Chapter 16

β1,4-Galactosyltransferases, Potential Modifiers of Stem Cell Pluripotency and Differentiation

Michael Wassler

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54376

1. Introduction

The ability of embryonic stem cells to self renew and, at a given signal, give rise to the multifaceted cell types normally observed in the body, is highly dependent on the complex interplay between both intrinsic (inside the cell) and extrinsic (outside of the cell) factors. Despite progress in analyzing the genome, proteome, and the transcriptome, challenges still exists to find the most efficient and specific conditions in which human embryonic stem cells (hESC) can maintain pluripotency and or/can be efficiently directed to differentiate towards a homogenous cell type. In a stem cell niche, the integrity of the cell matrix and the manifold of different cell-cell interactions and the ability of the cells to respond to a variety of cytokine cues from both interstitial fluids and from extracellular matrices, are crucial factors in giving the right signal signals to the cells internal machinery, in a space (spatio) and time (temporal) manner during different developmental stages. One of these molecules is the glycan. A glycan is a polysaccharide or oligosaccharide, that is attached to a glucoconjugate such as glycoprotein, glycolipid, and proteoglycan. Cell surface glycoproteins are abundant and constitute approximately 50% of all proteins in nature. For many years, the biological function of glycosylation in stem cell behavior/homeostasis was overlooked and thought of as a more or less redundant process with applications only limited to the identification and sorting of cells at different stages of pluripotency and during formation of induced pluripotent stem cells (iPSC). Markers such as stage specific embryonic antigen (SSEA1 and -3/4) and the tumor rejection antigens (TRA-1-60 and TRA-1-81) have been used to analyze the pluripotency and differentiation stages of embryonic stem cells and induced pluripotent stem cell (iPSC).The research of how glycosylation can impact stem cells has long been hampered by the structural complexities of glycosylation and the difficulties to identify and purify the enzymes, glycosyltransferases, responsible for these processes. This problem is partly due to

the fact that glycans are not encoded directly from the genome but rather depends on the collaboration of a limited number of both glycosyltransferases and glycosidases, whose expression are reliant upon both intracellular as well as extracellular changes. Furthermore, glycosyltransferases are expressed differentially between many cell types and disease states in a spatio- temporal manner during development. In this review, I will summarize research on what is known for glycosyltransferases in stem cell pluripotency and differentiation. I will specifically focus on one glycosyltransferase, N-acetylglucosamin β1,4- Galactosyltransferase 1 (β4Gal-T1), a unique galactosyltrasferase implicated in a variety of cellular processes such as cell-cell and cell-matrix adhesion, apoptosis, proliferation and differentiation, to mention a few. I will discuss its regulation and potential mechanism(s) in cell-cell, cell-matrix and cytokine signaling pathways. Finally, in the last section, I will talk about some diseases related to galactosyltransferase deficiency. All in all, this chapter is intended to evoke more interest in the field of stem cell glycobiology, both for the layman as well as for the bench scientist. Ultimately, the goal of this review is to encourage future research to find alternative therapeutic modalities for glycoprotein related diseases, such as cancer, congenital disease and even Alzheimer's.

2. What is glycosyltransferases?

Glycosyltransferases (GTs; EC 2.4.x.y) constitute a large protein family of about 200-300 enzymes that are involved in the biosynthesis of glycans. GTs are type II transmembrane proteins with large carboxy-terminal globular catalytic domains, that face the luminal side of the Golgi complex, and a short cytoplasmic domain. The sequential action of GTs results in the formation of both linear as well as highly branched glycan structures that are present in both prokaryotes and eukaryotes. Mammalian GTs utilize a variety of uridine diphosphate activated (UDP) sugars as donors: UDP-glucose, UDP-galactose, UDP-GlcNAc, UDP-GalNAc, UDP-xylose, UDP-glucuronic acid, GDP-mannose, GDP-fucose, and CMP-sialic acid. Glycosyl transfer can occur on protein residues, usually to asparagine, to give N-linked glycoproteins and on tyrosine, serine, or threonine to give O-linked glycoproteins [1]. The first step in N-linked glycosylation occurs in the endoplamic reticulum (ER) in which a "high mannose" oligosaccharide branch is added to an Asparagine (Asn) residue in the protein backbone (N-linkage). Another type of glycan linkage is the *O*-linked glycosylation, which occurs through serine/threonin residues in the protein back bone during transport within the Golgie complex [2]. Other GTs are responsible for extensive branching of glycan structures such as the galactosyltransferase family (GalTs) [3] which together with glycosidases give rise to more "complex" type sugar chains (Figure 1). These processes creates oligosaccharide structures of enormous diversity and whose functions spans from cell adhesion, inflammation, cancer metastasis, stem cell proliferation and development [4]. This exciting area of biology has resulted in an intensive research to unveil the function of individual GTs in during stem cell pluripotency and differentiation. Several studies have implicated a variety of GTs in stem cell biology, some of which are presented below:

http://dx.doi.org/10.5772/54376

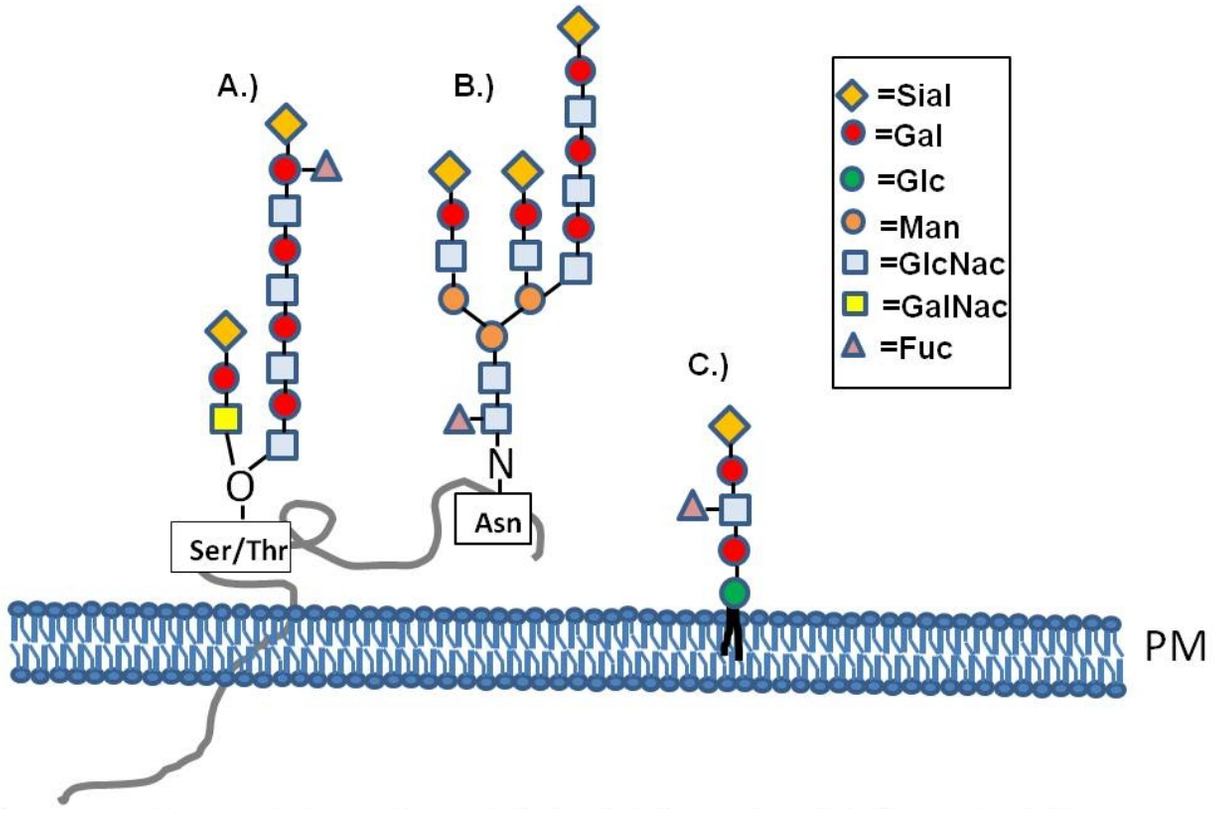

Figure 1. General view of an O-linked (A), and a (B) "complex" N-linked cell surface glycoprotein. A lactosylceramide-glycolipid (LacCer) (C) is also shown, located at the upper leaflet of the plasma membrane (PM). Ser; Serine, Thr;Threonine, Asn;Asparagine Sial;Sialic acid, Gal;Galactose, Glc;Glucose,Man;Mannose, GlcNac; N-Acetylglucoseamine, GalTNAc; N-Acetylglucoseamine, Fuc;Fucose

1. N-acetylglucosaminyl-1 phosphate transferase (GPT): The first steps in N-linked glycan synthesis begins on both the cytosolic and luminal side of the endoplasmic reticulum where nine mannosyl residues are sequentially added to a poly-isoprenoid lipid, dolychylmonophosphate by the activity of N-acetylglucosaminyl-1 phosphate transferase (GPT) and a number of mannosyltransferases. One inhibitor to GPT, tunicamycin (TM), inhibits N-linked glycosylation and has been reported affect cell proliferation, neu-vascularization and cancer progression, due to induced cell death from ER stress [4].

2. βGalNAc-T3: The cell surface glycan epitope LacdiNac (GalNac-β4GlcNAc) has been shown to be an important glycosylation modification of leukemia inhibitor factor receptor (LIFR) and its co-receptor, gp130.The addition of LacdiNac epitopes to LIFR was dependent on a specific transferase, β-3-N-acetyl-Galactosyl transferase 3 (βGalNac-T3). This modification is crucial for the localization of LIF to lipid rafts/ calveolar components, such as caveolin-1, in order to enhance its activity. Mouse and human stem cells (mESC, hESC) differ from each other in some aspects on how they respond to cytokines necessary for pluripotency. hESCs seem to be at a later developmental stage than mESCs, because of their independency of the LIF pathway for self renewal. Interestingly, the level of βGalNac-T3 was much lower in human versus mouse embryonic stem

indicating that LacdNac play an important role for adopting stem cells from a primed state (already programmed for germ line specification) to a more naïve state, e g fully pluripotent cells[5]

3. Ext1 and Ext2: Heparan sulphate is a large sulphated oligosaccharide chain located on proteoglycans impacting both the stability of pluripotency and differentiation into neural stem cell lineage. Ext1 and Ext2 encodes two bifunctional endoplasmic reticulum-resident type II transmembrane glycosyltransferase that are involved in the chain elongation and modification of HS biosynthesis. HS on embryonic stem cells has been shown to exhibit a lower amount of sulfated glycosaminoglycans relative to differentiated cells indicating that the ratio between nonsulphated versus sulphated HS is important in stem cell pluripotency [6-8]

4. O-GlcNac Transferase (OGT): O-GlcNAcylation is a O-β-glycosidic attachment of a single N-acetyl glucosamine to a serine or threonin residue in nucleoplasmic proteins. Some of these proteins are represented by the transcription factors Oct4, Klf4, Sox and Nanog, which are involved in the pluripotency network in stem cell self renewal and in the core proteins responsible for the production of induced pluripotent stem cells (iPSCs). Recently it was discovered that this specific O-linked modification of Oct4 and Sox was crucial for their transcriptional activities. Two enzymes are responsible for O-GlcNAcylation: O-GlcNac Transferase (OGT) adds the modification and O-glucNAcase removes it [9].

