# Lactoferrin in Malignant Human Tumours: New Insights from an Immunohistochemical Meta-Analysis

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**Abstract:** Lactoferrin (LF), an iron-binding glycoprotein, is well known to have different physiological activities in humans; in normal conditions, it has been found in milk, blood, urine as well as in many external and mucosal secretions. Herein we have performed a meta-analysis regarding LF immunohistochemical expression as well as its localization pattern in human malignant tumours obtained from personal observations and literature suggestions. The observed heterogeneity in LF immunoexpression between different malignancies as well as inside the same tumour was also discussed in order to acquire a possible explanation for its presence and function.

We suggest that neoplastic elements should be able to produce LF in order to make a greater amount of iron available for their turnover; alternatively, the localization of LF in malignant cells may not reflect an intracellular synthesis, reflecting instead the degree of trans-membranous iron transfer as the consequence of defective or functionally impaired LF-receptors, already documented elsewhere on the surface of target cells as well as in human neoplastic cell lines.

**Keywords:** Immunohistochemistry, Lactoferrin, Cancer, Humans.

## INTRODUCTION

Lactoferrin (LF) is an 80kDa glycosylated single chain protein, constituted of ca. 700 amino acids, with high homology among species, present in milk and colostrums as well as in many body fluids, such as blood plasma, amniotic fluid, tears, saliva, semen, bile, urine [1-5].

Several functions have been attributed to LF, although the corresponding mechanisms remain still controversial [6]; it appears involved in the regulation of iron homeostasis and absorption in the bowel [7] as well as in the antimicrobial activity against bacteria, viruses, fungi and parasites [8-13]. Moreover, immunomodulatory and anti-inflammatory effects of LF have been reported [4,5,14-19]. Finally, LF appears to show some enzymatic properties such as protease, DNAase, RNAase and ATPase [20-23].

Recently, it has been suggested that LF is involved in the regulation of some important processes, such as the cycle and the death of cells, fighting against the carcinogenesis and the development of metastases [17,19, 24, 25]. In particular, it has been hypothesized that LF inhibits cell proliferation and suppresses tumour growth, blocking the transition from G1 to S in the cell cycle of malignant cells, both in vitro and in vivo [3,6,19,25-27]. In the present study, to better understand the LF anticancer activity, we have performed a meta-analysis based on immunohistochemical data obtained from the literature in comparison to a large cohort of human neoplasms of different organs by us collected in the last 25 years.

## MATERIALS AND METHODS

During the last twenty-five years we have analyzed the immunohistochemical pattern of LF distribution in 948 human surgical and bioptic specimens of different organs, affected by neoplastic diseases as well as non-neoplastic counterparts (Table **1**). In detail, we report herein our experience about the appearance of LF, immunohistochemically detected, in 385 surgical samples obtained from a corresponding number of malignant tumours developed in prostate (30), thyroid (76), stomach (30), colon (39), gallbladder (32), brain (18), skin (22), endometrium (71), kidney (40), bone and cartilage (27) as well as in 25 metastatic bone specimens, occurred in carcinomas with the following primitive site: breast (10 cases), prostate (3 cases), kidney (4 cases), lung (3 cases), colon-rectum (3 cases), uterus (2 cases). The present study was conducted with the understanding and the consent of the human subjects; moreover, the local Ethical Committee has approved the experiments.

All samples have been fixed in 10% neutral formalin for 24 hrs at room temperature (RT) and then embedded in paraffin at 56°C. Moreover, the bone/cartilage specimens have been decalcified using

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**Table 1: Personal Casuistry of Human Non-Neoplastic and Neoplastic Samples Analyzed During the Last 25 Years**

| Site | Nr. Samples | Benign/Normal | Malignant | Nr. Reference |
|---|---|---|---|---|
| Prostate | 70 | 40 | 30 | 28 |
| Thyroid | 33 | 10 | 23 | 29 |
| Thyroid | 14 | 0 | 14 | 30 |
| Thyroid | 56 | 17 | 39 | 31 |
| Small intestine | 18 | 18 | 0 | 32 |
| Stomach | 60 | 30 | 30 | 33 |
| Colon-rectum | 71 | 32 | 39 | 34 |
| Gallbladder | 52 | 20 | 32 | 35 |
| Brain | 26 | 8 | 18 | 36 |
| Liver | 127 | 127 | 0 | 10 |
| Skin | 57 | 35 | 22 | 37 |
| Uterus | 71 | 0 | 71 | 38 |
| Kidney | 50 | 10 | 40 | 25 |
| Bone | 58 | 52 | 6 | 39 |
| Cartilage | 38 | 35 | 3 | 40 |
| Embryo-fetal tissues | 35 | 35 | 0 | 41 |
| Fetal and adult bone and cartilage | 92 | 82 | 10 | 42 |

