Expression of sialic acid α2-3 and α2-6 in MCF cells stimulated with lipopolysaccharide

ABSTRACT

Protein sialylation is an important post-translational modification of glycoproteins that is also involved in tumor progression and metastasis. Increased expression of sialic acid can disrupt receptor-ligand interactions and protect tumor cells from the immune system. In this work, the expression of sialic acid α2-3 and α2-6 in MCF-7 stimulated with LPS at a concentration of 20 ng/ml cells was determined, by flow cytometry, fluorescence microscopy, and RT- qPCR. An increase in the expression of sialic acid α2-3 and α2-6 recognized by *Maackia amurensis* and *Sambuccus nigra* lectins was observed. While the percentage of cells expressing α2, 3 sialic acid recognized by the *Maackia amurensis* lectin remained at 20%, in the case of α2, 6 sialic acid recognized by the *Sambucus nigra* lectin, it was observed that 44% of the population expressed it after 2h of stimulation. The expression of ST3GAL1 and ST6GAL1 was evaluated by RT- qPCR, and the results obtained showed that Lipopolysaccharide did not produce significant changes in ST6GAL1 mRNA levels, while for ST3GAL1 it is observed that Lipopolysaccharide causes an increase in mRNA at 4 hours while at 2 and 6 hours it decreases. Our results suggest that lipopolysaccharide causes an increase in the expression of α2, 3 sialic acid, while it does not cause changes in the expression of α2, 6 sialic acid, further studies are needed to evaluate the effect of protein sialylation caused by lipopolysaccharide.

Keywords: Lipopolysaccharide, Lectin, MCF-7, Sialic Acid.

1. INTRODUCTION

Protein sialylation plays a significant role in regulating cellular properties by interfering with the function of the associated proteins. Altered sialylation status is associated with cancer since sialylation of glycoproteins regulates cellular

properties such as cell–cell and cell–matrix adhesion, migration of cells, response towards extracellular stimuli, and interaction with immune cells (1). Expression of sialic acid is often upregulated in breast cancer, sialic acid shields the cancer cells from immune surveillance by masking tumor epitopes and thereby reduces the antitumor immunity and alternative complement activation (2,3). Sialic acid is involved in the regulation of immune cells by binding to receptors of the SIGLECS (sialic-acid-binding immunoglobulin-like lectins) family of immune effector cells such as NK and T cells by inducing inhibitory tyrosine motifs (4-6). Sialyltransferases play a seminal role in a variety of immunological processes through their modulation of surface receptors. For example, ST6Gal-I, a Golgi sialyltransferase that adds α2–6 linked sialic acids to N-glycosylated proteins, is vital for granulopoiesis, thymopoiesis, B cell proliferation, and antibody production (7-10). ST3GAL1 catalyzes the transfer of sialic acid in an α2,3 linkage to Galβ1-3-GalNAc-Ser/Thr, and thus terminates further chain elongation, except extension with sialic acids (11). The altered expression level or activity of this enzyme might lead to changes in the composition and length of O-glycans attached to mucin-type proteins. ST3GAL1 mRNA expression is elevated in primary breast carcinoma cells compared to normal or benign breast tissues (12). Regulation of sialyltransferase expression is mainly achieved at the transcriptional level according to the physiological or pathological states of the cells and several studies have indicated that the expression of Sialyltransferases could be controlled by steroid hormones in a tissue-specific manner (13). For example, the ST6Gal I) mRNA expression is enhanced by dexamethasone in rat fibroblasts (14). LPS is an important structural component of the outer membrane of Gram-negative bacteria, PS-induced inflammation has been shown to increase the growth of experimental metastases in a murine tumor model and increase angiogenesis in vivo and in vitro (15). Activation of Toll-like receptor 4 signaling and the nuclear factor-κB pathway are involved in increases in LPS-induced metastasis in each process, including tumor cell adhesion and invasion (16,17). The present study aimed to evaluate the expression of sialic acid α2-3 and α2-6 in MCF-7 cells stimulated with lipopolysaccharide.

2. MATERIALS AND METHODS

Chemicals and lectins

Lectins from *Maackia amurensis* and *Sambuccus nigra* labeled with fluorescein were obtained from Vector Laboratories (Burlingame, CA USA). Vectashield [mounting medium with diamidino-2-phenylindole (DAPI) fluorescent dye] was from Vector Laboratories Inc. (Burlingame, CA). Gibco™ Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin solution, and L-glutamine were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Hyclone™ fetal bovine serum (FBS) was obtained from GE Healthcare Life Sciences (Logan, UT, USA). GalNAc and Lipopolysaccharide LPS (from *Escherichia coli* 0111:B4; from Sigma, St Louis, MO, USA) were stored in a stock solution of 1 mg/ml at −20°C until use.

Cell culture

MCF-7 cells were purchased from the American Type Culture Collection and maintained in dulbecco modified Eagles minimal essential medium (DMEM), supplemented with 1% penicillin/streptomycin antibiotics (Gibco CA), 1% glutamine and 10% fetal bovine serum (Gibco CA) in 75 cm culture bottle, incubated at 37 ° C and 5% CO_2, until obtaining a confluence of 80%. For viability determination, cells were incubated with MTT reagent for nearly 3 h.

Cells stimulation with lipopolysaccharide

Total Cells (2×10^5) were seeded in six-well plates until obtaining a confluence of 80 and were treated for 2, 4, and 6 hours with lipopolysaccharide at 20 ng/ml after incubation cells were washed three times with phosphate-buffered saline (PBS) containing. Cells are detached with trypsin at 0.5% and placed in an Eppendorf fixed with 200 µl of 1% formaldehyde for 15 minutes and then washed again with PBS. After LPS stimulation, cells were incubated with *Maackia amurensis* lectin or *Sambuccus nigra* lectin at 5 µg/ml, for flow cytometry or immunofluorescence assays. Cell viability was 95%. Untreated cells were used as a control.

