lactoferrin expression. Mol Carcinog 20: 240-250.

92. Gregoriadis G (1973) Drug entrapment in liposomes. FEBS letters 36: 292-296.

93. Kramer PA (1974) Letter: Albumin microspheres as vehicles for achieving specificity in drug delivery. J Pharm Sci 63: 1646-1647.

94. Kanwar JR, Mohan RR, Kanwar RK, Roy K, Bawa R (2010) Applications of aptamers in nanodelivery systems in cancer, eye and inflammatory diseases. Nanomedicine (Lond) 5: 1435-1445.

95. Choe TB, Park IC, Hong SI (1998) Enhancement of cationic liposome-mediated transfection by lactoferrin. Biotechnol Tech 12: 577-581.

96. Takeuchi T, Jyonotsuka T, Kamemori N, Kawano G, Shimizu H, et al. (2006) Enteric-formulated lactoferrin was more effectively transported into blood circulation from gastrointestinal tract in adult rats. Exp Physiol 91: 1033-1040.

97. Gupta AK, Curtis AS (2004) Lactoferrin and ceruloplasmin derivatized superparamagnetic iron oxide nanoparticles for targeting cell surface receptors. Biomaterials 25: 3029-3040.

98. Kanwar JR, Mahidhara G, Kanwar RK (2011) A novel alginate enclosed chitosan-calcium phosphate- loaded iron saturated bovine lactoferrin nanocarriers for oral delivery in colon cancer. Nanomedicine In Press.

99. Baratchi S, Kanwar RK, Khoshmanesh K, Vasu P, Ashok C, et al. (2009) Promises of Nanotechnology for Drug Delivery to Brain in Neurodegenerative Diseases. Curr Nanosci 5: 15-25.

100. Huang R, Ke W, Han L, Liu Y, Shao K, et al. (2009) Brain-targeting mechanisms of lactoferrin-modified DNA-loaded nanoparticles. J Cereb Blood Flow Metab 29: 1914-1923.

101. Kanwar JR, Sun X, Punj V, Sriramoju B, Mohan RR, et al. (2011) Nanoparticles in the treatment and diagnosis of neurological disorders: untamed dragon with fire power to heal. Nanomedicine [Epub ahead of print].

102. Neuwelt EA, Maravilla KR, Frenkel EP, Rapaport SI, Hill SA, et al. (1979) Osmotic blood-brain barrier disruption. Computerized tomographic monitoring of chemotherapeutic agent delivery. J Clin Invest 64: 684-688.

103. Garcia-Garcia E, Andrieux K, Gil S, Couvreur P (2005) Colloidal carriers and blood–brain barrier (BBB) translocation: A way to deliver drugs to the brain? Int J Pharm 298: 274-292.

104. de Boer AG, Gaillard PJ (2007) Drug targeting to the brain. Annu Rev Pharmacol Toxicol 47: 323-355.

105. Zhang Y, Zhang YF, Bryant J, Charles A, Boado RJ, et al. (2004) Intravenous RNA interference gene therapy targeting the human epidermal growth factor receptor prolongs survival in intracranial brain cancer. Clin Cancer Res 10: 3667-3677.

106. Vinogradov SV, Batrakova EV, Kabanov AV (2004) Nanogels for oligonucleotide delivery to the brain. Bioconjug Chem 15: 50-60.

107. Kawamata T, Tooyama I, Yamada T, Walker DG, McGeer PL (1993) Lactotransferrin immunocytochemistry in Alzheimer and normal human brain. American J Pathol 142: 1574-1585.

108. Leveugle B, Faucheux BA, Bouras C, Nillesse N, Spik G, et al. (1996) Cellular distribution of the iron-binding protein lactotransferrin in the mesencephalon of Parkinson's disease cases. Acta Neuropathol 91: 566-572.

109. Leveugle B, Spik G, Perl DP, Bouras C, Fillit HM, et al. (1994) The iron-binding protein lactotransferrin is present in pathologic lesions in a variety of neurodegenerative disorders: a comparative immunohistochemical analysis. Brain Res 650: 20-31.

110. Siebert PD, Huang BC (1997) Identification of an alternative form of human lactoferrin mRNA that is expressed differentially in normal tissues and tumor-derived cell lines. Proc Natl Acad Sci USA 94: 2198-2203.

111. Faucheux BA, Nillesse N, Damier P, Spik G, Mouatt-Prigent A, et al. (1995) Expression of lactoferrin receptors is increased in the mesencephalon of patients with Parkinson disease. Proc Natl Acad Sci USA 92: 9603-9607.

112. Huang R, Ke W, Liu Y, Jiang C, Pei Y (2008) The use of lactoferrin as a ligand for targeting the polyamidoamine-based gene delivery system to the brain. Biomaterials 29: 238-246.