formic acid 5% or EDTA 5%, pH 7.4, for a period not longer than 48 hrs., depending on the size of mineralised samples. From each block of malignant neoplastic tissue, 4 μm-thick sections were stained with haematoxylin/eosin for the microscopic evaluation, but parallel sections were cut and mounted on silane-coated glasses, then dewaxed in xylene and rehydrated in graded ethanols. Antigen retrieval was performed before adding primary antibody by heating slides placed in 0.01 M citrate buffer, pH 6.0, in a microwave oven for three cycles x 5 min. For the immunohistochemical study, sections were treated in a moist chamber at room temperature: (1) with 0.1% $H_2O_2$ in methanol to block the intrinsic peroxidase activity (30 min); (2) with normal sheep serum to prevent unspecific adherence of serum proteins (30 min); (3) with the monoclonal primary antibody against anti-human LF (clone 1A1; Biodesign International, Saco, ME; w.d. 1:75; 60 min); (4) with sheep anti-mouse immunoglobulin antiserum (Behring Institute; w.d. 1:25; 30 min); (5) with mouse anti-horseradish peroxidase-antiperoxidase complexes (Dako Cyto-mation, w.d. 1:25; 30 min). For the demonstration of peroxidase activity, the sections were incubated in darkness for 10 min with 3-3' diaminobenzidine tetra hydrochloride (Sigma Chemical Co., St Louis, MO), in the amount of 100 mg in 200 ml 0.03% hydrogen peroxide in phosphate-buffered saline (PBS). The nuclear counterstain was performed by Mayer's haemalum.

Renal tubular structures within normal kidney samples as well as portions of parotid gland were utilized as LF positive controls; in addition, the LF immunoreactivity demonstrated in granules of poly-morphonuclear neutrophils inside the neoplastic lesions was utilized as additional positive control. Finally, to test the inter-run variability of LF immunostaining, the same LF-positive parotid sample was utilized in every run. To test the of LF immunoreaction in order to deny the possibility of non-specific reaction, serial sections of each affected specimen were tested by replacing the specific antiserum by either PBS, normal rabbit serum or absorbing with excess of purified human LF from human liver and spleen (Sigma Chemical Co.) as well as with pre-absorbed primary antibody: the results obtained were negative.

Immunostained sections were estimated by light microscopy using a 20x and 40x objective lens and 10x eyepiece. Two pathologists using a double-headed microscope performed the assessment of LF immunostained sections on a consensus basis. The percentage of stained neoplastic cells (area of staining

positivity, ASP) was graded as follows: 0 (no staining), 1 (>0% - 5%), 2 (>5% - 50%), and 3 (>50%). The intensity of staining (IS) (weak=1; moderate=2; strong= 3) was also taken into consideration. Successively, a LF intensity-distribution (ID) score was calculated for each case by multiplying the values of the ASP and the IS, according to that reported elsewhere [10].

## RESULTS

All neoplastic samples, routinely stained by haematoxylin and eosin, exhibited a good morphology, confirming the histopathological diagnosis; however, parallel sections were adequately stained by LF immunohistochemistry, with an immunoreactivity generally localized in the cytoplasm but occasionally in the nucleus.

In differentiated adenocarcinomas of the prostate, an intense and diffuse cytoplasmic LF immunopositivity was observed; tumour cells arranged in acini and ducts were also strongly stained, although positive and negative neoplastic elements were found in direct contact (Figure **1a**). Frequently, LF was noted sometimes in the secretory product inside the glandular lumina. Areas of papillary, mucinous and cribriform carcinomas also showed a positive LF reaction. Undifferentiated prostatic carcinomas exhibited a very slight cytoplasmatic LF positivity.

In thyroid tissue, follicular and papillary carcinomas exhibited various degrees of LF immunoreactivity localized in the cytoplasm; medullary carcinomas as well as anaplastic ones were always unstained, although incorporated organoid and follicular entrapped structures were positive for LF (Figure **1b**).

The mucous neck cells of the antrum and body of the stomach were positive for LF; moreover, an evident LF immunoreactivity was encountered in intestinal type carcinomas (Figure **1c**), whereas diffuse type ones were always unstained.

