The International Journal of Biochemistry & Cell Biology 44 (2012) 1244–1253

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Glycomic alterations are associated with multidrug resistance in human leukemia

Zhaohai Zhang[a,b,1], Yongfu Zhao[c,1], Lili Jiang[d], Xiaoyan Miao[a], Huimin Zhou[e], Li Jia[a,*]

[a] College of Laboratory Medicine, Dalian Medical University, Dalian 116044, Liaoning Province, China
[b] Institute of Clinical Laboratory, Jinshan Hospital of Benxi, Benxi 117000, Liaoning Province, China
[c] Department of General Surgery, the Second Affiliated Hospital of Dalian Medical University, Dalian 116044, Liaoning Province, China
[d] Department of Foreign Language, Dalian Medical University, Dalian 116044, Liaoning Province, China
[e] Department of Microbiology, Dalian Medical University, Dalian 116044, Liaoning Province, China

ARTICLE INFO

Article history:
Received 3 February 2012
Received in revised form 19 April 2012
Accepted 30 April 2012
Available online 11 May 2012

Keywords:
Glycomics
Glycan
FITC–lectin
Multidrug resistance
Human leukemia cell lines

ABSTRACT

Correlations of disease phenotypes with glycosylation changes have been analyzed intensively in tumor biology field. In this study we describe glycomic alterations of multidrug resistance in human leukemia cell lines. Using multiple glycan profiling tools: real-time PCR for quantification of glycogenes, FITC–lectin binding for glycan profiling, and mass spectrometry for glycan composition, we compared the glycomics of drug-resistant K562/ADR cells with parental K562 line. The results showed that the expression of glycogenes, glycan profiling and N-glycan composition were different in K562/ADR cells, as compared with those in K562 cells, whereas O-glycans of the two cell lines showed no different mass spectra. Further analysis of the N-glycan regulation by way of tunicamycin application or PNGase F treatment in K562/ADR cells showed partial inhibition of biosynthesis and increased sensitivity to chemotherapeutic drugs *in vitro*. We targeted glycogene B3GNT8 and ST8SIA4, which were over-expressed in K562/ADR cells, and silenced the expression levels of two glycogenes after using RNA interference approach. The results showed that the silencing of B3GNT8 or ST8SIA4 in K562/ADR cells resulted in increased chemosensitivity to anti-tumor drugs. In conclusion, glycomic alterations are responsible for the overcoming multidrug resistance in human leukemia therapy and the N-linked oligosaccharides are associated with the drug resistance of cancer cells.

1. Introduction

Protein glycosylation is one of the major types of post-translational modifications with profound biological implications (Bertozzi and Kiessling, 2001; Solís et al., 2001). Glycan interactions mediate biological events such as trafficking, signaling, folding and adhesion, in addition to processes such as cell development, and differentiation (Varki, 1993; Rudd et al., 2001; Dove, 2001; Haltiwanger and Lowe, 2004; Mitra et al., 2006). Aberrant glycosylation is known to be associated with various human diseases and cell surface sialylation as well as β1–6 branching of N-linked glycans are strongly correlated with metastatic potential of cancer cells (Dennis et al., 1982, 1987). Additionally, compared with O-glycans from normal cells, mucin type O-glycans from cancer cells can be highly sialylated but less sulfated (Brockhausen, 2006). Thus, alterations to N- and/or O-glycans found in glycoproteins derived from cancer cells are a common feature and can be used as biomarkers.

Multidrug resistance (MDR) and disease relapse are challenging clinical problems in the treatment of cancers (Sharom, 2008). Several mechanisms involving changes in the expression of drug transporting pumps (Kvackajova-Kisucka et al., 2001) and drug metabolizing enzymes (Bosch, 2008) as well as changes in apoptosis regulatory pathways (Inoue et al., 2001) have been described to induce MDR. Furthermore, a number of studies have aimed to identify those glycans that are expressed specifically by chemo resistant tumor cells (Nakahara et al., 2003; Kudo et al., 2007). Lattová et al. reported that N-glycomic changes in human breast carcinoma MCF-7 and T-lymphoblastoid cells occurred after treatment with herceptin and herceptin/Lipoplex (Lattová et al., 2010).

