Histol Histopathol (2019) 34: 995-1007

http://www.hh.um.es

Histology and Histopathology

From Cell Biology to Tissue Engineering

Review

Comparison of functional glycans between cancer stem cells and normal stem cells

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Summary. Cancer stem cells (CSCs) are a small group of cells within a tumor that preserve stemness and enhance regrowth of cancer cells. CSCs have important implications in resistance to conventional therapies and tumor relapse, although their detailed properties remain unknown. Thus, CSCs represent promising targets to improve cancer treatment. So far, a number of cell surface markers containing glycans have been exploited to identify and isolate CSCs. Cell surface glycans are well-known markers for specific cell types and also play important cellular roles, such as regulation of cell signaling. In normal stem cells, including embryonic and tissue stem cells, glycan markers in an undifferentiated state have been identified. These markers are mostly known to regulate signaling pathways required for maintenance of stemness. In contrast, CSC-specific glycans have not been well characterized yet. In this review, we summarize functional commonalities between CSCs and normal stem cells in glycan-mediated signaling pathways. Identification of CSC-specific glycans may lead to early diagnosis and radical treatment of cancer.

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DOI: 10.14670/HH-18-119

Key words: Cancer stem cell, Pluripotent stem cell, Tissue stem cell, Signaling pathway, Glycans

Introduction

Cancer cells present a wide variety of genetic and epigenetic characteristics even in the same patient (Ishiwata, 2016). Among these heterogeneous cancer cells, there are small populations (0.01-1%), defined as cancer stem cells (CSCs), that possess the capacity to self-renew and generate heterogeneous lineages of cancer cells (Clarke et al., 2006; Visvader and Lindeman, 2008). CSCs were first identified in human acute myeloid leukemia and were found to be capable of initiating tumors in non-obese diabetic/severe combined immunodeficiency mice, possessing self-renewal properties and the ability to differentiate into multiple lineages (Bonnet and Dick, 1997). Since then, CSCs have been isolated from various solid tumors, including breast (Al-Hajj et al., 2003), brain (Wilson et al., 2004), prostate (Collins et al., 2005), pancreas (Li et al., 2007), colon (O'Brien et al., 2007), stomach (Takaishi et al., 2009), ovary (Curley et al., 2009), liver (Terris et al., 2010), skin (Boiko et al., 2010) and lung cancer (Zhang et al., 2012). CSCs increase tumor resistance to radiotherapy and chemotherapy (Zhou et al., 2009; Singh and Settleman, 2010), which influences tumor recurrence and overall survival in patients (van Rhenen et al., 2005; Liu et al., 2012). Researchers have hypothesized that the elimination of CSCs can lead to the disappearance of specific types of cancer cells, based

on the concept that CSCs are the sole source of tumor self-renewal (Ishiwata et al., 2018). The development of therapeutic strategies targeting CSCs mainly relies on specific cell surface markers including proteins and/or glycans.

Glycans are displayed mainly on the surface of cells as components of glycoproteins, glycolipids and proteoglycans (Fig. 1) (Varki et al., 2017). Glycoproteins contain *N*-linked glycans (including three major types: high mannose, hybrid and complex glycans) synthesized on asparagine residues part of the Asn-X-Ser/Thr consensus sequence (X: any amino acid except proline) and *O*-linked glycans synthesized on serine or threonine residues. Glycolipids such as gangliosides, which are molecules composed of glycosphingolipids with one or more sialic acids linked, are composed of a glycan structure attached to a lipid tail containing the sphingolipid ceramide. Proteoglycans are macromolecules composed of a specific core protein with covalently linked glycosaminoglycan chains, such as chondroitin sulfate (CS) and heparan sulfate (HS). Glycosylation, which is the enzymatic reaction catalyzed by glycosyltransferases and glycosidases that binds glycans to proteins or lipids, takes place in the endoplasmic reticulum and the Golgi apparatus (Varki and Lowe, 2009). Cell surface glycans contribute to fundamental biological functions, such as cell differentiation, cell adhesion, cell-cell interaction,