A clear intense cytoplasmatic immunopositivity for LF was found in well and moderately differentiated colo-rectal adenocarcinomas (Figure **1d**) as well as colloid carcinomas, even if some undifferentiated cases were unreactive; the LF immunostaining was also

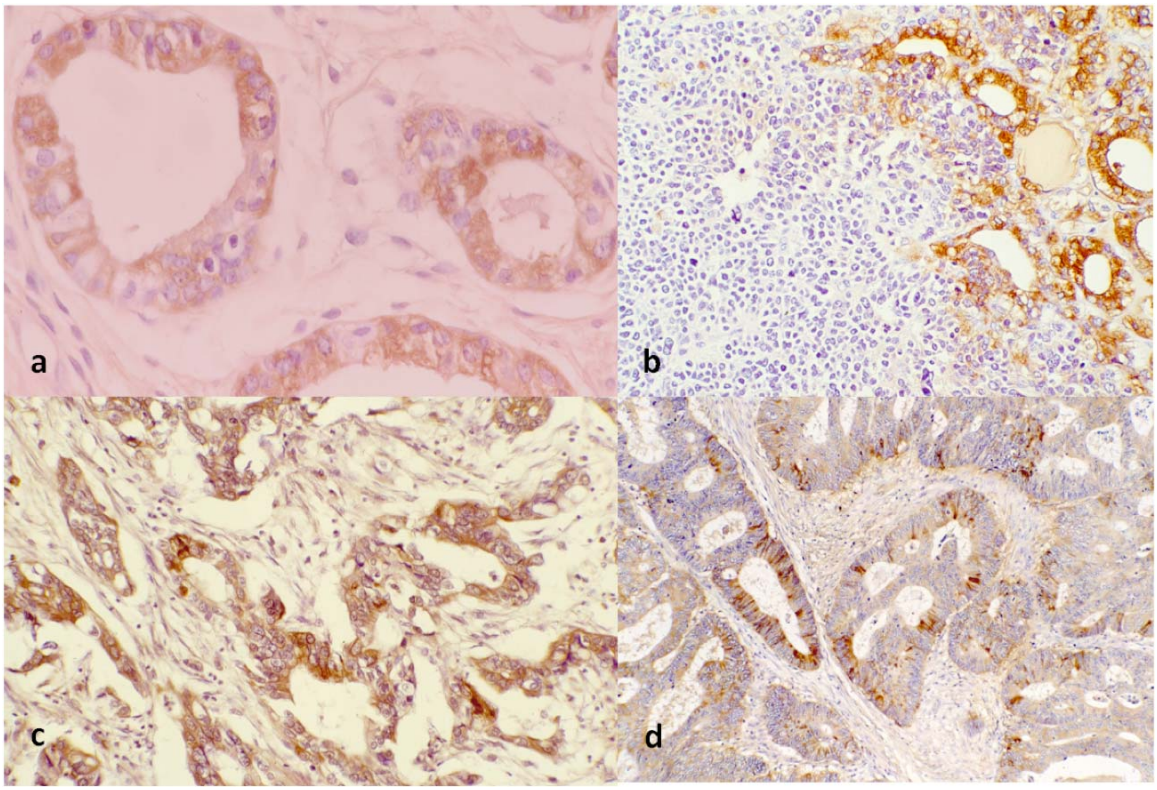


**Figure 1:** Lf immunoreactivity: (**a**) a cytoplasmic positivity was found in prostatic acinar elements adjacent to negative ones (160X); (**b**) immunostaining was limited to thyroid follicles entrapped inside anaplastic carcinoma (100X); (**c,d**) an evident immunostaining was appreciable in neoplastic glands of both stomach (160X) and large bowel (100X) (Immunoperoxidase, Mayer's haemalum counterstain).

encountered in neoplastic elements present in metastatic lymphnodes, when the primary cancer was stained.

In gallbladder, a positive LF immunoreactivity was found in a variable share of adenocarcinomas, mainly represented in papillary or glandular areas (Figure **2a**), while sarcomatoid, squamous or mucinous components were negative; the number of immunostained elements as well as the staining intensity showed some differences in the context of the same tumour.

In the central nervous system, a moderate to strong diffuse immunoreactivity for LF has been found in glial elements of astrocytomas, while the positivity was progressively reduced in anaplastic astrocytomas and multiforme glioblastomas, some of which were unstained.

In melanocytic proliferations of the skin, such as melanomas, an evident immunoreactivity for LF was encountered, although no appreciable difference in LF staining was appreciable between spindle and epithelioid cells (Figure **2b**). In basal cell carcinomas neoplastic elements, either organized as solid nests or palisading structures, were always unstained.

In endometrial adenocarcinomas, mainly the endometrioid histotype showed a variable immunoexpression of LF in comparison to the non-endometrioid; positive neoplastic epithelial cells were found in direct contact with negative ones, although in some cases LF was intensely expressed throughout the entire neoplastic mass (Figure **2c**).

In kidney, the pattern of LF positivity was different in clear cell, papillary or cromophobe variants of renal carcinomas: neoplastic clear cells exhibited a LF immunolocalization mainly evident at the cytoplasmic boundary (Figure **2d**), while immunoreactive cromophobe elements were found in direct contact with negative once, showing a diffuse granular LF cytoplasmic distribution.