Abbreviations: MDR, multidrug resistance; ADR, adriamycin; TM, tunicamycin; PBS, phosphate-buffered saline; PBST, PBS containing 0.1% Tween 20; TBS, tris buffered saline; FITC, fluorescein isothiocyanate; MALDI-TOF MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; P-gp, P-glycoprotein; DMSO, dimethyl sulfoxide; siRNA, small interfer RNA; MTT, methyl thiazolyl tetrazolium; PNGase F, peptide N glycosidase F; IC_{50}, 50% growth inhibition; Hex, hexose; HexNAc, N-acetylhexosamine; Man, mannose; GlcNAc, N-acetylglucosamine; NeuAc, N-acetylneuraminic acid.

* Corresponding author at: College of Laboratory Medicine, Dalian Medical University, 9 Lvshunnan Road Xiduan, Dalian 116044, Liaoning Province, China. Tel.: +86 411 86110386.
E-mail address: jiali@dlmedu.edu.cn (L. Jia).

[1] These two authors contributed equally to this work.

1357-2725/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biocel.2012.04.026

Table 1
Primer sets of real-time RT-PCR.

Glycogene	Primer sequence (forward, reverse)
CHST2	5′-GGGCGCAACCTCACCACG-3′; CCACGA AAGGCTTGGAGGAGG-3′
ST3GAL5	5′-GCACGGATTAGAACTGGG-3′; 5′-CGCCCTCTGGATAAGTCAT-3′
GCNT2	5′-AGACTTACAGATTTTGACGGT-3′; 5′-TAGATATTTTGGGGCATGTA-3′
GALNT1	5′-TTCCCAGCGACTCCAGAAACAC-3′; 5′-TGGGATAACCTGCATCCACGGA-3′
B3GALT1	5′-TCGCCCTACTTCTTCTTACA-3′; 5′-AGATTTGGCTCTCTTGCTCC-3′
ST3GAL1	5′-TTCCTCACCTCCTTCTTCCTGAACTAC-3′; 5′-TCTTCTCCAGCATAGGGTCCACATTCC-3′
ST8SIA4	5′-TCCGTCATTGAGACTTATTCAT-3′; 5′-CACATTTAATGTTTTGAATTCT-3′;
FUT8	5′-CCTGGCGTTGGATTATGCTCA-3′; 5′-CCCTGATCAATAGGGCCTTC-3′
B3GNT8	5′-GGCCTGACCTAGACTCACTAGTG-3′; 5′-CGCAGTGCGGTCTGCTGGCCAG-3′
ST3GAL4	5′-ATGAGCAGATCACGCTCAAGTCCA-3′; 5′-TCCCATCTCCAGCATCCGCTTAAT-3′
ST6GAL1	5′-GTGGGCACAAAAACTACCAT-3′; 5′-GGCTCTGGGCTCATAAACTG-3′
B4GALT1	5′-AACTTGACCTCGGTCCCAGTGC-3′; 5′-GGCCGCCCATCTTCACATTTG-3′
GAPDH	5′-CAGCGACACCCACTCCTC-3′; 5′-TGAGGTCCACCACCCTGT-3′

Increased levels and defective glycosylation of multidrug resistance associated with proteins (MRPs) in ovarian carcinoma oxaliplatin resistant cells have been reported (Beretta et al., 2010). Fiala et al. have also described considerably reduced levels of UDP-sugars in L1210/VCR cells compared to L1210 cells, which reflects the lower contents of glycoproteins and polysaccharides (Fiala et al., 2003) and N-glycan alterations are associated with drug resistance in human hepatocellular carcinoma (Kudo et al., 2007). Taken together, these indicate the existence of differences between sensitive and resistant cells in the content and composition of cell surface sugars.

Many studies have followed the evaluation of glycan changes in proteins during diseases from a variety of biological samples using different proteomic approaches. Glycogenes, which encode proteins, are involved in glycan synthesis and modification, and almost the entire human glycogenes have been cloned, including those encoding glycosyltransferases, glycolytic enzymes, sugar nucleotide synthetases, sugar nucleotide transporters and, in a broader sense, sugar-chain recognizing molecules, and glycoconjugates themselves (Mantelli et al., 2009). Real-time PCR, being both sensitive and accurate, is one of the most popular validation protocols for mRNA quantification analysis (Kudo et al., 2007) and we used it in this study to analyze the expression levels of glycogenes.

