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Galectin-1 upregulates glucose transporter-1 expression level via protein kinase C, phosphoinositol-3 kinase, and mammalian target of rapamycin pathways in mouse embryonic stem cells

Min Young Lee, Ho Jae Han*

Department of Veterinary Physiology, Biotherapy Human Resources Center, College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, Republic of Korea

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ABSTRACT

Although galectin-1 is expressed in various stem cells, our understanding of the functional roles of galectin-1 in embryonic stem (ES) cells is still fragmentary and incomplete. Thus, this study investigated the effect of galectin-1 on the 2-deoxyglucose (2-DG) uptake and its related signal cascades. Galectin-1 significantly increased 2-deoxyglucose uptake time- and dose-dependently. In addition, galectin-1-induced 2-deoxyglucose uptake was inhibited by glucose transporter-1 siRNA. Moreover, galectin-1 increased glucose transporter-1 mRNA and protein expression levels, which were inhibited by a disruption in transcription by actinomycin D and translation by the cycloheximide. Subsequently, the galectin-1-induced 2-deoxyglucose uptake was attenuated by these inhibitors. In investigation of signal transduction involved in this process, galectin-1 increased intracellular Ca^{2+} concentration and the protein kinase C activation, which induced extracellular signal regulated kinase1/2 phosphorylation. On the other hand, phosphoinositol-3-kinase/Akt activated by galectin-1 was not involved in extracellular signal regulated kinase1/2 pathway. Moreover, mammalian target of rapamycin signal pathway was stimulated in response to galectin-1. Finally, galectin-1-induced increase of glucose transporter-1 expression and 2-deoxyglucose uptake were inhibited by blocking of Ca^{2+}/protein kinase C/extracellular signal regulated kinase1/2, phosphoinositol-3-kinase/Akt, and mammalian target of rapamycin pathways. In conclusion, galectin-1 upregulates glucose uptake through Ca^{2+}/protein kinase C/extracellular signal regulated kinase1/2, phosphoinositol-3-kinase/Akt, and mammalian target of rapamycin pathways in mouse ES cells.

1. Introduction

Galectins are a family of carbohydrate-binding proteins with an affinity for β-galactoside containing glycoconjugates. To date, 15 members have been identified (Cooper and Barondes, 1999; Liu and Rabinovich, 2005), of which all can be classified according to the carbohydrate recognition domains (Barondes et al., 1994). Galectin-1 is lectin that preferentially binds to the lactosamine structure in glycans (Camby et al., 2006; Hirabayashi et al., 2002; Leffler et al., 2004). Galectin-1 is expressed by various stem cells, including embryonic, hematopoietic, and keratinocytes stem cells (Ivanova et al., 2002; Ramalho-Santos et al., 2002; Silva et al., 2003; Tumbar et al., 2004). To date, there has been little research into the functions of lectins in embryonic stem (ES) cells. Recently, Sakaguchi et al. (2006) reported that galectin-1 is expressed on adult neural stem cells and promotes their proliferation through its carbohydrate-binding ability. Moreover galectins also play important functional roles in determining cell fate such as self-renewal, prolif-

* Corresponding author. Tel.: +82 62 530 2831; fax: +82 62 530 2809.
E-mail address: hjhan@chonnam.ac.kr (H.J. Han).

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eration, and differentiation in stem cells (Chan et al., 2006; Yanagisawa and Yu, 2007). Recent studies clearly implicate the functional relevance of glycoconjugates in mediating signal transduction and cell–cell interaction and adhesion (Nakahara and Raz, 2006; Rabinovich, 1999). This evidence raises the possibility that galectins play roles in the functions of ES cells. However, at present, our understanding of the functional roles of glycoconjugates in ES cells is still fragmentary and incomplete.

Previous studies have established a direct relationship between glucose transporter and the energy requirements of some cell type, such as blastocyst, suggesting that glucose transporter is associated to the physiology of cells with a high energy demand. The growth of mouse ES cells requires an increased uptake of glucose and other substrates (Heilig et al., 2003). Facilitative glucose transporters allow the energy independent transport of glucose across the hydrophobic cell membrane down its concentration gradient. To date, 13 members of the mammalian facilitative glucose transporter family have been identified (Macheda et al., 2005). Of these, glucose transporter (GLUT)-1 expression has been described for mouse and human ES cells and the GLUT-1−/− cells were non-viable, indicating that GLUT-1 is the main glucose transporter isoform in embryonic stem cells. Its expression is detectable throughout preimplantation development from oocytes through the blastocyst stage (Aghyan et al., 1992; Morita et al., 1994), and increases 11-fold in developing embryos from the two-cell stage to the blastocyst stage (Morita et al., 1994). In contrast, other embryonic glucose transporters first appear at a later stage than GLUT-1. This may in part reflect the finding that GLUT-1 is the major glucose transporter in the inner cell mass (ES cells) of a preimplantation embryo. Indeed, the inner cell mass also relies on GLUT-1 to provide glucose from the blastocoel (Iyer et al., 1998). Therefore, it was hypothesized that GLUT-1 would be one of the efficient isoforms to supply glucose to mouse ES cells. Although these findings strongly suggest a role of galectin-1 in self-renewal of ES cells, there are few reports on the function of galectin-1 in the glucose transporter of mouse embryonic stem cells. Recent reports suggested that galectin-1 promotes the proliferation of neural stem cells (Sakaguchi et al., 2006) and hematopoietic stem cells (Vas et al., 2005). However, there is no report to elucidate the effect of galectin-1 on glucose transport and proliferation in ES cells until now. Therefore, this study examined the effect of galectin-1 on the 2-deoxyglucose (2-DG) uptake and its related signal cascades in mouse ES cells.