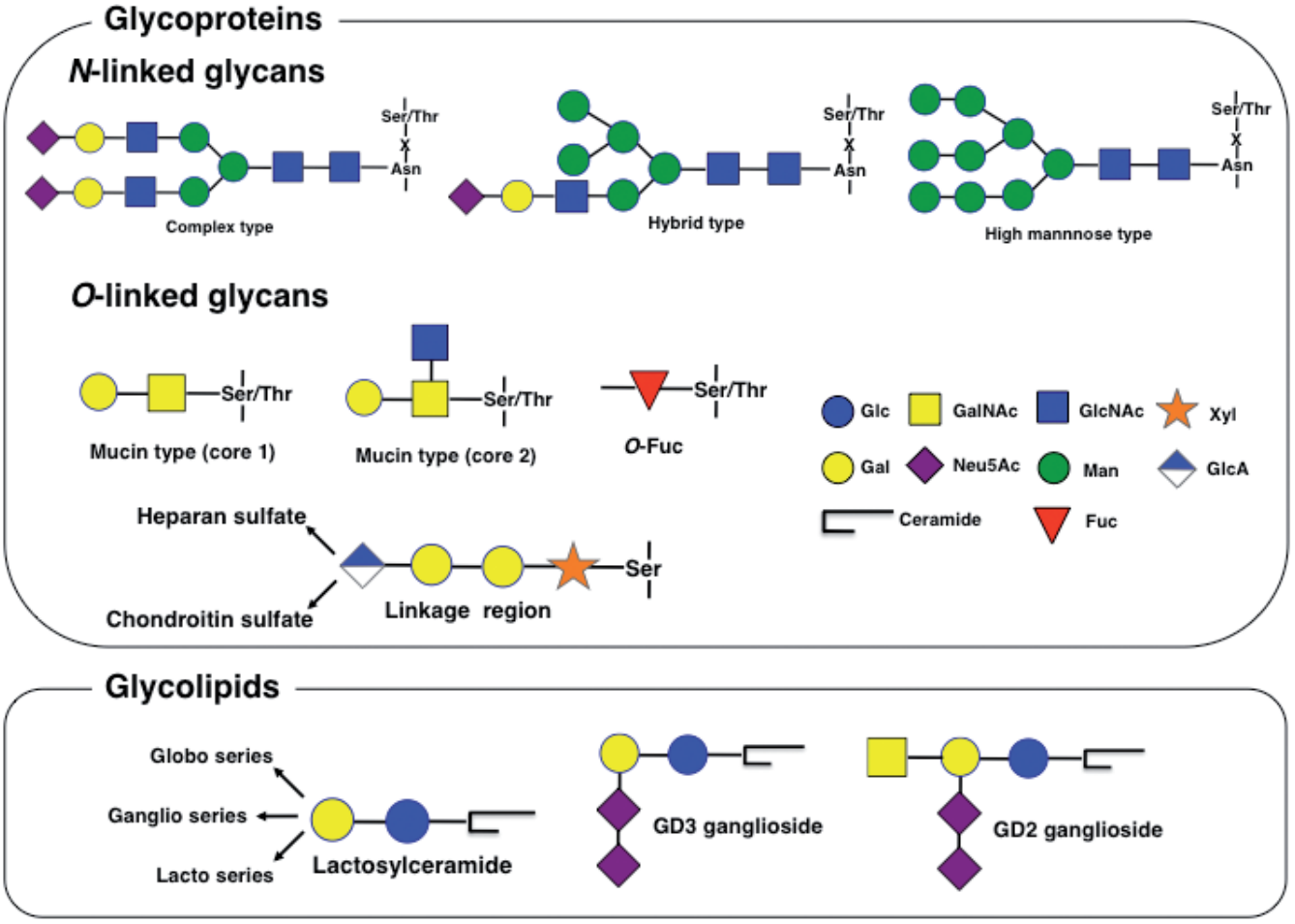

Fig. 1. Representative glycan structures of glycoproteins and glycolipids. Glycoproteins contain *N*- and *O*-linked glycans. *N*-linked glycans include three major types (high mannose, hybrid and complex glycans) and are synthesized on asparagine residues part of the Asn-X-Ser/Thr consensus sequence (X=any amino acid except proline). *O*-linked glycans are synthesized on serine or threonine residues. Mucin-type *O*-linked glycans include many core *O*-glycan structures, such as Core 1 and Core 2. Other *O*-linked glycans, such as proteoglycans, are macromolecules composed of a specific core protein with covalently linked glycosaminoglycan chains, such as chondroitin sulfate (CS) and heparan sulfate (HS). CS or HS chains contain common linkage regions. Glycolipids are composed of a glycan structure attached to a lipid tail containing the sphingolipid ceramide. Three major series of glycolipids (ganglio-series, globo-series and lacto-series) are synthesized from lactosylceramide. Gangliosides are ganglio-series molecules composed of glycosphingolipids with one or more sialic acids linked. Glc, Glucose; Gal, Galactose; GalNAc, *N*-acetylgalactosamine; Neu5Ac, *N*-acetylneuraminic acid; Fuc, Fucose; Xyl, Xylose; GlcNAc, *N*-acetylglucosamine; Man, Mannose; GlcA, Glucuronic acid.

pathogen-host recognition, toxin-receptor interactions, cancer metastasis, immune responses and regulation of signaling pathways (Varki, 2017). In addition, glycans are also used as cell surface markers (Varki, 2017).

Glycan markers are known to exhibit undifferentiated state in pluripotent stem cells. Stage-specific embryonic antigen 1 (SSEA-1, Lewis blood group-related carbohydrate antigen) is known as an undifferentiated glycan marker in mouse embryonic stem cells (ESCs) (Muramatsu and Muramatsu, 2004). In contrast, SSEA-3, SSEA-4, SSEA-5, the TRA-1-60 and TRA-1-81 antigens are generally used as undifferentiated glycan markers in human ESCs and induced pluripotent stem cells (International Stem Cell et al., 2007; Tang et al., 2011; Itakura et al., 2013). In this review, we describe glycans in CSCs and show functional similarities and differences in glycans between CSCs and normal pluripotent and tissue stem cells. We also highlight the clinical significance of glycans as potential therapeutic targets in CSC-targeting therapies.

