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ORIGINAL ARTICLE

Bovine holo-lactoferrin inhibits migration and invasion in MDA-MB-231 breast cancer cells

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Abstract

Purpose Breast cancer is the most common malignancy in developed countries and the main cause of deaths in women worldwide. Lactoferrin (Lf) is an iron-binding protein constituted for a single polypeptide chain that is folded into two symmetrical lobes that bind Fe^{2+} or Fe^{3+}. Lf has the ability to reversibly bind Fe^{3+} and is found free of Fe^{3+} (Apo-Lf) or associated with Fe^{3+} (Holo-Lf) with a different three-dimensional conformation. However, the role of bovine Apo-Lf (Apo-BLf) and bovine Holo-Lf (Holo-BLf) in the migration and invasion induced by linoleic acid (LA) and fetal bovine serum (FBS), as well as in the expression of mesenchymal and epithelial proteins in breast cancer cells has not been studied.

Methods and results Scratch wound assays demonstrated that Holo-BLf and Apo-BLf do not induce migration, however they differentially inhibit the migration induced by FBS and LA in breast cancer cells MDA-MB-231. Western blot, invasion, zymography and immunofluorescence confocal microscopy assays demonstrated that Holo-BLf partly inhibit the invasion, FAK phosphorylation at tyrosine (Tyr)-397 and MMP-9 secretion, whereas it increased the number and size of focal adhesions induced by FBS in MDA-MB-231 cells. Moreover, Holo-BLf induced a slight increase of E-cadherin expression in MCF-7 cells, and inhibited vimentin expression in MCF-7 and MDA-MB-231 breast cancer cells.

Conclusion Holo-BLf inhibits cellular processes that mediate the invasion process in breast cancer cells.

Keywords Breast cancer · Lactoferrin · Invasion · Migration · FAK · E-cadherin

Introduction

Breast cancer is the most common invasive cancer in developed countries and the leading cause of death among women worldwide [1]. The highest incidence of breast cancer occurs in high-income countries. However, an increase of breast cancer has been reported in low- to middle-income countries, which is the consequence of a variety of factors including the age and lifestyle changes, such as dietary factors [2–4].

Lactoferrin (Lf) is an iron-binding glycoprotein of 80 kDa constituted for a single polypeptide chain, which is folded into two symmetrical lobes that are connected by a hinge region constituted for α-helix regions. The lobes of Lf bind Fe^{2+} or Fe^{3+} ions, however Lf has the ability to reversibly bind Fe^{3+}, and then it is found free of Fe^{3+} (Apo-Lf) or associated with Fe^{3+} (Holo-Lf). The binding of Fe^{2+} or Fe^{3+} to Lf generates a different three-dimensional conformation of the protein [5, 6]. Lf has a high homology between species, transport iron in blood serum and is produced and secreted by mucosal epithelial cells in a variety of mammalian species including cows, goats, horses, humans and some rodents [5]. Moreover, Lf is present in mucosal secretions and body fluids, such as saliva, tears, vaginal fluids, semen, blood plasma, amniotic fluid and is abundant in milk and colostrum [7, 8].

Lf has antimicrobial activity against a variety of bacteria, fungi, yeast, virus and parasites, and regulates the absorption of iron and modulates the immune system [9–12]. Moreover, Lf has protective effects in gastrointestinal cancers, such as cancer of colon, stomach, liver and pancreas. Particularly, Lf inhibits proliferation and Akt activation in SGC-7901 stomach cancer cells, whereas bovine Lf (BLf) from milk decreases viability and proliferation and increase

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apoptosis in breast cancer cells HS578T and T47D [13–15]. Human Lf induces arrest at G1 to S transition of cell cycle, inhibition of Cdk2 kinase activity, Rb hypophosphorylation and reduction of Cdk2 and cyclin E protein levels in breast cancer cells MDA-MB-231 [16, 17]. However, the role of Apo-BLf and Holo-BLf in the inhibition of migration and invasion induced by linoleic acid (LA) and fetal bovine serum (FBS) in breast cancer cells has not been studied.

We demonstrate here that Holo-BLf and Apo-BLf differentially inhibit migration induced by FBS and LA in breast cancer cells MDA-MB-231. Holo-BLf partly inhibit invasion, FAK phosphorylation at tyrosine (Tyr)-397 and metalloproteinase (MMP)-9 secretion, and it also increases the number and size of focal adhesions induced by FBS in MDA-MB-231 cells. Moreover, Holo-BLf induces a slight increase of E-cadherin expression in MCF-7 cells, and inhibits vimentin expression in MCF-7 and MDA-MB-231 breast cancer cells.

