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Carbohydrate antigens expressed on stem cells and early embryonic cells

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Lewis X antigen (Le^X) is a marker of embryonic stem cells, embryonal carcinoma cells and multipotential cells of early embryos in the mouse. Le^X is carried by branched, high-molecular weight poly-*N*-acetyllactosamines (embryoglycan). While embryoglycan is present in human embryonal carcinoma cells, Le^X is not expressed in human embryonic stem cells, embryonal carcinoma cells or inner cell mass cells. Instead, these cells express SSEA-3 and SSEA-4, both of which are carried by globo-series glycolipids. Le^X is a marker of primordial germ cells or multipotential stem cells derived from primordial germ cells both in the mouse and human. In other species of vertebrates, Le^X is widely expressed in early embryonic cells and primordial germ cells, but the mode of expression is not completely conserved among species. Le^X is expressed in neural stem cells from both humans and mice. Hematopoietic stem cells are not reported to express the above carbohydrate markers. A marker of these cells is CD34, a membrane-bound sialomucin. Another sialomucin, CD164 (MGC-24v) is expressed in hemotopoietic progenitor cells. As a function of Le^X in stem cells, the promotion of integrin action is proposed, based on analyses of glycoproteins with the marker, cDNA transfection experiments and the inhibitory effects of an anti-Le^X antibody. Most probably, Le^X antigen as well as poly-*N*-acetyllactosamines play roles in the interactions on the same membrane. On the other hand, *O*-linked oligosaccharides on CD34 and CD164 are probably involved in the regulation of cell adhesion and proliferation via intercellular recognition.
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Introduction

The isolation and long-term cultivation of various stem cells, such as embryonic stem (ES) cells, neural stem cells and hematopoietic stem cells, has led to new possibilities for regenerative therapy and also studies on the mechanism of differentiation [1]. Carbohydrate antigens on stem cells are helpful in the isolation and identification of these cells, and are also expected to have functional significance. Since ES cells are equivalent to multipotential cells of early embryos, ES cells and early embryonic cells share carbohydrate antigens. Here, we review the nature and function of carbohydrate antigens expressed on stem cells. For a detailed description of carbohydrate antigens and their developmentally regulated expression, readers are referred to a previous review [2].

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ES cells and related cells in the mouse

ES cells are derived from the inner cell mass of blastocysts [1]. Embryonal carcinoma (EC) cells are stem cells of teratocarcinomas, which develop spontaneously in the gonad or are induced by graft of early embryos [1]. Both ES and EC cells differentiate to a number of cell types, and are equivalent to multipotential cells of early embryos, *i.e.* cells of inner cell masses and primitive ectoderm (Figure 1). Consistent with their biological resemblance, ES, EC and multipotential embryonic cells share common carbohydrate antigens. SSEA-1(Stage-Specific Embryonic Antigen-1), which is detected by a monoclonal antibody against EC cells [3], is an established marker of these cells in the mouse. SSEA-1 first appears in late eight-cell embryos, and is expressed in the embryonic ectoderm, the visceral endoderm and trophoblasts in early postimplantation embryos (Figure 1). In inner cell mass, SSEA-1 expression is weak in early stage and becomes increased in later stage (Table 1). The antigenic epitope of SSEA-1 corresponds to that of Lewis X antigen (Le^X), namely Galβ1-4 (Fucα1-3)GlcNAc [4]. *Lotus tetragonolobus* agglutinin (LTA) recognizes both Le^X and H

Table 1. Expression of Le^X in stem cells and early embryonic cells of mice and human

Cells	*Mice*	*Human*
ES cells	+	−
EC cells	+	−
EG cells	+	+
Neural stem cells	+	+
Hematopoietic stem cells	−	−
Inner cell mass cells	±+	−
Primitive ectoderm cells	+	?
Primordial germ cells	+	?