The immunohistochemical LF distribution pattern in malignant neoplastic bone and cartilage samples was very heterogeneous with an immunolocalization confined to chondroblastomas, myeloma as well as

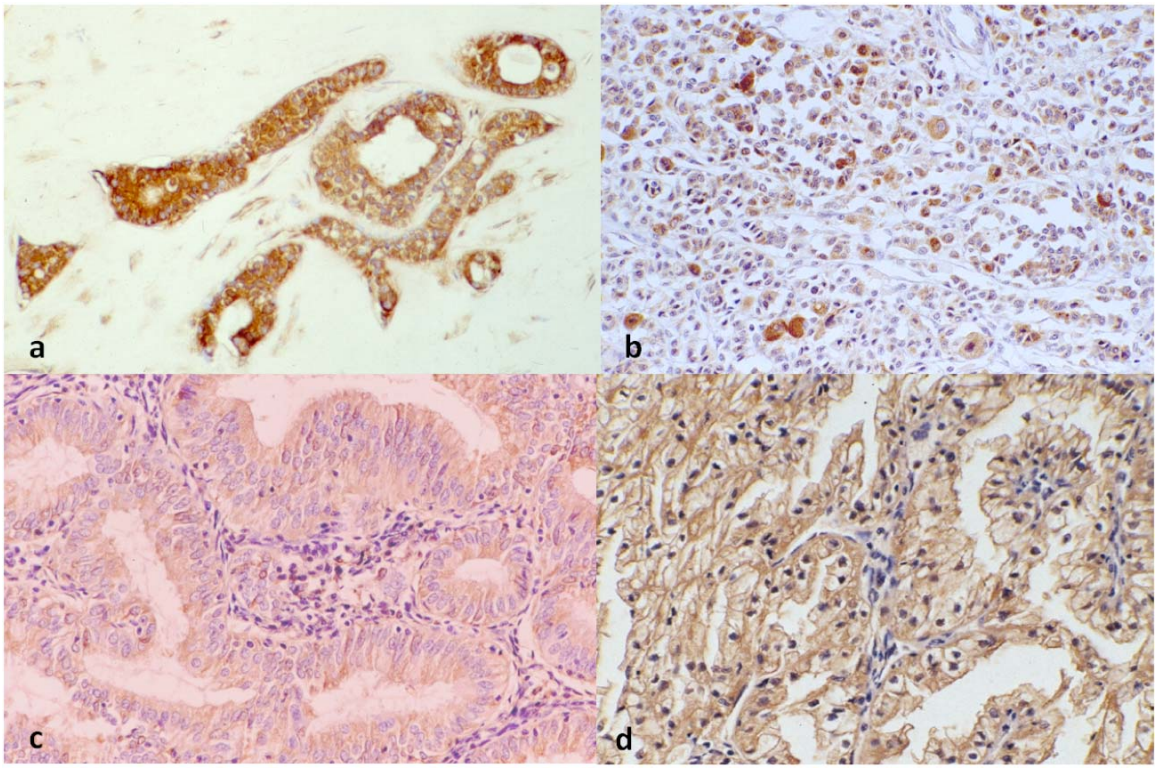


**Figure 2:** Lf immunoreactivity: (**a**) neoplastic glands are strongly stained (100X); (**b**) epithelioid and spindle cells of melanomas showed a cytoplasmic localization (100X); (**c**) diffuse immunostaining was noted in well differentiated endometriod adenocarcinoma (100X); (**d**) a cytoplasmic positivity was found at the periphery of clear cells in carcinomas of the kidney (100X) (Immunoperoxidase, Mayer's haemalum counterstain).

adamantinoma; no LF immunoexpression was detected in chondrosarcomas as well osteosarcomas.

## DISCUSSION

In the present study, we have firstly reported the LF immunoexpression in a series of human malignant tumours, showing that LF presence was not exclusively localized to the cytoplasm, but also in the nucleus. However, the site of LF immunolocalization in both the nucleus and cytoplasm has not been considered surprising since this glycoprotein has been thought to be involved in ribosomal biogenesis [43,44] and, after its transport into the nucleus, LF is able to bind specific DNA sequences, thus activating transcription [44,45]. Moreover, we have shown that heterogeneity in LF immunoexpression between different malignancies as well as inside the same tumour was not infrequent; if this observation could reflect different cell subpopulations, the stage in the cell cycle or instead some metabolic abnormalities should be verified by methods other than morphological analysis.