Materials and methods

Materials

Apo-BLf (97% purity) was from NutriScience Innovations, LCC (Connecticut, USA). LA sodium salt and tetramethylrhodamine (TRITC)-conjugated phalloidin were from Sigma-Aldrich (St. Louis MO, USA). Anti-vimentin antibody (Ab), anti-E-cadherin Ab and anti-focal adhesion kinase (FAK) Ab were from Santa Cruz Biotechnology (Sta. Cruz, CA, USA). Anti-paxillin Ab was from Abcam® (Waltham, MA, USA). Phospho-specific Ab to tyrosine (Tyr)-397 of FAK (anti-FAK-p-Tyr397) was from Invitrogen (Camarillo, CA, USA). Anti-actin Ab was from R&D Systems, Inc (Minneapolis, MN, USA). FBS was from ByProductos (Mexico).

Preparation of Holo-BLf

Holo-BLf was obtained by saturation of Apo-BLf with iron according to a method previously described [18]. Quantification of iron in Holo-BLf was determined by an enzymatic automated method, (MicroTech Laboratories, Mexico), and it was of 93%.

Cell lines and culture

Human breast cancer cells MDA-MB-231 and MCF-7 were acquired from American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) complemented with 5% FBS, 3.7 g/l sodium bicarbonate and antibiotics. Cultures were incubated under a humidified atmosphere with 5% CO_2 and 95% air at 37 ºC. MDA-MB-231 cells were starved with DMEM without FBS for 24 h, and MCF-7 cells were starved in DMEM without FBS for 18 h before treatment with BLf, FBS and/or LA.

Cell stimulation

Cultures of MDA-MB-231 and MCF-7 cells were washed twice with phosphate-buffered saline (PBS), equilibrated in DMEM for 30 min and then untreated or treated with Apo-BLf, Holo-BLf, FBS or LA. After stimulation, conditioned media were obtained and cells were solubilized in 500 µl ice-cold RIPA buffer (50 mM HEPES pH 7.4, 150 mM NaCl, 1 mM EGTA, 1 mM sodium orthovanadate, 100 mM NaF, 10 mM sodium pyrophosphate, 10% glycerol, 1% Triton X100, 1% sodium deoxycholate, 1.5 mM $MgCl_2$, 0.1% SDS and 1 mM PMSF). Protein concentration of lysates was determined by the micro-Bradford protein assay (Bio-Rad, USA).

Western blot (WB)

Proteins were separated by using 10% SDS-PAGE separating gels, and then proteins were transferred to nitrocellulose membranes. Membranes were blocked with 5% non-fat dried milk in PBS pH 7.2/0.1% Tween 20 (wash buffer) for 2 h at room temperature. Primary Abs were incubated with membranes overnight at 4 ºC, and washed three times with wash buffer. Secondary Abs conjugated to horseradish peroxidase were incubated with membranes for 2 h at room temperature and were washed three times with wash buffer. Immunoreactive bands were visualized using WB luminol reagent and an autoradiography film. Autoradiograms were scanned and bands were analyzed by using the ImageJ software v. 1.52e (NIH, USA).

Scratch-wound assay

Cell cultures were treated with 12 µM mitomycin C for 2 h to inhibit proliferation. Cultures were scratched, washed with PBS and supplemented with serum-free DMEM without or with BLf, FBS and LA for 48 h at 37 ºC. Cultures were photographed with an inverted microscope coupled to a camera. Images from at least three fields per experimental condition were obtained and analyzed using the ImageJ software v. 1.52e (NIH, USA).

Invasion assays

Inserts of 24-well plates were covered with 30 µl Matrigel (3 mg/ml) and incubated overnight at 37 ºC. MDA-MB-231 cells (1×10^5) in FBS-free DMEM were plated on Matrigel

of each insert (Upper chamber). Lower chamber contained 600 µl DMEM without or with Holo-BLf and/or FBS. Plates were incubated for 48 h at 37 °C under a humidified atmosphere with 5% CO_2 and 95% air. After incubation, cells and Matrigel on the upper surface of membranes were removed with cotton swabs, and cells on the lower surface of membranes were washed with PBS and fixed with methanol at room temperature for 5 min. Quantification of invaded cells was obtained by staining of membranes with a solution of crystal violet (0.5%) for 15 min at room temperature and elution of dye with 300 µl acetic acid (10%). Absorbance of samples was measured at 600 nm.

