The International Journal of Biochemistry & Cell Biology 44 (2012) 830–833

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Molecules in focus

LewisX: A neural stem cell specific glycan?

Eva Hennen, Andreas Faissner*

Department of Cell Morphology and Molecular Neurobiology, Ruhr-University, Bochum, Germany

ARTICLE INFO

Article history:
Received 22 December 2011
Received in revised form 28 February 2012
Accepted 28 February 2012
Available online 13 March 2012

Keywords:
Glycans
Glycoproteins
Neural stem cells
Nervous system development
CD15

ABSTRACT

LewisX (LeX) detecting antibodies are routinely used for cell sorting of neural stem- and progenitor cells (NSPCs). Applications include the enrichment of NSPCs after neural differentiation of human induced pluripotent- or embryonic stem cells, as well as their direct isolation from mouse neural tissue. Nevertheless, only little is known about the role of LeX in the central nervous system. Here we review the current knowledge on LeX-containing glycans expressed by neural stem cells and their progeny. New LeX-carrier proteins and ligands have recently been identified which reveal further insights into the potential function(s) of LeX-glycans. Moreover, evidence accumulates that individual LeX detecting antibody clones vary in their suitability as neural stem cell specific biomarker. Each antibody clone detects a unique LeX-containing glycan epitope. This allows a versatile utilization of anti-LeX antibodies that goes beyond neural stem cell sorting applications.

1. Introduction

Glycosylation is a common modification of proteins and lipids. Glycans serve a wide range of different biological functions. A large number of glycan motifs have been identified. LewisX (LeX) is a glycan motif linked to glycoproteins, proteoglycans or lipids on the cell surface or associated with secreted extracellular matrix (ECM) proteins. LeX, also known as CD15 or stage-specific embryonic antigen (SSEA-1), is a member of the Lewis blood group antigens, a set of structurally related glycan moieties that all contain α1-3 or α1-4 fucosylated N-acetyllactosamine. This glycan family also includes the sialylated or sulfated forms of LeX: sialyl LewisX (sLeX) and sulfoLeX. The function of sLeX and sulfoLeX is well characterized in the context of lymphocyte rolling (Taylor and Drickamer, 2007) and cancer metastasis (Kannagi et al., 2004). In the central nervous system (CNS), up to now, only the expression of non-sialylated and sulfated LeX-glycans has been studied, using various monoclonal antibodies (mAbs) detecting the unmodified LeX motif. Overall, only little is known about the function of LeX-containing glycans in the CNS. Nevertheless, anti-LeX antibodies are routinely used to label and isolate neural stem cells and their lineage committed progeny such as glioblasts and neuroblasts, herein referred to as neural stem/progenitor cells (NSPCs).

In this review, we summarize the current knowledge on LeX-containing glycans, focussing on their expression in the mouse CNS, their function for neural stem cells and their potential as biomarker.

2. Structure

On the structural level, LeX-containing glycans are defined by a minimal LeX motif that consists of galactose β1-4-linked and fucose α1-3-linked to N-acetylglucosamine [Galβ1-4(Fucα1-3)GlcNAc] (Fig. 1A). This distinguishes LeX from Lewis A or B antigens where the galactose is β1-3-linked and fucose α1-4-linked. The minimal LeX motif can be further modified to create LewisY, sLeX or sulfoLeX by the addition of fucose, N-acetylneuraminic acid, or sulfate respectively. LeX-containing carbohydrates are part of large glycan chains linked via N- or O-glycosylation to glycoproteins or attached to glycolipids.