Flow Cytometry analysis

Cells with lipopolysaccharide treatment or without treatment were analyzed by indirect immunofluorescence. In brief, cells were permeabilized with perm/wash buffer (BD Biosciences US) according to the manufacturer's instructions. Total Cells (2 x 10^5) were incubated with 200 µl of *Maackia amurensis* or *Sambuccus nigra* lectins at 5 µg/ml for 90 min at 37°, subsequently, the cells were washed with PBS for 5 minutes and resuspended in 500 µl of PBS. Analysis of the MCF-7 region was performed according to cell size and granularity, Debris and dead cells were excluded using side scatter and forward scatter plots. Ten thousand events were acquired in Attune NxT Cytometer (Applied Biosystems). The FlowJo® software package (version 10.0) was used for analyzing flow cytometry data. Control experiments consisted of incubation of lectin with GalNAc at 200 mM for 30 minutes before adding it to the cells

Immunofluorescence Microscopy

Total Cells (2 x 10^5) were seeded in eight well chamber slides (LAB-TEK Brand Products Thermo Fischer Scientific) until obtaining a confluence of 80% and were treated with lipopolysaccharide at 20 ng/ml during 2, 4, and 6 hrs after incubation cells were washed three times with PBS and permeabilized with perm/wash buffer (BD Biosciences US) according to the manufacturer's instructions. Cells were incubated with 200 µl of Maackia amurensis or Sambuccus nigra lectins at 5 µg/ml for 90 min at 37°, after incubation cells were washed with PBS for 5 minutes. The slides were removed from the incubator and 1µl of DAPI was added and covered with a coverslip. The lectins binding was visualized using a green filter and DAPI was visualized using a blue filter. The slides were viewed under an Axioscop 40 microscope (Zeiss, Germany) and micrographs were analyzed using ZEN 2011 Software (Zeiss, Germany). Control experiments consisted of incubation of lectin with GalNAc at 200 mM for 30 minutes before adding it to the cells.

Design of in-silicon qPCR primers for the ST3GAL1 and ST6GAL1 genes.

Open-access bioinformatics tools such as PIMER 3 PLUS, and PRIMER-BLAST were used. First, the nucleotide sequence of the ST3GAL1 (NM_003033.4) and ST6GAL1 (NM_173216.2) gene was searched in the GENEBANK database, then

the gene sequence in FASTA format was entered in the main menu of the PRIMER 3 PLUS software (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and optimal values for primer design were assigned: Banding temperature: 60˚ C, Prime length. 17-28b, Elongation temperature and time: 70-72 °C for 0.5 to 3 minutes, Number of cycles: 25-30, G-C content 50-60%. For the rest of the parameters, we used the default values provided by the software. The software provides us with our forward and reverse primer pairs. The quality and specificity of the resulting amplicons were analyzed using the Nucleotide-BLAST website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

The primers used are for sialyltransferase α2,3 ST3GAL1 5´-ACA AAG ATG GGC TGA TGA GG-3´F and 5´-AAA TCT GCC CTC CTT TCT CC- 3´R, for sialyltransferase α2,6 ST6GAL1 5´-TGA ATG GGA GGG TTA TCT GC-3´F and 5'-AGA GTT CAT CAG GCG AAT GG-3´R and hypoxanthine phosphoribosyltransferase HPRT 5'-GCC AGA CTT TGT TGG ATT TG-3'F and 5'-CTC TCA TCT TAG GCT TTG TAT TTTG-3'R-

RNAm extraction

MCF-7 cells were detached washed with trypsin 0.25%, and incubated for two minutes at 37°C, then the cells were transferred to an RNAase-free polypropylene centrifuge tube and centrifuged at 2000 rpm for 2 minutes. Cells were lysed with 350 µl of RLT Plus Buffer. The lysate was transferred to a gDNA Eliminator spin column, centrifuged for 30 seconds at 10000rpm, and 350µl of 70% ethanol was added, the sample was then transferred to an RNeas centrifuge column and placed in a 2ml tube and centrifuged for 15 seconds at 10 000 rpm. Washes were then performed to remove contaminants first with 700 µl of RW1 buffer and then with 500µl of RPE buffer. Finally, to elute the RNA, the RNeasy spin column was placed in a new 1.5ml collection tube, 30-50µl RNAase-free water was added directly to the column membrane and centrifuged for 1 min at 10,000 rpm. The concentration and purity of the RNA samples were estimated by absorbance (Abs)

measurements at 260 and 280 nm in the nanophotometer. The determination of the RNA purity of the samples was estimated with the ratio of Abs 260/Abs 280 nm, considering an adequate purity at a ratio higher than 1.7. RNA integrity was verified by 1% agarose gel electrophoresis stained with Biotin Red Gel.

cDNA synthesis.

cDNA synthesis was performed using the RevertAid™ H Minus First Strand cDNA Synthesis kit from Thermo Scientific according to the manufacturer's recommendations. The reaction is performed in a final volume of 20 µL, consisting of oligo dT (1µL), nuclease-free water (20 µL), total RNA (3µg), 5X reaction buffer (4µL), RNAase inhibitor (1 µL), dNTPs mix (2 µL, 10mM) and reverse transcriptase (1µL). The sample was transferred to a thermal cycler and incubated for 60 minutes at 42°C, 5 minutes at 70°C, and finally 5 minutes at 4°C.

RT-PCR

RT-PCR was carried out in a 10 µL volume, in a MiniAmp Plus Thermal Cycler (Applied Biosystems technology, Thermo Fisher Scientific, MOD: A37029, Singapore). PCR Master Mix 2X (5µL), sense and antisense oligos of ST3GAL1 and ST6GAL1 (1 µL, 10mM), cDNA (1µL), and nuclease-free water (2µL) were incorporated into the reaction mixture. The thermal parameters of the reaction were an incubation at 95°C for 2 minutes, 30 cycles of denaturation at 95°C for 30 seconds, an alignment at 57°C for 30 seconds, and an extension at 72°C for 30 seconds, plus a final extension at 72°C for 5 minutes. RT-PCR products were analyzedanalyzed on 1% agarose gels, stained with Biotin Red Gel (0.3µL), and visualized on a UV transilluminator.