Zymography

Conditioned media were obtained and concentrated with 3,000 NMWL Amicon Ultra Centrifugal filters (Merck Millipore). Equal volumes of non-heated conditioned medium and sampler buffer (2% sucrose ,2.5% SDS, 4 µg/ml phenol red) were mixed and loaded into 8% polyacrylamide gels copolymerized with gelatin (1 mg/ml). Electrophoresis was performed at 72 V for 2 h and gels were rinsed three times with 2.5% Triton X-100 for 30 min, and incubated in assay buffer (50 mM Tris–HCl pH 7.4, 5 mM $CaCl_2$) at 37 °C for 48 h. Gels were fixed and stained with developer solution (0.125% Coomassie Brilliant Blue G-250, 50% acetic acid, 10% methanol). Proteolytic activity was identified as clear bands against the background stain of undigested substrate. Controls of MMP-2 and MMP-9 secretion were obtained by treatment of MDA-MB-231 cells with 400 mg/dl ethanol or 100 ng/ml PDB for 24 h at 37 ºC respectively [19, 20]. Controls were included.

Immunofluorescence confocal microscopy

MDA-MB-231 cells were grown on coverslips, washed with PBS, equilibrated in FBS-free DMEM for 30 min and treated with Holo-BLf and/or FBS for 20 min at 37 °C. Cells on coverslips were fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.5% Triton X-100 for 20 min and blocked with gelatin solution (0.5% gelatin, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$) for 20 min at room temperature. Staining was performed by incubation of cells on coverslips with anti-paxillin Ab (1:1000) for 12 h at 4 °C followed by FITC-labeled anti-mouse secondary Ab for 2 h at room temperature. Fibrillar actin was stained by incubation of cells with TRITC-conjugated phalloidin for 2 h at room temperature. Cells were mounted on glass slides with Vectashield and analyzed by confocal microscopy (Model TCS SP2; Leica Microsystems, Inc). Analysis of images was performed by using the ImageJ software v. 1.52e (NIH, USA).

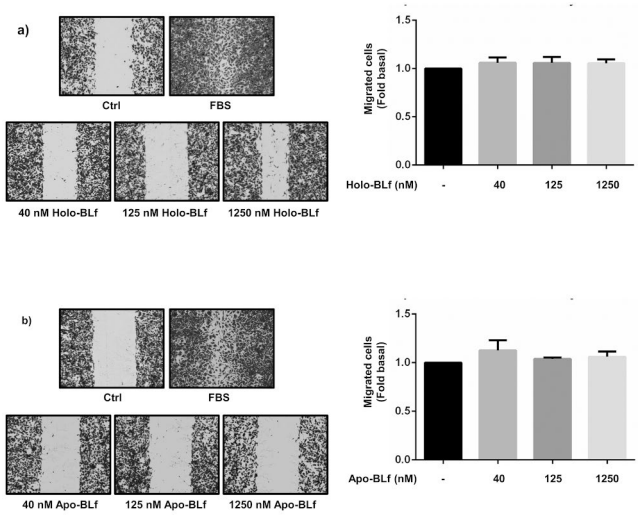

Fig. 1 BLf does not induce migration in MDA-MB-231 breast cancer cells. a and b Migration assays of MDA-MB-231 cells treated with increasing concentration of Holo-BLf and Apo-BLf. One positive control of migration was included (FBS). Graphs are the mean ± S.D. of at least three independent experiments, and indicate the fold of migration above unstimulated cells (Control, Ctrl) value

Statistical analysis

Data are expressed as mean ± SD of at least three independent experiments. Analysis of data was performed by one-way ANOVA and Dunnett's multiple comparison test. Statistical probability of $P < 0.05$ was considered significant.

Results

BLf does not induce migration in MDA-MB-231 breast cancer cells

We determined whether Holo-BLF and Apo-BLf induced migration in breast cancer cells MDA-MB-231. Cultures of MDA-MB-231 cells were scratch-wounded and untreated or treated for 48 h with increasing concentrations of Holo-BLf or Apo-BLf. As illustrated in Fig. 1 A and B, treatment with Holo-BLf and Apo-BLf did not induce migration in breast cancer cells MDA-MB-231.

