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Immunohistochemical Evidence of Lactoferrin in Malignant Gastrointestinal Tumors': A Mini Review

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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. We report herein a mini-review regarding the LF immunohistochemical pattern in gastrointestinal malignant tumours obtained from different human districts in order to acquire a possible explanation for its presence and function.

Keywords: Immunohistochemistry; Lactoferrin; Gastrointestinal; Cancer;

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 [1,2]. Several functions have been attributed to LF, although the corresponding mechanisms remain still controversial [3]; it appears involved in the regulation of iron homeostasis and absorption in the bowel as well as in the antimicrobial activity against bacteria, viruses, fungi and parasites [4]. Moreover, immunomodulatory and anti-inflammatory effects of LF have been reported [3,4]. Finally, LF appears to show some enzymatic properties such as protease, DNAase, RNAase and ATPase [5].

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 [6]. 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* [7,8]. Therefore, to better understand the LF anticarcinogenic activity, we report herein an immunohistochemical analysis of LF in human neoplasms of different gastrointestinal district in the attempt to elucidate also its possible pathogenetic role.

Materials and Methods

We have reviewed the immunohistochemical pattern of LF distribution in 101 surgical samples obtained from a corresponding number of malignant tumours developed in the stomach (30), colon (39) and gall bladder (32). The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki and approved by local ethics committee.

All samples have been fixed in 10% neutral formalin for 24hrs at room temperature (RT) and then embedded in paraffin at 56 °C. From each block of malignant neoplastic tissue, 4μm-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 5min.

For the immunohistochemical study, sections were treated in a moist chamber at room temperature:

i. with 0.1% H_2O_2 in methanol to block the intrinsic peroxidase activity (30min);

ii. with normal sheep serum to prevent unspecific adherence of serum proteins (30min);

iii. with the monoclonal primary antibody against anti-human LF (clone 1A1; Biodesign International, Saco, ME; w.d. 1: 75; 60min);

iv. with sheep anti-mouse immunoglobulin antiserum (Behring Institute; w.d. 1: 25;30min);

v. with mouse anti-horseradish peroxidase-antiperoxidase complexes (Dako Cytomation, w.d. 1: 25; 30min).

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 utilised as additional positive controls, as elsewhere suggested [7,8]. The LF immunoreactivity demonstrated in granules of polymorphonuclear neutrophils was utilised as positive control. Finally, in order to test the inter-run variability of LF staining, the same LF-positive parotid sample was utilised in every run. To test the specificity of LF immunostaining in order to deny the possibility of non-specific reaction, serial sections of each neoplastic 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 an x20 and x40 objective lens and x10 eyepiece; the assessment of LF immunostained sections was performed on a consensus basis by two pathologists using a double-headed microscope.

Results and Discussion

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

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, whereas diffuse type ones were always unstained. A clear intense cytoplasmatic immunopositivity for LF was found in well and moderately differentiated colo-rectal adenocarcinomas as well as mucinous carcinomas, even if some undifferentiated cases were unreactive; the LF immunostaining was also encountered in neoplastic elements present in metastatic lymphnodes, when the primary cancer was stained.

In gastric and colonic cancer, a positive LF immunoreactivity was found in a variable share of adenocarcinomas, mainly represented in papillary or glandular areas, 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. (Figure 1)