The origin of LF in human malignant tumours has not yet been fully elucidated. It is well known that LF has a high affinity for iron, which has been considered an essential nutrient for cells that are dividing rapidly such as tumour cells, taking part in various metabolic processes such as oxydative phosphorylation and RNA and DNA synthesis [4,19, 22,46]. Therefore, neoplastic elements should be able to produce LF in order to make a greater amount of iron available for their turnover, similarly to that elsewhere suggested [25, 35, 39]. Alternatively, the localization of LF in malignant cells may not reflect an intracellular synthesis, reflecting instead the degree of trans-membranous iron transfer as the consequence of defective or functionally impaired LF-receptors, already documented on the surface of target cells as well as in human neoplastic cell lines [47-49].

In our casuistry, the LF immunostaining was never founded in relation to the site as well as the stage of malignant tumours, excluding thus its role as predictive or prognostic neoplastic markers. Nevertheless, the immunohistochemical evidence of LF was largely confined to differentiated carcinomatous histotypes, such as prostatic adenocarcinomas, follicular and papillary thyroid carcinomas, differentiated glandular carcinomas of the stomach, colon and gallbladder, endometrioid as well as renal adenocarcinomas, while anaplastic and undifferentiated carcinomas were always unstained; consequently, it may be suggested a role for LF as marker of glandular or acinar differentiation, similarly to that already pointed out in other malignancies [25, 38,50-52].

However, the protective effects of LF have been demonstrated on chemically induced tumors of rodents; moreover, it has been previously reported that LF is able to inhibit the development of experimental metastases in mice, mainly by an increase of NK cells and T lymphocytes expressing CD8, CD4 and IFNγ [19,53,54]. Meanwhile, other potential mechanisms have been suggested regarding the role of LF in the process of human carcinogenesis, including induction of programmed cell death, prevention of angiogenesis and regulation of cell cycle protein expression [5,7,55,56]. In fact, LF is able to trigger the apoptotic process by the activation of caspases 3 and 8 as well as the FAS signaling pathway [19,57]; on the other hand, LF was also shown to inhibit tumour-initiated angiogenesis *in vitro* and *in vivo*, possibly by blocking endothelial function and inducing IL-18 production [55,58,59]. In addition, it has been reported that LF promoted growth arrest either at the $G_1$ to S transition in breast cancer cells [26] as well as at the $G_0$-$G_1$ checkpoint in oral and neck cancer cells [60]; finally, LF demonstrated its ability to regulate cell growth by controlling the level of retinoblastoma protein, a key tumour suppressor involved in cell cycle progression [61]. Nevertheless, whatever was the mechanism of action of LF in tumours, we probably still require additional investigations about the opportunity for new applications of LF in cancer, mainly regarding its nutraceutical function as well as its ability to potentiate chemotherapy.

## REFERENCES

[1] Steijns JM, Van Hooijdonk AC. Occurrence, structure, biochemkical properties and technological characteristics of lactoferrin. Brit J Nutr 2000; 84 Suppl 1: 11-17. http://dx.doi.org/10.1017/S0007114500002191

[2] Van der Strate BW, Beljaars L, Molema G, Harmsen MC, Meijer DK. Antiviral activities of lactoferrin. Antiviral Res 2001; 52: 225-39. http://dx.doi.org/10.1016/S0166-3542(01)00195-4

[3] Oztas Yesim ER, Ozgunes N. Lactoferrin: a multifunctional protein. Adv Mol Med 2005; 1: 149-54. http://dx.doi.org/10.1007/s10238-011-0156-3

[4] García-Montoya IA, Cendón TS, Arévalo-Gallegos S, Rascón-Cruz Q. Lactoferrin a multiple bioactive protein: an overview. Biochim Biophys Acta 2012; 1820: 226-36. http://dx.doi.org/10.1016/j.bbagen.2011.06.018

[5] Gonzalez-Chavez SA, Arevalo-Gallegos S, Rascon-Cruz Q. Lactoferrin: structure, function and applications. Int Journal Antimicrob Agents 2009; 33: 301-9. http://dx.doi.org/10.1016/j.ijantimicag.2008.07.020

[6] Brock JH. Lactoferrin 50 years on. Biochem Cell Biol 2012; 90: 245-51. http://dx.doi.org/10.1139/o2012-018

[7] Lonnerdal B, Iyer S. Lactoferrin: Molecular structure and biological function. Ann Rev Nutr 1995; 15: 93-110. http://dx.doi.org/10.1146/annurev.nu.15.070195.000521

[8] Cirioni O, Giacometti A, Barchesi F, Scalise G. Inhibition of growth of Pneumocystis carinii by loactoferrins alone and in combination with pyrimethanime, clarithromycin and minocycline. J Antimicrob Chemother 2000; 46: 577-82. http://dx.doi.org/10.1093/jac/46.4.577