3. β-1,4-Galactosyltransferases

β-1,4-Galactosyltransferases (β4GalTs) are type II membrane proteins of the glycosyltransferase family that have the exclusive specificity to transfer an active UDP-galactose in a β1,4 linkage to acceptor sugars such as N-acetylglucosamine (GlcNAc), Glucose (Glc), Galactose (Gal) and even Xylose (Xyl). Each β4-GalTs have a distinct function in the biosynthesis of different glycoconjugates and disaccharide structures.The most common structure, the Galβ1-β4GlcNAc, or N-Acetyllactosamine, exists as disaccharide repeats within linear or branched poly-N-acetyllactoseamine chains, but also at the terminal ends of oligosaccharide chains where they become sialyllated. These structures are formed by a combined action of UDP-GlcNac:Mannosyl N-acetylglucosaminyltransferases and β-1,4-galactosyltransferases (β4GalTs) [10]. The first galactosyltransferase, β4GalT-1, was cloned in 1986 due to its function of transfer galactosyl residues to β-1,4-linked GlcNac found in glycoconjugates [11]. Targeted inactivation of mouse β4Gal-T1 gene, however, revealed that both tissue and serum glycoproteins still contained residual β4GalT-1 activity towards glycoprotein acceptors [10]. To date there are currently seven members of the β4GalT gene family designated β4Gal-T1-T7. Even though, β4Gal-T1 to -T6 shares various homologies (30-50%) to β4GalT-1 at the amino acid level, their substrate affinities and end products appear to be slightly different, depending on nature of the branched oligosaccharide structure tissue expression and the cellular milieu for the enzymes, e.g. lipid -rich environment [12, 13]. Both β4Gal-T1 and

β4Gal-T2 preferentially transfer galactose to the GlcNacβ1-2Manα and the GlcNAcβ1-4Man1-3 branch. β4Gal-T4 and β4Gal-T5 catalyzes the addition of galactose to GlcNAcβ1-6Man and the GlcNAcβ1-4 Man, respectively (Figure 2). The β4Gal-T1, β4Gal-T2, and β4Gal-T3 can also transfer galactose residues to tetra-antenna oligosaccharides. In addition being involved in glycoconjugate synthesis, β4Gal-T2, -3, -4 and -6, are also important catalysts for glycolipid biosynthesis. β4Gal-T2 and -3 prefers a glycolipid intermediate, Lc3Cer, as a substrate and β4Gal-T4 uses GlcNac-6-sulphate, a common constituent of keratin sulphate, as a substrate [14]. β4Gal-T6 has been shown to have Lactosyl Ceramide synthase activity. Finally, β4Gal-T7, transfers a Galactose to an O-linked Xylose on a serine residue to start the synthesis of the linker region between glycosaminoglycans (GAG) and proteoglycans [15]. A general summary or the chromosomal location, tissue expression, glycosidic linkage and potential biological function of currently known β, 4-GalTs is summarized in Table 1.

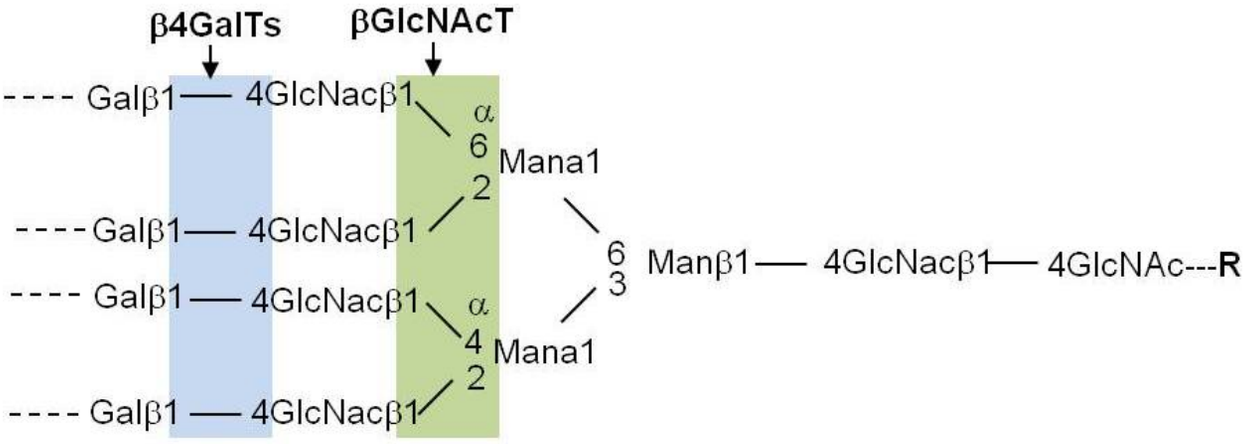

Figure 2. An example of a tetra antenna structure in a complex-type N-glycan. The numbers indicate the glycosidic linkages. The arrows and the boxed areas represent the bonds catalyzed by β1,4-galactosyltransferase (β4GalT, blue area) and βGlcNAc Transferases (βGlcNAcT, green area), respectively. Gal; Galactose, Man: Mannose, GlcNAc; N-Acetylglucosamin, R; glycoprotein back bone.

4. β-1,4-Galactosyltransferase 1 (β4Gal-T1)

One member of the β4galactosyltransferase family, that has got increased attention in stem cell biology, is the β4Gal-T1. β4Gal-T1 catalyze the transfer of galactose (Gal) from uridine diphosphate-galactose (UDP-Gal) to terminal N-Acetylglucosamine (GlcNac) residues of oligosaccharide chains in a β1,4 linkage, to form N-acetyllactosamine. β4Gal-T1 and βal-T2 are unique among the β4galactosyltransferases (β4GalTs) genes that they form a heterodimer with alpha-lactalbumin and changes substrate specificity from GlcNac towards Glucose (Glc) as a substrate, forming lactose, a very common protein in the mammary glands. Interestingly, β4Gal-T1 is constitutively expressed. However, apart from β4Gal-T1, β4Gal-T2 is only expressed in fetal brain. β4GalT-2 is a key regulator of glycosylation of the proteins in-

volved in neuronal development [16] and is responsible for the synthesis of complex-type N-linked oligosaccharides in many glycoproteins, as well as the carbohydrate moieties of glycolipids. Like the β4Gal-T1 enzyme, its substrate specificity is affected by alpha-lactalbumin but is not expressed in lactating mammary tissue Apart from the other βGalTs, βGal-T1 encodes two protein isoforms produced by differential translation initiation at the 5′ end of the mRNA transcript: a long isoform, containing a 24 amino acid cytoplasmic domain, and a short isoform with only an 11 amino acid domain [24]. Both isoforms are localized to trans-Golgi network and are able to function as glycoprotein processing enzymes (Fig.3). However, a small fraction of the long isoform of βGaT-1, preferentially targets the cell surface of various cells [25]. The specific signal sequence in β4GalTs that regulate the differential localization between cell surface and the Golgi complex, has been shown to consist of a short N-terminal hydrophobic sequence in the cytoplasmic domain, adjacent to the plasma membrane. This observation was further extended by the findings that the 13 amino acid sequence in the cytoplasmic domain of long Gal-T1, could be phophorylated by p58 (CDK11), a GalT1 associated and cell cycle related Serine/Threonin kinase and, hence, could act as a retention signal for β4Gal-T1 in the Golgi complex [26, 27, 28, 38, 55] (Fig.3). Apart from being involved in a variety of physiological activities, such as, for example mouse gamete interaction, neurite extension, epithelial mesenchymal transition and neural crest cell migration [29], cell surface GalT1 is also responsible for late morula compaction during development [30]. For more than a decade ago, β4Gal-T1 was found to facilitate cell migration on laminin 1, an important constituent of the extra cellular matrix (ECM) and during development [31, 32]. Furthermore, addition of β4Gal-T1 perturbants to F9 embryonic carcinoma led to an arrest in cell growth and morphological changes of embryoid bodies (EB) during differentiation [33]. Eckstein et. al., showed that cell surface β4Gal-T1 needed to associate with intact actin cytoskeleton in order for its cell surface activity [34] Interestingly, the intracellular domain of long form of β4Gal-T1 has been shown to bind to an array of signal transduction molecules such as a trimeric G-proteins (Gi) [35], Src Suppressed C-kinase Substrate (SSECKs) [36, 37], CDK11 (p58) [26, 38] and a novel ubiquitin conjugating enzyme [39]. The β4Gal-T1 interaction with SSeCKS was detected using the two hybrid system with the amino terminal 13 amino acid long cytoplasmic domain of β4GalT-1 [37]. The β4Gal-T1 association with SSeCKS is interesting since both proteins show similar subcellular distributions and share important cellular functions, such as cell proliferation, actin dynamics, and cell migration during development [36, 40]. For example, ectopic expression of both cell surface β4Gal-T1 and SSeCKS has been reported to induce a transient tyrosine phosphorylation of focal adhesion kinase (FAK) and rearrangement of filamentous actin [41]. Furthermore, SSeCKs also control the G1 to S phase progression through regulation of cyclin D1 expression and localization. Since SSeCKS is a scaffolding molecule that can binds to several signaling proteins, such as PKC, Rho family members, and FAK, to mention a few, it is possible that most effects attributed to cell surface GalT1 in stem cell growh and differentiation may be mediated through SSeCKS. However it is unclear if cell surface β4GalT-1 performs in a similar manner as a lectin for its biological function [42, 105] or whether it utilizes its enzymatic activity to modify and release its galactosylated product [31].

http://dx.doi.org/10.5772/54376

(β4GalTs) (Chrom. #)	Expression	Glycosidic linkage and Acceptor substrates	Function in stem cell, cancer and/or development	References
β4Gal-T1 (9p13)	Heart, liver, lung, testis, ovary, placenta, fetal brain	Galβ1-4GlcNac-R	Morula compaction, cell growth, laminin dependent migration	[17], [18]
β4Gal-T2 (1p32-33)	Restricted in brain, testis	Galβ1-4GlcNac-R Glycolipid	Neuronal development, spermatogenic differentiation	[16], [18]
β4Gal-T3 (1q23)	Constitutively expressed, high in fetal brain.	Galβ1-4GlcNac-R Glycolipid	N/A	[14]
β4Gal-T4 (3q13.3)	Testis, ovary, placenta, pancreas	Galβ1-4GlcNac-R GlcNac-6-sulphate LacCer	Testicular development, tumor metastasis, keratin sulfate synthesis.	[19], [14], [20, 21]
β4Gal-T5 (11)	heart, lung, liver, kidney, testis, Restricted in brain	Galβ1-4GlcNac-R LacCer	Self renewal of glioma cells, astrocytoma, extraembryonic development	[18], [22]
β4Gal-T6 (18q)	Restricted to adult brain	Galβ1-4GlcNac-R LacCer	Extra embryonic development	[18, 19]
β4Gal-T7 (5q35.1-35.3)	Heart, Brain, Placenta, Liver, kidney, pancreas	GlcAβ1-3Galβ1-3Galβ1-4 Xylβ1-R	Glycosaminoglycan (GAG) biosynthesis	[15, 23]

Table 1. Table depicting the chromosomal region, the glycosidic linkage, substrate, and the function for the β4Galactosyltransferase family, related to stem cells and development.

