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The expression and function of β-1,4-galactosyltransferase-I in dendritic cells ☆

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ABSTRACT

β-1,4-Galactosyltransferase-I (GalTI) is unusual among the galactosyltransferase family, which has two isoforms that differ only in the length of their cytoplasmic domains [1]. In this study, we found that both the long and short isoforms of GalTI were expressed in human monocyte-derived dendritic cells (MoDCs), and localized in the cytoplasm near nucleus and cytomembrane. The expression level of GalTI and cellular adhesion ability was increased when DCs continued to mature. We also demonstrated that the cellular adhesion ability of DCs was inhibited by α-lactalbumin (α-LA) via interference with cell surface GalTI function, suggesting that the adhesion ability was positively correlated with the expression of cell surface long GalTI. α-LA also could inhibit DC-T clustering and $CD4^+$ T cell proliferation. Collectively, the data suggests that GalTI might act as a key adhesion molecular participating in T cells–DCs contacts.

1. Introduction

β-1,4-Galactosyltransferase-I (GalTI) is one of the most deeply studied glycosyltransferases, and is also the first mammalian glycosyltransferase whose cDNA has been cloned and whose crystal structure has been resolved [2]. GalTI gene encodes two mRNAs of 4.1 and 3.9 kb with different in-frame AUGs that are transcripted from two different start sites with the interval of about 200 bp. Translation from the longer and shorter mRNAs produces two GalTI isoforms of 399 and 386 amino acids designated as the long and short protein isoforms, respectively [3]. Both protein isoforms are type II membrane-bound glycoproteins with a relatively short amino-terminal cytoplasmic domain, a signal sequence/transmembrane domain, and a large carboxy-terminal luminal/extracellular catalytic domain. They have identical catalytic and transmembrane domains but differ in their cytoplasmic domains. The shorter protein has a cytoplasmic tail of only 11 amino acids, whereas the longer protein has a 24-amino acid cytoplasmic domain [4]. The additional 13-amino acid extension unique to the long form serves as a targeting signal for expression of GalTI on the plasma membrane [5].

GalTI exists in two subcellular compartments where it performs two distinct functions. The majority of long and short GalTI work as a biosynthetic enzyme in Golgi apparatus, where it catalyzes the transfer of galactose from uridine diphosphate-galactose (UDP-Gal) donors to terminal N-acetyl glucosamine (GlcNAc) residues [6]. On the other hand, a portion of long GalTI could work as a recognition molecule on the cell surface, and participate in cellular interactions by binding to appropriate glycoside substrates on the adjacent cell surface and in the extracellular matrix [7]. As a result, GalTI is involved in a variety of physiological or pathological phenomena, including sperm-egg interactions, embryonic maturation, embryonal carcinoma cell spreading, neurite extension, tumor metastasis, etc. [8–14].

Currently, few studies have been reported about GalTI in the immunologic system, DCs as the most potent antigen-presenting cells (APCs), can initiate T cells-mediated immune responses upon direct cell–cell contact with T cells, which is a dynamic process of adhesion and segregation [15]. However, the precise molecular basis of cellular adhesion remains unclear. It is thus of significance to examine whether GalTI participates in the adhesion process of IS formation and plays a pivotal role in immune responses.

This study is to identify the expression and localization of both long and short GalTI proteins in human monocyte-derived dendritic cells, and to further investigate the role of GalTI in T cells–DCs contacts.

2. Materials and methods

2.1. Cell culture and identification

2.1.1. DCs

Monocytes were isolated from normal human peripheral blood by Nycoprep™ 1.068 (AXIS, Belgien), the purity of which was more than 90%. 4×10^5 isolated cells per ml were incubated in RPMI-1640 complete medium (Gibco BRL, Fort Worth, TX) for 2 h, 37 °C, 5% CO_2. Nonadherent cells were washed away and adherent

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cells continued to be incubated in RPMI-1640 complete medium supplemented with 10% fetal bovine serum (FCS), 1 mM L-glutamine, rhGM-CSF (1000U/ml), rhIL-4(500U/ml) and 200 U/ml Amikacin, 37 °C, 5% CO_2. Immature DCs were harvested in the 7th day, and mature DCs were harvested in the 10th day, adding LPS in the 5th day. DCs surface molecules HLA-DR, CD80, CD83 and CD86 were detected by FACS to identify the maturation of DCs.

2.1.2. $CD4^+$ T cells

Normal human peripheral blood $CD4^+$ T cells were isolated from the peripheral blood monocytes preparation by negative selection with Dynal CD4 negative isolation kit (Dynal Biotech, Oslo, Norway). The purity of $CD4^+$ T cells was more than 95% assessed by flow cytometry staining with PE-anti-CD4 (eBioscience, San Diego, CA) (Fig. 7A). The $CD4^+$ T cells were cultured at 1×10^6 cells per ml in RPMI-1640 complete medium (Gibco BRL, Fort Worth, TX) supplemented with 10% fetal bovine serum (FCS), 1 mM L-glutamine and 200 U/ml Amikacin.