BLf inhibits migration induced by FBS

Since Holo-BLf and Apo-BLf did not induce migration, we studied whether Holo-BLf and Apo-BLf inhibited migration

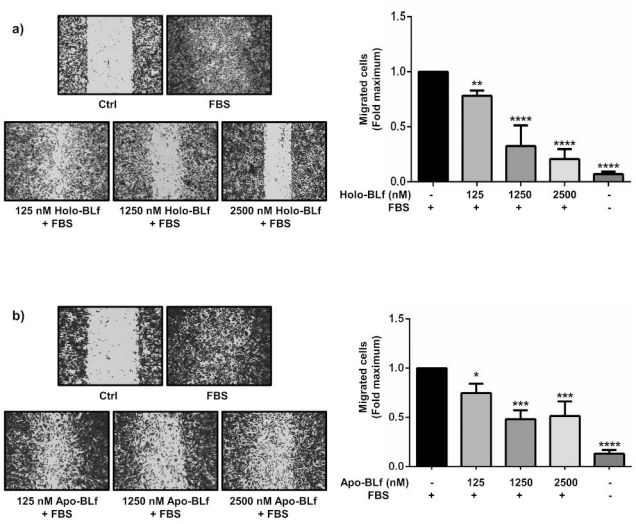

Fig. 2 BLf inhibits migration induced by FBS. a and b Migration assays of MDA-MB-231 cells cotreated with increasing concentrations of Holo-BLf and Apo-BLf and 5% FBS. One positive control of migration was included (FBS). Graphs represent the mean ± S.D. of at least three independent experiments, and indicate the fold of migration above Ctrl value. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

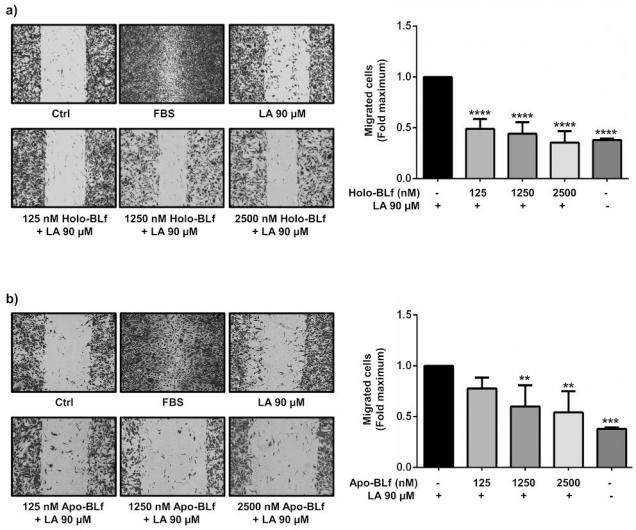

Fig. 3 BLf inhibits migration induced by LA. a and b Migration assays of MDA-MB-231 cells cotreated with increasing concentrations of Holo-BLf and Apo-BLf and 90 µM LA. One positive control of cell migration was included (FBS). Graphs represent the mean ± S.D. of at least three independent experiments, and indicate the fold of migration above Ctrl value. **$P < 0.01$. ****$P < 0.0001$

induced by FBS. Cultures of MDA-MB-231 cells were scratch-wounded and treated without or with 5% FBS and increasing concentrations of Holo-BLf or Apo-BLf for 48 h. Results showed that treatment with Holo-BLf and Apo-BLf partly inhibited the migration induced by FBS in MDA-MB-231 cells. Particularly, treatment with 2500 nM Holo-BLf inhibited FBS-induced migration by ~90%, whereas treatment with 2500 nM Apo-BLf inhibited FBS-induced migration by ~50% in MDA-MB-231 cells (Fig. 2 A and B).

BLf inhibits migration induced by LA

LA induces migration and invasion of MDA-MB-231 cells [21, 22]. We determined whether Holo-BLf and Apo-BLf inhibited migration induced by LA. Cultures of MDA-MB-231 cells were scratch-wounded and treated without or with 90 µM LA and increasing concentrations of Holo-BLf or Apo-BLf for 48 h. Findings demonstrated that treatment with 125 nM and 1250 nM Holo-BLf inhibited LA-induced migration by 90%, whereas treatment with 2500 nM Holo-BLf inhibited LA-induced migration to basal levels in MDA-MB-231 cells. Moreover, treatment with 1250 nM and 2500 nM Apo-BLf inhibited LA-induced migration by 50% in MDA-MB-231 cells (Fig. 3 A and B).

BLf inhibits invasion induced by FBS

Since, Holo-BLf inhibited migration to basal levels, we determined whether Holo-BLf inhibited invasion and secretion of MMP-2 and MMP-9 induced by FBS. Invasion assays were performed with MDA-MB-231 cells cotreated with 2500 nM Holo-BLf and 5% FBS for 48 h. Results demonstrated that treatment with Holo-BLf partly inhibited invasion induced by FBS (Fig. 4 A).