LeX represents a strong immunogen. In the past decades, several laboratories have independently generated antibodies that recognize LeX-containing glycans. By immunizations with F9 teratocarcinoma cells Solter and Knowles generated one of the first anti-LeX monoclonal antibodies (mAb). The antigen was named SSEA-1 (Solter and Knowles, 1978). (For an overview on anti-LeX clones, identified LeX-glycoconjugates and corresponding references see Table 1). The individual anti-LeX antibody clones differ in their affinity for complex LeX-containing glycans. Clone MC-480, usually referred to as SSEA-1 detects LeX exposed on long neo-lacto backbones (Fukui et al., 2002). Anti-LeX clone 487/L5 binds with high affinity to short terminal LeX-glycan motifs in glycan arrays, whereas clone 5750 detects the LeX motif if present within a glycan chain (Hennen et al., 2011). The glycan structures recognized by mAbs SSEA-1, 487/L5 and 5750 are depicted in Fig. 1(B)–(D). Note that these antibodies do not detect sLeX or sulfoLeX. Examples of cell sorting applications using the different anti-LeX clones are given in Sections 3 and 5.

* Corresponding author. Tel.: +49 234 3223851.
E-mail address: andreas.faissner@rub.de (A. Faissner).

1357-2725/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biocel.2012.02.019

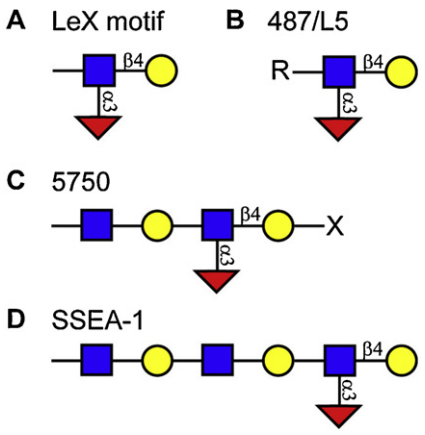

Fig. 1. Symbolic representation of LeX-containing antigens. (A) Structure of the LeX-glycan motif consisting of galactose β1-4-linked, and fucose α1-3-linked to N-acetylglucosamine. **(B-D)** LeX-containing glycan epitopes that are recognized by the anti-LeX mAbs 487/L5 (B), 5750 (C) or SSEA-1 (D). Note that 487/L5 detects all glycans containing a terminal LeX motif. 5750 requires internal LeX motifs whereby x may represent galactose or lactosamine but not sulfate or sialic acid. SSEA-1 (clone MC-480) recognizes LeX on long neo-lacto backbones. Symbolic representation: fucose (red), galactose (yellow), N-acetylglucosamine (blue), R: any glycan(s).

3. Expression in the CNS

Reports on the expression of the LeX-carbohydrates are not completely congruent. This is most likely due to the fact that different anti-LeX antibodies have been used for individual studies. Using anti-LeX clone MMA, strong LeX expression can be observed during embryonic development on neural stem cells in neurogenic brain regions, e.g. the hippocampal primordium, the isthmus and the embryonic cerebral cortex (Capela and Temple, 2006). MAb 5750 confirmed a prominent LeX expression on cortical neuroepithelial cells and radial glia which constitute the neural stem cell population in the embryonic brain (Hennen et al., 2011). In the adult mouse, LeX expression as detected by mAb MMA is present on a subpopulation of GFAP-positive cells in the SVZ and the dentate gyrus of the hippocampus, which comprises the neural stem cell population in the adult brain (Capela and Temple, 2002). Indeed, cell sorting with clones MMA and 5750 from embryonic tissue (Hennen et al., 2011; Capela and Temple, 2006), as well as with clone MMA from adult tissue (Capela and Temple, 2002) enriches for multipotent, sphere forming cells, characterizing LeX-positive cells as NSPCs. However, LeX does not exclusively label neural stem cells, since the glycan is also present on immediate progeny such as glioblasts and neuroblasts (Capela and Temple, 2006). Interestingly, not all NSPCs express LeX. LeX-negative cells isolated from E14 ganglionic eminence (Hennen et al., 2011) or spinal cord (Kelly et al., 2009) can give rise to neurospheres in vitro. Whether LeX-positive and -negative neural stem cells differ in their properties has not been investigated so far (see also Section 5).

In vitro, clones detecting the LeX-containing antigens SSEA-1 and forebrain-surface-embryonic-antigen-1 (FORSE-1) are used to isolate NSPCs derived from human embryonic stem cells (hESCs). LeX-glycans are present on early neuroectodermal precursor cells but not on hESCs or differentiated neurons (Pruszak et al., 2007). In contrast, mouse ESCs express LeX already at pluripotent stages.

