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SCIENCE DIRECT

Experimental Cell Research 303 (2005) 14–21

Experimental Cell Research

www.elsevier.com/locate/yexcr

Nuclear CD38 in retinoic acid-induced HL-60 cells

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Received 7 August 2004, revised version received 7 September 2004
Available online 26 October 2004

Abstract

The cell surface antigen, CD38, is a 45-kDa transmembrane protein which is predominantly expressed on hematopoietic cells during differentiation. As a bifunctional ectoenzyme, it catalyzes the synthesis of cyclic ADP-ribose (cADPR) from NAD^+ and hydrolysis of either NAD^+ or cADPR to ADP-ribose. All-*trans*-retinoic acid (RA) is a potent and specific inducer of CD38 in myeloid cells. In this report, we demonstrate that the nuclei of RA-treated human HL-60 myeloblastic cells reveal enzymatic activities inherent to CD38. Thus, GDP-ribosyl cyclase and NAD^+ glycohydrolase activities in the nuclear fraction increased very significantly in response to incubation with RA. With Western blotting, we detected in the nuclear protein fraction from RA-treated cells a ~43-kDa protein band which was reactive with the CD38-specific monoclonal antibody OKT10. The expression of CD38 in HL-60 nuclei was also shown with FACScan analysis. RA treatment gave rise to an increase in in vitro ADP ribosylation of the ~43-kDa nuclear protein. Moreover, nuclei isolated from RA-treated HL-60 cells revealed calcium release in response to cADPR, whereas a similar response was not observed in control nuclei. These results suggest that CD38 is expressed in HL-60 cell nuclei during RA-induced differentiation.

Keywords: CD38; cGDP-ribose; NAD^+ glycohydrolase; ADP ribosylation

Introduction

Human CD38, a surface antigen of 45 kDa, is a type II transmembrane glycoprotein with a short N-terminal cytoplasmic and long C-terminal extracellular domain. Its expression is widely used as a phenotypic marker of differentiation as well as activation in T and B lymphocytes [1–4], and it is also present in a number of other cell types including erythrocytes [5,6]. CD38 has been shown to be a bifunctional enzyme with NAD^+ glycohydrolase and ADP-ribosyl cyclase activities [6–9]. Cyclic ADP-ribose (cADP-ribose), the product of cyclase activity, has gained considerable interest as an inositol 1,4,5-trisphosphate (IP3)-independent Ca^{+2} mobilizer [10,11]; in addition, CD38 has been demonstrated to catalyze a number of base exchange reactions that include conversion of $NADP^+$, under specific conditions and in the presence of nicotinic acid, to an additional calcium mobilizer, $NAADP^+$ [12,13]. The calcium stores mobilized by cADPR copurify with those sensitive to IP3, which are mainly located in the endoplasmic reticulum (ER) [14–16], whereas those stores mobilized by NAADP have been localized to lysosome-related organelles [17]. Recent evidence suggests that the nuclear envelope may also be an important location of Ca^{+2} stores. This is shown to be the case in isolated liver nuclei [18–20], which appear to contain CD38 as well [18,19,21]. Such a strategic localization of cADPR-signaling machinery in the nuclear envelope implicates an involvement in the pathway to regulation of gene expression.

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doi:10.1016/j.yexcr.2004.09.010

In human HL-60 myeloblastic cells and K562 erythroleukemia cells, differentiation induced by retinoic acid (RA) and hemin, respectively, is accompanied with increases in NAD^+ glycohydrolase and ADP-ribosyl cyclase activities, which are attributed to CD38 antigen [22–25]. Other inducers of granulocyte maturation, such as dimethyl sulfoxide, granulocyte colony-stimulating factor, and granulocyte macrophage colony-stimulating factor, fail to prompt induction of CD38 [26]. Recently, Kishimoto et al. [27] provided direct evidence that RA-induced expression of CD38 is mediated by direct transcriptional regulation via activation of a RAR/RXR heterodimer interacting with a retinoic acid response element located in the first intron of the CD38 gene. The physiological relevance of CD38 expression upon RA induction is presently not known. CD38 expression may be an event accompanying RA-dependent cell differentiation. Alternatively, however, CD38 may be a component actively involved in the later stages of the differentiation process. Thus, in view of the reports indicating localization of fully functional CD38 in the nuclear membrane, it appeared to be of interest to investigate whether the induction of HL-60 cells by RA results in nuclear association of de novo expressed CD38.