RT-qPCR.

Real-time quantitative PCR was performed using Thermo Scientific PCR Master Mix SYBR Green/ROX qPCR (2X) kit #K0221 in a total volume of 10 µl, using PCR master mix SYBR Green/ROX qPCR (2X) which contains: TaqDNA polymerase, reaction buffer (contains KCl and (NH4)2SO4), passive reference dye ROX, as well as 0. 3 mM of each dNTPs (dATP, dCTP, dGTP, and dTTP) and SYBR Green I.

The forward and reverse oligonucleotide sequences of ST6GAL1 and ST3GAL1, each at 10 mM concentration, cDNA and nuclease-free water were added. HPTR was used as an endogenous gene to normalize the expression of transcripts of interest. The reaction mixture was placed in the Applied Biosystems StepOne thermal cycler (StepOne™ Real-Time PCR System, Thermo Fisher Scientific, mod. 4376357), using the following cycling program: An initial denaturation cycle at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 25 seconds, alignment at 60°C for 30 seconds and an extension phase at 70°C for 30 seconds. In addition, to verify that there were no non-specific products or primer dimers, a Melting curve or dissociation curve of 40 cycles at 95°C for 15 seconds and 60°C for 1 minute per cycle was integrated at the end of each amplification program.

Statistical analysis

All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) software (version 16; SPSS Inc., Chicago, IL, USA) to demonstrate that the data groups came from the same population; a unidirectional-univariate analysis performed using the non-parametric Kruskal-Wallis test (from William Kruskal and W. Allen Wallis). To isolate the group or groups that differ from the others (statistically significant difference), a multiple comparison procedure (Tukey's test) was used.

3. RESULTS

Figure 1 shows the results of α2,3-sialic acid expression in MCF-7 cells stimulated with LPS at 2,4 and 6 h. The results showed that α2,3-sialic acid recognized by MAA lectin (*Maackia amurensis*) was expressed in the plasma membrane of MCF-7 cells, in cells that did not receive stimuli and in cells stimulated with LPS at 2, 4, and 6 hours showed an increase α2,3-sialic acid expression recognized by lectin

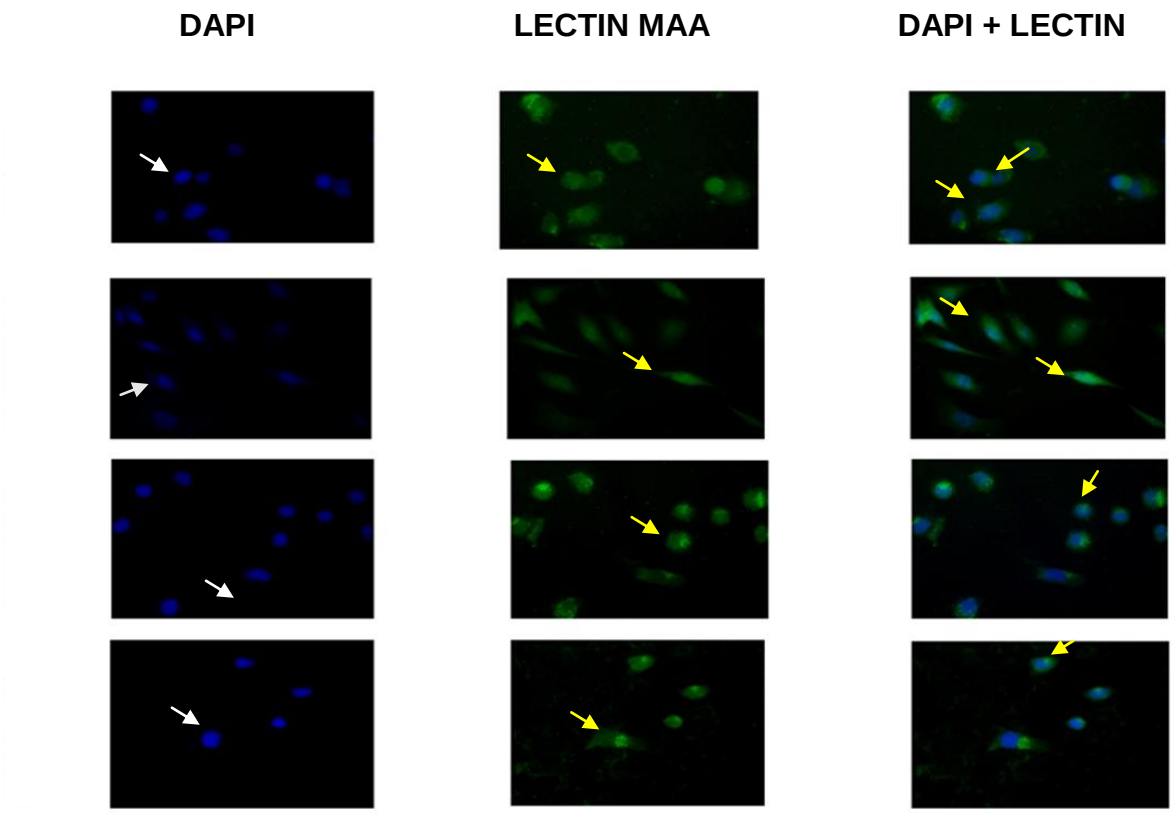

Figure 1 Effect of lipopolysaccharide (LPS) on α2,3-sialic acid expression in MCF-7 cells. MCF-7 cells were stimulated with LPS 20 ng/ml at 2.4 and 6 hours, then incubated with *Maackia amurensis* lectin (MAA), and finally MCF-7 cells were stained with DAPI (4',6-diamidino-2-phenylindole) to mark nuclei, which are shown in blue. The first column of the image shows the DAPI-labelled nuclei, the second column shows the lectin labeling in green and the third column shows the colocalization. The white arrow indicates nucleus recognition. The yellow arrow indicates lectin recognition. Micrographs at 40X.