[9] Andersen JH, Osbakk SA, Vorland LH, Traavik T, Gutteberg TJ. Lactoferrin and cyclic lactoferricin inhibit the entry of human cytomegalovirus into human fibroblasts. Antiviral Res 2001; 51: 141-49. http://dx.doi.org/10.1016/S0166-3542(01)00146-2

[10] Tuccari G, Villari D, Giuffrè G, Simone A, Squadrito G, Raimondo G, Barresi G. Immunohistochemical evidence of lactoferrin in hepatic biopsies of patients with viral or cryptogenetic chronic liver disease. Histol Histopathol 2002; 17: 1077-83. http://www.hh.um.es/pdf/Vol_17/17_4/Tuccari-17-1077-1083-2002.pdf

[11] Takakura N, Wakabayashi H, Ishibashi H, Teraguchi S, Tamura Y, Yamaguchi H et al. Oral lactoferrin treatment of experimental oral candidiasis in mice. Antimicrob Agents Chemother 2003; 47: 2619-23. http://dx.doi.org/10.1128/AAC.47.8.2619-2623.2003

[12] Yamauchi K, Wakabayashi H, Shin K, Takase M. Bovine lactoferrin: benefits and mechanism of action against infections. Biochem Cell Biol 2006; 84: 291-96. http://dx.doi.org/10.1139/o06-054

[13] Zarember KA, Sugui JA, Chang YC, Kwon-Chung KJ, Gallin JI. Human polymorphonuclear leukocytes inhibit Aspergillus fumigatus conidial growth by lactoferrin-mediated iron depletion. J Immunol 2007; 178: 6367-73. http://www.jimmunol.org/content/178/10/6367

[14] Brock JH, Lamont A, Boyle DJ et al. Antibodies to lactoferrin. A possible link between cow's milk intolerance and autoimmune disease. Adv Exp Med Biol 1998; 443: 305-11.

[15] Baveye S, Elass E, Mazurier J, Spik G, Legrand D. Lactoferrin: a multifunctional glycoprotein involved in the modulation of the inflammatory process. Clin Chem Lab Med 1999; 37: 281-86.

[16] Guillen C, McInnes IB, Vaughan D, Speekenbrink AB, Brock JH. The effects of local administration of lactoferrin on inflammation in murine autoimmune and infectious arthritis. Arthr Rheum 2000; 43: 2073-80. http://dx.doi.org/10.1002/1529-0131(200009)

[17] Legrand D, Pierce A, Elass E, Carpentier M, Mariller C, Mazurier J. Lactoferrin structure and functions. Adv Exp Med Biol 2008; 606: 163-194. http://dx.doi.org/10.1007/978-0-387-74087-4_6

[18] Lonnerdal B. Nutritional roles of lactoferrin. Curr Op Clin Nutr Metab Care 2009; 12: 293-7. http://dx.doi.org/10.1097/MCO.0b013e328328d13e

[19] Pierce A, Legrand D, Mazurier J. Lactoferrin: a multifunctional protein. Med Sci 2009; 25: 361-9. http://dx.doi.org/10.1051/medsci/2009254361

[20] Furmanski P, Li ZP, Fortuna MB, Swamy CV, Das MR. Multiple molecular forms of human lactoferrin. Identification of a class of lactoferrins that possess ribonuclease activity and lack iron-binding capacity. J Exp Med 1989; 170: 415-29. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2189405/pdf/je1702415.pdf

[21] Qiu J, Hendrixson DR, Baker EN, Murphy TF, St Geme JW 3rd, Plaut AG. Human milk lactoferrin inactivates two putative colonization factors expressed by Haemophilus influenzae. Proc Natl Acad Sci USA 1998; 95: 12641-6. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC22884/pdf/pq012641.pdf

[22] Ye XY, Wang HX, Liu F, Ng TB. Ribonuclease, cell-free translation-inhibitory and superoxide radical scavenging activities of the iron-binding protein lactoferrin from bovine milk. Int J Biochem Cell Biol 2000; 32: 235-41. http://dx.doi.org/10.1016/S1357-2725(99)00131-4

[23] Kanyshkova TG, Babina SE, Semenov DV, Isaeva N, Vlassov AV, Neustroev KN et al. Multiple enzymatic activities of human milk lactoferrin. Eur J Biochem 2003; 270: 3353-61. http://dx.doi.org/10.1046/j.1432-1033.2003.03715.x

[24] Ward PP, Paz E, Conneelly OM. Multifunctional roles of lactoferrin: a critical overview. Cell Mol Life Sci 2005; 62: 2540-8. http://dx.doi.org/10.1007/s00018-005-5369-8

[25] Giuffrè G, Barresi V, Skliros C, Barresi G, Tuccari G. Immunoexpression of lactoferrin in human sporadic renal cell carcinomas. Oncology Reports 2007; 17: 1021-6.