5. β4GalTs in cancer

Glycosylation of cell surface glycoproteins and glycolipids changes dramatically upon the malignant transformation of cells [43]. β4GalTs have been reported to be increased in a fair amount of cancer. However, is not currently known if the elevated expression of β4GalTs contributes to the induction of cancer/malignancy, by affecting the cell surface landscape of glycans, or is an indirect effect of cancer progression or metasisis. β4Gal-T1 has been detected in highly metastatic lung cancer by transcription factor E1AF activation of the β4Gal-T1 promoter [17, 51]. Furthermore, siRNA interference of surface β4Gal-T1 function, inhibited cell adhesion on laminin, the invasive potential in vitro,and tyrosine phosphorylation of focal adhesion kinase [17]. The relative level of β4Gal-T1 has been reported to be important in melanoma invasiveness. For example, increasing cell surface β4Gal-T1 expression in cells of low metastatic potential promoted their invasive potential [44]. Other β4GalTs such as β4Gal-T5, function as an important growth regulator in glioma cells using both the E1AF and Sp1 transcription factors for its metastatic potential [17, 45]. Furthermore, clinically over expressed β4Gal-T4 and β4Gal-T6 have been shown to increase E2F1 and cyclin D3 transcription in colorectal cancer, respectively [18, 19]. Moreover, β4Gal-T1, -T2 and -T5 levels

are higher in astrocytoma [18]. The expression of the β4Gal-T5 gene has also been shown to be regulated by transcription factors Sp1 and Ets-1 in cancer cells. Both these transcription factors regulate the gene expression levels of not only glycosyltransferases, but also key molecules involved in tumor growth, invasion and metastisis. Finally, small molecules that increase expression of GalTs could have beneficial effects during treatment of various cancer forms [45].

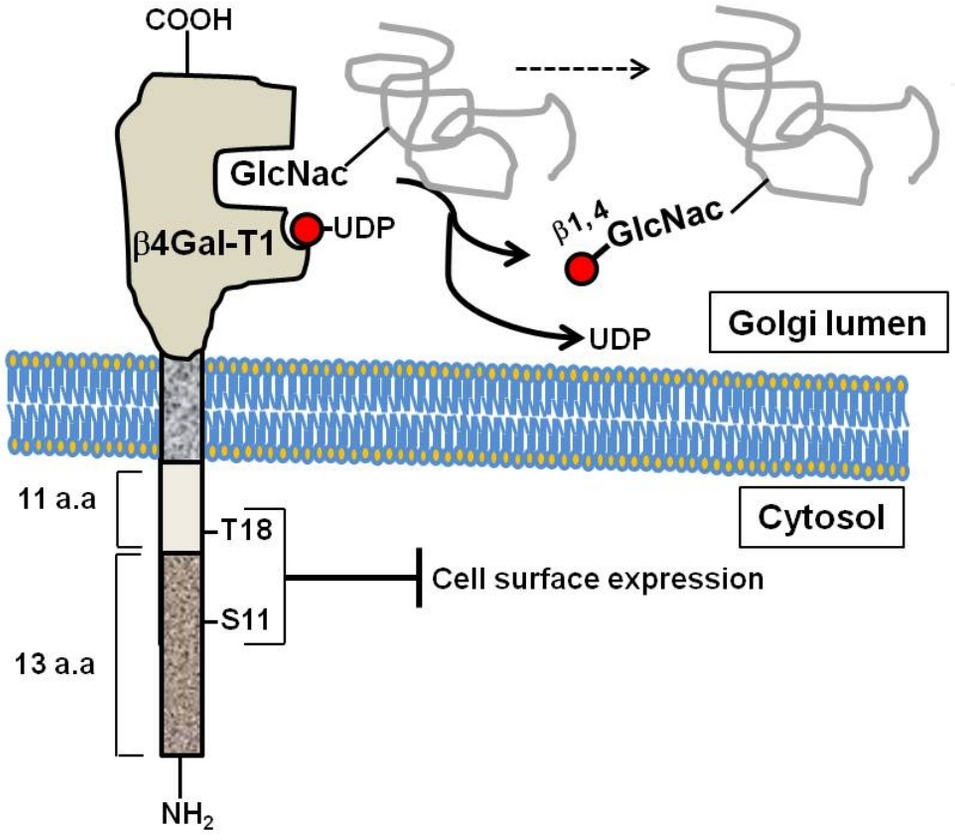

Figure 3. The long isomer of β1,4Galactosyltransferase 1 (β4GalT-1). β4GalT-1 catalyzes the transfer of UDP-galactose (red circle) to a terminal N-Acetylglycosamine (GlcNAc) residue in a newly synthesized glycoprotein in the golgie lumen. The cytosolic domain of the long β4Gal-T1 consists of 11 amino acids (a.a) together with a 13 a.a extension (24 a.a in total). Phosphorylation of Serine 11 (S11) and/or Theonine 18 (T18) in the cytoplasmic domain negatively regulate the localization and function GalT-1 as a cell surface receptor. The figure is not in scale.

6. β4Gal-T1 in cell cycle

The observation that some, or maybe all, of the β4GalTs have relevancy in cancer progression and/or metastasis, has highlighted the idea that stem cell pluripotency and differentiation may also depend on N-glycan structures [46]. One decisive factor in pluripotency and stem cell differentiation is the speed by which cells goes through the G1 phase in the cell cycle [47]. The cell cycle in pluripotent stem cells is remarkable for the shortness of the G1 phase, permitting rapid proliferation and reducing the duration of differentiation signal sen-

sitivity associated with G1 phase. Changes in the length of G1 phase are understood to accompany the differentiation of human embryonic stem cells (hESCs), but the timing and extent of such changes are poorly defined. Terminally differentiated cells usually have a longer G1 phase than those of stem cell and progenitor cells. Understanding the early steps governing the differentiation of hESCs will facilitate better control over differentiation for regenerative medicine and drug discovery applications. To avoid that cells with genetic aberrations are expanded in the population, stem cells have adapted to their harsh environment by shutting off specific checkpoints normally activated in somatic cells. This will result in cell death as a default pathway for stem cells exhibiting chromosomal deveations, without slowing down proliferation of otherwise healthy cells. Since the upstream promoter region of the 4.1 kb β-GalT1 transcript is mainly occupied by the Sp1 transcriptional factor, GalT1 was long believed to be another "house keeping" gene. However, several laboratories have shown that β4GalT-1 is regulated during cell cycle [28, 48, 49]. Interestingly, experiments in F9 embryonic carcinoma cells and in 3T3 cells have indicated that the cell surface bound and the Golgi related forms of β4GalT-1 are regulated differently, in which the long form is induced much earlier than the short and Golgie bound form. β4Gal-T1 showed the highest activity during G1-S phase and during interphase of the cell cycle [50]. There are many transcription factors important during the G1-S transition. The E2F family members of transcription factors serve as key regulators of the cell cycle progression by inducing activators of S-phase related genes. Normally, during the onset of G1/S transition in cell cycle, the cyclic dependent kinases (CDKs) phosphorylate the retina blastoma (Rb) protein, resulting in a conformational change in Rb and subsequent release of active E2F from the Rb-E2F complex. This event results in transcription of G1-phase activating proteins such as e.g Cyclin D3. Interestingly, E2F1, one of the best characterized members of this family, also binds to a promoter element in β4Gal-T1 transcript and positively regulates its activity. Moreover, cells subjected to a short hairpin RNA (shRNA) to β4Gal-T1 became less responsive for E2F1 activation [51].The effect of E2F1 on the expression of the other family members of β4GalTs, however, (β4Gal-T1, -T7) has not been exclusively determined. Another cell cycle related protein that has been found to regulate β4GalT1 expression is the p16 protein. This protein is a product of a tumor suppressor gene called CDKN2A that inhibits the cyclin-dependent kinases (CDK)-4 and 6 which are responsible for the G1 checkpoint in cell cycle. Transfection of A549 human lung cancer with p16 led to down regulation of βGalT1 activity [53]. Thus, inactivation of either p16 or pRb function allows the cells to enter the S-phase only after a brief pause at the G1 checkpoint, leading to accelerated cell proliferation. Similar results for GalT1 expression was obtained in hepatocarcinoma SMMC-7721 cells after blocking endogenous activity of TGFβ, a known regulator of the G1 to S-phase transition of cell cycle, by arresting cells in G1 phase [54]. Over expressing β4Gal-T1 has also been shown to exasperate cyclohexamid induced apoptosis of [45]. This process is partly dependent on the activity of the CDK11(p58), a CDK11 family Ser/Thr kinase, a G2/M specific protein that contributes to regulation of cell cycle [55]. Recently GalT1 has been shown to interact with CDK11(p58) [26, 38] where it has an important function during cell cycle in stem cells progression [28, 56]. Furhtermore, β4Gal-T1 contributes to HBx-induced cell cycle progression In hepatoma cells [57]. All these findings have led to the conclusion that β4Gal-T1 may be

directly or indirectly connected to cell cycle progression and could be a potential reason for the growth impeded phenotype observed earlier in knock out β4Gal-T1 mice [52]

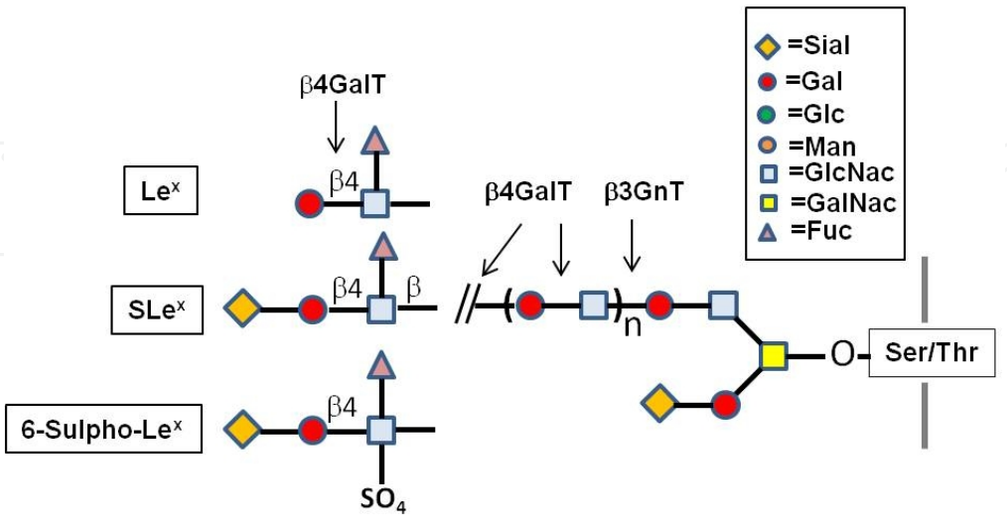

Figure 4. Biosynthesis of a core 2 O-glycan with Lewis X, (LeX,SSEA-1), Sialyl-Lewis (SLex) or 6-sulpho Sialyl (6-Sulpho-Lex synthesized at the terminus of poly-N-Acetyllactosamine chains. The action of β4Ga|Ts and β3GnT are indicated with arrows. Sia|;Sia|ic acid, Gal;Ga|act0se, G|c;G|uc0se, Man;Mann0se, G|cNac; N-Acetylglucoseamine, Ga|TNAc; N-Acetylgalactoseamine, β4Ga|act0syltransferases; β4GalT, β1,3-N- Acetylglucosaminyltransferase; β3GnT

