FEBS Letters 587 (2013) 3986–3994

journal homepage: www.FEBSLetters.org

Regulation of galectin-3-induced apoptosis of Jurkat cells by both *O*-glycans and *N*-glycans on CD45

Jing Xue [1], Xiqiang Gao [1], Chunyan Fu, Zhe Cong, Hong Jiang, Wei Wang, Ting Chen, Qiang Wei *, Chuan Qin *

Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (CAMS) and Comparative Medicine Center, Peking Union Medical College (PUMC), Key Laboratory of Human Disease Comparative Medicine, Beijing, China

ARTICLE INFO

Article history:
Received 18 June 2013
Revised 24 October 2013
Accepted 28 October 2013
Available online 5 November 2013

Edited by Beat Imhof

Keywords:
CD45
Galectin-3
Binding
Apoptosis
N-Glycans
O-Glycans

ABSTRACT

Galectin-3 has been reported to induce apoptosis of Jurkat cells through binding receptors such as CD45. CD45RABC is heavily *O*-glycosylated and *N*-glycosylated, while CD45RO is only *N*-glycosylated. In this study, no apoptosis induced by galectin-3 was detected in CD45RO-transfected cells, whereas apoptosis of CD45RABC-transfected cells was observed, implying that *O*-glycans on CD45 might play roles in galectin-3-induced apoptosis. *O*-Glycosylation inhibition assay further suggests the role of *O*-glycans on CD45 in regulation of galectin-3-induced apoptosis. We also found that deglycosylation at N327 of CD45RO resulted in increased binding to galectin-3 without affecting apoptosis, while deglycosylation at N36 or N109 of CD45RO enhanced galectin-3-induced apoptosis. These data demonstrate that galectin-3-induced apoptosis of Jurkat cells is regulated by both *O*-glycans and *N*-glycans on CD45.

1. Introduction

Galectin is a family of soluble lectins that binds β-galactoside-containing glycans and forms supramolecular structures on cell surface. Galectins are characterized by a highly conserved carbohydrate recognition domain (CRD) with a specificity of binding to carbohydrate and have a structural folding in common [1,2]. To date, fifteen galectins have been identified in mammals and they are thought to mediate diverse biological processes involved in the regulation of the innate and the adaptive immune responses, such as cell activation, differentiation, cytokine secretion, and apoptosis [3,4]. Among members of the galectin family, galectin-1 and galectin-3 are the most ubiquitously expressed and the most extensively studied [5–8]. Galectin-1 and galectin-3 have been shown to induce cell death by binding the β-galactoside-containing glycans of cell surface glycoproteins. The identified receptors for both galectin-1 and galectin-3 are CD29, CD43, and CD45, whereas only galectin-3 binds CD71, and only galectin-1 binds CD7 and CD2 [9].

CD45 is a type I glycoprotein, containing a single transmembrane domain. Its cytoplasmic domain consists of tandem protein tyrosine phosphatase (PTPase) domains and a C-terminal tail of approximately 80 amino acids [10]. The extracellular domain proximal to the cell membrane covers three tandem fibronectin type III domains which are heavily *N*-glycosylated. The N-terminal of the extracellular part can include 1, 2 or 3 additional domains termed A, B, and C. These contain numerous serine and threonine residues with core 1 or core 2 *O*-glycosylation. Between the C- and N-terminal domains, a cysteine-rich region is present [11,12]. At the cell surface, CD45 can exist as multiple isoforms resulting from alternative RNA splicing of exons 4, 5, and 6 which code for A, B, and C domains in the extracellular domain. CD45RABC contains all the fibronectin type III domains, the cysteine-rich region, and A, B and C domains. CD45RO lacks the three A, B and C domains and has only the fibronectin type III domains and the cysteine-rich region [12]. While the *N*-glycosylated region of the extracellular domain is conserved, the main variation exists in the length of the *O*-glycosylated region (A, B, or C domains). In addition, the number of *O*-glycans on CD45 can vary significantly among different isoforms and the number of *N*-glycans on CD45RO can vary by different lymphocyte subsets at distinct developmental stages [13–16].

Abbreviations: PBST, phosphate buffered saline with Tween 20; MW, molecular weight; DMEM, Dulbecco's modified Eagle's medium; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis

* Corresponding authors. Address: Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (CAMS) and Comparative Medicine Center, No 5 Panjiayuan Nanli, Chaoyang District, Beijing 100021, China. Fax: +86 10 67761136.