+, expression; −, no expression; ±+ , weak expression ; ?, unknown

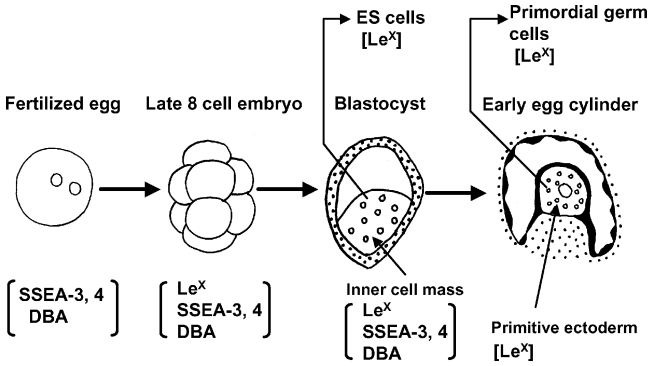

Figure 1. Expression of carbohydrate markers in early embryos and stem cells in mice. Expressed markers are written in parenthesis. More detailed profile of their expression is described in a review [2]: in the inner cell mass, Le^X expression changes from ± to + and DBA expression changes from + to ± during blastocyst development. Le^X(SSEA-1, 4C9, LTA): Galβ1-4(Fucα1-3)GlcNAc, SSEA-3: R-3Gal NAcβ1-3Galα1-4R′, SSEA-4: NeuAcα2-3Galβ1-3GalNAcβ1-3Galα1-4R, DBA(Sd^a antigen): NeuAcα2-3(GalNAcβ1-4)Galβ1-4GlcNAc.

antigenic structures. However, in histochemical analyses, the expression of LTA binding sites is more similar to that of SSEA-1 [5]. Thus, LTA has been used to isolate Le^X-carrying glycoproteins from EC cells [6,7].

Mouse EC cells and early embroyonic cells have large amounts of branched poly-*N*-acetyllactosamines with a molecular weight of 10, 000 or more [8,9]. This class of glycan carries various carbohydrate markers expressed on early embryonic cells, but not ABH blood group antigen, and is called embryoglycan [2,6]. Le^x and an α-galactosyl antigen, ECMA-2, are typical antigens expressed on embryoglycan [2]. The branched domain of poly-N-acetyllactosamine is the epitope of I antigen, and the linear domain is that of *i* antigen. Consistent with the abundance of embryoglycan in early embryonic cells, I antigen is expressed throughout preimplantation and early postimplantation embryogenesis of the mouse [10]. On the other hand, i antigen first appears in the parietal and visceral endoderm of early postimplanation embryos [10].

Species difference of carbohydrate antigens on early embryonic cells

Surprisingly, human ES and EC cells do not express SSEA-1 [11] (Table 1). Instead, they express SSEA-3, SSEA-4, TRA-1-60 and TRA-181. The inner cell mass of human blastocysts exhibits the same expression profile for these antigens [11].

Generally speaking, the onset of SSEA-1 expression in early embryogenesis is not conserved in vertebrates. Thus, *Xenopus* embryos do not express Le^X at the early embryonic stage; its expression starts at the tail bud stage [12]. The Medaka fish expresses Le^X from the one-cell stage [13]. Inner cell mass cells of porcine embryos are SSEA-1 positive [14], but those of bovine embryos are not [15]. ES cells established from rhesus monkeys and common marmosets have antigenic profile identical to human ES cells [16].

Even though human EC and ES cells lack Le^X, the expression of large amounts of embryoglycan is conserved between human and mouse EC cells [17,18]. In chicken embryos, branched long chain of poly-*N*-acetyllactosamines is expressed in early embryonic ectoderm [19]. Therefore, branched and high molecular weight poly-*N*-acetyllatosamines appear to be evolutionally conserved during early embryogenesis, and variations occur in the non-reducing terminal epitopes.