In this study, we show that the induction of cell differentiation by RA, indeed, gives rise to expression of CD38 not only on the cell surface, but also on the nuclear membrane. The nucleus in RA-treated cells also displays an increase in sensitivity to cADP-ribose-induced calcium release, which suggests that changes in calcium concentration in the nucleus could regulate the expression of particular proteins. Furthermore, the activity of CD38 on the nucleus is implicated to be regulated by ADP ribosylation.

Materials and methods

Materials

εNAD^+, NGD^+, cGDPR, RA, protein A, protein markers, bovine serum albumin, NAD^+ glycohydrolase from pig brain, all chemicals of analytical grade, and cell culture media were purchased from the Sigma Company. [Adenine-^{14}C]NAD, specific activity 534 Ci/mol, was obtained from DuPont (NEN). Nitrocellulose blotting membranes (0.45-μm pore size) were purchased from Sartorius. CD38-specific monoclonal antibody, OKT10 (anti-CD38, IgG1), was isolated from the culture supernatant of the mouse hybridoma cell line (ATCC CRL 8022) grown in RPMI 1640, supplemented with 10% fetal calf serum, by ammonium sulfate precipitation [28]. Monoclonal anti Na^+-K^+-ATPase (α6F) was obtained from the Developmental Studies Hybridoma Bank at the University of Iowa.

Treatment of HL-60

Human promyelocytic leukemia (HL-60) cells (ATCC CCL240) were grown in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin/streptomycin. The cells were cultured by dilution in fresh medium to a density of about 2.10^5 cells/ml. RA was added at a final concentration of 1 μM by dilution from a 5-mM stock solution prepared in ethanol and incubated for 48 h. Control cells were treated in parallel with similar dilution of ethanol which was found to exert no effect on differentiation. Cell differentiation was determined by NBT-reducing activity [29].

Isolation of nuclei

HL-60 cells (3×10^6) were washed twice with cold PBS, resedimented in cold hypotonic buffer (TKM; 50 mM Tris–HCl, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$), and resuspended and kept for 5 min in 1 ml of the same buffer at 4°C prior to the addition of 1 ml of TKM containing 1% Nonidet P-40. The mixture was incubated with occasional vortexing for 5 min on ice, and cells were broken with approximately 25 strokes of a type S pestle of a Dounce homogenizer. Subsequently, two volumes of 2.3 M sucrose were added to bring the sucrose concentration to 1.62 M, and the mixture was layered over a 3-ml cushion of 2.3 M sucrose in TKM. By ultracentrifugation for 30 min at 130,000 × *g* in a SW41 rotor of a Beckman centrifuge, the nuclei were sedimented through the cushion to the bottom of the tube, whereas membranes remained at the interface [30].

For isolation of CD38, the nuclear pellet was lysed in nuclear lysis buffer [50 mM Tris–HCl, pH 7.4; 150 mM NaCl; 0.3 M sucrose; 2 mM EGTA; 20% glycerol; 2% Triton X-100; and 2 mM phenylmethylsulfonyl (PMFS)], leupeptin (10 μg/ml), and soybean trypsin inhibitor (50 μg/ml). This was allowed to stand for 2 h on ice with gentle stirring and centrifuged for 30 min at 12,000 × *g*. The supernatant containing solubilized nuclear proteins at the final step was then used as the starting material for the subsequent enrichment steps [31].

Marker enzyme assays

A typical assay mixture for glucose-6-phosphatase activity consisted of 100 μg of protein in 1 ml of assay buffer (36.7 mM Tris–HCl, pH 6.5; 1.67 mM $MgCl_2$; 4 mM glucose-6-phosphate; and 55.5 mM KCl) and was incubated at 37°C for 20 min. The reaction was stopped with cold 10% trichloroacetic acid. The changes in absorbance at 660 nm were monitored spectrophotometrically [32]. Cytochrome oxidase activity was assayed with the method of Sottocasa et al. [33]. The changes in absorbance at 550 nm were monitored spectrophotometrically. Monoclonal anti-Na^+-K^+-ATPase was used in an immunoblot assay for assessment of contamination by cell surface elements.