2. Materials and methods

2.1. Materials

The mouse ES cells were obtained from the American Type Culture Collection (ES-E14TG2a). The fetal bovine serum was purchased from Biowhittaker (Walkersville, MD, USA). The recombinant mouse galectin-1 was obtained from R&D systems (Minneapolis, MN, USA). The bisindolylmaleimide I, LY294002, Akt inhibitor, PD98059, rapamycin, A23187, fluorescence isothiocyanate-conjugated (FITC-conjugated) goat-anti-rabbit IgM, and β-actin were acquired from Sigma Chemical Company (St. Louis, MO, USA). The H-7 was supplied by Calbiochem (LaJolla, CA, USA). The [^{3}H]-2-deoxyglucose and [^{3}H]-thymidine were purchased from NEN (Boston, MA, USA). Fluo-3-AM was obtained from Molecular Probes, Inc. (Eugene, OR, USA). The anti-GLUT-1, protein kinase C (PKC)-α, -β1, -γ, -δ, -ξ antibodies were acquired from Santa Cruz Biotechnology (Delaware, CA, USA). The phospho-PKC, phospho-Akt308, phospho-Akt473, phospho-ERK1/2, phospho-mTOR, phospho-p70S6K, phospho-4E-BP1, and phospho-STAT3 antibodies were supplied by New England Biolabs (Herts, UK). The goat anti-rabbit IgG was purchased from Jackson Immunoresearch (West Grove, PA, USA). All other reagents were purchased commercially and were of the highest purity available.

2.2. ES cell culture

The mouse ES cells were cultured for 5 days in Dulbecco's modified eagle medium (DMEM) (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 3.7 g/l of sodium bicarbonate, 1% penicillin and streptomycin, 1.7 mM L-glutamine, 0.1 mM β-mercaptoethanol, 5 ng/ml mouse leukemia inhibitory factor, and 15% fetal bovine serum (FBS) without a feeder layer. The cells were grown in an incubator maintained at 37 °C in an atmosphere containing 5% CO_2. The medium was exchanged with serum-free DMEM containing LIF for 24 h before the experiments.

2.3. [^{3}H]-2-Deoxyglucose uptake

The [^{3}H]-2-deoxyglucose uptake experiments were performed using a modification of the method reported by Henriksen et al. (1990). The culture medium was removed and the cells were gently washed with an uptake buffer (140 mM NaCl, 2 mM KCl, 1 mM KH_2PO_4, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM glucose, 5 mM L-alanine, 5 μM indomethacin, and 10 mM HEPES/Tris, pH 7.4). After the washing, the cells were then incubated in an uptake buffer containing 1 μCi/ml [^{3}H]-2-DG at 37 °C for 30 min. After incubation, the cells were washed, and dissolved in 1 ml 0.1% SDS. 900 μl of each sample was used for measuring the radioactivity using a liquid scintillation counter (LS6500, Beckman Instruments, Fullerton, CA). The remainder of each sample was used to determine the protein level (Bradford, 1976). The radioactivity counts for each sample were then normalized to the protein and corrected for zero-time uptake per mg of protein.

2.4. Transfection of mouse GLUT-1 small interfering RNA

The cells were grown to 75% confluence in each dish and transfected for 36 h with either a SMART-pool of the small interfering RNAs specific to mouse GLUT-1 [3′-GAUCACUGCAGUUCGGCUAUU (sense) and 5′-UAGCCGAACUGCAGUGAUCUU (antisense); 3′-CCUCUUUG-UUAAUCGCUUUUU (sense) and 5′-AAAGCGAUUAACA-AAGAGGUU (antisense); 3′-CAACGAGCAUCUUCGAGAAUU (sense) and 5′-UUCUCGAAGAUGCUCGUUGUU (antisense); 3′-GCUGUUUGUUGUAGAGCGAUU (sense) and 5′-UCGCUCUACAACAAACAGCUU (antisense)] (200 pmol/l;