[26] Damiens E, El Yazidi I, Mazurier J, Duthille I, Spik G, Boilly-Marer Y. Lactoferrin inhibits G1 cyclin-dependent kinases during growth arrest of human breast carcinoma cells. J Cell Biochem 1999; 74: 486-98. http://dx.doi.org/10.1002/(SICI)1097-4644(19990901)

[27] Norrby K, Mattsby-Baltzer I, Innocenti M, Tuneberg S. Orally administered bovine lactoferrin systematically inhibits VEGF165-mediated angiogenesis in the rat. Int J Cancer 2001; 91: 236-40. http://dx.doi.org/10.1002/1097-0215(200002)

[28] Barresi G, Tuccari G. Lactoferrin in benign hypertrophy and carcinomas of the prostatic gland. Virch Arch Pathol Anat Histol 1984; 403: 59-66.

[29] Tuccari G, Barresi G. Immunohistochemical demonstration of lactoferrin in follicular adenomas and thyroid carcinomas. Virch Arch Pathol Anat Histol 1985; 406: 67-74.

[30] Merlino G, Barresi G, Tuccari G, Bussolati G. Histogenesis of anaplastic carcinomas of the thyroid, giant cell variety: immunocytochemical investigation. Appl Pathol 1985; 3: 150-8.

[31] Barresi G, Tuccari G Iron-binding proteins in thyroid tumours. An immunocytochemical study. Pathol Res Pract 1987; 182: 344-51.

[32] Tedeschi A, Tuccari G, Magazzù G, Arena F, Ricciardi R, Barresi G. Immunohistochemical localization of lactoferrin in duodenojejunal mucosa from celiac children. J Pediatr Gastroenterol Nutr 1987; 6: 328-34.

[33] Tuccari G, Barresi G, Arena F, Inferrera C. Immunocyto-chemical detection of lactoferrin in human gastric carcinomas and adenomas. Arch Pathol Lab Med 1989; 113: 912-5.

[34] Tuccari G, Rizzo A, Crisafulli C, Barresi G. Iron-binding proteins in human colorectal adenomas and carcinomas: an immunocytochemical investigation. Histol Histopathol 1992; 7: 543-7.

[35] Tuccari G, Rossiello R, Barresi G. Iron binding proteins in gallbladder carcinomas. An immunocytochemical investigation. Histol Histopathol 1997; 12: 671-6.

[36] Tuccari G, Giuffrè G, Crisafulli C, Barresi G. Immunohisto-chemical detection of lactoferrin in human astrocytomas and multiforme glioblastomas. Eur J Histochem 1999; 43: 317-22.

[37] Tuccari G, Giuffrè G, Scarfì R, Simone A, Todaro P, Barresi G. Immunolocalization of lactoferrin in surgically resected pigmented skin lesions. Eur J Histochem 2005; 49: 33-8.

[38] Giuffrè G, Arena F, Scarfì R, Simone A, Todaro P, Tuccari G. Lactoferrin immunoexpression in endometrial carcinomas: relationships with sex steroid hormone receptors (ER and

PR), proliferation indices (Ki-67 and AgNOR) and survival. Oncology Reports 2006; 16: 257-63.

[39] Ieni A, Barresi V, Grosso M, Rosa MA, Tuccari G. Lactoferrin immuno-expression in human normal and neoplastic bone tissue. J Bone Min Metab 2009a; 27: 364-71. http://dx.doi.org/10.1007%2Fs00774-009-0044-z

[40] Ieni A, Barresi V, Grosso M, Rosa MA, Tuccari G. Immunolocalization of lactoferrin in cartilage-forming neoplasms. J Orthopaed Sci 2009b; 14: 732-7. http://dx.doi.org/10.1007%2Fs00776-009-1396-x

[41] Ieni A, Barresi V, Grosso M, Rosa MA, Tuccari G. Immunohistochemical evidence of lactoferrin in human embryo-fetal bone and cartilage tissues. Cell Biol Int 2010; 34: 845-9. http://dx.doi.org/10.1042/CBI20090358

[42] Ieni A, Barresi V, Grosso M, Speciale G, Rosa MA, Tuccari G. Does lactoferrin behave as an immunohistochemical oncofetal marker in bone and cartilage human neoplasms? Path Oncol Res 2011; 17: 287-93. http://dx.doi.org/10.1007%2Fs12253-010-9311-5

[43] Garré C, Bianchi-Scarrá G, Sirito M, Musso M, Ravazzolo R. Lactoferrin binding sites and nuclear localization in K562(S) cells. J Cell Physiol 1992; 153: 477-82.