7. β4GalTs involvement in Lewis X, glycosphingolipids and embryoglycans

Lewis X: As mentioned in the beginning of this chapter, β4GalTs are Important for the synthesis of linear or branched poly-N-acetyllactoseamines chains. They are attached to N-glycan, O-Glycans or glycolipids and are synthesized by the repeating and alternate action of N-acetylglucosaminyltransferases (β3GnT or β4GnT) and β4Gal-T1 [58]. These structures often carry various functional epitopes important in stem cell homeostasis and inflammation [59]. One of these antigen is called the Lewis X antigen (Le^x) and constitutes the core structure from which other antigens are synthesized. Le^x epitope consists of a trisaccharide, Galβ1-4(Fucα1-3) GlcNAcβ1 which is produced by the action of β4Gal-T1 and α-1,3-Fucosyltransferase (FUT). Other examples of epitopes formed from this core, are the Sialyl-Lewis (SLe^x) and 6-sulpho Sialyl (6-Sulpho-Lex) epitopes (Figure 3), in which the latter involve the activity of β4Gal-T4 (Table 1). These epitopes are implicated in biospecific interactions with selectins and other glycan-binding proteins during inflammatory processes [59] as well as in important regulatory functions during development [60]. Also, Le^x structures has been implicated in specific differentiation, such as myocardial differentiation from embryonic stem cells [60, 61].

Glycosphingolipids: Glycosphingolipids, or sometimes called glycolipids (GLS) have been found in the upper leaflet of the plasma membrane in both lower and higher eukaryotic

http://dx.doi.org/10.5772/54376

sources. Several members of β4GalT family seem to be important enzymes in the synthesis of GSL [62]. The basic structure for GLS is a monosaccharide, usually glucose, attached directly to a ceramide molecule, mediated through the action of ceramide glucosyltransferase (Ugcg), resulting in a glycosylceramide (glucocerebroside;GlcCer) (Figure 5). βGalT-2 then transfer a UDP-Galactose to the GlcCer moiety, forming Lactosylceramide (LacCer) [62] (Figure 5). A variety of structural subclasses of GLS may then be synthesized from LacCer by the addition of other mono and disaccharides, resulting in the synthesis of structural subclasses of GLS such as ganglio-, lacto/neolacto-, globo,- -isoglo, and ganglioseries-series [63]. Many of these structures are important for various biological functions, such as for example cell growth, myocardial differentiation cell migration and during development of the nervous system[60, 61, 64]. When the Le^x epitope is attached to a lactosylceramid it is identical to stage specific antigen (SSEA-1). This antigen is highly regulated during embryogenesis, expressed at the morula stage in embryos and is considered to function as a cell-cell interaction ligand in the compaction process [65].

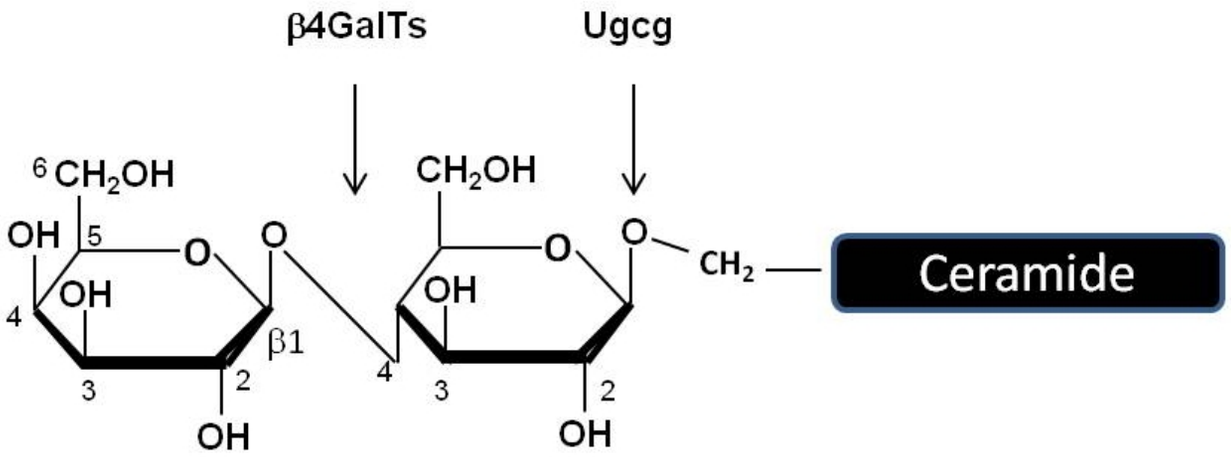

Figure 5. Core 2 structure of the glycoplipid, Lactosylceramide (LacCer) synthesized by UDP-glucose ceramide glucosyl transferase (Ugcg) and by β1,4Ga|act0sy|transferases (β4Ga|Ts) forming the β1,4-glycosidic linkage to ceramide.

Embryoglycans: Most developmentally regulated epitopes identified on embryonal carcinoma cells and murine preimplantation embryos are associated with a glycoprotein-bound and large glycans, called embryoglycans. Embryoglycans consists of linear of branched poly-N-acetyllactoseamines with high molecular weight that carries a number of different developmentally regulated carbohydrate epitopes, such as e. g. Le^x, described above (Figure 6). Apart from the mouse, where SSEA-1 is abundant from the 8-cell morula stage, SSEA-1 in human is not expressed until the germ cell line and in neural stem cells. Interestingly, β4Gal-T1 is expressed during the morula stage and has been shown to affect the compaction process [30]. Furthermore, human ES cells express SSEA-3 and -4 SSEA-1. SSEA-1 is also expressed in undifferentiated F9 teratocarcinoma cells. After induction of differentiation the expression of SSEA-1 decreases. This is caused by the upregulation of alpha-1,3-galactosyltransferase that is responsible for masking of the Le^x structure [66, 67]. The stage specific

embryonic antigens 3 and 4, (SSEA-3,-4) are from the globo-series of glycosphingolipids (GL-5 and GL-7) and have not been found on linear poly-N-lactosamines [68].

Glycoseaminoglycans (GAG): GAGs are long unbranched polysaccharides containing a repeating disaccharide unit. The disaccharide units contain either one of two modified sugars, *N*-acetylgalactosamine (GalNAc) or *N*-acetylglucosamine (GlcNAc), and a uronic acid such as glucuronate or iduronate, forming heparin sulphate and hondroitin sulphate, respectively [69]. GAGs are highly negatively charged molecules, and are located primarily on the surface of cells or in the extracellular matrix (ECM). GAGs are normally attached to soluble or membranes bound core proteins to form proteoglycans which carries various carbohydrate markers expressed on early embryonic cells [60]. In the few past years it has become clear that many growth factor such as EGF and FGF has been shown to bind specific pentasaccharides within GAGs efficiently affect signaling during development [70]. The integrity of proteoglycans is important. One of the β4galactosyltransferase, β4Gal-T4, is one has recently been shown to be involved in the biosynthesis of keratin sulphate (KS), in which TRA-1-60 and TRA-1-80 epitopes are found, [14]. Furthermore, β4GalT-7 is involved in the synthesis of the GAG linkage region to proteoglycans, by catalyzing the transfer UDP-Gal to an *O*-linked Xylose/Ser residue in the sequence, GlcAcβ1-3Galβ1-3Galβ1-4Xylβ1-O-ser [23].

8. βGalTs and ESC signaling pathways

A number of reports have suggested β4GalTs to be direct or indirect mediators and regulators of cytokine signaling during stem cell and/or cancer development. As discusses below, many signal transduction pathways, such as EGF, FGF, Wnt and the Notch pathway, that utilize Le^x-containing carbohydrates are potential targets for aberrations in β4GalTs activities:

8.1. Epidermal Growth Factor (EGF)

EGF is involved in the regulation of cell proliferation and exerts its effects in the target cells by binding to the plasma membrane located EGF receptor. The EGF receptor is a transmembrane protein tyrosine kinase. Binding of EGF to the receptor causes activation of receptor autophosphorylation, which is essential for the interaction of the receptor with its cytosolic substrates. In mouse embryonic stem cells (mESC), EGF has been shown to stimulate proliferation of mouse ES cells via PLC/PKC, Ca2+influx and p44/42 MAPK signal pathway through EGF tyrosine kinase phosphorylation [71]. Altering the core components of *N*-linked glycans will change the EGF binding, the transport and the receptor endocytosis meanwhile substitution of the outer chain or terminal glucosides have been shown to affect the phosphorylation state and the dimerization of the receptor [72, 73]. Cell surface βGalT1 has been suggested to associate with and disrupt autophophorylation of EGF receptor Hinton et. al, showed that when a dominant negative form of long β4GalT-1 was over expressed in F9 embryonic carcinoma cells, the endogenous and active cell surface GalT-1 is displaced from its association to actin cytoskeleton. This

inhibition of cell surface β4GalT-1 resulted in increased tyrosine phophorylation of the EGF receptor and attenuated cell proliferation, while the shorter form of βGal-T1 did not have any effect [48]. These results implies that cell surface β4Gal-T1 has an inhibitory effect on EGF activity. Later, several groups substantiated this observation by showing that knock-down of β4GalT1 activity in SMMC7721 hepatocarcinoma cells, elevated the autophosporylation of EGFR. Reversibly, the level of tyrosine phosphorylation was attenuated if cell surface βGal-T1 was over expressed [74]. Interestingly, EGF treatment of HeLa cells has been shown to increase the β4Gal-T1 mRNA level, suggesting that β4GalT1 also act in a negative feedback loop on EGF activity [17]. In another elegant experiment, using mutant Chinese hamster ovary cells (CHO), where the levels of six beta β4Galactosyltransferases (βGalT1-6) were reduced, the protein level of active and surface-located EGFR was greatly attenuated without affecting the transcriptional level and activity of EGF receptor [75]. β4Gal-T1 has also been shown to positively affect EGFR activity. Isoprenaline, a β-adrenergic receptor has a dramatic growth stimulating activation on the salivary glands of rat and mice, eventually leading to hyperplastic and hypertrophic gland enlargement. This effect has been suggested to be mediated in part by cell surface β4Gal-T1 by mimicking EGF receptor mediated receptor ligand binding and activation [76]. In any case, the specific β4GalT1 binding site on the EGF receptor has not, as yet, been investigated but it is possible that the recently discovered extracellular location of O-linked GlcNac moieties on the EGF receptor, could act as a recognition signal, as has been observed for other membrane anchored extracellular proteins, such as Notch and Dumpy receptor [77, 78]. In this scenario, β4GalT1 could act as a lectin like molecule, using its substrate, GlcNAc [79, 80].There are also possibilities for other, more indirect and β4Gal-T1 dependent effects on EGF receptor function, such as the ganglioside GM3. The synthesis of this glycolipid is dependent on β4Gal-T2 activity, and has been shown to inhibit ligand-induced tyrosine phophorylation of EGF receptor through its sialyllactose carbohydrate moiety by interacting with the GlcNAc termini [72, 81].

