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The association between oxidative stress-induced galectins and differentiation of human promyelocytic HL-60 cells

James R. Vinnai, Robert C. Cumming, Graham J. Thompson, Alexander V. Timoshenko*

Department of Biology, The University of Western Ontario, London, ON, Canada, N6A 5B7

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ABSTRACT

Galectins are multifunctional β-galactoside-binding proteins that are involved in the regulation of cellular stress responses and differentiation. The relationship between these processes is unclear and we report here that galectins display oxidative-stress specific expression patterns in neutrophil-like differentiated HL-60 cells. Three galectins (−1, −3, and −10) are upregulated in response to either menadione or DMSO exposure whereas galectins −9 and −12 exhibited a stimulus-dependent downregulation. Changes in galectin expression are oxidant dependent based on the observations that 1) oxidative stress biomarkers *HMOX1* (heme oxygenase-1) and *NCF1* (neutrophil cytosolic factor 1, which is also a biomarker of neutrophil differentiation) are elevated in both cases, and 2) the antioxidant N-acetyl-L-cysteine restores basal expression of galectin-3 following oxidant exposure. In addition, our results suggest that the regulation of oxidative stress-sensitive galectins involves DNA hypomethylation mechanisms. Expression of galectin-3 and galectin-12 exhibits an opposite relationship to the expression of *HMOX1/NCF1*, suggesting a stimulatory and inhibitory role of these galectins in neutrophil-like differentiation of HL-60 cells. We also show that the inhibition of galectins reduces the growth rate of HL-60 cells, and facilitates their neutrophil-like differentiation. Collectively, our findings indicate that the process of cellular differentiation implicates, in part, oxidative stress-sensitive galectins, which further highlights a biological significance of galectin network remodeling in cells.

1. Introduction

Galectins are an evolutionary ancient family of soluble β-galactoside-binding proteins that were discovered over 40 years ago [1]. However, only recently have we started to understand their roles in cells, considering that galectins appear to be uniquely poised to regulate a wide variety of biological activities at different levels [2]. Galectins are multifunctional proteins capable of modulating cell differentiation, proliferation, survival, death, adhesion and migration. These cellular events are critical in biological processes such as embryogenesis, angiogenesis, neurogenesis and immunity. Sixteen different galectin genes (gene symbol *LGALS*) have been identified in the animal kingdom and 12 of which are expressed in human cells, not counting different splicing variants [1–4]. Expression of galectin genes varies significantly in different cells and tissues, as evident by galectin profiling analyses [4–6]. A well-established role for galectins is to modulate both pro-survival and pro-apoptotic signaling pathways [1,2,7]. This biological function is essential for cellular homeostasis and cell survival under challenging microenvironmental conditions that may range from metabolic imbalance and local hypoxia to the accumulation of toxic oxidants [8]. Cells cope with environmental stress by increasing expression of stress response genes [9], including galectins as biomarkers of an overall cellular stress response (CSR). Indeed, many microenvironmental stressors such as hypoxia, hyperthermia, oxidants, and UV light have been reported to alter the expression of galectins [3]. The expression pattern of galectin genes and proteins changes substantially in tumor tissues and cancer cells [3,5,6] and in tissues associated with inflammation [10], which implicates galectins as regulators of the CSR. Galectins can be considered as “alarmin” molecules, which signal tissue damage and elicit an effector response from immune cells [11,12]. Tumor-derived galectins, in particular, may help to escape immune surveillance through immunosuppression [13]. Recently, we have found that chemical stimuli eliciting endoplasmic reticulum stress, mimicking hypoxia and inducing cellular differentiation, can change the galectin expression profiles in HL-60 cells [14]. These findings suggest there are two groups of galectins: stress-sensitive and stress-resistant. The regulation and requirement of either type is, however, not yet clear. Oxidative stress signaling and DNA methylation are two potential mechanisms that may regulate the differential expression of galectins. First, there are binding sites for the oxidative stress-induced transcription factor Sp1 and, second, there is an abundance of CpG islands within the promoter regions of some human galectin genes [15].