113. Huang R, Ke W, Han L, Liu Y, Shao K, et al. (2010) Lactoferrin-modified nanoparticles could mediate efficient gene delivery to the brain *in vivo.* Brain Res Bull 81: 600-604.

114. Silbergeld DL, Chicoine MR (1997) Isolation and characterization of human malignant glioma cells from histologically normal brain. J Neurosurg 86: 525-531.

115. Pang Z, Feng L, Hua R, Chen J, Gao H, et al. (2010) Lactoferrin-conjugated biodegradable polymersome holding doxorubicin and tetrandrine for chemotherapy of glioma rats. Mol Pharm 7: 1995-2005.

116. Chen H, Qin Y, Zhang Q, Jiang W, Tang L, et al. (2011) Lactoferrin modified doxorubicin-loaded procationic liposomes for the treatment of gliomas. Eur J Pharm Sci 44: 164-173.

117. Kanwar JR, Singh N, Kanwar RK (2011) Role of nanomedicine in reversing drug resistance mediated by ATP binding cassette transporters and P-glycoprotein in melanoma. Nanomedicine (Lond) 6: 701-714.

118. Roseanu A, Florian PE, Moisei M, Sima LE, Evans RW, et al. (2010) Liposomalization of lactoferrin enhanced its anti-tumoral effects on melanoma cells. Biometals 23: 485-492.

119. Onishi H, Koyama K, Sakata O, Machida Y (2010) Preparation of chitosan/alginate/calcium complex microparticles loaded with lactoferrin and their efficacy on carrageenan-induced edema in rats. Drug Dev Ind Pharm 36: 879-884.

120. Onishi H, Machida Y, Koyama K (2007) Preparation and *in vitro* characteristics of lactoferrin-loaded chitosan microparticles. Drug Dev Ind Pharm 33: 641-647.

121. Welling MM, Paulusma-Annema A, Balter HS, Pauwels EK, Nibbering PH (2000) Technetium-99m labelled antimicrobial peptides discriminate between bacterial infections and sterile inflammations. Eur J Nucl Med 27: 292-301.

122. Welling MM, Lupetti A, Balter HS, Lanzzeri S, Souto B, et al. (2001) 99mTc-labeled antimicrobial peptides for detection of bacterial and Candida albicans infections. J Nucl Med 42: 788-794.

123. Welling MM, Nibbering PH, Paulusma-Annema A, Hiemstra PS, Pauwels EK, et al. (1999) Imaging of bacterial infections with 99mTc-labeled human neutrophil peptide-1. J Nucl Med 40: 2073-2080.

124. Koyama K, Onishi H, Sakata O, Machida Y (2009) Preparation and *in vitro* evaluation of chitosan-coated alginate/calcium complex microparticles loaded with fluorescein-labeled lactoferrin. Yakugaku Zasshi 129: 1507-1514.

125. Pan D, Caruthers SD, Hu G, Senpan A, Scott MJ, et al. (2008) Ligand-directed nanobialys as theranostic agent for drug delivery and manganese-based magnetic resonance imaging of vascular targets. J Am Chem Soc 130: 9186-9187.

126. Stark DD, Weissleder R, Elizondo G, Hahn PF, Saini S, et al. (1988) Superparamagnetic iron oxide: clinical application as a contrast agent for MR imaging of the liver. Radiology 168: 297-301.

127. Weissleder R, Elizondo G, Wittenberg J, Rabito CA, Bengele HH, et al. (1990) Ultrasmall superparamagnetic iron oxide: characterization of a new class of contrast agents for MR imaging. Radiology 175: 489-493.

128. Weissleder R, Stark DD, Engelstad BL, Bacon BR, Compton CC, et al. (1989) Superparamagnetic iron oxide: pharmacokinetics and toxicity. AJR Am J Roentgenol 152: 167-173.

129. Xie H, Zhu Y, Jiang W, Zhou Q, Yang H, et al. (2011) Lactoferrin-conjugated superparamagnetic iron oxide nanoparticles as a specific MRI contrast agent for detection of brain glioma *in vivo*. Biomaterials 32: 495-502.

130. Sahu SC, Casciano DA (Eds) (2009) Nanotoxicity. Nanotoxicity: from *in vivo* and *in vitro* models to health risks, John Wiley & Sons Ltd: West Sussex, UK.

131. Nel A, Xia T, Madler L, Li N (2006) Toxic potential of materials at the nanolevel. Science 311: 622-627.

132. Huang X, Peng X, Wang Y, Shin DM, El-Sayed MA, et al. (2010) A reexamination of active and passive tumor targeting by using rod-shaped gold nanocrystals and covalently conjugated peptide ligands. ACS Nano 4: 5887-5896.

133. Kukowska-Latallo JF, Candido KA, Cao Z, Nigavekar SS, Majoros IJ, et al. (2005) Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. Cancer Res 65: 5317-5324.