Table 1. List of cell surface CSC markers containing glycans.

CSC marker	Type of tumor	References
ABCB1	Gastric cancer	Jiang et al., 2012
ABCG2	Gastric cancer	Jiang et al., 2012
ABCB5	Melanoma	Schatton et al., 2008
CD9	ALL[a]	Nishida et al., 2009
CD10	Head and neck squamous cell carcinoma	Fukusumi et al., 2014
CD13	Liver cancer	Haraguchi et al., 2010
CD15 (SSEA-1)	Glioblastoma	Son et al., 2009
CD19	Multiple myeloma	Ghosh and Matsui, 2009
CD20	Melanoma	Fang et al., 2005
CD24	Pancreatic cancer	Li et al., 2007
CD26 (dipeptidyl peptidase-4)	Colorectal cancer	Pang et al., 2010
CD34	AML[b] T-ALL[c]	Bonnet and Dick, 1997 Cox et al., 2007
CD44	Colorectal cancer Gallbladder carcinoma Hepatocellular carcinoma Pancreatic cancer Breast cancer	Du et al., 2008 Shi et al., 2010 Zhu et al., 2010 Li et al., 2007 Hu et al., 2017
CD90 (Thy-1)	Liver cancer T-ALL	Yang et al., 2008 Yamazaki et al., 2009
CD105 (Endoglin)	Renal cell carcinoma	Bussolati et al., 2008
CD110	T-ALL	Yamazaki et al., 2009
CD117 (c-kit)	Ovarian cancer	Luo et al., 2011
CD123 (interleukin-3 R alpha)	AML	Jin et al., 2009
CD133	Medulloblastoma Colon cancer Gallbladder carcinoma Hepatocellular carcinoma Lung cancer Melanoma Pancreatic cancer	Shu et al., 2008 Ricci-Vitiani et al., 2007 Shi et al., 2010 Zhu et al., 2010 Eramo et al., 2008 Monzani et al., 2007 Li et al., 2007
CD166 (ALCAM)	Non-small cell lung cancer	Zhang et al., 2012
CD184 (CXCR4)	Renal cell carcinoma	Gassenmaier et al., 2013
CD271 (NGFR)	Melanoma	Boiko et al., 2010
CD326 (EpCAM)	Pancreatic cancer Breast cancer	Li et al., 2007 Al-Hajj et al., 2003
SSEA-3 (glycolipid)	Breast cancer	Cheung et al., 2016
Ganglioside GD2 (glycolipid)	Breast cancer	Battula et al., 2012
Ganglioside GD3 (glycolipid)	Breast cancer	Liang et al., 2013