We determined whether Holo-BLf inhibited MMP-2 and MMP-9 secretion induced by FBS. Cultures of MDA-MB-231 cells were cotreated with 2500 nM Holo-BLf and 5% FBS for 48 h, and conditioned media were obtained. Secretion of MMP-2 and MMP-9 was analyzed by gelatin zymography of conditioned media. Findings showed that treatment with Holo-BLf partly inhibited the secretion of active MMP-9 induced by FBS in MDA-MB-231 cells. In contrast, treatment with Holo-BLf did not inhibit secretion of Pro-MMP-9 and MMP-2 induced by FBS in MDA-MB-231 cells (Fig. 4B).

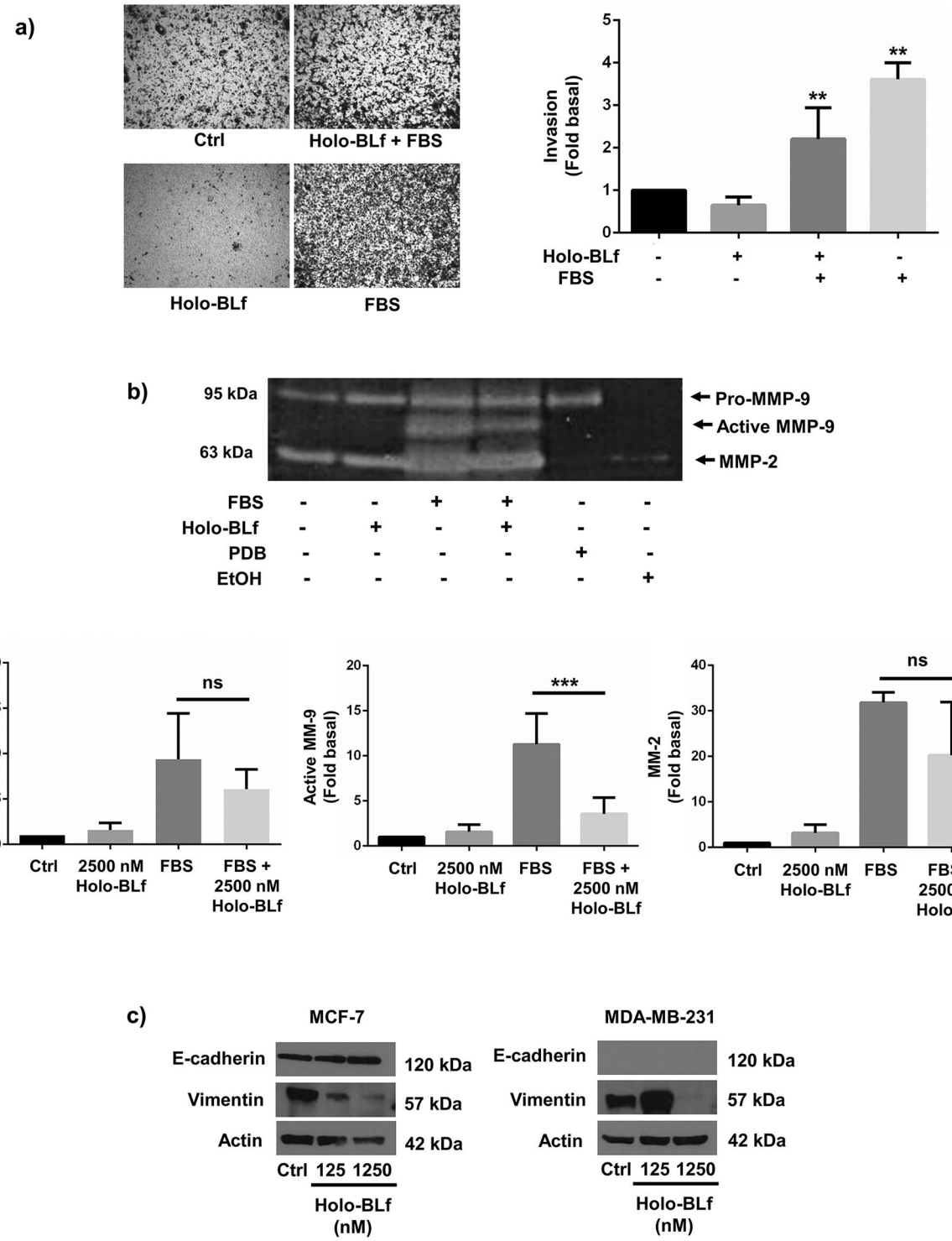

Fig. 4 BLf inhibits invasion and the expression of mesenchymal proteins. **a** Invasion assays of MDA-MB-231 cells cotreated with 2500 nM Holo-BLf and 5% FBS. One positive control of invasion was included (FBS). **b** Analysis of MMP-2 and MMP-9 secretion from MDA-MB-231 cells cotreated with 2500 nM Holo-BLf and 5% FBS. Positive controls of MMP-2 (EtOH) and MMP-9 (PDB) were included. **c** Analysis of E-cadherin and vimentin expression in lysates from MDA-MB-231 and MCF-7 cells treated with 125 nM and 1250 nM Holo-BLf. Graphs represent the mean ± S.D. of at least three independent experiments, and indicate the fold of invasion or MMP-2 and −9 secretion above Ctrl value. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{***}P < 0.001$

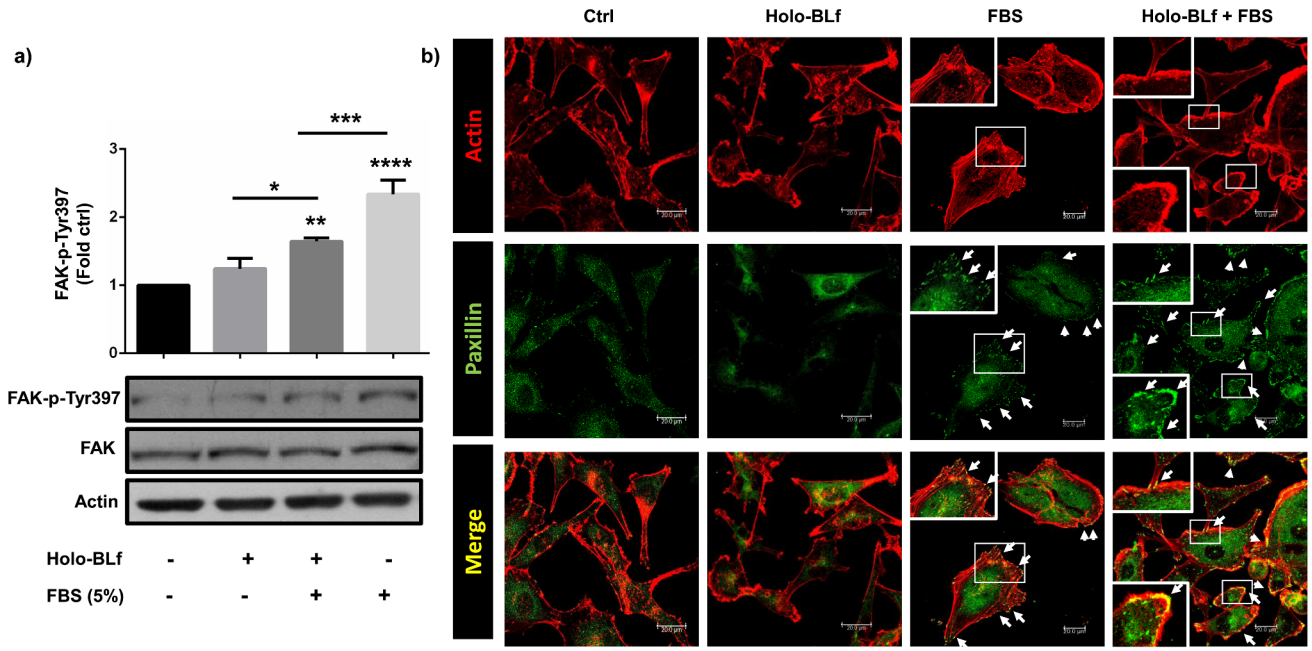