Figure 2 shows the results of α2,6-sialic acid expression in MCF-7 cells stimulated with LPS at 2,4 and 6 hours. The results showed that α2,6-sialic acid recognized by MAA lectin SNA (Sambuccus nigra) was expressed in the plasma membrane of MCF-7 cells, in cells that did not receive stimuli and in cells stimulated with LPS at 2, 4, and 6 hours showed an increase α2,6-sialic acid expression recognized by lectin

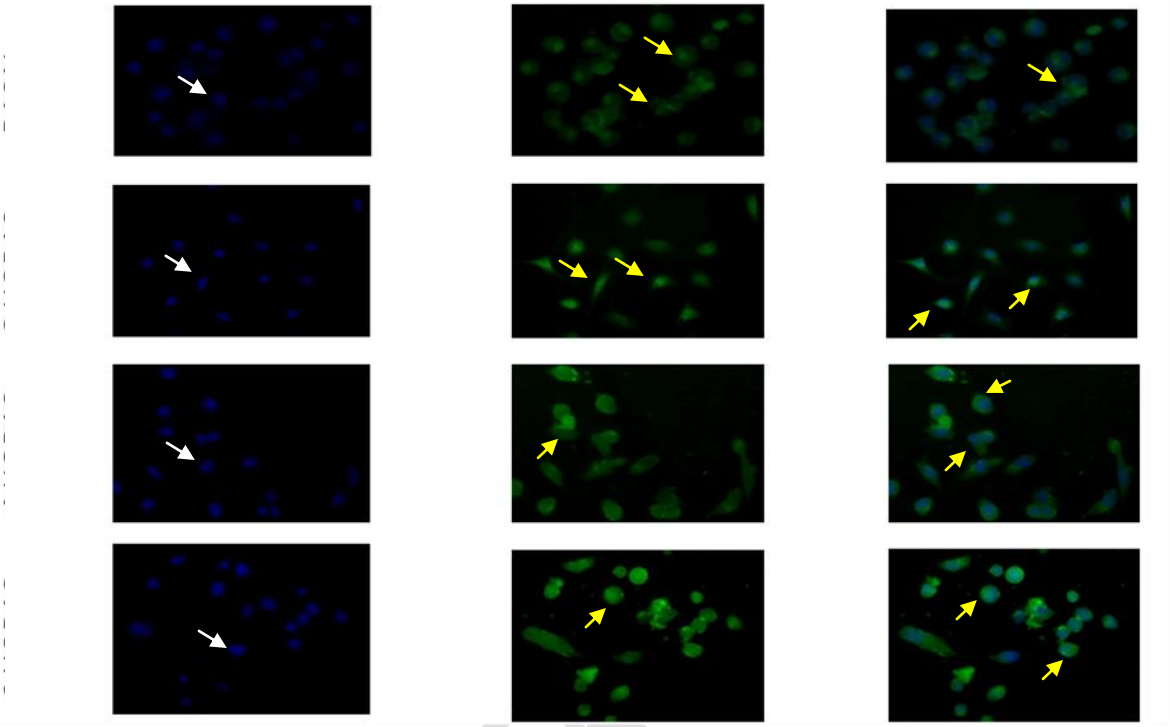

Figure 2 Effect of lipopolysaccharide (LPS) on α2,6-sialic acid expression in MCF-7 cells. MCF-7 cells were stimulated with LPS 20 ng/ml at 2.4 and 6 hours, then incubated with *Sambuccus nigra* lectin (SNA), and finally MCF-7 cells were stained with DAPI (4',6-diamidino-2-phenylindole) to mark nuclei, which are shown in blue. The first column of the image shows the DAPI-labelled nuclei, the second column shows the lectin labeling in green and the third column shows the colocalization. The white arrow indicates nucleus recognition. The yellow arrow indicates lectin recognition. Micrographs at 40X.

Figure 3**.** Shows the flow cytometry results for the expression of α2,3 sialic acid recognized by Maackia amurensis lectin (MAA) and α2, 6 sialic acid recognized by Sambucus nigra lectin (SNA). In the case of *Maackia amurensis* lectin (MAA), the results showed that on average 18.6% of the population expressed sialic acid in unstimulated cells, while at 2 hours 20.8% of the cells expressed sialic acid, at 4 hours of 18% the cells expressed sialic acid and at 6 hours 37.5% % of the cells expressed sialic acid (Figure 3 A).

In the case of *Sambucus nigra* lectin (SNA), the results showed that on average 19.1% of the population expressed sialic acid in unstimulated cells, while at 2

hours 44.9% of the cells expressed sialic acid, at 4 hours 42.8% of the cells expressed sialic acid and at 6 hours 51.1% of the cells expressed sialic acid. (Figure 3 B). Statistical analysis revealed no significant changes when cells stimulated at 2, 4, and 6 hours with LPS were compared.