[a]Acute lymphoblastic leukemia. [b]Acute myeloid leukemia. [c]T-cell acute lymphoblastic leukemia.

Cell surface CSC markers containing glycans

To date, unique cell surface markers have been exploited to identify CSCs. CSC markers, such as membrane glycoproteins and glycolipids, are present in various cancer and tissue types (Table 1) and have been reported to play functional roles. CD24 is a mucin-like glycosylphosphatidylinositol-anchored protein including *N*- and *O*-glycans, which was originally discovered as a leucocyte-expressed ligand for P-selectin (Aigner et al., 1997). In pancreatic ductal adenocarcinoma (PDAC), surface CD24 was identified as a CSC marker (Li et al., 2007). Overexpression of CD24 in the PDAC cell line MIA PaCa2 harboring mesenchymal features induced mesenchymal-epithelial transition, indicating that CD24 accumulation at the cell surface stabilizes an epithelial phenotype (Lubeseder-Martellato et al., 2016). CD44 is a transmembrane *N*- and *O*-glycosylated protein that binds hyaluronic acid and contributes to cell-cell and cell-matrix adhesion, cell growth, trafficking and tumor progression (Orian-Rousseau, 2010). Due to the presence of variable exons, CD44 has multiple isoforms (CD44v1–10) possessing stem cell potential. In colorectal cancer, CD44v6 is expressed in CSCs and plays a functional role in metastasis (Todaro et al., 2014). In breast CSCs, CD44v3–10 respond to osteopontin in the lung environment to enhance cancer cell invasiveness and promote lung metastasis (Hu et al., 2017). CD133 is a membrane glycoprotein modified with biantennary complex-type and high-mannose type *N*-linked glycans, with the terminal modified via α2,3-sialylation. In colorectal CSCs, CD133 contributes to cell motility and apoptosis resistance (Elsaba et al., 2010). Moreover, Jang et al. reported that CD133 promotes CSC-like properties by stabilizing epidermal growth factor receptor (EGFR)-Akt signaling in hepatocellular carcinoma (Jang et al., 2017). Liu et al. demonstrated that loss of *N*-glycosylation at Asn548 in CD133 reduced cell proliferation and decreased the ability of CD133 to associate with β-catenin and activate β-catenin signaling in liver cancer (Liu et al., 2015). The functional roles of glycan modifications on glycoproteins in CSCs have been largely unknown. In breast cancer, ganglioside GD2 was identified as a CSC marker (Battula et al., 2012). Reduction of GD2 by inhibition of GD3 synthase in breast cancer cells reduced the CSC population and CSC-associated properties including tumor formation in vivo (Battula et al., 2012). Another report has shown that both GD2 and GD3 are expressed in breast CSCs and contribute to sphere formation and cell motility (Liang et al., 2013).

Relationship between glycans and signaling pathways required for maintenance of CSCs

Signaling mediators such as leukemia inhibitory factor, Wnt, fibroblast growth factor (FGF), bone morphogenetic protein (BMP) and Notch contribute to maintenance of normal stem cells including ESCs, and the contribution of glycans to signaling mechanisms has mostly been clarified. These pathways and related glycans play important roles in maintenance of stemness of CSCs, although the detailed mechanisms still have to be characterized. The contributions of these pathways and related glycans to normal stem cells and CSCs are described below (see also Fig. 2).