Fig. 5 BLf inhibits FAK activation and induces assembly of focal adhesions. **a** Analysis of FAK-p-Tyr397, FAK and actin in lysates from MDA-MB-231 cells cotreated with 2500 nM Holo-BLf and 5% FBS **b** Immunofluorescence analysis of paxillin (green) and F-actin (red) in MDA-MB-231 cells cotreated with 2500 nM Holo-BLf and 5% FBS. Graph represents the mean ± S.D. of at least three independent experiments, and indicates the fold of FAK phosphorylated at Tyr397 (p-FAk) above Ctrl value. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

BLf regulates the expression of mesenchymal markers associated with the epithelial to mesenchymal transition (EMT) process

EMT process involves migration, invasion and secretion of MMPs [23]. We studied whether Holo-BLf regulates the expression of the epithelial protein E-cadherin and the mesenchymal protein vimentin in breast cancer cells. MDA-MB-231 and MCF-7 breast cancer cells were treated with 125 nM and 1250 nM Holo-BLf for 24 h and cells were lysed. Cell lysates were analyzed by WB with anti-E-cadherin Ab, anti-vimentin Ab and anti-actin Ab as loading control. Findings showed that treatment with Holo-BLf induced a slight increase of E-cadherin expression in MCF-7 cells, however Holo-BLf did not induce E-cadherin expression in MDA-MB-231 cells. In contrast, treatment with 125 nM Holo-BLf partly inhibit vimentin expression in MCF-7 cells, and 1250 nM Holo-BLf completely inhibited expression of vimentin in MCF-7 and MDA-MB-231 cells (Fig. 4 C).