NSPCs are not the only LeX-positive neural cell population. LeX-containing glycans detected by clone 487/L5 are expressed by rat hippocampal neurons in vitro (Brito et al., 2009), by astrocytes (Streit et al., 1993) and by oligodendrocyte precursor cells (OPCs) (Hennen et al., 2011). In the adult CNS, LeX expression is also present on some neurons in the cortex (Nishihara et al., 2003), and on cells in the septum, the striatum and the corpus callosum (Capela and Temple, 2002).

In conclusion, LeX is not exclusively expressed by neural stem cells, but also by their differentiated progeny. Therefore, LeX in general should not be considered as stem cell specific biomarker. Nevertheless, a subtype of LeX-containing glycans, detectable by specific anti-LeX mAbs, seems to be characteristic for neural stem cells and allows their isolation. How exactly the LeX-containing structures and their carrier proteins change upon differentiation will have to be addressed in future studies.

4. Biological function

The function of sLeX and sulfoLeX has been thoroughly investigated in the immune system and in the context of cancer cell migration, which is reviewed in detail elsewhere (Taylor and Drickamer, 2007; Kannagi et al., 2004). The functional relevance of LeX-glycosylation in the CNS is far less well understood. In this section, we will discuss LeX-expressing proteins that are of functional relevance for neural stem cells. A list of identified LeX-glycoconjugates is given in Table 1.

In vitro, the addition of anti-LeX antibodies to the culture medium can disturb migration, aggregation, adhesion and process formation of various cell types including astrocytes (Streit et al., 1993) and neurons (Lieberoth et al., 2009). Evidence for a role of LeX in stem cell migration comes from a study that demonstrated that migration of NSPCs is disturbed on Fibronectin in an integrin dependent mechanism in the presence of anti-LeX antibody AK97 (Yanagisawa et al., 2005). This study indentified β1-integrin as LeX-carrier protein in NSPC cultures (Yanagisawa et al., 2005). Other LeX-carrying proteins expressed by neural stem cells are the extra cellular matrix protein tenascin-C (Hennen et al., 2011), and the receptor protein tyrosine phosphatase beta/zeta (RPTPβζ) and its secreted splice variant phosphacan (Allendoerfer et al., 1995; Garwood et al., 1999). All of these proteins are capable of interacting with a large number of different ligands. LeX is expressed only on some glycoforms of these carrier proteins. A potential function of LeX-glycosylation could therefore be to modulate the affinity for certain ligands. Homotypic, calcium mediated LeX-LeX dimerization can facilitate the interaction of LeX-glycoproteins with each other (Luo et al., 2008). Neurons and glia express TAG-1 (contactin-2) and contactin-1, which contain putative fucose-binding lectin domains (Lieberoth et al., 2009). These proteins interact with tenascin-C and RPTPβζ/phosphacan (Shimoda and Watanabe, 2009). However, whether LeX is involved in this interaction remains to be investigated.

Table 1
Anti-LeX mAbs.

mAb	Reference	Identified glycoconjugates
487/L5	Streit et al. (1990)	L1-CAM, Thy-1, Streit et al. (1990); CD24, Lieberoth et al. (2009); Synapsin I, Wang et al. (2011); Tenascin-C, Hennen et al. (2011)
5750	Hennen et al. (2011)	Phosphacan, Tenascin-C, L1-CAM, Hennen et al. (2011)
AK97	Yanagisawa et al. (1999)	β1-Integrin, glycolipids, Yanagisawa et al. (2005); LAMP-1, Yagi et al. (2010)
FORSE-1	Tole et al. (1995)	Phosphacan, neutral glycolipids, Allendoerfer et al. (1995)
MC-480 (SSEA-1)	Solter and Knowles (1978)	Unknown glycolipids and glycoproteins
MMA	Hanjan et al. (1982)	Unknown >200 kDa protein, Capela and Temple (2002)