E-mail addresses: weiqiang@cnilas.pumc.edu.cn (Q. Wei), qinchuan@pumc.edu.cn (C. Qin).

[1] This work was contributed equally by Jing Xue and Xiqiang Gao.

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.10.034

Thus, CD45 has been described as being able to positively or negatively regulate galectin-1-induced cell death depending on cell types. Both *N*-glycans and *O*-glycans are reported to be involved in CD45-mediated apoptosis induced by galectin-1 [11,14]. However, CD45 expression is not absolutely required for galectin-1-induced T cell death and CD45RO expression even exhibited reduced susceptibility to galectin-1-induced apoptosis in murine BW5147, BW5147(Rev1.1), and T200$^-$ cell lines [17].

CD45 involvement has also been reported in galectin-3-mediated cell death. Stillman et al. observed that galectin-3 induced apoptosis of CD45$^+$ Jurkat cells, whereas cells lacking CD45 were not susceptible to galectin-3. Interestingly, CD45RABC transfection in these CD45$^-$ cells restored CD45 expression and more importantly, susceptibility to galectin-3 [9]. Clark et al. reported the importance of specific glycosylation of CD45 in the regulation of galectin-3-mediated signaling. They found that galectin-3 could bind to CD45 on diffuse large B-cell lymphoma cells, regulating susceptibility to cell death modified by C2GnT-1 glycosyltransferase [18]. However, the roles of *O*-glycans and/or *N*-glycans on CD45 in cell death induced by galectin-3 remain unknown. Using CD45RABC containing both *O*-glycans and *N*-glycans and CD45RO containing *N*-glycans only, the regulation of galectin-3-induced cell death was investigated. The role of *O*-glycans and/or *N*-glycans on CD45 in the regulation of cell death was examined by transfection of CD45RABC or CD45RO with individual removal of *N*-glycosylation sites into CD45$^-$ J45.01 cells. We observed galectin-3-induced cell death in CD45RABC-J45.01 cells but not in CD45RO-J45.01 cells. Moreover, death of CD45RABC-J45.01 cells induced by galectin-3 could be modified by *O*-glycosylation inhibitor Benzyl-GalNAc, demonstrating roles played by *O*-glycans on CD45 in the regulation of galectin-3-induced Jurkat cell death. Meanwhile, we also found that site-specific depletion of *N*-glycans in CD45RO cells affected galectin-3-binding and the initiation of apoptosis, indicating that galectin-3-induced Jurkat cell death was also regulated by *N*-glycans on CD45.

2. Materials and methods

2.1. Cell culture and transfection

The human leukemia cell line, Jurkat (Clone E6-1), and the CD45-deficient cell line, J45.01, were obtained from the American Type Culture Collection. Cells were maintained in RPMI 1640 medium containing 100 U of penicillin per ml and 0.1 mg of streptomycin per ml and 10% fetal bovine serum (FBS) in 5% CO_2 at 37 °C. HEK 293T cells were grown in DMEM containing 10% FBS and antibiotics at 37 °C in 5% CO_2. J45.01 cells stably expressing WT-CD45RO, CD45RABC, and mutated-CD45RO were established by lentiviral transduction and evaluated for receptor expression by flow cytometry. cDNA of WT-CD45RO, CD45RABC or mutated-CD45RO was inserted into lentiviral vector pWPXL between *Mlu*I and *Spe*I. Lentiviruses produced in 293T cells by co-transfection of pWPXL-CD45, packaging vector psPAX$_2$ and envelope vector pMD2.G were used to infect J45.01 cells. Target cells were infected with supernatants from packing cells supplemented with 10 μg/ml polybrene.

2.2. Reagents

Recombinant human galectin-3 was obtained from R&D Systems (Minneapolis, MN). Benzyl-GalNAc was purchased from Sigma–Aldrich (St. Louis, MO). APC-labeled CD43, PE-labeled CD29, FITC-labeled CD71, PE-labeled CD45RO mAb and FITC-labeled CD45RA mAb were obtained from BioLegend (San Diego, CA). Anti-CD45 antibody for Western blot was obtained from Abcam (Cambridge, UK). HRP-labeled goat anti-rabbit IgG polyclonal antibody was purchased from Zhongshan Biotechnology Co (Beijing, China). PerCP-labeled CD3 and PE-labeled mouse anti-human galectin-3 Set (B2C10) were purchased from BD Pharmingen (San Jose, CA).