During mouse embryogenesis, SSEA-3 and SSEA-4 are expressed earlier than SSEA-1, namely in early cleavage embryos [20] (Figure 1). SSEA-3 and SSEA-4 are on globo-series glycolipids [20] and are not observed on poly-*N*-acetyllactosamines. The epitopes of SSEA-3 and -4 are R-3GalNAcβ1-3Galα1-4R′ and NeuAcα 2-3Galβ1-3GalNAcβ1-3Galα1-4R, respectively. Sialylated structures probably serve as non-reducing epitopes on embryoglycan in human EC/ES cells. Indeed, a disialyl structure, namely NeuAcα 2-9NeuAc, is present on embryoglycan of human EC cells [18]. Furthermore, TRA-1-60 and TRA-1-81 have been found on keratan sulfate, which is a sulfated linear poly-*N*-acetyllactosamine. Sialic acid is present in the epitope of TRA-1-60 but not TRA1-81 [20]. It is possible that the TRA antigens are epitopes on poly-*N*-acetyllactosamines. In the mouse, *Dolicos biflorus* agglutinin binding site (DBA, also Sd^a antigen) is expressed in cleavage embryos (Figure 1) and extraembryonic endoderm as in the case of SSEA-3 [2]. The epitope is the terminal structure of GM2 linked to *N*-acetyllactosamine [NeuAcα 2-3 (GalNAcβ1-4)Galβ1-4GlcNAc][22] and is carried by embryoglycan [2]. To the best of our knowledge, the expression of DBA in human EC/ES cells has not been reported.

Primordial germ cells and neural stem cells

Primordial germ cells, which originate from primitive ectoderm, migrate to the genital ridge and differentiate into germ cells [1]. The resemblance of primordial germ cells to multipotential cells of early embryos is shown by the fact that ectopic transplantation of the genital ridge with primordial germ cells can induce teratocarcinomas.

Mouse primordial germ cells express Le^x (SSEA-1 and 4C9) [2, 23](Figure 1). Since the distribution of 4C9 is more restricted than SSEA-1, 4C9 has been used as a marker of primordial germ cells [24–27]. Multipotential cells with properties similar to ES cells have been estblished form primordial germ cells and are called embryonic germ cells (EG cells). EG cells from human [28] chicken [29] and goat [30] and primordial germ cells in turkey embryos [31] also express SSEA-1. However, primordial germ cells from bovine embryos lack SSEA-1 [15]. Human EG cells also express SSEA-4, TRA-1-60 and TRA-1-81. The difference of SSEA-1 expression between human ES an EG cells suggests that human ES cells are related to inner cell mass cells and human EG cells to primitive ectoderm cells, although antigenic profile of human primitive ectoderm cells is not yet known.

Neural stem cells derived from adult brain or from embryos yield both neurons and glia cells, and are expected to be valuable in therapy of neurodegenerative diseases. Both human and mouse neural stem cells express Le^X (CD15) [32–34] (Table 1). This property is helpful for the concentration of neural stem cells. However, because Le^X is also expressed in other cells of the nerve tissue, its use alone is not efficient to isolate neural stem cells [33]. Neural stem cells also express CD34, a marker of hematopoietic stem cells [34].

Hematopoietic stem cells

Hematopoietic stem cells yield progenitor cells, which have a more restricted differentiation potential, and eventually generate various blood cells such as erythrocytes and lymphocytes. CD 34 is a marker of hematopoietic stem cells and progenitor cells [1]. During hematopoietic differentiation, CD34 is progressively decreased and disappears from the fully differentiated cells. One function of CD34 is to suppress differentiation of the stem cells and the progenitor cells. CD34 is a transmembrane glycoprotein, and belongs to a sialomucin.

CD164 (MGC-24V) is also expressed on hematopoietic progenitor cells [35]. It was initially found as a soluble sialomucin (MGC-24) in human gastric carcinomas [36], and subsequently as a transmembrane form. The two forms are generated by differential splicing. Inhibition experiments with monoclonal antibodies to CD164 have suggested that its first mucin domain is involved in the promotion of cell adhesion and proliferation [35]. The exact functions of oligosaccharides on CD34 and CD164 are not known. However, the presence of ligands for selectins on hematopoietic stem and progenitor cells [37] suggests that the ligand structures are present on CD 34 and CD 164.

Function of carbohydrate antigens

Among the carbohydrate antigens mentioned above Le^x has attracted the most attention. Since Le^X first becomes detectable at the late 8-cell stage in mouse embryogenesis [3], it was postulated that the structure is involved in tight adhesion of the blastomeres, a process called compaction. Indeed, divalent Le^X was reported to inhibit compaction [38]. Le^X has weak binding activity for other Le^X molecules [39], and the carbohydrate to carbohydrate interaction was thought to be important in the compaction process [38]. However, the physiological significance of the role of Le^X in compaction is yet to be established.