* Corresponding author.
E-mail address: atimoshe@uwo.ca (A.V. Timoshenko).

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Oxidative stress, a disturbance in the balance between reactive oxygen species (ROS) production and antioxidant detoxification, is a contributing factor to many diseases. These include acute and chronic inflammation, and cancer, among other diseases [16]. Galectins are associated with oxidative stress through reciprocal molecular mechanisms. First, many galectins can directly stimulate a respiratory burst in phagocytes by activating superoxide- and hydrogen peroxide-producing plasma membrane NADPH oxidase [17–19]. Second, oxidative stress is known to upregulate some galectins, as reported for galectin-3 in lung macrophages [20] and again in breast cancer cells [21]. In addition, functions of galectins depend on the redox microenvironment [22] and oxidative stress–sensitive signaling associated with MAPK, MEK1/ERK, mTOR, and JNK pathways [23–25]. However, the details of this regulation remain elusive, especially with regard to the complex galectin networks in cells. Regulation of redox status is an important feature of cellular homeostasis, and changes in the production of ROS have been linked to controlling the fundamental processes of cell survival, proliferation, and differentiation [26]. As such, oxidative stress has been proposed to enhance differentiation of different cell types, including dimethyl sulfoxide (DMSO)-induced differentiation of human HL-60 promyelocytic leukemia cells into granulocytes [27]. Remarkably, myeloid differentiation of HL-60 cells into three different lineages (eosinophil-, monocyte-, and neutrophil-like cells) was accompanied by differential changes in the expression of galectins at both mRNA and protein levels [28]. The individual role of stress-sensitive and stress-resistant galectins in differentiation and oxidative stress response is poorly understood. In light of the apparent interplay between oxidative stress and differentiation, it would be interesting to determine which galectins potentially govern the signaling cascade ROS → altered galectin expression → cellular differentiation.

In this study, we used HL-60 cells as a model system to test molecular mechanisms mediating the involvement of stress-sensitive galectins in myeloid differentiation. HL-60 cells express six of the 12 known human galectin genes (*LGALS1, LGALS3, LGALS8, LGALS9, LGALS10,* and *LGALS12*) and are readily differentiated into neutrophil-like cells when exposed to DMSO [14,28]. Using menadione as an oxidative stress stimulus [29,30], we compared the galectin expression profiles of differentiated cells against those treated with drugs that modulate oxidative stress-associated JNK/Sp1 signaling and DNA methylation. Moreover, the effects of galectin inhibitors on HL-60 cell growth and differentiation were tested to assess functional significance of upregulated galectins.

2. Materials and methods

2.1. Chemicals

N-acetyl-L-cysteine (NAC), N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), horseradish peroxidase (HRP), lactose, D-mannose, menadione sodium bisulfite, methyl α-D-mannopyranoside (α-MM) and scopoletin were purchased from Sigma-Aldrich Canada (Oakville, ON). Anisomycin was from StressMarq Biosciences (Victoria, BC), 5-aza-2'-deoxycytidine (decitabine) was from Cayman Chemical (Ann Arbor, MI), lactobionic acid (LBA) was from Toronto Research Chemicals (Toronto, ON), OTX008 was from Axon Medchem (Groningen, The Netherlands), SP600125 was from LC Laboratories (Woburn, MA), and thiodigalactoside (TDG) was from Carbosynth (San Diego, CA).