2.3. Site-directed mutagenesis

Site-directed polymerase chain reaction (PCR) mutagenesis was used for mutation of Asp-X-Ser/Thr to Glu-X-Ser/Thr in CD45RO cDNA. The template cDNA of CD45RO and CD45RABC were purchased from OriGene (Rockville, MD). PCR was performed with CD45 upstream primer 5′-CGA CGC GTA CCA TGT ATT TGT GGC TTA AAC TCT-3′ and downstream primers with a stop codon 5′-GGA CTA GTC TAT GAA CCT TGA TTT AAA GCT GGA-3′. For each site, two reactions with primers overlapping the desired amino acid exchange were performed. The two products were annealed and amplified with the flanking CD45RO primers (Table 1). The final products were individually inserted into *Mlu*I/*Spe*I site of pWPXL vector. Successful mutagenesis was confirmed by sequencing.

2.4. Western blot

Cell were lysed with ice-cold lysis buffer, run on 6% SDS–PAGE, and then transferred onto a nitrocellulose membrane. After blocking with 5% skimmed milk for 1 h at room temperature, membranes were incubated with rabbit anti-human CD45 antibody overnight at 4 °C. Then, membranes were washed with PBST and incubated with HRP-labeled goat anti-rabbit antibody. Membranes were visualized by Super Signal West Pico chemiluminescent substrate (Pierce, Rockford, IL).

2.5. RT-PCR

Total RNA was extracted using RNAeasy Mini kit (Qiagen, Valencia, CA). Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed on 1 μg RNA using Super Script One-Step RT-PCR Kit (Invitrogen, Carlsbad, CA). PCR was performed to amplify CD45RO and CD45RABC.

2.6. Flow cytometry

Cells were incubated with APC-labeled CD43, PE-labeled CD29, FITC-labeled CD71, PerCP-labeled CD3, PE-labeled CD45RO or FITC-labeled CD45RA, and then evaluated by flow cytometry (FACS Aria; BD, CA). For galectin-3 binding assay on cell surface, 180 nM

Table 1
Primers used for site-directed mutagenesis of CD45RO.

N36Q	5′-GGTTGTTTCAGAGGCCTGAAGGTAGGCATCAG-3′
	5′-CTGATGCCTACCTTCAGGCCTCTGAAACAACC-3′
N71Q	5′-GATGAAAAATATGCACAGATCACTGTGGATTAC-3′
	5′-GTAATCCACAGTGATCTGTGCATATTTTTCATC-3′
N99Q	5′-GTTTGTGCAAGTATTCTGTCCACATTCCACATTC-3′
	5′-GAATGTGGAATGTGGACAGAATACTTGCACAAAC-3′
N109Q	5′-CATTTTTACATTCTGTAAGCTGATGCACCTCATTG-3′
	5′-CAATGAGGTGCATCAGCTTACAGAATGTAAAAATG-3′
N115Q	5′-GGAAACAGACGCCTGTTTACATTCTGTAAG-3′
	5′-CTTACAGAATGTAAACAGGCGTCTGTTTCC-3′
N174Q	5′-CTGAAATCTGTAGGTAATCTGCTGTGTATCACAAG-3′
	5′-CTTGTGATACACAGCAGATTACCTACAGATTTCAG-3′
N217Q	5′-GTTTTAATAATTTTACTTGCCTGAGTAAACTTGTGG-3′
	5′-CCACAAGTTTACTCAGGCAAGTAAAATTATTAAAAC-3′
N258Q	5′-CAGAGGGTAAACTGATGAAATGATCTTTG-3′
	5′-CAA AGATCATTTCATCAGTTTACCCTCTG-3′
N307Q	5′-GGTTGTTTCAGAGGCCTGAAGGTAGGCATCAG-3′
	5′-CTGATGCCTACCTTCAGGCCTCTGAAACAACC-3′
N327Q	5′-GGAGACAGTCATCTGCCAGACCTGGCTTGG-3′
	5′-CCAAGCCAGGTCTGGCAGATGACTGTCTCC-3′
N368Q	5′-GCAATTCTTATGCGACTCCTGTCTAACCAGAGTATTTC-3′
	5′-GAAATACTCTGGTTAGACAGGAGTCGCATAAGAATTGC-3′