We have obtained a monoclonal antibody, 4C9, which inhibits cell-substratum adhesion of EC cells [40]. The epitope of 4C9 is Le^x [24]. Transfection and expression of FUT4 cDNA , which can form Le^X structure, in L cells increase integrin-dependent cell-substratum adhesion of these cells [41]. Expression of FUT4 in ES cells promotes the myocardial differentiation of these cells [42]; the importance of integrin in myocardial differentiation has been well established.

We have recently isolated Le^X-carrying glycoproteins from EC cells by LTA affinity chromatography, and identified them to be $\alpha 6$ integrin and embigin (Muramatsu H., Oda Y. and Muramatsu T., unpublished results). Embigin, a member of the IgG superfamily, is known to enhance integrin-dependent cell-substratum adhesion [43]. It is expressed both in extraembryonic endoderm and embryonic ectoderm [44]. We have also identified basigin as a Le^X-carrying glycoprotein in EC cells [45,46]. Basigin has extensive sequence homology to embigin and is also known as an integrin-associated protein [45]. Another function of basigin is also interaction within the membrane, namely to associate with monocarboxylic acid transporters (MCTs) and to transfer them to the plasma membrane [45]. In early postimplantation embryos, basigin is expressed in all three germ layers [44].

Based on all these findings we propose that Le^X structure enhances integrin activity. It is possible that Le^X in both intergrin and embigin or basigin promotes the interaction of these proteins via Le^X-Le^X interaction.; Le^X epitope in α_6 integrin is located in embryoglycan; the multivalent Le^X in the branched poly-*N*-acetyllactosamines is expected to enhance the Le^X activity (Figure 2). Although the above proposal still lacks

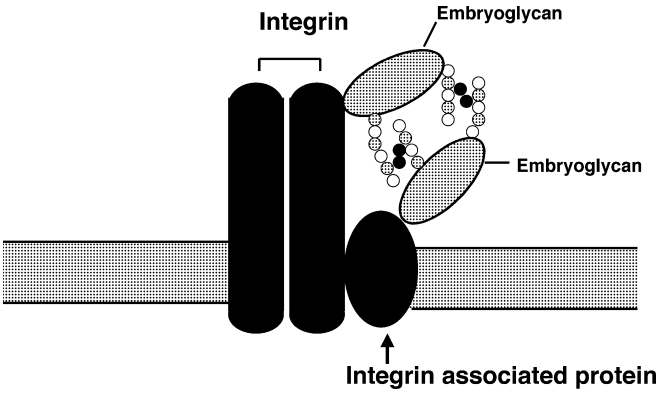

Figure 2. A scheme illustrating possible function of Le^X epitopes on embryoglycan. Sugars in terminal portions of embryoglycan are shown by circles. Closed circles show fucose residues in Le^X.

in vivo evidence, many experiments can be performed along this line.

The branching of poly-*N*-acetyllactosamines is carried out by multiple β-1, 6-*N*-acetylglucosaminyltransferases (IGnTs). We found that there are two classical IGnTs [47], and recently another IGnT has been found. These three IGnTs share a common exon both in humans and mice. We have knocked out the common exon of IGnTs. The knockout mice exhibited some abnormalities, probably related to cell differentiation or survival (Chen G, Muramatsu H and Muramatsu T, unpublished results). Lysosomes have been shown to participate in the repair of the plasma membrane by interacting with it [46], and a lysosomal major glycoprotein, LAMP, is a carrier of poly-*N*-acetyllactosamines [48]. Thus, it is possible that branched poly-*N*-acetyllactosamines participate in cell survival by enhancing the interaction of membrane molecules within the membrane.

Generally speaking, carbohydrates on membrane-bound glycoproteins are recognized either by proteins on membranes of other cells (*trans* recognition) or by proteins in the same membrane including the glycoprotein itself (*cis* recognition). Recognition of the ligand by selectin is a typical example of *trans* recognition. The expression of selectin ligands on hematopoietic stem and progenitor cells suggests the importance of *trans* recognition in the regulation of adhesion and proliferation of these cells. However, our studies to date suggest the importance of *cis* recognition of carbohydrates in the function of other stem cells mentioned here.

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