Changes in O- and N-glycosylation of cytosolic proteins in proliferating HL-60 cells and in those induced to granulocytic differentiation

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Glycoproteins are classically regarded as components of the plasma membrane and extracellular spaces. The notion that glycoproteins occur in the nuclear and/or cytoplasmic portions of the cell has only recently gained wide acceptance. The studies reported here were designated to evaluate the effect of tunicamycin (Tu) and isopropylthiogalactoside (IPTG) alone and in combination with retinoic acid (RA) on HL-60 cell growth and differentiation and cytosolic protein glycosylation. Tu was used to inhibit protein N-glycosylation. O-linked acetylglucosamine was inhibited by treatment with IPTG. We have shown that only the inhibitor of N-glycosylation markedly decreased the viability of HL-60 cells. The level of differentiation increased slightly (up to 20%) when the inhibitor of N-glycosylation was used. We found that both O- and N-linked glycosylation of cytosolic proteins was increased in RA-treated HL-60 cells. The degree of both O- and N-glycosylation of HL-60 cell cytosolic proteins was RA-dependent.

Key words: glycosylation, cytosolic proteins, RA, tunicamicyn, isopropylgalactoside, HL-60

INTRODUCTION

Dynamic glycosylation on nuclear and cytoplasmic proteins is abundant in all multicellular eukaryotes. The major glycans of glycoproteins can be classified into two groups according to their glycan–peptide linkage regions. Those that are linked to the asparagine residues of polypeptides are termed N-glycans, while others which are linked to serine or threonine residues are called O-glycans. N-glycans are essential for the normal development of mammalians [1, 2]. O-linked modification occurs on numerous cellular proteins. Like phosphorylation, O-linked N-acetylglucosamine (GlcNAc) is highly dynamic, transiently modifying proteins. Only a small portion of these proteins has been identified, including several RNA polymerase II transcription factors, heat-shock, cytoskeletal proteins, etc. [3]. It has been suggested that O-glycosylation can compete with phosphorylation on serines and threonines. Many glycoproteins contain one or more glycan chain per molecule. In many cases that depend on differentiation and malignant transformation of cells [4]. For these studies we have used the human promyelocytic HL-60 cell line, which was induced for granulocytic differentiation with retinoic acid (RA).

Here we examine the glycosylation of cytosolic proteins upon induction of granulocytic differentiation of HL-60 cells. Studies involving the viability and differentiation of HL-60 cells treated with inhibitors of glycosylation (Tu, IPTG) alone and its synergistic effect with granulocytic differentiation are also described. We showed that Tu and IPTG alone did not affect a viability of HL-60 cells. However, the percentage of viable cells decreased when RA was used together with glycosylation inhibitors. Also, in RA-treated culture O- and N-glycosylation was less inhibited by IPTG or Tu, respectively, compared to untreated controls, suggesting that RA may have an impact on both O- and N- glycosylation of cytosolic proteins.

MATERIALS AND METHODS

Cell culture. Human promyelocytic leukemia HL-60 cells were cultured in RPMI 1640 medium (Gibco

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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 isopropylgalactoside (IPTG). N-glycosylation was inhibited with 0.5 μM tunicamycin (Tu). The extent of differentiation was assayed by the ability of the cells to reduce NBT to insoluble blue-black formazan on stimulation with PMA [5]. The viability of cells was determined by the exclusion of 0.2% trypan blue.

Isolation of cytosolic proteins. Cytosolic proteins were isolated as described by Kulyte et al. [6].

Gel electrophoresis and immunoblotting. The cytosolic 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), was 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). To analyze the total glycosylation of cytosolic proteins, the proteins after fractionation on 2-DE gels were transferred onto an Immobilon PVDF membrane and analyzed with WGA (Sigma) according to the manufacturer's instructions. The immunoreactive spots were detected by enhanced chemiluminescence (Amersham, Life Science) following the manufacturer's instructions.

RESULTS AND DISCUSSION

Viability and differentiation of proliferating HL-60 cells and those after treatment with RA and glycosylation inhibitors. Typically, the viability of the cells remained above 95–90% up to 72 h and then decreased to 65% between 72 and 96 h (Fig. 1A). The number of differentiated cells increased from 6–8% at 48 h to 50–55% at 96 h after induction of differentiation (Fig. 1B). HL-60 cells were also treated with 0.5 μM Tu and 1 mM IPTG alone and in combination with 1 μM RA to evaluate the viability and differentiation of these cells. As one can see in Fig. 1A, IPTG did not affect the viability of HL-60 cells. The inhibition of N-glycosylation inhibited HL-60 cell growth and viability in comparison with proliferating cell culture. After a 4-day treatment of HL-60 cells with RA and Tu or IPTG, their viability decreased by to 65% and 80%, respectively. The level of granulocytic differentiation increased after inhibition of N-glycosylation to 15% (Figure 1B). We also showed that Tu with RA synergistically affected the granulocytic differentiation