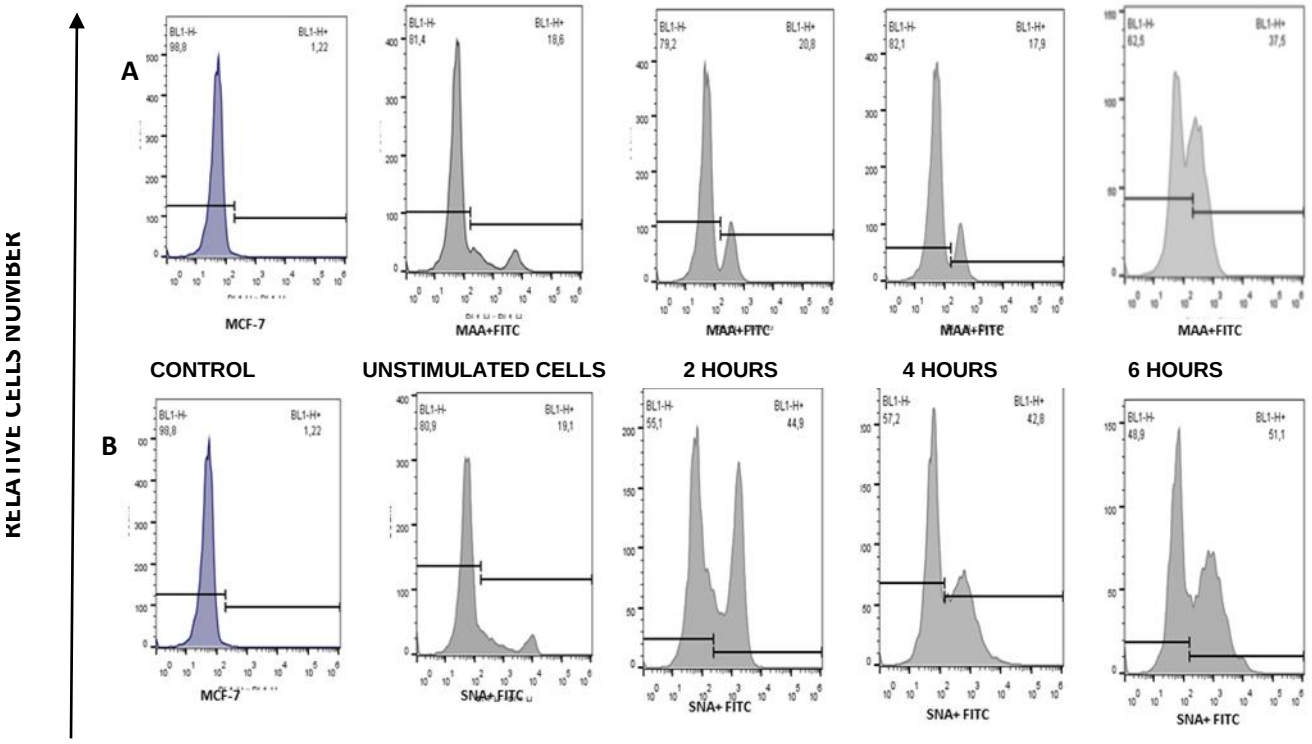

Figure 3 Effect of LPS on α2,3 and α2,6 sialic acid expression in the breast cancer cell line MCF-7 by flow cytometry. MCF-7 cells were treated with 20 ng/ml LPS for 2,4 and 6 h. Control and treated cells were incubated for 1.5 h under dark conditions with FITC-conjugated *Maackia amurensis* (MAA) lectin and FITC-conjugated *Sambucus nigra* (SNA) at 5 µg/ml. In A results for the expression of α2,3 sialic acid recognized by *Maackia amurensis* lectin (MAA). 18.6% of the population expressed sialic acid in unstimulated cells, while at 2 hours 20.8% of the cells expressed sialic acid, at 4 hours 18% the cells expressed sialic acid and at 6 hours 37.5% % of the cells expressed sialic acid. In B 19.1% of the population expressed sialic acid in unstimulated cells, while at 2 hours 44.9% of the cells expressed sialic acid, at 4 hours 42.8% of the cells expressed sialic acid, and at 6 hours 51.1% of the cells expressed sialic acid. The results are from a representative experiment of three independent experiments.

Figure 4 shows the amplification of ST6GAL1 and the amplification of ST3GAL1 by RT-PCR. A) Shows the amplifications of ST6GAL1 B) shows the amplifications of ST3GAL1. In both cases lane 1 shows the molecular weight markers, lane 2 shows the negative control, Lane 3 the positive control (reaction mixture with primers), lane 4 shows unstimulated cells MCF-7 cells, lanes 5, 6, and 7 show MCF-7 cells stimulated with LPS incubated for 2, 4 and 6 hours respectively. In both cases, the band size was as expected, for ST3GAL1 180 bp and ST6GAL1 156 bp.

A) B)

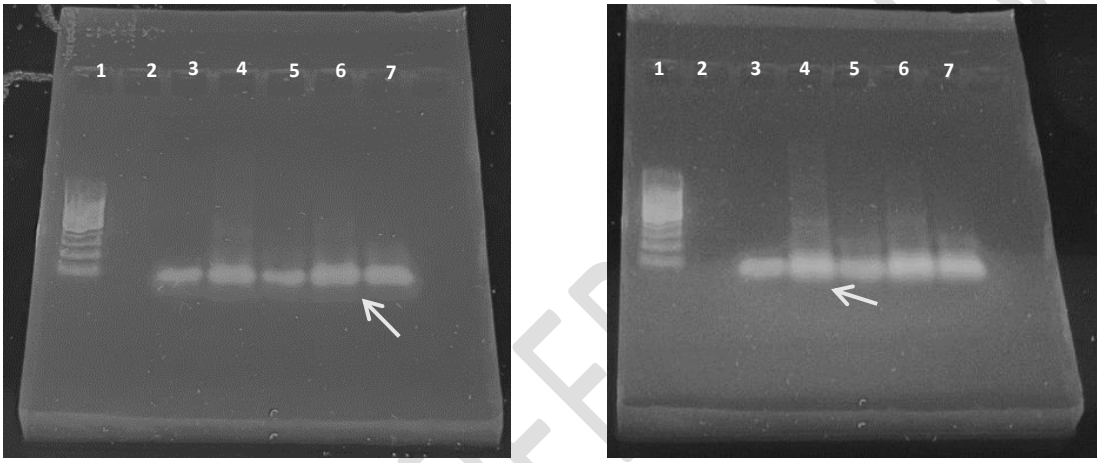

Figure 4. Agarose gel electrophoresis of polymerase chain reaction products of the ST6GAL1 and ST3GAL1. A) Shows the amplifications of ST6GAL1. B) shows the amplifications of ST3GAL1. In both cases lane 1 shows the molecular weight markers, lane 2 shows the negative control, Lane 3 the positive control (reaction mixture with primers), lane 4 shows unstimulated cells MCF-7 cells, lanes 5, 6, and 7 show MCF-7 cells stimulated with LPS incubated for 2, 4 and 6 hours respectively. In both cases, the band size was as expected, for ST3GAL1 180 bp and ST6GAL1 156 bp. The white arrow indicates the position of the amplified product. The results are from a representative experiment of three independent experiments.