8.2. FGF-2

Fibroblast growth factor (FGF) functions as a natural inducer of mesoderm, regulator of cell differentiation and autocrine modulator of cell growth and transformation of various cell types. FGF is activated by ligand-receptor interaction that results in tyrosine phosphorylation of the intracellular domain of the FGF receptor [82]. FGF-2 is often used as a key player in regulating self renewal and proliferation of human embryonic stem cells. Recently FGF-2 has been shown to regulate the transition from one pluripotent state to another. It has been speculated that human embryonic stem cells, due to their precautious ability to differentiate in culture, are identical to a later or "primed" developmental stage of mouse embryonic stem cells, EpiESC. LIF signaling is dispensable for this state, but instead relies on FGF signaling. Inhibition of FGF signaling with inhibitors in the presence of human LIF can "rescue" human embryonic stem cells from a primed state to a more naïve state, e g full pluripotency [83].This difference is still unclear but there are indication that extracellular proteoglycans, such as heparin sulphate (HSPG) acts as key co-activators of FGF receptors. Furthermore, during development, oligosaccharides from embryoglycans are often shed in-

to the extra cellular environment where they can influence cytokine and mitogen signaling. Lewis x epitopes on embryoglycans acts as a recognition molecule for FGF2 and plays an active role in the formation of FGF ligand receptor complexes. Free and soluble sulphated Lewis X was most prominent to activate the FGF-2 mitogenic acitivity [84, 85]. Also exogenous and free glycolipids in the form of gangliosides, can interact with the FGF-2. Gangliosides are derivatives of LacCer with a neuraminic acid (NeuAc) attached to the core, and seem to have dual roles in affecting both EGF and FGF proliferative action; soluble gangliosides and sulphated heparin act in a negative manner meanwhile membrane bound gangliosides increase the receptor activity. It seems clear that the close interplay between Le^x epitopes, adhesion molecules and cytokines has an important impact on the efficiency by which ligands are presented, and ultimately results in receptor oligomerization of the receptors and signalling [70]. It is therefore possible that β4GalTs could mediate some aspect of FGF receptor signalling, as described below.

8.3. Wnt pathway

The Wnt family of growth/differentiation factors has important developmental roles in embryonic stem cells. They act through the complex of Frizzled receptor and LPR co-receptor with effect on β-catenin transcriptional activity [86]. Similarly to EGF, the activity of Wnt also depends on association with HSPG for activity. HSPG is a rich source for developmentally regulated Le^x epitopes. Furthermore, Wnt-1 has been shown to interact directly with Le^x epitopes [87]. These observations suggests that surface bound and secreted Le^x have a regulatory function in stabilizing the stem cell niche, where they binds to and present appropriate factors, important for cell proliferation and self renewal.

8.4. Notch pathway

In a stem cell niche, stem cells and a variety of progenitor cells have to receive both temporal and spatial signals in order to differentiate or stay pluripotent. Also, during development and differentiation, cells have to decipher their precise localization in the dorso-ventral plane in order to form distinct and proper boundaries with other cell types in the tissue. These processes are governed by the Notch/ Delta system [88]. Notch is an essential developmental glycoprotein that plays key roles in both growth control and cell fate decisions. It is a transmembrane glycoprotein with a large extracellular domain made up of 29-36 EGF repeats, which can contain both N-linked and O-linked EGF repeats [90]. When Notch receptor is activated by a ligand on adjacent cells it is proteolytically cleaved, disposing the extracellular domain, followed by a second cleavage resulting in the released of the intracellular domain into the cytosol where it translocates to the nucleus and activates the transcription of numerous developmental genes. There are two ligands to Notch receptor, Delta and Jagged. Even though Notch receptor is ubiquitously expressed, Delta and Jagged are not usually located in the same cells but rather in different parts of the tissue during development where they exert their effect dependent on cell type and/or the environment. To avoid ubiquitous activation, Notch undergoes a post translational modification in which Fucose is first attached to certain EGF repeats on the extracellular domain of the receptor by *O*-Fuco-

syltransferase (O-FucT1). An N-acetyl glucosamine (GlcNac) and a Galactose (Gal) residue are then sequentially added to the fucosyl residue by the action of Fringe, a O-fucose β1,3-N-Acetyl glucosaminyl transferase and β4Gal-T1, respectively. The addition of Gal is necessary for the enhancement of Delta dependent signaling but not sufficient for the inhibition of Jagged induced Notch activation [89]. Recently, another layer of regulation of Delta induced Notch signaling was discovered in which the two Fringe genes, Lunatic Fringe (LFNG) and Manic Fringe (MFNG), seem to exhibit differential activity toward Delta dependent Notch activation. Gal was required for enhancement of Notch activation through LFNG and inhibited the enhancement of Delta induced signaling [90, 91]. Apart from O-linked Fucosylation, an O-linked GlcNAc modification of Notch EGF repeats was recently discovered [77]. Although the O-GlcNac modification is known to regulate a wide range of cellular processes, the list of known modified proteins has previously been limited to intracellular proteins in animals. Thus, this novel finding predicts a distinct glycosylation process associated with a novel regulatory mechanism for Notch receptor activity that may include a variety of βGalTs [77]. Furthermore, continuous hypoxic culturing conditions have been shown to activate Notch signaling to allow long-term propagation of human embryonic stem cells without spontaneous differentiation. Stem cells isolated and cultured under low oxygen tension (hypoxia) condition have been shown to maintain a stable pluripotency potential because of Notch activation [92]. Recently, it was also shown that β4GalT1 derived Lewis X epitopes on N-linked glycans was necessary for Notch activity and in the propagation of neural stem cells (NSC) [93].

9. β4GalTs deficiency in fish

It has been a challenge to get a consensus of the mechanisms by which complex carbohydrates control aspects of mammalian development and early differentiation. Some of the information has been available from knock-down experiment of individual galactosyltransferases. However, since many carbohydrate functions during early development in mammals are confined to " *in utero*", further analysis of the physiological effects of galactosyltransferases has not been possible. An attractive model using a more efficient "high-throughput " a assay system, is the zebrafish system. **β4Gal-T1:** The zebrafish β4Gal-T1 has the highest sequence homology to β4Gal-T1 among the human β4GalT family. β4Gal-T1 morpholino treated embryos had a truncated anterior-posterior axis phenotype that was a result of a defect in convergent extension [94]. Convergent extension is a developmental process that relies on coordinated cell migration to elongate and narrow a field of cells. Laminin is an extracellular substrate for cell surface β4Gal-T1 and constitutes one of the major components of the basement membrane upon which cell adhesion and migration occur during development [29]. Interestingly, in the mopholino treated embryos, laminin was hypo-galactosylated and hence could explain the decreased in ectodermal cell migration of [94]. **β4Gal-T2:** Tonoyama, et al. showed that β4Gal-T2 was indispensable for mediolateral cell intercalation and thus extension movement during gastrulation [95]. The specific substrates for β4Gal-T2 activity in glycoproteins responsible for these ef-

fects are currently not known but has been speculated to be related with N-glycosylated FGF receptor signaling. FGF signaling pathway is dependent on its N-glycans in the interaction with heparin co-receptor, regulating the efficiency of signaling [96]. **β4Gal-T5:** Transforming Growth factor (TGFβ) and bone morphogenic protein (BMP) are polypeptide members of the transforming growth factor beta (TGF) super family of cytokines. They are both secreted protein that performs many cellular functions, including the control of cell growth, cell proliferation, cell differentiation and apoptosis. In this context, knock-down of β4Gal-T5 using morpholino-injected zebrafish resulted in embryos with an elongated dorso-ventral axis and a defective tail bud [97]. This effect was suggested to be mediated through a decreased BMP-2 (a TGFβ family member) binding to proteoglycan due to defective glycosylation, and subsequent attenuation of SMAD signaling.

10. β4GalTs deficiency in mouse and human

Many diseases such as disorders of blood clotting, congenital disorder of glycosylation, diseases of blood vessels, cancer, angiogenesis essential for breast and other solid tumor progression and metastasis, are all associated with a dysfunctional *N*-glycan expression. The expression of many galactosyltransferases is under control of cytokines and could therefore become altered in various disease states. In order to find physiological functions for each galactosyl transferases, researchers have used both mouse and rat knockout models. **β4Ga-T1:** β4Ga-T1 was the first galactosyltransferase that indicated potential relevance in physiology. About 50% of β4Gal-T1, knock-out mice died prematurely because of pituitary deficiency [10].The surviving animals showed growth retardation, elevated proliferation of skin epidermis, and delayed wound healing due to attenuated leukocyte recruitment and infiltration [59]. Recently, some diseases in humans due to aberrations in β4Gal-T1 have emerged. For example, congenital disorders of glycosylation (CDGs) comprise a group of inherited disorders associated with psychomotor and mental disorders. One of these groups, CDGII, comprises all defects in trimming and elongation of N-linked oligosaccharides. CDGIId fall into a group in which β4Gal-T1 is mutated in its catalytic domain. This resulted in an aberrant translation product that was 15 kDa shorter than normal. Since β4GalT-1 has been shown to be is important during the early development of the brain, the phenotype from this mutation is mental retardation [98]. **β4Gal-T5:** Furthermore, knock-out β4Gal-T5 in mouse resulted in growth retardation and early lethality of embryos due to hematopoietic and/or placental defects [99]. Also the expression of β4Gal-T5 strongly increased during embryonic stem (ES) cell differentiation [22]. Both β4Gal-T5 and β4Gal-T6 are lactosylceramid synthases. However, β4Gal-T5 is more restricted to the early embryogenisis than β4Gal-T6, which is more limited to adult brain. β4GalT-5 deficient animals showed abnormal extra embryonic structures that led to embryonic lethal phenotype at day E10.5. **β4Gal-T7:** A rare genetic mutation of β4Gal-T7, believed to be the consequence of two missense mutations in the active domain resulted defective GAG chain formation [15] gives rise to Ehlers-Danlos disease. This is a disorder in which patients exhibit phenotypes such as aged appear-

ance, developmental delay, dwarfism, craniofacial disproportion, delayed wound healing, loose skin, and general ostopenia [15, 100].

11. Potential treatments

The involvement of β4GalTs in cancer, inflammation and during development / stem cell homeostasis has encouraged research to come up with new modalities that can either boost or inhibit the expression/activity of endogenous glycosyltransferases. I will briefly discuss potential therapeutic models for treatment that will inhibit or activate specific galactosyltransferases.