Lectins are carbohydrate-binding proteins or glycoproteins of non-immune origin that recognize and reversibly bind to glycans without altering their covalent structure. Plant lectins are important tools in cell biology and immunology, with potential for clinical application (Kim et al., 2009; Naeem et al., 2007). In this study, FITC–lectin binding was used for glycan profiling of cell membrane glycoproteins in K562 and K562/ADR cell lines. Additionally, a high sensitive mass spectrometry was used to provide the most effective means of both identification and quantitation of N-glycans or O-glycans in glycomics studies (Zaia, 2004).

The objective of the present study was to examine glycomic alterations between the K562 and the K562/ADR cell lines by using multiple glycan profiling tools, namely real-time PCR, FITC–lectin binding and mass spectrometry, in order to investigate whether glycans participate in the regulation of tumor multidrug resistance. Our results provide further evidence that the differential expressions of glycans are responsible for tumor MDR through alternated regulation of glycans in human MDR K562/ADR cells.

2. Materials and methods

2.1. Cell culture

Human leukemia cell line K562 was obtained from the KeyGEN Company (China). The cell line was cultured in 90% RPMI 1640 (Gibco) supplemented with antibiotics (1× penicillin/streptomycin 100 U/ml, Gibco) and 10% heat-inactivated fetal bovine serum (Gibco). Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Adriamycin (Sigma) was added to K562 cell cultures in stepwise increasing concentrations to develop a drug resistant cell subline (K562/ADR). To maintain the MDR phenotype, the medium of the K562/ADR cells was supplemented with 1.0 mg/l adriamycin. The K562/ADR cells were cultured for one week in drug-free medium prior to their use in each experiment.

2.2. Analysis of glycogenes

To investigate the expression profiles of genes related to glycan synthesis, a real time RT-PCR analysis was performed. Total RNA were isolated from the two cell lines by way of an RNeasy Mini Kit (QIAGEN) and cDNA was synthesized by QuantiTect Reverse Transcription Kit (QIAGEN) from 5 μg of total RNA according to the manufacturer's instruction. Real-time PCR amplification and analysis were performed on 7500 fast Real-time PCR System (Applied Biosystems) for 40 cycles (15 s at 95 °C, 15 s at 60 °C and 30 s at 72 °C). All reactions were performed with QuantiTect SYBR Green PCR Kit (QIAGEN) according to the manufacturer's instruction. Primer sequences were summarized in Table 1. Expression levels of each glycogene were normalized using either the expression level of GAPDH mRNA and compared between K562 and K562/ADR cell lines. Real-time RT-PCR analysis was performed in triplicate.

To further investigate the expression profiles of enzyme, a western blot analysis was performed. Whole cell proteins were electrophoresed under reducing conditions in 10% polyacrylamide gels. The separated proteins were transferred to a polyvinylidene difluoride membrane. After blocking with 5% skimmed milk in PBS containing 0.1% Tween 20 (PBST), the membrane was incubated with antibody (1/200 diluted, Santa Cruz Biotech and Abcam), and then with peroxidase-conjugated anti-rabbit IgG (1/10,000 diluted; GE Healthcare UK Ltd., Little Chalf-ont, UK). A GAPDH antibody (1/200 diluted; Santa Cruz Biotech) was used as a control. All bands were detected using ECL Western blot kit (Amersham Biosciences, UK), according to the manufacturer's instruction. The bands were analyzed with LabWorks (TM ver4.6, UVP, BioImaging systems).

2.3. Membrane protein extract

A total of 1×10^7 cells were washed with phosphate buffered saline (PBS) and lysed on a plate with lysis and separating buffer containing a protease inhibitor cocktail. Cell membrane proteins were extracted from the cell suspension using a CelLytic MEM Protein Extraction kit (Sigma, St. Louis, MO, USA). The membrane protein concentration was measured with a Micro BCA Protein Assay kit (PIERCE, Rockford, IL) and used for further experiments as described below.