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Identification of O- and N-glycosylated nuclear proteins of HL-60 cells induced to granulocytic differentiation

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Many eukaryotic proteins contain both O- and N-linked N-acetylglucosamine groups. The effects of O- and N-glycosylation on the growth and differentiation of the human promyelocytic leukemia cell line HL-60 have been studied to examine whether the glycosylation of nuclear proteins is needed for HL-60 cells to differentiate into granulocyte-like cells. All-trans-retinoic acid was used for differentiation induction. O- and N-glycosylation of nuclear proteins was inhibited by HL-60 cell treatment with tunicamycin and isopropylgalactoside, respectively. We demonstrated that the level of both O- and N-glycosylation increased during granulocytic differentiation. In addition, by MALDI-TOF we identified five nuclear proteins that underwent glycosylation upon inducing the granulocytic differentiation of HL-60 cells.

Key words: nuclear proteins, glycosylation, granulocytic differentiation, HL-60 cells

INTRODUCTION

The post-translational modification of O-linked and N-linked glycosylations is found in nuclear and cytoplasmic proteins. The first modification occurs on serine and threonine residues and the second one on asparagine residue [1]. It is known [2] that O-glycosylation is an important regulatory modification that may have a reciprocal relationship with O-phosphorylation and modulate many biological processes in eukaryotes. Activation of protein kinase A or C, for example, results in reduced levels of O-GlcNAc specifically in the fraction of cytoskeletal and cytoskeleton-associated proteins, while inhibition of the same kinases results in increased levels of O-glycosylation [3]. N-Linked sugar chains were first released quantitatively as oligosaccharides by enzymatic and chemical means. It was found that expression of many glycoproteins was altered by aging. However, the role of glycosylations is not yet clear.

To study changes of glycosylation of nuclear proteins during the differentiation process, we chose proliferating and terminally differentiated HL-60 cells. The HL-60 cell line was isolated from a patient with promyelocytic leukemia [4]. The majority of this cell population is at the promyelocytic stage of development, but they can be chemically induced to differentiate into several pathways [4, 5]. Continuous incubation with retinoic acid (RA) promotes granulocytic differentiation of HL-60 cells [6].

In the present study, we examined O- and N-linked glycosylations of nuclear proteins in proliferating and differentiated HL-60 cells. Also, we identified by MALDI-TOF five nuclear proteins that underwent glycosylation after induction of granulocytic differentiation of HL-60 cells.

MATERIALS AND METHODS

Cell culture. Human promyelocytic leukemia HL-60 cells were cultured in RPMI 1640 medium (Gibco BRL, Life Technologies) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (NordCell, Sweden) in a 5% CO_2-supplemented incubator at 37 °C. Granulocytic differentiation was induced with 1 μM retinoic acid (RA, Sigma). O-glycosylation was inhibited with 1 mM isopropylthiogalactoside (IPTG). N-glycosylation was inhibited with 0.5 μM tunicamycin (Tu).

Isolation of nuclear proteins. Nuclear proteins were isolated as described by Kulyte et al [7].

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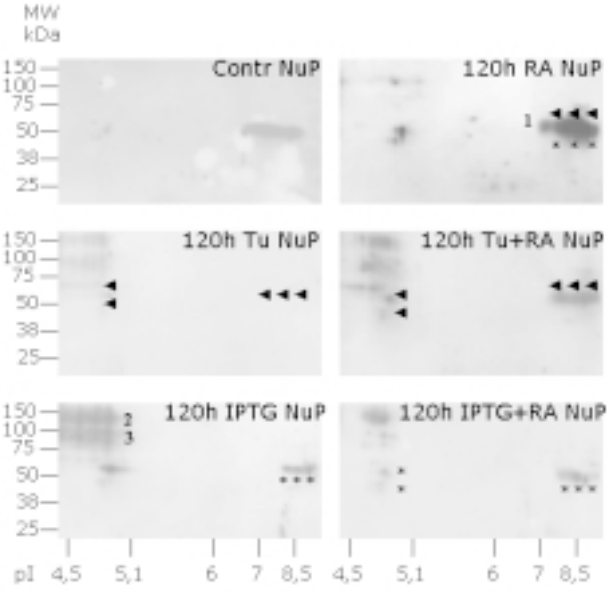
Gel electrophoresis and immunoblotting. The nuclear proteins were resolved by two-dimensional gel electrophoresis (IEF/SDS). Immobiline DryStrip Kit, pH range 3–10, and Exel Gel SDS, gradient 8–18% (Pharmacia Biotech, Uppsala, Sweden) were used for 2-DE. It was performed according to the manufacturer's instructions (Immobiline DryStrip Kit for 2-D Electrophoresis with Immobiline DryStrip and ExelGel SDS Instructions, Pharmacia Biotech). For analysis of glycosylated nuclear proteins after two-dimensional electrophoresis, the proteins were transferred on an Immobilon™ PVDF transfer membrane (Millipore) and then blocked with 3% gelatine dissolved in TBS containing 0.1% Tween-20 (TTBS) by incubation 1 h at room temperature. After washing in TBS-Tween-20, the filters were probed with WGA, 1:500 in TTBS for 1 h at room temperature. Afterwards the filters were incubated with streptavidin-HRP in TTBS 1:4000 for 1 h at room temperature. The immunoreactive spots were detected by enhanced chemiluminescence (Amersham, Life Science) following the manufacturer's instructions.