11.1. Protein ubiquitination

A potential regulator of a galactosyltransferase, GTAP, was discovered 2008 in a two hybrid screen of a mouse embryonic library, using the cytoplasmic domain of cell surface Gal-T1 as bait. Ectopically expressed GTAP down regulated the expression of cell surface bound GalT-1 and negatively affected both laminin dependent stem cell migration and embryonic body formation during differentiation. GTAP is an ubiqutin conjugating enzyme that is expressed during early development of the inner cell mass and in embryonic stem cells but also in highly proliferative tissues, such as, such as kidney, lung and testis. This effect was not due to a proteasome dependent degradation of βGal-T1 but an increase of ubiquitin dependent lysosomal activity. So far this is the only report on ubiquitin related regulation of a cell surface galactosyltranferase and may be important for the development of more effective and specific inhibitors of various glycosyltransferases in glycan related diseases. The only known ubiquitin/proteaseome regulated system of glycans so far, is the endoplasmic reticulum assisted degradation (ERAD).This system helps cells to avoid stress and cell death by degradation of missfolded proteins in the ER [101]

11.2. Analogues to GalT donor and acceptor

A limited number of GalT-1 inhibitors have been described. Most of them have been analogues of either the donor substrate (e.g Gal) or the acceptor (GlcNac) molecules to galactosyltransferases. E. g. a modified GlcNac acceptor, called compound 612, was recently discovered showing differential affinities for β4Gal-T1 and β4Gal-T5, two galactosyl transferases with similar acceptor specificities [102]. Also, in contrast to other β4galactosyltransferases, β4Gal-T7 has the ability to bind, but not actively transfer Mannose or GalNAc to an acceptor substrate, implying that these donors can be used as potential inhibitors to GAG synthesis [103]

11.3. Lectins

During recent years, several laboratories, using specific cell lines that either over express or lack different glycosyltransferases in combination with high density lectin microarrays.

In order to entangle the mechanism by which the cellular glycome can influence stem cell pluripotency and differentiation. Lectins are proteins that bind to particular carbohydrate epitopes in a similar manner as an antibody. Glycans are located at the cell surface where many signal transduction pathways, cell-cell interaction and cell-to cell recognition are constantly active. Interactions between glycans and endogenous lectins may influence self renewal, maintenance of pluripotency and differentiaon of iPS/ESC. Such an approach has already been tested in which synthetic substrates, mimicking endogenous lectins, can facilitate the formation of induced pluripotent cell(iPSC) and help sustain long term culture of human ESCs [104]

12. Conclusion and perspectives

It is clear that both N-linked and O-linked glycans are implicated in many intricate and complex processes during development, differentiation and in many diseases. For many years glycosyltransferases were thought of as just redundant enzymes acting solely in the ER and Golgie, creating oligosaccharide structure mostly important for transport and solubility of secreted proteins. However, in the last decades, the functions of glycosyl transferases have been expanded to involve receptor oligomerization, antigen presentation, endocytosis, ligand-receptor binding, and even signal transduction. These observations have attracted attention in the stem cell biology field. Several markers for pluripotency, such as Lewis X antigen, e.g. SSEA-1, -3 and -4, and the keratin sulphate related markers, TRA-1-60 and TRA-1-80, are all dependent on functional galactosylation for their synthesis and functionality. The levels and modifications of these embryonic derived antigens are changing upon differentiation. These markers have mainly been used, and are still used, as markers for isolation and propagation of different stem cell populations. With recent technological advances and the development of more efficient lectin microarrays and HPLC systems, more and more details of the functional and structural requirements of early epitopes during stem cell self renewal and differentiation, are emerging. These techniques, combined with specific knock- down models and ectopical expression of individual galactosyltransferases, would eventually reveal the molecular mechanisms by which glycans influence stem cell and cancer progression. The complex interplay between members of the galactosyltransferase family, does not only affect the core structures of glycans but are also extensively involved in the synthesis of other bioactive compounds, such as glycolipids and the Lexis X antigens that affect a variety of biological systems spanning from cell migration to signal transduction. The presence of the long form of β4Gal-T1 at the cell surface raises many interesting questions on how this receptor, or maybe other glycosyltranferases as well, can influence so many different signal transduction pathways in the regulation of cell cycle, cell death, proliferation and differentiation. Apart from being located to the Golgi complex, where it is responsible for creating complex oligosaccharide structures on proteoglycans and glycolipid, the cell surface β4Gal-Ts also affect intracellular signal transduction pathways. As seen in Figure 6, cell surface β4GalTs can indirectly affect many cell specific functions because of its involvement in the synthesis of glycolipids, embryoglycans and many embryonal epitopes,

http://dx.doi.org/10.5772/54376

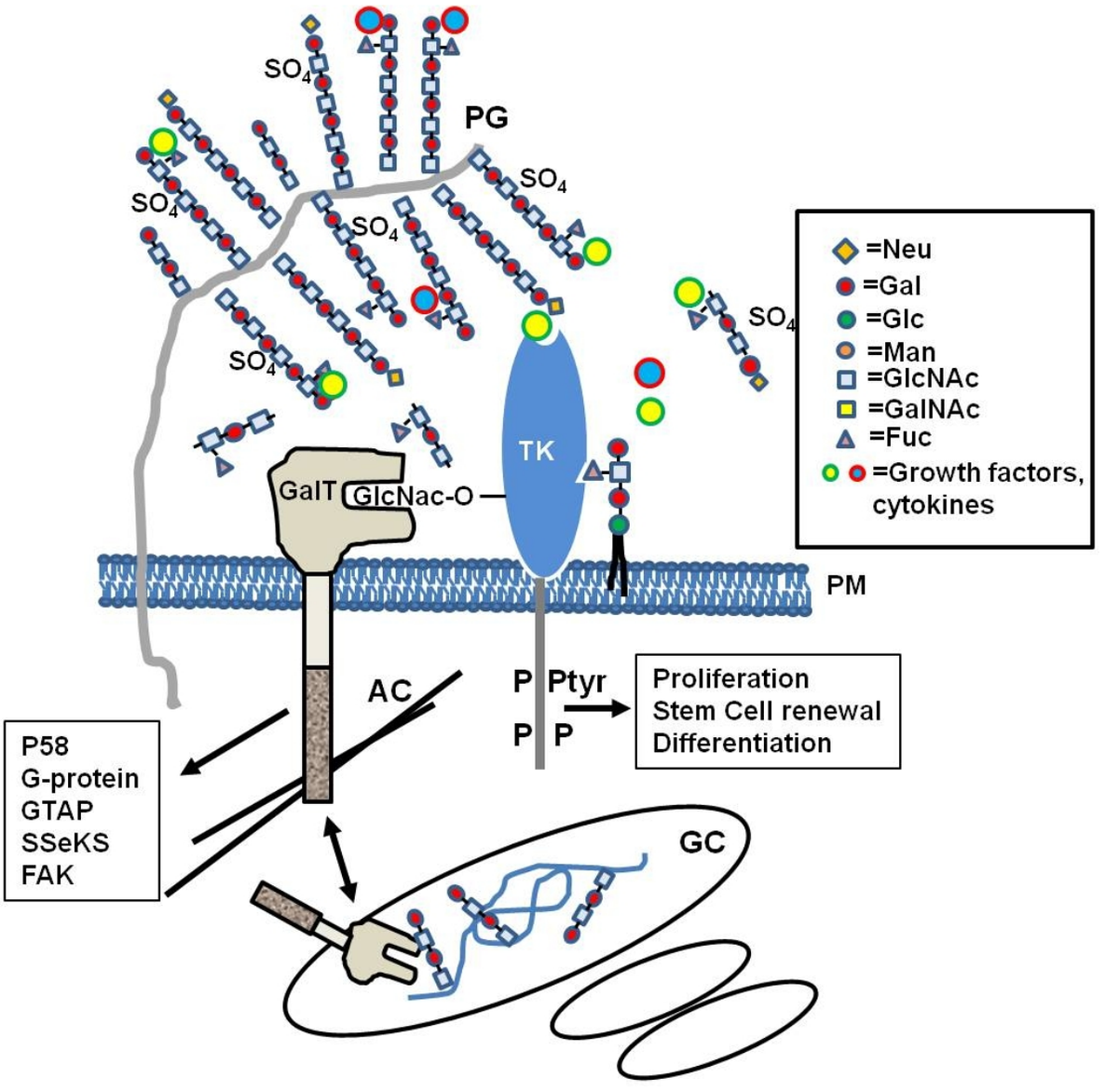

Figure 6. Schematic view of cell surface β4GalTs potential functions. Cell surface as well as Golgi bound long β34Galactosyltransferase (GalTs) can influence stem cell homeostatis. TK; Tyrosin kinase. AC; actin, GC; Golgi complex, GL;Glycolipid, PM; Plasma membrane, Ptyr; Tyrosine phosphorylation, PG; Proteoglycan, S04; sulphate, Neu;Neuramic acid, Gal;Galactose, Glc;Glucose, Man;Mannose, GalNAc; N-Acetylgalactosamine, GalNAc; N-Acetylgalactoseamine, Fuc;Fucose.

such Lewis X antigens. These complexes will either stabilize growth factor or cytokine-receptor complexes or, after shedded into the extracellular matrix during differentiation, inhibit receptor function. A change in galactosyltransferase activity could therefore indirectly affect the stem cell nitch by hinder effective glycolipid, proteoglycan/GAG synthesis and signal transduction through tyrosin kinase (TK) receptors. Secondly, apart from binding to the extracellular matrix, such as laminin, the cell surface β4Gal-T1 could also act directly as a lectin-like molecule that bind to tyrosine receptors (EGF, FGF or Notch), either on the same cells, or on adjacent cells, as long as a terminal GlcNAc are presented. This could ei-

ther create a block or enhancement of the TK receptor- ligand complexes, or even hinder dimerization and activation of the receptors. Furthermore, the β4GalT-receptor binding could lead to aggregation of cell surface β4Gal-T1, increasing its association to actin, and subsequently lead to increase in intracellular signal transduction through FAK, SSeCKS and other signalling molecules. In this scenario, it is plausible that β4GalTs, control a myriad regulatory feedback loops. It is clear that so much more of the biological function of GalTs has to be understood in order to unravel attractive and potential therapies for cancer and in regenerative medicine.

Author details

Michael Wassler*

Address all correspondence to: Michael.Wassler@uth.tmc.edu

The center for cardiovascular Biology and Atherosclerosis Research, Department of Internal Medicine, The University of Texas Medical School at Houston, Houston, TX, USA

References

[1] Taniguchi, N. and H. Korekane, Branched N-glycans and their implications for cell adhesion, signaling and clinical applications for cancer biomarkers and in therapeutics. BMB Rep., 2011. 44 (12) p. 772-81.

[2] Weerapana, E. and B. Imperiali, Asparagine-linked protein glycosylation: from eukaryotic to prokaryotic systems. Glycobiology, 2006. 16(6): p. 91R-101R.

[3] Amado, M., et al., Identification and characterization of large galactosyltransferase gene families: galactosyltransferases for all functions. Biochim Biophys Acta, 1999. 1473(1): p. 35-53.