Real-time RT- qPCR.

RT-qPCR was performed to validate the endogenous gene and to quantify the st3gal1 and st6gal1 genes in MCF-7 cells stimulated with LPS. Quantification of gene expression was analyzed using the delta. Delta Ct($\Delta\Delta$Ct)- method, where ΔCt represents the difference between the cycle threshold of the gene of interest and that of the endogenous control genes; based on these results, one-way analysis of

variance (ANOVA) was performed using Tukey's multiple comparison test, with a significant difference of $P<0.05$ and a 95% confidence interval. The results obtained indicate that LPS did not produce significant changes in the mRNA levels of the st6gal1 gene in the MCF-7 cell line. For the st3gal1 gene, LPS decreased mRNA expression at 2 and 6 hours, however, at 4 hours in MCF-7 cells, an increase in the level of st3gal1 mRNA was observed (Figure 5).

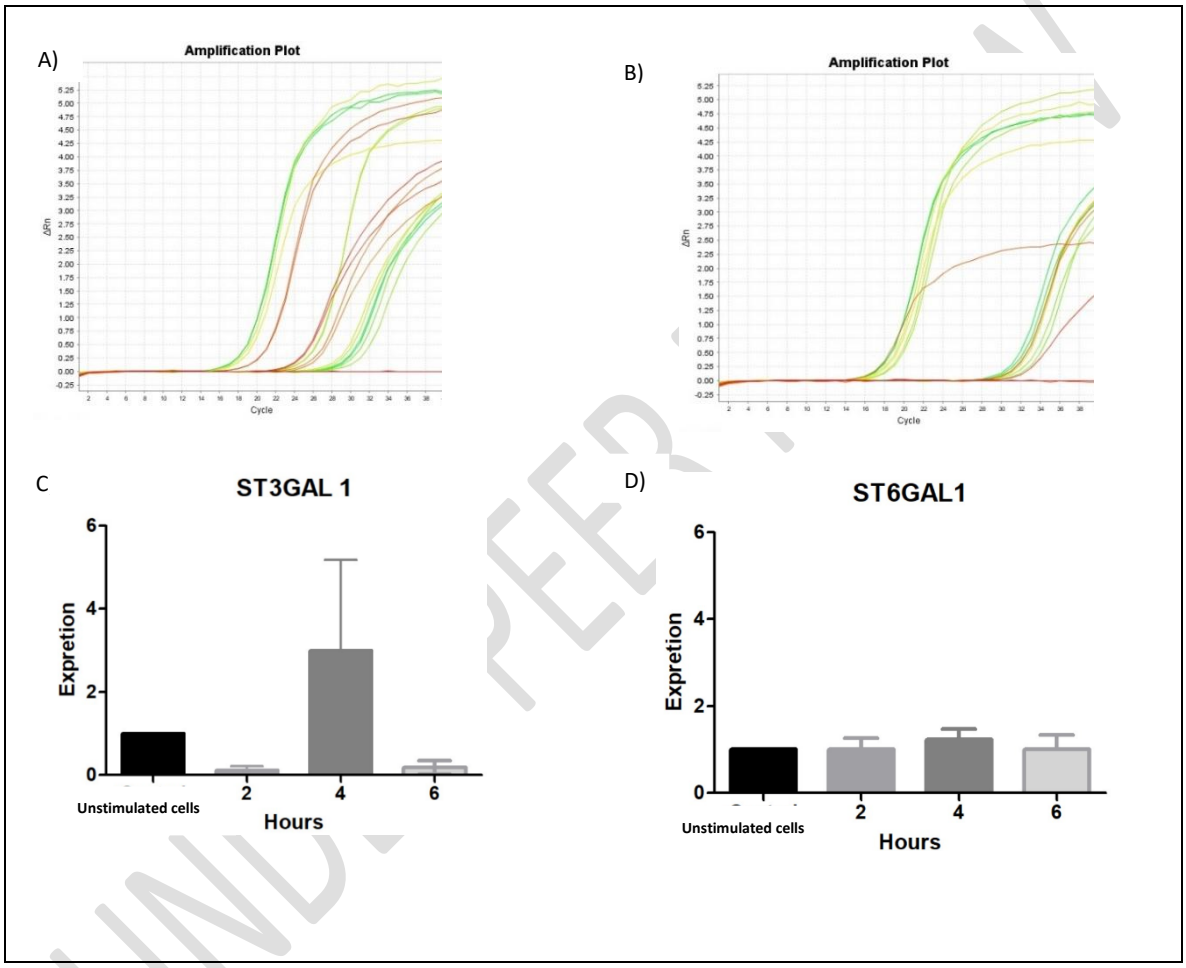

Figure 5. Effect of LPS on ST3GAL1 and ST6GAL1 expression in the MCF-7 cells. ST3GAL1 and ST6GAL1 mRNA expression was determined by real-time qPCR. In the image A and B the amplification curve of ST3GAL1 and ST6GAL1 is represented respectively, the maximum amplification threshold for each experimental condition as well as the amplification of the endogenous HPRT gene; in the ¨Y¨ axis the amplification of the mRNA is indicated, and in the ¨X¨ axis the cycles of the reaction. In images C and D the results of the statistical analysis are shown, the TC data for each gene were normalized to HPRT expression and compared to untreated cells. Each bar represents the mean +/- standard deviation. For n= 3 experiments: $p < 0.05$.

4. DISCUSSION

Cell surface carbohydrates are characteristic of the cell type and are specifically expressed according to the stage of development of the cells or tissues (18). In neoplasms, this normal cell expression is modified, which favors tumor progression in some cases due to interactions of carbohydrates with ligands that promote dissemination or alter the normal recognition by components of the immune response against tumor (19). Sialylation of glycoconjugates is mediated by different sialyltransferase enzymes that can establish different types of bonds, for example, an α2-3 or α2-6 bond to galactose (Gal); through an α2-6 bond with N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc); or an α2-8 bond with another sialic acid, forming poly(sialic acid) (20). The mechanisms that lead to altered glycan structures in cancer cells involve changes in epigenetics, genetic mutations, dysregulated expression of glycosyltransferase and chaperone genes, and mislocalization of glycosyltransferases (21). These changes can be studied using sialic acid-specific lectins.