2.2. Suspension cell culture and treatments

Human promyelocytic leukemia HL-60 cells (ATCC® CCL240™) were cultured in Corning™ cellgro™ Iscove's Modification of DMEM (IMDM) supplemented with 10% charcoal stripped fetal bovine serum (Wisent Bioproducts, St-Bruno, QC), 100 IU/mL penicillin, and 100 μg/mL streptomycin in a humidified incubator at 37 °C and 5% CO_2. Suspension cell cultures were maintained under culture conditions not exceeding $1x10^6$ cells/mL for all treatments and passaged accordingly. To induce oxidative stress, HL-60 cells were treated with 10 μM menadione for 24 h. HL-60 neutrophilic differentiation was accomplished by treating cells with 1.3% DMSO for 72 h unless otherwise stated. Inhibition and activation of JNK was induced by exposure to SP600125 (25 μM) and anisomycin (400 nM), respectively for 24 h. Global hypothemylation was accomplished by daily treatments of cell cultures with 50 nM decitabine (a DNA methyltransferase inhibitor) over 72 h. NAC was used as an antioxidant at concentrations of 1 mM and 2.5 mM for oxidative stress (24 h) and differentiation experiments (72 h), respectively. Galectin inhibitors (lactose, LBA, OTX008 and TDG) and non-inhibitory sugars (mannose and α-MM) were used at millimolar and sub-millimolar concentrations to test their dose-dependent effects on cell proliferation and differentiation.

2.3. RNA extraction, cDNA synthesis, and PCR analysis

Cells were washed with Ca^{2+}/Mg^{2+}-free Dulbecco's phosphate-buffered saline and the total mRNA was extracted from the cell pellet using TRIzol® reagent from Ambion (Carlsbad, CA) as per the manufacturer's protocol. cDNA was reverse transcribed in 20 μL from 2 μg of RNA using the Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Scientific, cat. # K1642). Oligonucleotide PCR primers were either designed using Primer-BLAST (NCBI) software or based on previous publications and verified by nucleotide BLAST analysis (Supplementary Material, Table S1). SensiFAST™ SYBR® No-ROX Kit (Bioline, cat. # BIO-98005) was used to run real-time PCR in 20 μL of PCR mix containing 400 nM forward/reverse primers and 0.5 μL of undiluted cDNA template, which were amplified in a CFX96™ Real-Time PCR Detection System (Bio-Rad). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control and the quantification of relative gene expression was calculated using $2^{-\Delta\Delta Ct}$ methodology [31]. RT^2 Profiler™ PCR Array (Qiagen, cat. # PAHS-065ZD-2) was used to assess the expression of 84 genes related to oxidative stress as per the manufacturer's real-time PCR protocol.

2.4. Protein extraction, western blotting, and antibodies

Cells were washed with Ca^{2+}/Mg^{2+}-free Dulbecco's phosphate-buffered saline and the cell pellet was homogenized in the SDS extraction buffer (2% SDS, 50 mM Tris/pH 7.5, 1 mM PMSF, 1 mM AEBSF, 5 mM EDTA, 50 μM leupeptin and 1 μM pepstatin). Samples were then incubated on ice for 10 min before further homogenization via sonication with a Microson™ XL-2000 Ultrasonic Liquid Processor (Qsonica) for 15 s at the amplitude setting 2. Protein concentration was determined using the *DC* Protein Assay™ (Bio-Rad, cat. # 5000111) as per the manufacturer's protocol and absorbance was measured at 595 nm in a Model 3550 Microplate Reader (Bio-Rad). Protein extract (25 μg) was resolved by 15% SDS-PAGE and transferred onto PVDF membrane (Bio-Rad). Membranes were blocked with 5% BSA and 1% skim milk in TBS-T buffer (50 mM Tris, 150 mM NaCl and 0.05% Tween-20) at room temperature for 1 h before being probed with primary antibody overnight at 4 °C. Membranes were then incubated for 1 h with an appropriate HRP-conjugated secondary antibody, treated with SuperSignal™ West Pico Chemiluminescent Substrate (ThermoFisher Scientific, cat. # 34080) and imaged with a ChemiDoc XRS system (Bio-Rad). Densitometry was performed using ImageLab™ software (Bio-Rad). Primary antibodies were rabbit polyclonal antibodies against galectin-1 (sc-28248), galectin-3 (sc-20157), galectin-12 (sc-67294), and mouse monoclonal antibody against β-actin (sc-47778) from Santa Cruz Biotechnology, monoclonal rabbit antibody against galectin-10 from Abcam (ab-157475), polyclonal rabbit antibodies against SAPK/JNK (#9252) and phospho-SAPK/JNK (#9251) from Cell Signaling Technology. Secondary antibodies were polyclonal goat anti-rabbit IgG-HRP (sc-2004) and goat anti-