2.2. Immunofluorescence and confocal microscopy

DCs were transferred to a poly-L-lysine coated slide, and fixed with 4% paraformaldehyde for 20 min at room temperature and washed three times with phosphate buffered solution (PBS). The cells were permeabilized for 5 min at room temperature in PBS with 0.2% saponin, 0.03 M sucroseand 1% BSA. After being blocked for 15 min with 5% normal goat serum (NGS) in PBS/1% BSA, the cells were incubated with goat anti-human β-1,4-GalT-I Poly antibody (N-20, Santa Cruz biotechnology, Inc., CA) for 1 h. Cells were washed with PBS/BSA and blocked with 5% NGS in PBS/BSA for 15 min and then were incubated with FITC labeled second antibody in PBS/BSA for 30 min. The cells were stained with 5 μg/ml Hoechst (H33342, Vector Laboratories, Burlingame, CA) for 30 min at 37 °C. After being washed three times with PBS, the glycerol-mounted slides were observed and photographed under a confocal laser scanning fluorescence microscope (Leica Microsystems, Heidelberg, Germany).

2.3. Semiquantitative RT-PCR analysis

Total RNA was extracted from 1×10^6 cells using Trizol reagent (Invitrogen, Carlsbad, CA), and 2 μg of total RNA was reverse transcribed into cDNA with oligodT primers using Omniscript reverse transcriptase (QIAGEN, Hilden, Germany) according to the manufacture's protocol. Hot start PCR was performed to analyze expression levels with the following primers.

- Total GalTI: Sense 5′-GCAGAACCCAAATGTGAAG-3′andantisense 5′-TGTGCCGTGGCTGTGAAAA-3′.
- Long of GalTI: Sense 5′-CGGCGGGAAGATGAGGC-3′ and antisense 5′-ACAGTGCGGTGGTGTGGGG-3′.
- GAPDH: Sense 5′-TGATGACATCAAGAAGGTGGTGAAG-3′ and antisense 5′-TCCTTGGAGGCCATGTGGGCCAT-3′.

The PCR products were subjected to agarose gel electrophoresis followed by staining with ethidium bromide. The band optical density of GalTI, relative to that of GAPDH (internal control) was quantified by image analysis system (Leica Q550I W, Cambridge, UK).

2.4. Flow cytometry analysis

2.4.1. Cell surface staining

DCs were incubated with GalTI Poly antibody (N-20, Santa Cruz) for 20 min at 4 °C and washed three times with PBS, and incubated with FITC labeled second antibody for 30 min at room temperature. The cell pellets were washed and resuspended in 200 μl of PBS, and then flow cytometry analysis was performed with a FACScalibur (BD Bioscience, San Jose, CA) to measure the fluorescence intensity.

2.4.2. Intracellular staining

DCs were permeabilized for 5 min before antibody incubation at room temperature in PBS with 0.2% saponin, 0.03 M sucroseand and 1% BSA. Cells were washed with PBS and incubated with FITC labeled second antibody for 30 min at room temperature. The cell pellets were washed and resuspended in 200 μl of PBS, and flow cytometry analysis was performed with a FACScalibur (BD Bioscience, San Jose, CA) to measure the fluorescence intensity.

2.5. Western blotting

Western blotting was performed with membrane protein extraction and whole cell lysate. Aliquots of total protein (50 μg per lane) were electrophoresed on a 12% SDS–polyacrylamide gradient gel and transferred to nitrocellulose membranes (Millipore). Washed in rinse buffer at room temperature for 15 min and incubated in blocking buffer (5% fat-free milk in rinse buffer) for 30 min, the membranes were incubated for 2 h at room temperature with antibody. Further washed with rinse buffer, the membranes were incubated with 1:1000 diluted horseradish peroxidase-conjugated anti-mouse immunoglobulin antibody (Santa Cruz) followed by developing with enhanced chemiluminescence reagents (Amershame, Little Chalfont Buckinghamshire, UK).

2.6. Adhesion assay

A 96-well microtiter plate was precoated with laminin averaging 1 μg/100 μl per well overnight at 4 °C. Nonspecific binding sites were blocked at room temperature for 1 h using 1% bovine serum albumin (BSA)/PBS, and washed extensively with PBS. DCs were resuspended in RPMI-1640 serum-free medium and distributed into the plates averaging 1×10^5 cells in 100 μl were plated per well (in triplicate), followed by incubation for 2 h at 37 °C under 5% CO_2 atmosphere. Both Immature DCs and mature DCs were divided into two groups. One group was washed gently with PBS to remove nonadherent cells; and the other was served as a control for the count of total cells. Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) was utilized to determine the number of adherent cells according to the manufacturer's protocol. Cell Counting Kit-8 is a one-bottle solution which allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays by utilizing WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium,monosodiumsalt]. WST-8 is reduced by dehydrogenases in cells to give a yellow colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells. Optical density (OD) of cell numbers in triplicate wells was measured at 450 nm. The cellular adhesion ability was evaluated by percentage of adherent cells to total cells.

2.7. α-lactalbumin interference test

After being treated with α-lactalbumin (α-LA, Sigma–Aldrich, St Louis, MO) at 10 mg/ml, the DCs were incubated for 4 h at 37 °C under 5% CO_2 atmosphere. Then the cellular adhesion ability was determined by adhesion assay as described above.

2.8. DC-T clustering test

DCs or purified $CD4^+$ T cells or both were pretreated with 10 mg/ml α-LA respectively for 4 h at 37 °C and washed three