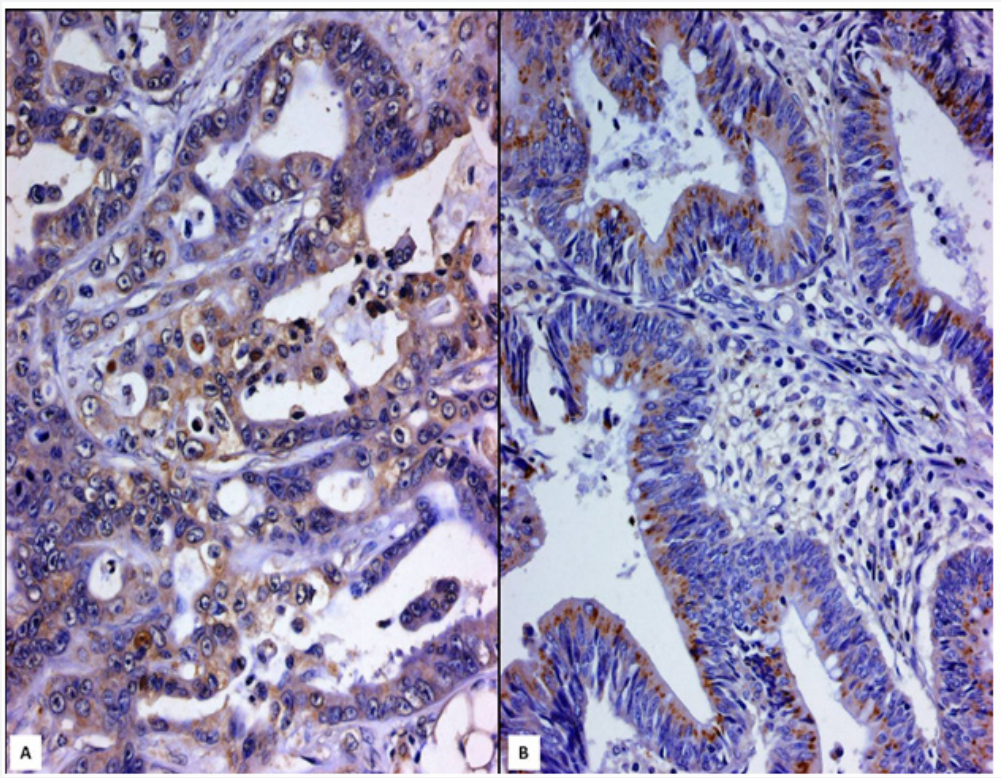

Figure 1: An evident diffuse or granular LF immune reactivity was encountered either in gastric intestinal type carcinoma (A, X200) as well as in colonic well differentiated carcinoma (B, X200) (Mayer's Haemalum nuclear counter stain).

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In the present study, we have analyzed LF immunoexpression in a series of human gastrointestinal 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 and after its transport into the nucleus; LF is able to bind specific DNA sequences, thus activating transcription [9]. Moreover, we have shown that heterogeneity in LF immunoexpression between different malignancies as well as inside the same tumour were 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 [10-19].

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 [1,5]. 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 [7,20]. Alternatively, the localization of LF in malignant cells may not reflect an intracellular synthesis, reflecting instead the degree of transmembranous 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 [21].

In our casuistry, the LF immunostaining was never founded in relation to the site, grade and 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 differentiated glandular carcinomas of the stomach, colon and gallbladder, 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 [20,22,23].

Conclusion

The protective effects of LF have been demonstrated on chemically induced tumors of rodents [1]; 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 [24]. 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 [25,26]. 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 [24]; 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 [25]. Moreover, it has been reported that LF promoted growth arrest either at the G1 to S transition in breast cancer cells [8] as well as at the G0-G1 checkpoint in oral and neck cancer cells [27]; 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 28-30. 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 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 nutraceutical function as well as ability to potentiate chemotherapy..

References

1. Adlerova L, Bartoskova A, Faldyna A (2008) Lactoferrin: a review. Veterinarni Medicina 53: 457-468.
2. Gonzalez-Chavez SA, Arévalo-Gallegos S, Rascon-Cruz Q (2009) Lactoferrin: structure, function and applications. Int Journal Antimicrob Agents 33(4): 301-309.
3. Brock JH (2002) The physiology of lactoferrin. Biochim Biol Cell 80(1): 1-6.
4. Lonnerdal B, Iyer S (1995) Lactoferrin: Molecular structure and biological function. Ann Rev Nutr 15: 93-110.
5. Kanyshkova TG, Babina SE, Semenov DV, Isaeva N, Vlassov AV, et al (2003) Multiple enzymatic activities of human milk lactoferrin. Eur J Biochem 270(16): 3353-3361.
6. Ward PP, Paz E, Conneely OM (2005) Multifunctional roles of lactoferrin: a critical overview. Cell Mol Life Sci 62(22): 2540-2548.
7. Giuffrè G, Barresi V, Skliros C, Barresi G, Tuccari G (2007) Immunoexpression of lactoferrin in human sporadic renal cell carcinomas. Oncology Reports 17(5): 1021-1026.
8. Damiens E1, El Yazidi I, Mazurier J, Duthille I, Spik G, et al. (1999) Lactoferrin inhibits G1 cyuclin-dependent kinases during growth arrest of human breast carcinoma cells. J Cell Biochem 74(3): 486-498.
9. Penco S1, Scarfi S, Giovine M, Damonte G, Millo E, et al. (2001) Identification of an import signal for, and the nuclear localization of, human lactoferrin. Biotechnol Appl Biochem 34(Pt 3): 151-159.
10. Barresi G, Tuccari G (1984) Lactoferrin in benign hypertrophy and carcinomas of the prostatic gland. Virch Arch Pathol Anat Histol 403(1): 59-66.
11. Tuccari G, Barresi G (1985) Immunohistochemical demonstration of lactoferrin in follicular adenomas and thyroid carcinomas. Virch Arch Pathol Anat Histol 406(1): 67-74.
12. Merlino G, Barresi G, Tuccari G, Bussolati G (1985) Histogenesis of anaplastic carcinomas of the thyroid, giant cell variety: immunocytochemical investigation. Appl Pathol 3(3): 150-158.
13. Barresi G, Tuccari G (1987) Iron-binding proteins in thyroid tumours. An immunocytochemical study. Pathol Res Pract 182(3): 344-351.
14. Tuccari G, Giuffrè G, Crisafulli C, Barresi G (1999) Immunohistochemical detection of lactoferrin in human astrocytomas and multiforme glioblastomas. Eur J Histochem 43(4): 317-322.
15. Tuccari G, Giuffrè G, Scarfì R, Simone A, Todaro P, Barresi G (2005) Immunolocalization of lactoferrin in surgically resected pigmented skin lesions. Eur J Histochem 49(1): 33-38.