[4] Banerjee, D.K., N-glycans in cell survival and death: Cross-talk between glycosyltransferases. Biochim Biophys Acta, 2012. 1820(9): p. 1338-46.

[5] Sasaki, N., et al., LacdiNAc (GalNAcbeta1-4GlcNAc) contributes to self-renewal of mouse embryonic stem cells by regulating leukemia inhibitory factor/STAT3 signaling. Stem Cells, 2011. 29(4): p. 641-50.

[6] Kraushaar, D.C., Y. Yamaguchi, and L. Wang, Heparan sulfate is required for embryonic stem cells to exit from self-renewal. J Biol Chem, 2010. 285(8): p. 5907-16.

[7] Sasaki, N., et al., Heparan sulfate regulates self-renewal and pluripotency of embryonic stem cells. J Biol Chem, 2008. 283(6): p. 3594-606.

[8] Smith, R.A., et al., Glycosaminoglycans as regulators of stem cell differentiation. Biochem Soc Trans, 2011. 39(1): p. 383-7.

[9] Jang, H., et al., O-GlcNAc Regulates Pluripotency and Reprogramming by Directly Acting on Core Components of the Pluripotency Network. Cell Stem Cell, 2012. 11(1): p. 62-74.

[10] Furukawa, K. and T. Sato, Beta-1,4-galactosylation of N-glycans is a complex process. Biochim Biophys Acta, 1999. 1473(1): p. 54-66.

[11] Shaper, N.L., et al., Bovine galactosyltransferase: identification of a clone by direct immunological screening of a cDNA expression library. Proc Natl Acad Sci U S A, 1986. 83(6): p. 1573-7.

[12] Guo, S., et al., Galactosylation of N-linked oligosaccharides by human beta-1,4-galactosyltransferases I, II, III, IV, V, and VI expressed in Sf-9 cells. Glycobiology, 2001. 11(10): p. 813-20.

[13] Lee, J., et al., Chinese hamster ovary (CHO) cells may express six beta 4-galactosyltransferases (beta 4GalTs). Consequences of the loss of functional beta 4GalT-1, beta 4GalT-6, or both in CHO glycosylation mutants. J Biol Chem, 2001. 276(17): p. 13924-34.

[14] Seko, A., et al., Beta 1,4-galactosyltransferase (beta 4GalT)-IV is specific for GlcNAc 6-O-sulfate. Beta 4GalT-IV acts on keratan sulfate-related glycans and a precursor glycan of 6-sulfosialyl-Lewis X. J Biol Chem, 2003. 278(11): p. 9150-8.

[15] Bui, C., et al., Molecular characterization of beta1,4-galactosyltransferase 7 genetic mutations linked to the progeroid form of Ehlers-Danlos syndrome (EDS). FEBS Lett, 2010. 584(18): p. 3962-8.

[16] Sasaki, N., et al., beta4GalT-II is a key regulator of glycosylation of the proteins involved in neuronal development. Biochem Biophys Res Commun, 2005. 333(1): p. 131-7.

[17] Zhu, X., et al., Elevated beta1,4-galactosyltransferase I in highly metastatic human lung cancer cells. Identification of E1AF as important transcription activator. J Biol Chem, 2005. 280(13): p. 12503-16.

[18] Xu, S., et al., Over-expression of beta-1,4-galactosyltransferase I, II, and V in human astrocytoma. J Cancer Res Clin Oncol, 2001. 127(8): p. 502-6.

[19] Chen, W.S., et al., Tumor beta-1,4-galactosyltransferase IV overexpression is closely associated with colorectal cancer metastasis and poor prognosis. Clin Cancer Res, 2005. 11(24 Pt 1): p. 8615-22.

[20] Chatterjee, S., A. Kolmakova, and M. Rajesh, Regulation of lactosylceramide synthase (glucosylceramide beta1-->4 galactosyltransferase); implication as a drug target. Curr Drug Targets, 2008. 9(4): p. 272-81.

[21] Schwientek, T., et al., Cloning of a novel member of the UDP-galactose:beta-N-acetylglucosamine beta1,4-galactosyltransferase family, beta4Gal-T4, involved in glycosphingolipid biosynthesis. J Biol Chem, 1998. 273(45): p. 29331-40.

[22] Nishie, T., et al., Beta4-galactosyltransferase-5 is a lactosylceramide synthase essential for mouse extra-embryonic development. Glycobiology, 2010. 20(10): p. 1311-22.

[23] Almeida, R., et al., Cloning and expression of a proteoglycan UDP-galactose:beta-xylose beta1,4-galactosyltransferase I. A seventh member of the human beta4-galactosyltransferase gene family. J Biol Chem, 1999. 274(37): p. 26165-71.

[24] Shur, B.D., S. Evans, and Q. Lu, Cell surface galactosyltransferase: current issues. Glycoconj J, 1998. 15(6): p. 537-48.

[25] Shur, B.D., Cell surface beta 1,4 galactosyltransferase: twenty years later. Glycobiology, 1991. 1(6): p. 563-75.

[26] Bunnell, B.A., D.E. Adams, and V.J. Kidd, Transient expression of a p58 protein kinase cDNA enhances mammalian glycosyltransferase activity. Biochem Biophys Res Commun, 1990. 171(1): p. 196-203.

[27] Hathaway, H.J., et al., Mutational analysis of the cytoplasmic domain of beta1,4-galactosyltransferase I: influence of phosphorylation on cell surface expression. J Cell Sci, 2003. 116(Pt 21): p. 4319-30.

[28] Zhang, S.W., et al., Effect of p58GTA on beta-1,4-galactosyltransferase 1 activity and cell-cycle in human hepatocarcinoma cells. Mol Cell Biochem, 2001. 221(1-2): p. 161-8.

[29] Shur, B.D., Glycosyltransferases as cell adhesion molecules. Curr Opin Cell Biol, 1993. 5(5): p. 854-63.

[30] Bayna, E.M., J.H. Shaper, and B.D. Shur, Temporally specific involvement of cell surface beta-1,4 galactosyltransferase during mouse embryo morula compaction. Cell, 1988. 53(1): p. 145-57.

[31] Begovac, P.C., et al., Evidence that cell surface beta 1,4-galactosyltransferase spontaneously galactosylates an underlying laminin substrate during fibroblast migration. J Biol Chem, 1994. 269(50): p. 31793-9.

[32] Maillet, C.M. and B.D. Shur, Uvomorulin, LAMP-1, and laminin are substrates for cell surface beta-1,4-galactosyltransferase on F9 embryonal carcinoma cells: comparisons between wild-type and mutant 5.51 att- cells. Exp Cell Res, 1993. 208(1): p. 282-95.

[33] Maillet, C.M. and B.D. Shur, Perturbing cell surface beta-(1,4)-galactosyltransferase on F9 embryonal carcinoma cells arrests cell growth and induces laminin synthesis. J Cell Sci, 1994. 107 (Pt 6): p. 1713-24.

[34] Eckstein, D.J. and B.D. Shur, Cell surface beta-1,4-galactosyltransferase is associated with the detergent-insoluble cytoskeleton on migrating mesenchymal cells. Exp Cell Res, 1992. 201(1): p. 83-90.

[35] Gong, X., et al., Activation of a G protein complex by aggregation of beta-1,4-galactosyltransferase on the surface of sperm. Science, 1995. 269(5231): p. 1718-21.

[36] Lin, X., P. Nelson, and I.H. Gelman, SSeCKS, a major protein kinase C substrate with tumor suppressor activity, regulates G(1)-->S progression by controlling the expression and cellular compartmentalization of cyclin D. Mol Cell Biol, 2000. 20(19): p. 7259-72.

[37] Wassler, M.J., et al., Functional interaction between the SSeCKS scaffolding protein and the cytoplasmic domain of beta1,4-galactosyltransferase. J Cell Sci, 2001. 114(Pt 12): p. 2291-300.

[38] Bunnell, B.A., et al., Increased expression of a 58-kDa protein kinase leads to changes in the CHO cell cycle. Proc Natl Acad Sci U S A, 1990. 87(19): p. 7467-71.

[39] Wassler, M.J., et al., Characterization of a novel ubiquitin-conjugating enzyme that regulates beta1,4-galactosyltransferase-1 in embryonic stem cells. Stem Cells, 2008. 26(8): p. 2006-18.

[40] Gelman, I.H., E. Tombler, and J. Vargas, Jr., A role for SSeCKS, a major protein kinase C substrate with tumour suppressor activity, in cytoskeletal architecture, formation of migratory processes, and cell migration during embryogenesis. Histochem J, 2000. 32(1): p. 13-26.

[41] Wassler, M.J. and B.D. Shur, Clustering of cell surface (beta)1,4-galactosyltransferase I induces transient tyrosine phosphorylation of focal adhesion kinase and loss of stress fibers. J Cell Sci, 2000. 113 Pt 2: p. 237-45.

[42] Zeng, F.Y., et al., Differential response of the epidermal growth factor receptor tyrosine kinase activity to several plant and mammalian lectins. Mol Cell Biochem, 1995. 142(2): p. 117-24.

[43] Dennis, J.W., M. Granovsky, and C.E. Warren, Glycoprotein glycosylation and cancer progression. Biochim Biophys Acta, 1999. 1473(1): p. 21-34.

[44] Johnson, F.M. and B.D. Shur, The level of cell surface beta1,4-galactosyltransferase I influences the invasive potential of murine melanoma cells. J Cell Sci, 1999. 112 (Pt 16): p. 2785-95.

[45] Shen, J., et al., Two specific inhibitors of the phosphatidylinositol 3-kinase LY294002 and wortmannin up-regulate beta1,4-galactosyltransferase I and thus sensitize SMMC-7721 human hepatocarcinoma cells to cycloheximide-induced apoptosis. Mol Cell Biochem, 2007. 304(1-2): p. 361-7.

[46] Satomaa, T., et al., The N-glycome of human embryonic stem cells. BMC Cell Biol, 2009. 10: p. 42.

[47] Calder, A., et al., Lengthened G1 Phase Indicates Differentiation Status In Human Embryonic Stem Cells. Stem Cells Dev, 2012.

[48] Hinton, D.A., S.C. Evans, and B.D. Shur, Altering the expression of cell surface beta 1,4-galactosyltransferase modulates cell growth. Exp Cell Res, 1995. 219(2): p. 640-9.

[49] Pouncey, L., et al., Beta 1-4-galactosyltransferase gene expression is regulated during entry into the cell cycle and during the cell cycle. Somat Cell Mol Genet, 1991. 17(5): p. 435-43.

[50] Wu, G.Q., S.M. Jiang, and J.X. Gu, Studies on the beta(l-4)Galactosyltransferase of Cell Surface in Induced HL60 Cells. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai), 1997. 29(4): p. 402-408.

[51] Wei, Y., et al., Regulation of the beta1,4-Galactosyltransferase I promoter by E2F1. J Biochem, 2010. 148(3): p. 263-71.

[52] Asano, M., et al., Growth retardation and early death of beta-1,4-galactosyltransferase knockout mice with augmented proliferation and abnormal differentiation of epithelial cells. EMBO J, 1997. 16(8): p. 1850-7.