BLf promotes the formation of focal adhesions

We studied whether Holo-BLf regulates FAK activation induced by FBS. FAK activation was analyzed by determination of its phosphorylation at Tyr-397. MDA-MB-231 cells were treated for 1 h with 1250 nM Holo-BLf and then cells were stimulated with 5% FBS for 60 min and lysed. Cell lysates were analyzed by WB with anti-FAK-p-Tyr397 Ab and anti-actin Ab as loading control. Results demonstrated that Holo-BLf partly inhibited FAK phosphorylation at Tyr-397 induced by FBS in MDA-MB-231 cells (Fig. 5 A).

Next, we studied whether Holo-BLf inhibited the formation of focal adhesions induced by FBS. Focal adhesions were analyzed by immunofluorescence analysis of paxillin, because the paxillin protein is concentrated in focal adhesions [24]. MDA-MB-231 cells cultured on coverslips were treated without or with 1250 nM Holo-BLf for 1 h and stimulated with 5% FBS for 20 min. Results showed that FBS induced the assembly of focal adhesions, whereas treatment with Holo-BLf and FBS increased the number and size of focal adhesions in MDA-MB-231 cells (Fig. 5B).

Discussion

Metastasis is a process that implicate the dissemination of cancer cells from primary tumors to distant organs in adenocarcinomas, and it requires the migration and invasion of cancer cells into adjacent tissues and then intravasation into blood and/or lymphatic vessels [25, 26]. Therefore, migration and invasion play a pivotal role in metastasis. BLf (1 µM) enhances migration of WI-38 human fetal fibroblasts, whereas recombinant human Lf (50–200 µg/ml) induces migration of human dermal fibroblasts [27, 28]. In contrast, we demonstrate that Holo-BLf and Apo-BLf do not induce migration in MDA-MB-231 breast cancer cells. Since, human fetal fibroblasts WI-38 and human dermal fibroblasts are not cancerous cells, we propose that BLf induces migration in non-cancer cells but it does not induce migration in breast cancer cells.

An association between diets containing high levels of omega-6 polyunsaturated fatty acids, particularly LA, and an increased risk of developing breast cancer has been suggested [29–32]. LA is an essential fatty acid and represents the main polyunsaturated fatty acid in most Western diets, and these diets increase the risk for development of chronic diseases [29]. Particularly, LA induces FAK and PLD activation, migration and invasion via a FFAR1-, FFAR4- and PI3K/Akt-dependent pathway in MDA-MB-231 cells [21, 22, 33]. We demonstrate that treatment with 2500 nM Holo-BLf completely inhibit the migration induced by LA, whereas treatment with 2500 nM Holo-BLf inhibit the migration induced by FBS by ~90% in MDA-MB-231 cells. However, treatment with 2500 nM Apo-BLf inhibit the migration induced by LA and FBS by ~50% in MDA-MB-231 cells. Since, Apo-BLf and Holo-BLf have a different three-dimensional conformation depending on its binding to Fe^{3+} [5], our results demonstrate that Holo-BLf conformation has a bigger capacity than Apo-BLf conformation to inhibit migration induced by FBS and LA in MDA-MB-231 breast cancer cells. In contrast, it has been reported that treatment with 10 nM Apo-BLf has a bigger capacity than Holo-BLf to inhibit migration induced by FBS in MDA-MB-231 and MCF-7 breast cancer cells [34]. We propose that Apo-BLf and Holo-BLf and their different concentrations used in the different studies show different capacities of inhibition of specific cell processes in breast cancer cells. Supporting our proposal, Holo-BLf has a bigger capacity than Apo-BLf to inhibit proliferation induced by FBS in MDA-MB-231 cells, whereas Holo-BLf and Apo-BLf inhibit proliferation induced by FBS to similar levels in MCF-7 cells [34]. Moreover, Apo-BLf is more cytotoxic than Holo-BLf in MDA-MB-231 and MCF-7 cells [34].