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16. Giuffrè G, Arena F, Scarfì R, Simone A, Todaro P, Tuccari G (2006) Lactoferrin immunoexpression in endometrial carcinomas: relationships with sex steroid hormone receptors (ER and PR), proliferation indices (Ki-67 and AgNOR) and survival. Oncology Reports 16(2): 257-263.

17. Ieni A, Barresi V, Grosso M, Rosa MA, Tuccari G (2009) Lactoferrin immuno-expression in human normal and neoplastic bone tissue. J Bone Min Metab 27(3): 364-371.

18. Ieni A, Barresi V, Grosso M, Rosa MA, Tuccari G (2009) Immunolocalization of lactoferrin in cartilage-forming neoplasms. J Orthopaed Sci 4(6): 732-737.

19. Ieni A, Barresi V, Grosso M, Tuccari G (2010) Immunohistochemical evidence of lactoferrin in human embryo-fetal bone and cartilage tissues. Cell Biol Int 34(8): 845-849.

20. Tuccari G, Rossiello R, Barresi G (1997) Iron binding proteins in gallbladder carcinomas. An immunocytochemical investigation. Histol Histopathol 12(3): 671-676.

21. Suzuki YA, Lopez V, Lönnerdal B (2005) Mammalian lactoferrin receptors: structure and function. Cell Mol Life Sci 62(22): 2560-2575.

22. Tuccari G, Barresi G, Arena F, Inferrera C (1989) Immunocytochemical detection of lactoferrin in human gastric carcinomas and adenomas. Arch Pathol Lab Med 113(8): 912-915.

23. Tuccari G, Rizzo A, Crisafulli C, Barresi G (1992) Iron-binding proteins in human colorectal adenomas and carcinomas: an immunocytochemical investigation. Histol Histopathol 7(4): 543-547.

24. Pierce A, Legrand D, Mazurier J (2009) Lactoferrin: a multifunctional protein. Med Sci 25(4): 361-369.

25. Rodrigues L, Texeira J, Schmitt F, Paulsson M, Lindmark H (2009) Lactoferrin and cancer disease prevention. Crit Rev Food Sci Nutr 49(3): 203-217

26. de Mejia EG, Dia VP (2010) The role of nutraceutical proteins and peptides in apoptosis, angiogenesis, and metastasis of cancer cells. Cancer Met Rev 29(3): 511-528.

27. 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(24): 8683-8686.

28. Son KN, Park J, Chung CK (2002) Human lactoferrin activates transcription of IL-beta gene in mammalian cells. Biochem Biophys Res Comm 290(1): 236-241.

29. Damiens E, El Yazidi I, Mazurier J et al (1999) Lactoferrin inhibits G1 cyuclin-dependent kinases during growth arrest of human breast carcinoma cells. J Cell Biochem 74(3): 486-498.

30. Penco S, Scarfì S, Giovine M (2001) Identification of an important signal for, and the nuclear localization of human lactoferrin. Biotechnol Appl Biochem 34 (Pt 3): 151-159.

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