In the present study, the expression of α2,3 and α2,6 sialic acid in MCF-7 cells when treated with 20 ng LPS was assessed using fluorescence microscopy and cytometry. α2,3 and α2,6 sialic acid was expressed in the plasma membrane of MCF-7 cells at 2 hours, while lower amounts could be observed in unstimulated cells, as well as LPS-treated MCF-7 cells at 4 and 6 hours, similar results were reported by Akasov R (22). The expression of α2,3 sialic acid recognized by *Maackia amurensis* lectin (MAA) 18.6% of the population expressed sialic acid in unstimulated cells, while at 2 hours 20.8% of the cells expressed sialic acid, at 4 hours of 18% the cells expressed sialic acid and at 6 hours 37.5% % of the cells expressed sialic acid. The expression of α2,3 sialic acid recognized *by Sambucus nigra* lectin (SNA), 19.1% of the population expressed sialic acid in unstimulated cells, while at 2 hours 44.9% of the cells expressed sialic acid, at 4 hours 42.8% of the cells expressed sialic acid and at 6 hours 51.1% of the cells expressed sialic

acid. Populations expressing α2,3-sialic acid increase at six hours of LPS treatment, while populations expressing α2,6-sialic acid increase at two hours of LPS treatment, decreasing at four and six hours of treatment, identifying the possibility of differential expression of the sialyltransferases

The relevance of sialyltransferases in cancer is assessed at the mRNA level, so qPCR in this study shows that LPS decreases ST3GAL1 mRNA expression at 2 and 6 hours, but at 4 hours in MCF-7 cells an increase in ST3GAL1 mRNA level is detected, while no significant changes in ST6GAL1 mRNA expression were observed. ST3GAL1 mRNA expression is elevated in primary breast carcinoma cells compared to normal or benign breast tissues (23). High expression of α2,3-sialic acid residues in breast cancer is associated with metastatic potential (24). Cell surface alpha 2,6-sialylation contributes to cell-cell and cell-extracellular matrix adhesion of tumor cells (25). GFRA1 has been identified as a substrate of ST3GAL1, which mediates O-glycan sialylation, facilitating its interaction with RET, thereby regulating phosphorylation and signaling of the GDNF/GFRA1/RET pathway in breast cancer cells (26).

LPS enhances the invasiveness and metastasis of breast cancer cells by activating multiple inflammatory and oncogenic signaling pathways (27). Sialylation at the α2-6 position of TLR4 enhances LPS-dependent signaling, in U937 and BMDM cells, ST6Gal-I activity was observed to selectively promote the sustained activation of three signaling nodes, TNF/NFkB, LPS/NFkB, and LPS/STAT3 (28). Sialic acid at position α2-3 in TLR4 is important for TLR signaling via TLR (29). TLR4 as well as MD2 contain α2,6-linked sialic acid residues while CD14 is sialylated with α2,3-glycosidic linkage (30).

Further studies are needed to determine the mechanisms promoted by LPS to favor ST3Gal I and ST6Gal I expression, as well as the correlation with TLR4 sialylation and integrins related to adhesion, metathesis, and invasion.

5. CONCLUSIONS

Our results suggest that LPS induces expression of ST3Gal I, which may lead to sialylation of TLR4 or adhesion-promoting integrins, metastasis, and invasion.

REFERENCES

1. - Nardy A.F.F.R., Freire-De-Lima L., Freire-De-Lima C.G., Morrot A. The Sweet Side of Immune Evasion: Role of Glycans in the Mechanisms of Cancer Progression. Front. Oncol. 2016; 6:207691. doi: 10.3389/fonc.2016.00054**.**

2.- Zhang Z., Wuhrer M., Holst S. Serum sialylation changes in cancer. Glycoconj. J. 2018; 35:139–160. doi: 10.1007/s10719-018-9820-0

3.- Lübbers J., Rodríguez E., Van Kooyk Y. Modulation of Immune Tolerance via Siglec-Sialic Acid Interactions. Front. Immunol. 2018; 9:2807. doi: 10.3389/fimmu.2018.02807.

4.- Pearce O., Läubli H. Sialic acids in cancer biology and immunity. Glycobiology. 2015; 26:111–128. doi: 10.1093/glycob/cwv097

5.- Rodriguez E., Schetters S.T.T., Van Kooyk Y. The tumour glyco-code as a novel immune checkpoint for immunotherapy. Nat. Rev. Immunol. 2018; 18:204–211. doi: 10.1038/nri.2018.3

6.- Tanida S., Akita K., Ishida A., Mori Y., Toda M., Inoue M., Ohta M., Yashiro M., Sawada T., Hirakawa K., et al. Binding of the Sialic Acid-binding Lectin, Siglec-9, to the Membrane Mucin, MUC1, Induces Recruitment of β-Catenin and Subsequent Cell Growth. J. Boil. Chem. 2013; 288:31842–31852. doi: 10.1074/jbc.M113.471318.

7.- Dougher CWL, Buffone A Jr., Nemeth MJ, Nasirikenari M, Irons EE, Bogner PN, et al. The blood-borne sialyltransferase ST6Gal-1 is a negative systemic regulator of granulopoiesis. J Leukoc Biol. 2017; 102(2):507–16.

8.- Marino JH, Tan C, Davis B, Han ES, Hickey M, Naukam R, et al. Disruption of thymopoiesis in ST6Gal I-deficient mice. Glycobiology. 2008; 18:719–26.

9.- Hennet T, Chui D, Paulson JC, Marth JD. Immune regulation by the ST6Gal sialyltransferase. Proc Natl Acad Sci U S A. 1998; 95(8):4504–9.

10.- Jones MB, Nasirikenari M, Lugade AA, Thanavala Y, Lau JT. Anti-inflammatory IgG production requires functional P1 promoter in beta-galactoside alpha2,6-sialyltransferase 1 (ST6Gal-1) gene. J Biol Chem. 2012; 287(19):15365–70.