[53] Zhang, S.W., et al., Down-regulation of beta1,4-galactosyltransferase gene expression by cell-cycle suppressor gene p16. Biochim Biophys Acta, 1999. 1444(1): p. 49-54.

[54] Zhang, S.W., et al., Effect of suppression of TGF-beta1 expression on cell-cycle and gene expression of beta-1,4-galactosyltransferase 1 in human hepatocarcinoma cells. Biochem Biophys Res Commun, 2000. 273(3): p. 833-8.

[55] Li, Z., et al., Downregulation of beta1,4-galactosyltransferase 1 inhibits CDK11(p58)-mediated apoptosis induced by cycloheximide. Biochem Biophys Res Commun, 2005. 327(2): p. 628-36.

[56] Ohno, S., et al., Polypyrimidine tract-binding protein regulates the cell cycle through IRES-dependent translation of CDK11(p58) in mouse embryonic stem cells. Cell Cycle, 2011. 10(21): p. 3706-13.

[57] Wei, Y., et al., Identification of beta-1,4-galactosyltransferase I as a target gene of HBx-induced cell cycle progression of hepatoma cell. J Hepatol, 2008. 49(6): p. 1029-37.

[58] Ujita, M., et al., Poly-N-acetyllactosamine synthesis in branched N-glycans is controlled by complemental branch specificity of I-extension enzyme and beta1,4-galactosyltransferase I. J Biol Chem, 1999. 274(24): p. 16717-26.

[59] Asano, M., et al., Impaired selectin-ligand biosynthesis and reduced inflammatory responses in beta-1,4-galactosyltransferase-I-deficient mice. Blood, 2003. 102(5): p. 1678-85.

[60] Muramatsu, T. and H. Muramatsu, Carbohydrate antigens expressed on stem cells and early embryonic cells. Glycoconj J, 2004. 21(1-2): p. 41-5.

[61] Sudou, A., et al., Le(X) structure enhances myocardial differentiation from embryonic stem cells. Cell Struct Funct, 1997. 22(2): p. 247-51.

[62] Ma, R., et al., Post-translational and transcriptional regulation of glycolipid glycosyltransferase genes in apoptotic breast carcinoma cells: VII. Studied by DNA-microarray after treatment with L-PPMP. Glycoconj J, 2009. 26(6): p. 647-61.

[63] Biellmann, F., et al., The Lc3-synthase gene B3gnt5 is essential to pre-implantation development of the murine embryo. BMC Dev Biol, 2008. 8: p. 109.

[64] Yamashita, T., et al., A vital role for glycosphingolipid synthesis during development and differentiation. Proc Natl Acad Sci U S A, 1999. 96(16): p. 9142-7.

[65] Rastan, S., et al., Cell interactions in preimplantation embryos: evidence for involvement of saccharides of the poly-N-acetyllactosamine series. J Embryol Exp Morphol, 1985. 87: p. 115-28.

[66] Cho, S.K., et al., Transcriptional regulation of alpha1,3-galactosyltransferase in embryonal carcinoma cells by retinoic acid. Masking of Lewis X antigens by alpha-galactosylation. J Biol Chem, 1996. 271(6): p. 3238-46.

[67] Patil, S.A., et al., Scaling down the size and increasing the throughput of glycosyltransferase assays: activity changes on stem cell differentiation. Anal Biochem, 2012. 425(2): p. 135-44.

[68] Liang, Y.J., et al., Changes in glycosphingolipid composition during differentiation of human embryonic stem cells to ectodermal or endodermal lineages. Stem Cells, 2011. 29(12): p. 1995-2004.

[69] Hacker, U., K. Nybakken, and N. Perrimon, Heparan sulphate proteoglycans: the sweet side of development. Nat Rev Mol Cell Biol, 2005. 6(7): p. 530-41.

[70] Raman, R., V. Sasisekharan, and R. Sasisekharan, Structural insights into biological roles of protein-glycosaminoglycan interactions. Chem Biol, 2005. 12(3): p. 267-77.

[71] Heo, J.S., Y.J. Lee, and H.J. Han, EGF stimulates proliferation of mouse embryonic stem cells: involvement of Ca2+ influx and p44/42 MAPKs. Am J Physiol Cell Physiol, 2006. 290(1): p. C123-33.

[72] Kawashima, N., et al., Tyrosine kinase activity of epidermal growth factor receptor is regulated by GM3 binding through carbohydrate to carbohydrate interactions. J Biol Chem, 2009. 284(10): p. 6147-55.

[73] Takahashi, M., et al., Role of N-glycans in growth factor signaling. Glycoconj J, 2004. 20(3): p. 207-12.

[74] Li, Z., et al., Cell surface beta 1, 4-galactosyltransferase 1 promotes apoptosis by inhibiting epidermal growth factor receptor pathway. Mol Cell Biochem, 2006. 291(1-2): p. 69-76.

[75] Gabius, H.J., et al., Down-regulation of the epidermal growth factor receptor by altering N-glycosylation: emerging role of beta1,4-galactosyltransferases. Anticancer Res, 2012. 32(5): p. 1565-72.

[76] Purushotham, K.R., et al., A novel mechanism for isoprenaline-stimulated proliferation of rat parotid acinar cells involving the epidermal growth factor receptor and cell surface galactosyltransferase. Biochem J, 1992. 284 (Pt 3): p. 767-76.

[77] Matsuura, A., et al., O-linked N-acetylglucosamine is present on the extracellular domain of notch receptors. J Biol Chem, 2008. 283(51): p. 35486-95.

[78] Sakaidani, Y., et al., O-linked-N-acetylglucosamine on extracellular protein domains mediates epithelial cell-matrix interactions. Nat Commun, 2011. 2: p. 583.

[79] Hazan, R., L. Krushel, and K.L. Crossin, EGF receptor-mediated signals are differentially modulated by concanavalin A. J Cell Physiol, 1995. 162(1): p. 74-85.

[80] Hebert, E., Endogenous lectins as cell surface transducers. Biosci Rep, 2000. 20(4): p. 213-37.

[81] Rusnati, M., et al., Interaction of fibroblast growth factor-2 (FGF-2) with free gangliosides: biochemical characterization and biological consequences in endothelial cell cultures. Mol Biol Cell, 1999. 10(2): p. 313-27.

[82] Basilico, C. and D. Moscatelli, The FGF family of growth factors and oncogenes. Adv Cancer Res, 1992. 59: p. 115-65.

[83] Lanner, F. and J. Rossant, The role of FGF/Erk signaling in pluripotent cells. Development. 2010.137 (20) p. 3351-60.

[84] Dvorak, P., et al., Embryoglycan ectodomains regulate biological activity of FGF-2 to embryonic stem cells. J Cell Sci, 1998. 111 (Pt 19): p. 2945-52.

[85] Jirmanova, L., et al., O-linked carbohydrates are required for FGF-2-mediated proliferation of mouse embryonic cells. Int J Dev Biol, 1999. 43(6): p. 555-62.

[86] Katoh, M., WNT signaling pathway and stem cell signaling network. Clin Cancer Res, 2007. 13(14): p. 4042-5.

[87] Capela, A. and S. Temple, LeX is expressed by principle progenitor cells in the embryonic nervous system, is secreted into their environment and binds Wnt-1. Dev Biol, 2006. 291(2): p. 300-13.

[88] Haltiwanger, R.S., Regulation of signal transduction pathways in development by glycosylation. Curr Opin Struct Biol, 2002. 12(5): p. 593-8.

[89] Chen, J., D.J. Moloney, and P. Stanley, Fringe modulation of Jagged1-induced Notch signaling requires the action of beta 4galactosyltransferase-1. Proc Natl Acad Sci U S A, 2001. 98(24): p. 13716-21.

[90] Haltiwanger, R.S. and P. Stanley, Modulation of receptor signaling by glycosylation: fringe is an O-fucose-beta1,3-N-acetylglucosaminyltransferase. Biochim Biophys Acta, 2002. 1573(3): p. 328-35.

[91] Hou, X., Y. Tashima, and P. Stanley, Galactose differentially modulates lunatic and manic fringe effects on Delta1-induced NOTCH signaling. J Biol Chem, 2012. 287(1): p. 474-83.

[92] Prasad, S.M., et al., Continuous hypoxic culturing maintains activation of Notch and allows long-term propagation of human embryonic stem cells without spontaneous differentiation. Cell Prolif, 2009. 42(1): p. 63-74.

[93] Yagi, H., et al., Lewis X-carrying N-Glycans Regulate the Proliferation of Mouse Embryonic Neural Stem Cells via the Notch Signaling Pathway. J Biol Chem, 2012. 287(29): p. 24356-64.

[94] Machingo, Q.J., A. Fritz, and B.D. Shur, A beta1,4-galactosyltransferase is required for convergent extension movements in zebrafish. Dev Biol, 2006. 297(2): p. 471-82.

[95] Tonoyama, Y., et al., Essential role of beta-1,4-galactosyltransferase 2 during medaka (Oryzias latipes) gastrulation. Mech Dev, 2009. 126(7): p. 580-94.

[96] Duchesne, L., et al., N-glycosylation of fibroblast growth factor receptor 1 regulates ligand and heparan sulfate co-receptor binding. J Biol Chem, 2006. 281(37): p. 27178-89.

[97] Machingo, Q.J., A. Fritz, and B.D. Shur, A beta1,4-galactosyltransferase is required for Bmp2-dependent patterning of the dorsoventral axis during zebrafish embryogenesis. Development, 2006. 133(11): p. 2233-41.

[98] Hansske, B., et al., Deficiency of UDP-galactose:N-acetylglucosamine beta-1,4-galactosyltransferase I causes the congenital disorder of glycosylation type IId. J Clin Invest, 2002. 109(6): p. 725-33.

[99] Kumagai, T., et al., Early lethality of beta-1,4-galactosyltransferase V-mutant mice by growth retardation. Biochem Biophys Res Commun, 2009. 379(2): p. 456-9.

[100] Talhaoui, I., et al., Identification of key functional residues in the active site of human {beta}1,4-galactosyltransferase 7: a major enzyme in the glycosaminoglycan synthesis pathway. J Biol Chem, 2010. 285(48): p. 37342-58.

[101] Mallinger, A., et al., Using a ubiquitin ligase as an unfolded protein sensor. Biochem Biophys Res Commun, 2012. 418(1): p. 44-8.

[102] Gao, Y., et al., Specificity of beta1,4-galactosyltransferase inhibition by 2-naphthyl 2-butanamido-2-deoxy-1-thio-beta-D-glucopyranoside. Glycoconj J, 2010. 27(7-9): p. 673-84.

[103] Daligault, F., et al., Thermodynamic insights into the structural basis governing the donor substrate recognition by human beta1,4-galactosyltransferase 7. Biochem J, 2009. 418(3): p. 605-14.

[104] Tateno, H., et al., Glycome diagnosis of human induced pluripotent stem cells using lectin microarray. J Biol Chem, 2011. 286(23): p. 20345-53.

[105] Roth, S., E.J. McGuire, and S. Roseman, Evidence for cell-surface glycosyltransferases. Their potential role in cellular recognition. J Cell Biol, 1971. 51(21): p. 536-47.