EMT is a cellular process implicated in tumor progression, because through EMT the epithelial cells acquire mesenchymal properties, and then the ability to execute the steps implicated in invasion and metastasis [23]. EMT process is mediated by the loss of apico-basal polarity, disassembly of adherens junctions, MMPs secretion, expression of mesenchymal proteins including vimentin, N-cadherin, α-smooth muscle actin, myosin isoforms, fibronectin and FSP-1, and the loss of epithelial characteristics, such as downregulation of E-cadherin expression [23, 35]. In this study, we analyzed MCF-7 breast cancer cells because they are non-invasive with a low metastatic potential that still have epithelial characteristics including expression of E-cadherin, estrogen and progesterone receptors. However, MCF-7 cells express some mesenchymal markers, such as vimentin. In contrast, MDA-MB-231 breast cancer cells are invasive with high metastatic potential and have mesenchymal characteristics, such as the expression of vimentin and the absence of E-cadherin, estrogen and progesterone receptors expression [36–38]. We demonstrate that Holo-BLf induces downregulation of vimentin expression in MDA-MB-231 and MCF-7 cells. Moreover, Holo-BLf induces a slight increase of E-cadherin expression in MCF-7 cells, however it does not induce E-cadherin expression in MDA-MB-231 cells. We propose that treatment with Holo-BLf promotes the expression of epithelial characteristics in breast cancer cells, such as the expression of E-cadherin and downregulation of vimentin, and then Holo-BLf is able to inhibit the EMT process in breast cancer cells. Supporting our proposal, Holo-BLf induces a higher inhibition of vimentin expression than Apo-BLf in human glioblastoma GL-15 cells, whereas high concentrations of BLf (10 and 100 µg/ml) induce upregulation of E-cadherin expression and downregulation of vimentin expression in malignant oral squamous carcinoma cells HOC3313 [39, 40].

EMT induces the activation of signal transduction pathways that mediate the invasion process through extracellular matrix. Particularly, EMT induces a variety of specific cellular processes, including migration, invasion, focal adhesion assembly and secretion of some MMPs, including MMP-2 and MMP-9 (gelatinases) [35]. We demonstrate that Holo-BLf partly inhibits invasion and MMP-9 secretion induced by FBS in MDA-MB-231 cells. In agreement with our findings, BLf partly inhibits invasion in HOC3313 and SCCVII oral squamous carcinoma cells [39]. Our findings support the proposal that Holo-BLf inhibits the EMT process in breast cancer cells.

Focal adhesions are structures wherein integrin receptors mediate the interaction between the actin cytoskeleton of cells and the extracellular matrix. The composition of focal adhesion is varied and includes scaffolding proteins, adaptor proteins, GTPases, phosphatases and kinases, such as FAK and Src [41, 42]. FAK is a protein tyrosine kinase of 125 kDa, which is activated by a variety of agonists and regulates cell

spreading, differentiation, proliferation, migration, invasion survival and angiogenesis [43, 44]. FAK activation is given by its phosphorylation at Tyr-397, which is a binding site for SH2 domains of proteins including Src family kinases. Particularly, formation of FAK/Src complex, mediated by a SH2 domain, participates in the regulation of assembly and disassembly of focal adhesions, and then it mediates migration, invasion, and then the EMT process [43, 45, 46]. We demonstrate that Holo-BLf inhibits phosphorylation of FAK at Tyr-397, and promotes an increase in the number and size of focal adhesions induced by FBS in MDA-MB-231 cells. We propose that Holo-BLf induces inhibition of migration and invasion through inhibition of FAK activation and the inactivation of proteins that participate in the disassembly of focal adhesions. Supporting our proposal, a peptide corresponding to residues 17–25 of BLf induces paxillin tyrosine phosphorylation in intestinal epithelial cells IEC-6 [47]. These findings support our proposal that Holo-BLf inhibits the EMT process in breast cancer cells.

In conclusion, our findings demonstrate Holo-BLf exhibit a higher capacity of inhibition of migration than Apo-BLf in MDA-MB-231 breast cancer cells. In addition, Holo-BLf inhibits migration and invasion in MDA-MB-231 cells, and the expression of epithelial characteristics in MDA-MB-231 and MCF-7 cells. We propose that Holo-BLf inhibit the EMT process in breast cancer cells.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-022-07943-8.

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Author contributions N R-O, K R-R: design, conduction, data analysis, methodology, validation and manuscript preparation. P C-R, MG, EPS: Conceptualization, Methodology, Writing-Review and editing, Project administration, Funding acquisition, Visualization, Supervision.

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Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no conflicts of interests.

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