11.- Harduin-Lepers A, Vallejo-Ruiz V, Krzewinski-Recchi M.A, Samyn-Petit B, Julien S, Delannoy P. The human sialyltransferase family, Biochimie. 2001; 727-737

12.- Burchell J, Poulsom R, Hanby A, Whitehouse C, Cooper L, H. Clausen D, Miles, Taylor-Papadimitriou J. An alpha 2,3 sialyltransferase (ST3Gal I) is elevated in primary breast carcinomas, Glycobiology. 1999; 1307-1311.

13.- Coughlan CM, Seckl JR, Fox DJ, Unsworth R, Breen KC. Tissue-specific regulation of sialyltransferase activities in the rat by corticosteroids in vivo. Glycobiology. 1996 Jan; 6(1):15-22. doi: 10.1093/glycob/6.1.15.

14.- Vandamme V, Pierce A, Verbert A, and Delannoy, P. (1993), Transcriptional induction of β-galactoside α-2,6-sialyltransferase in rat fibroblast by dexamethasone. European Journal of Biochemistry, 211; 135-140. doi.org/10.1111/j.1432-1033.1993.tb19879.x

15.- Wang EL, Qian ZR, Nakasono M, Tanahashi T, Yoshimoto K, Bando Y, Kudo E, Shimada M, Sano T. High expression of Toll-like receptor 4/myeloid differentiation factor 88 signals correlates with poor prognosis in colorectal cancer. Br J Cancer. 2010; 102(5):908-15. doi: 10.1038/sj.bjc.6605558.

16.- Liu X, Liang J, Li G. Lipopolysaccharide promotes adhesion and invasion of hepatoma cell lines HepG2 and HepG2.2.15. Mol Biol Rep. 2010; 37(5):2235-9. doi: 10.1007/s11033-009-9710-4.

17.- Brown DM, Ruoslahti E. Metadherin, a cell surface protein in breast tumors that mediates lung metastasis. Cancer Cell. 2004; 5(4):365-74. doi: 10.1016/s1535-6108(04)00079-0

18. Lis H, Sharon N. Protein glycosylation. Structural and functional aspects. Eur J Biochem. 1993; 218(1):1-27. doi: 10.1111/j.1432-1033.1993.tb18347.x.

19. Varki A. Biological roles of glycans. Glycobiology. 2017; 27(1):3-49. doi: 10.1093/glycob/cww086

20. Videira PA. ST3Gal.I sialyltransferase relevance in bladder cancer tissues and cell lines. BMC cancer. 2009; 9, 357. doi.org/10.1186/1471-2407-9-357.

21. Munkley J, Elliott DJ. Hallmarks of glycosylation in cancer. Oncotarget. 2016; 7;7(23):35478-89. doi: 10.18632/oncotarget.8155.

22. Akasov R, Haq S, Haxho F, Samuel V, Burov SV, Markvicheva E, Neufeld RJ, Szewczuk MR. Sialylation transmogrifies human breast and pancreatic cancer cells into 3D multicellular tumor spheroids using cyclic RGD-peptide induced self-assembly. Oncotarget. 2016; 4;7(40):66119-66134. doi: 10.18632/oncotarget.11868.

23. Burchell J, Poulsom R, Hanby A, Whitehouse C, Cooper L, Clausen H, Miles D, Taylor-Papadimitriou J. An alpha2,3 sialyltransferase (ST3Gal I) is elevated in primary breast carcinomas. Glycobiology. 1999; 9(12):1307-11. doi: 10.1093/glycob/9.12.1307

24. Cui H, Lin Y, Yue L, Zhao X, Liu J. Differential expression of the α2,3-sialic acid residues in breast cancer is associated with metastatic potential. Oncol Rep. 2011; 25(5):1365-71. doi: 10.3892/or.2011.1192.

25. Lin S, Kemmner W, Grigull S, Schlag PM. Cell surface alpha 2,6 sialylation affects adhesion of breast carcinoma cells. Exp Cell Res. 2002; 276(1):101-10. doi: 10.1006/excr.2002.5521

26. Fan TC, Yeo HL, Hsu HM, Yu JC, Ho MY, Lin WD, Chang NC, Yu J, Yu AL. Reciprocal feedback regulation of ST3GAL1 and GFRA1 signaling in breast cancer cells. Cancer Lett. 2018; 434:184-195. doi: 10.1016/j.canlet.2018.07.026.

27. Wilkie T, Verma AK, Zhao H, Charan M, Ahirwar DK, Kant S, Pancholi V, Mishra S, Ganju RK. Lipopolysaccharide from the commensal microbiota of the breast enhances cancer growth: role of S100A7 and TLR4. Mol Oncol; 2022 16(7):1508-1522. doi: 10.1002/1878-0261.12975.

28. Holdbrooks AT, Britain CM, Bellis SL. ST6Gal-I sialyltransferase promotes tumor necrosis factor (TNF)-mediated cancer cell survival via sialylation of the TNF receptor 1 (TNFR1) death receptor. J Biol Chem. 2018; 293(5):1610-1622. doi: 10.1074/jbc.M117.801480.

29. Amith SR, Jayanth P, Franchuk S, Finlay T, Seyrantepe V, Beyaert R, Pshezhetsky AV, Szewczuk MR. Neu1 desialylation of sialyl alpha-2,3-linked beta-galactosyl residues of TOLL-like receptor 4 is essential for receptor activation and cellular signaling. Cell Signal. 2010; 22(2):314-24. doi: 10.1016/j.cellsig.

30. Feng C, Stamatos NM, Dragan AI, Medvedev A, Whitford M, Zhang L, Song C, Rallabhandi P, Cole L, Nhu QM, Vogel SN, Geddes CD, Cross AS. Sialyl residues modulate LPS-mediated signaling through the Toll-like receptor 4 complex. PLoS One. 2012; 7(4):e32359. doi: 10.1371/journal.pone.