134. Pan XQ, Wang H, Lee RJ (2003) Antitumor activity of folate receptor-targeted liposomal doxorubicin in a KB oral carcinoma murine xenograft model. Pharm Res 20: 417-422.

135. Riviere K, Huang Z, Jerger K, Macaraeg N, Szoka FC Jr (2011) Antitumor effect of folate-targeted liposomal doxorubicin in KB tumor-bearing mice after intravenous administration. J Drug Target 19: 14-24.

136. Leamon CP, Cooper SR, Hardee GE (2003) Folate-liposome-mediated antisense oligodeoxynucleotide targeting to cancer cells: evaluation *in vitro* and *in vivo*. Bioconjug Chem 14: 738-747.

137. Zhang Y, Zhang J (2005) Surface modification of monodisperse magnetite nanoparticles for improved intracellular uptake to breast cancer cells. J Colloid Interface Sci 283: 352-357.

138. Farokhzad OC, Jon S, Khademhosseini A, Tran TN, Lavan DA, et al. (2004) Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. Cancer Res 64: 7668-7672.

139. Farokhzad OC, Cheng J, Teply BA, Sherifi I, Jon S, et al. (2006) Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy *in vivo*. Proc Natl Acad Sci USA 103: 6315-6320.

140. Choi CH, Alabi CA, Webster P, Davis ME (2010) Mechanism of active targeting in solid tumors with transferrin-containing gold nanoparticles. Proc Natl Acad Sci USA 107: 1235-1240.

141. Wu J, Lu Y, Lee A, Pan X, Yang X, et al. (2007) Reversal of multidrug resistance by transferrin-conjugated liposomes co-encapsulating doxorubicin and verapamil. J Pharm Pharm Sci 10: 350-357.

142. Bellocq NC, Pun SH, Jensen GS, Davis ME (2003) Transferrin-containing, cyclodextrin polymer-based particles for tumor-targeted gene delivery. Bioconjug Chem 14: 1122-1132.

143. Sahoo SK, Ma W, Labhasetwar V (2004) Efficacy of transferrin-conjugated paclitaxel-loaded nanoparticles in a murine model of prostate cancer. Int J Cancer 112: 335-340.

144. Kirpotin DB, Drummond DC, Shao Y, Shalaby MR, Hong K, et al. (2006) Antibody targeting of long-circulating lipidic nanoparticles does not increase tumor localization but does increase internalization in animal models. Cancer Res 66: 6732-6740.

145. Pan X, Wu G, Yang W, Barth RF, Tjarks W, et al. (2007) Synthesis of cetuximab-immunoliposomes via a cholesterol-based membrane anchor for targeting of EGFR. Bioconj Chem 18: 101-108.

146. Tseng CL, Wang TW, Dong GC, Yueh-Hsiu Wu S, et al. (2007) Development of gelatin nanoparticles with biotinylated EGF conjugation for lung cancer targeting. Biomaterials 28: 3996-4005.

147. Murphy EA, Majeti BK, Barnes LA, Makale M, Weis SM, et al. (2008) Nanoparticle-mediated drug delivery to tumor vasculature suppresses metastasis. Proc Natl Acad Sci USA 105: 9343-9348.

148. Danhier F, Vroman B, Lecouturier N, Crokart N, Pourcelle V, et al. (2009) Targeting of tumor endothelium by RGD-grafted PLGA-nanoparticles loaded with paclitaxel. J Control Release 140: 166-173.

149. Hood JD, Bednarski M, Frausto R, Guccione S, Reisfeld RA, et al. (2002) Tumor regression by targeted gene delivery to the neovasculature. Science 296: 2404-2407.

150. Nasongkla N, Shuai X, Ai H, Weinberg BD, Pink J, et al. (2004) cRGD-functionalized polymer micelles for targeted doxorubicin delivery. Angew Chem Int Ed Engl 43: 6323-6327.

151. Gosk S, Moos T, Gottstein C, Bendas G (2008) VCAM-1 directed immunoliposomes selectively target tumor vasculature *in vivo*. Biochim Biophys Acta 1778: 854-863.

152. Leuschner C, Kumar CS, Hansel W, Soboyejo W, Zhou J, et al. (2006) LHRH-conjugated magnetic iron oxide nanoparticles for detection of breast cancer metastases. Breast Cancer Res Treat 99: 163-176.

153. Banerjee R, Tyagi P, Li S, Huang L (2004) Anisamide-targeted stealth liposomes: a potent carrier for targeting doxorubicin to human prostate cancer cells. Int J Cancer 112: 693-700.

154. Physorg-Instablogs Network (2007), viewed March 6 2012, http://www.scienceahead.com/entry/now-remote-controlled-nanoparticles-to-treat-tumors-directly-on-location/.