MALDI-TOF analysis. In-gel digestion and mass spectrometry analysis were performed as described in [8].

RESULTS AND DISCUSSION

The lectin wheat germ agglutinin (WGA) recognizes clustered terminal N-acetylglucosamine (GlcNAc) [9]. After nuclear protein fractionation on 2-DE we examined them with WGA. As can be seen in Fig. 1, after 120 h of RA treatment glycosylation increased in comparison with control proliferating HL-60 cells. For elucidation of the origin of glycosylated nuclear proteins we used tunicamycin (inhibitor of N-glycosylation) and IPTG (inhibitor of O-glycosylation). We have shown that during granulocytic differentiation nuclear proteins with 45 kDa, pI 4.9, 52 kDa, pI 4.95 and 65 kDa, pI 7.2–9.0 undergo O-glycosylation (Figure, marked by arrows-head), others with 45 kDa, pI 4.9, 52 kDa, pI 4.95 and 47–52 kDa, pI 8.0–9.0 (Figure, marked by stars) undergo N-glycosylation. As one can see from molecular masses and pI, the same nuclear proteins with acidic pI undergo both O- and N-linked glycosylations. We found that inhibition of O- and N-glycosylations did not effect the differentiation of HL-60 cells (data not shown), suggesting the importance of glycosylation for granulocytic differentiation of HL-60 cells.

In addition, nuclear proteins specifically bound to WGA were supplied for MALDI-TOF analysis.



Two-dimensional electrophoretic patterns of glycosylated nuclear proteins of proliferating, differentiating and treated with inhibitors of glycosylation HL-60 cells. Total nuclear proteins were isolated from proliferating (Contr) cells, HL-60 cells induced to differentiation with retinoic acid (120 h RA), cells treated with inhibitors of N-glycosylation (120 h Tu) and O-glycosylation (120 h IPTG) and HL-60 cells treated both for differentiation and inhibition of glycosylation (Tu + RA, IPTG + RA). The proteins were fractionated by 2-DE and then analyzed with WGA as described in "Materials and Methods". Arrowhead show proteins which are O-glycosylated, stars – N-glycosylated. Proteins which were supplied for MALDI-TOF MS are indicated by numbers.

Several proteins (Figure, marked by numbers) were identified: 1) myeloperoxidase heavy chain, 53 kDa, pI 9.4; 2) nucleoporin NUP 159 kDa; 2) nucleoporin NUP 157, 156 kDa, pI 4.5–5.1; 3) thrombospondin 2, 127 kDa, pI 4.62 and/or translation initiation factor, 122 kDa, pI 5.25. Other glycosylated proteins were impossible to identify because of a very small amount of the protein; even the glycosylation signal after analysis with WGA was strong.

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O- IR N-GLIKOZILINTŲ BRANDUOLIO BALTYMŲ IDENTIFIKAVIMAS HL-60 LĄSTELĖSE

S a n t r a u k a

Tirtas HL-60 ląstelių branduolio baltymų O- ir N-glikozilinimas granuliocitinės diferenciacijos metu. Granuliocitinės diferenciacijos indukcijai buvo panaudota retinoinė rūgštis. Glikozilinimo kilmė nustatyta O- ir N-glikozilinimo inhibitoriais. Taip pat, naudojant MALDI-TOF MS metodą, buvo identifikuoti keli glikozilinti branduolio baltymai.