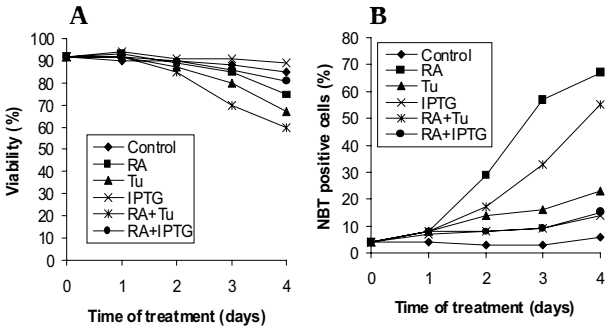

Fig. 1. HL-60 cell differentiation and viability after treatment with retinoic acid (RA) and glycosylation inhibitors. HL-60 cells were exposed to 1 μM RA or/and 0.5 μM Tu, or/and 1 mM IPTG and the viability was estimated after staining with trypan blue (A) and tested for the ability of mature granulocytes to reduce nitroblue tetrazolium (B). Each point represents the mean of 3 separate experiments

of HL-60 cells (up to 85% after 4 days of treatment).

O- and N-glycosylation of cytosolic proteins of HL-60 cells. We examined changes in glycosylation of cytosolic proteins of HL-60 cells treated for granulocytic differentiation and with inhibitors of N- and O-glycosylation. Figure 2 demonstrates that af-

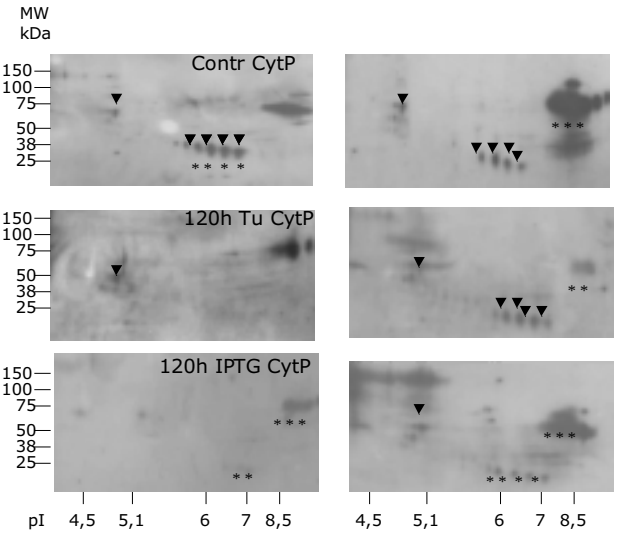

Fig. 2. Two-dimensional electrophoretic patterns of glycosylated nuclear proteins of HL-60 cells (proliferating, differentiating and treated with glycosylation inhibitors). Total nuclear proteins were isolated from proliferating (Contr) cells, HL-60 cells induced to differentiation with retinoic acid (120 h RA), cells treated with inhibitors of N-glycosylation (120 h Tu) and O-glycosylation (120 h IPTG) and HL-60 cells treated for both differentiation and inhibiton of glycosylation (Tu+RA, IPTG+RA). The proteins were fractionated by 2-DE and then analyzed with WGA as described in Materials and Methods. Arrow-heads show O-glycosylated and stars N-glycosylated proteins

ter induction of granulocytic differentiation cytosolic protein with 60 kDa, pI 7–8.5 underwent abundant N-glycosylation, and proteins with molecular mass 25–30 kDa, pI 5.7–7.0 and 60 kDa, pI 5.0 underwent O-glycosylation. From the experiments when N- and O-glycosylation inhibitors were used (Fig. 2, Tu+RA, IPTG+RA) but still N- and O-glycosylated proteins, respectively, were detected, we conclude that this effect was caused by retinoic acid, which might act through the mechanisms of glycosylation.

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CITOZOLIO BALTYMŲ O- IR N-GLIKOZILINIMO POKYČIAI PROLIFERUOJANČIOSE IR INDUKUOTOSE GRANULIOCITINEI DIFERENCIACIJAI HL-60 LĄSTELĖSE

S a n t r a u k a

Šiame darbe įvertintas proliferuojančių, indukuotų granuliocitinei diferenciacijai ir paveiktų su glikozilinimo inhibitoriais HL-60 ląstelių augimas bei diferenciacija. Nustatyta, jog N-glikozilinimo lėtinimas labai sumažina ląstelių augimą, tuo tarpu ląstelių diferenciacija išauga iki 20%. Taigi HL-60 ląstelių citozolio baltymų O- ir N-glikozilinimas sustiprėja paveikus retinoine rūgštimi.