[44] Penco S, Scarfi S, Giovine M, Damonte G, Millo E, Villaggio B et al. Identification of an import signal for, and the nuclear localization of, human lactoferrin. Biotechnol Appl Biochem 2001; 34: 151-9. http://www.babonline.org/bab/034/0151/0340151.pdf

[45] Legrand D, Vigié K, Said EA, Elass E, Masson M, Slomianny MC et al. Surface nucleolin participates in both the binding and endocytosis of lactoferrin in target cells. Eur J Biochem 2004; 271: 303-17.

[46] Weinberg ED. Iron withholding: a defense against infection and neoplasia. Physiol Rev 1984; 64: 65-102.

[47] Roiron D, Amouric M, Marvaldi J, Figarella C. Lactoferrin-binding sites at the syurface of HT29-D4 cells. Comparison with transferrin. Eur J Biochem 1989; 186: 367-73. http://dx.doi.org/10.1111/j.1432-1033.1989.tb15218.x

[48] Suzuki Y, Lonnerdal B. Characterization of mammalian receptors for lactoferrin. Biochem Cell Biol 2002; 80: 75-80.

[49] Suzuki YA, Lopez V, Lonnerdal B. Mammalian lactoferrin receptors: structure and function. Cell Mol Life Sci 2005; 62: 2560-575. http://dx.doi.org /10.1007%2Fs00018-005-5371-1

[50] Caselitz J, Jaup T, Seifert G. Lactoferrin and lysozyme in carcinomas of the parotid gland. A comparative immunocytochemical study with the occurrence in normal and inflamed tissue. Virch Arch Pathol Anat Histol 1981; 394: 61-73.

[51] Charpin C, Lachard A, Pourreau-Schneider N, Jacquemier J, Lavaut MN, Andonian C et al. Localization of lactoferrin and nonspecific cross-reacting antigen in human breast carcinomas. An immunohistochemical study using the avidin-biotin-peroxidase complex method. Cancer 1985; 55: 2612-7.

[52] Cabaret V, Vilain MO, Delobelle-Deroide A, Vanseymortier L. Immunohistochemical demonstration of ceruloplasmin and lactoferrin in a series of 59 thyroid tumors. Ann Pathol 1992; 12: 347-52.

[53] Wang WP, Iigo M, Sato J, Sekine K, Adachi I, Tsuda H. Activation of intestinal mucosa immunity in tumor-bearing mice by lactoferrin. Jpn J Cancer Res 2000; 91: 1022-7.

[54] Wolf JS, Li D, Taylor RJ, O'Malley BW Jr. Lactoferrin inhibits growth of malignant tumors of the head and neck. J Oto-Rhino-Laryngol Rel Spec 2003; 65: 245-9. http://dx.doi.org/10.1159/000075220

[55] Rodrigues L, Teixeira J, Schmitt F, Paulsson M, Månsson HL. Lactoferrin and cancer disease prevention. Crit Rev Food Sci Nutr 2009; 49: 203-17. http://dx.doi.org/10.1080/10408390701856157

[56] de Mejia EG, Dia VP. The role of nutraceutical proteins and peptides in apoptosis, angiogenesis, and metastasis of cancer cells. Cancer Met Rev 2010; 29(3): 511-28. http://dx.doi.org/10.1007/s10555-010-9241-4

[57] Fujita K, Matsuda E, Sekine K, Iigo M, Tsuda H. Lactoferrin enhances Fas expression and apoptosis in the colon muciosa of azoxymethane-treated rats. Carcinogenesis 2004; 25: 1961-6. http://dx.doi.org/10.1093/carcin/bgh205

[58] Norrby K. Human apo-lactoferrin enhances angiogenesis mediated by vascular endothelial growth factor A in vivo. J Vasc Res 2004; 41: 293-304. http://dx.doi.org/10.1159/000078927

[59] Shimamura M, Yamamoto Y, Ashino H, Oikawa T, Hazato T, Tsuda H et al. Bovine lactoferrin inhibits tumor induced angiogenesis. Int J Cancer 2004; 111: 111-6. http://dx.doi.org/10.1002/ijc.20187

[60] Xiao Y, Monitto CL, Minhas KM, Sidransky D. Lactoferrin down-regulates G1 cyclin-dependent kinases during growth arrest of head and neck cancer cells. Clin Cancer Res 2004; 10: 8683-6. http://dx.doi.org/10.1158/1078-0432.CCR-04-0988

[61] Son KN, Park J, Chung CK, Chung DK, Yu DY, Lee KK et al. Human lactoferrin activates transcription of IL-beta gene in mammalian cells. Biochem Biophys Res Comm 2002; 290: 236-41. http://dx.doi.org/10.1006/bbrc.2001.6181

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http://dx.doi.org/10.6000/1927-7229.2013.02.01.3