LIF/signal transducers and activator of transcription 3 (STAT3) signaling

The LIF/STAT3 pathway is mediated by the LIF receptor (LIFR), which consists of two subunits, gp130 and LIFRβ. LIF induces heterodimerization and phosphorylation of these subunits, leading to rapid phosphorylation of intracellular non-receptor janus kinases (JAKs) that phosphorylate STAT3 on tyrosine residues (Davis et al., 1993). Activated STAT3 is required for maintenance of mouse ESCs (Matsuda et al., 1999). It has been demonstrated that LacdiNAc (GalNAcβ1-4GlcNAc) glycan structures on LIFRβ and gp130 are required for LIF/STAT3 signaling (Sasaki et al., 2011). The presence of LacdiNAc structures on LIFRβ and gp130 is also required for their localization in lipid rafts/caveolae and their heterodimerization upon LIF stimulation followed by LIF/STAT3 signal transduction. In contrast, primed pluripotent stem cells, such as mouse epiblast stem-like cells and human induced pluripotent stem cells, have lower levels of LacdiNAc glycans and thus no LIF/STAT3 signaling occurs in these cells (Sasaki et al., 2011). In breast CSCs, the interleukin (IL)-6/JAK2/STAT3 pathway is preferentially active and suggested to contribute to maintenance of CSC properties (Marotta et al., 2011). Penuelas et al. reported that TGF-β increases glioma-initiating cell self-renewal through induction of LIF in human glioblastoma (Penuelas et al., 2009). To date, it has not been reported that LIF/STAT3 signaling regulates properties of CSCs in other types of cancer. However, it is known that LIF is present in various types of cancer cells (Kamohara et al., 1994). Therefore, we assume that LIF/STAT3 signaling might be involved in the maintenance of other CSCs and mechanisms similar to those required for the maintenance of naïve-state mouse ESCs mediated by glycans might be occurring in other CSCs.

Wnt/β-catenin signaling

The canonical Wnt/β-catenin pathway is initiated by the binding of Wnt proteins to their cognate receptors of the frizzled and low density lipoprotein receptor-related protein/α2-macroglobulin receptor families on the cell surface. The signal transduction mediated by several cytoplasmic components induces the stabilization and nuclear accumulation of β-catenin, leading to the activation of Wnt target genes (Clevers and Nusse, 2012). Wnt/β-catenin signaling has an essential role in regulating many biological processes, including embryonic development, tissue homoeostasis and

maintenance of stem cells. In ESCs, Wnt/β-catenin signaling is required for establishment and self-renewal of ESCs (Merrill, 2012; Clevers et al., 2014). HS on the surface of ESCs is predicted to bind Wnt3a, given the observed strong interaction between Wnt3a and heparin (Sasaki et al., 2008). A genetic study targeting *EXT1*, which is required for synthesis of HS (Lind et al., 1998), has shown that HS on mouse ESCs contributes to regulation of Wnt/β-catenin signaling (Sasaki et al., 2008; Kraushaar et al., 2012). Wnt/β-catenin signaling is also involved in the regulation of CSCs in many other tissue types (Clevers and Nusse, 2012; Hoffmeyer et al., 2012; Holland et al., 2013). In breast cancer, leucine-rich repeat-containing G-protein-coupled receptor 5-expressing CSC-like cells form self-renewing spheres with high tumorigenicity through activation of Wnt/β-catenin signaling (Yang et al., 2015). Wnt3a can promote self-renewal of CSCs in acute lymphocytic leukemia and prostate cancer (Kawaguchi-Ihara et al., 2008; Bisson and Prowse, 2009). However, the contribution of HS to Wnt/β-catenin signaling in the maintenance of CSCs is still unknown.

FGF signaling

FGFs are heparin-binding growth factors including FGF-1 to FGF-23 (Itoh and Ornitz, 2004). Each FGF binds to transmembrane FGF receptors (FGFRs, FGFR-

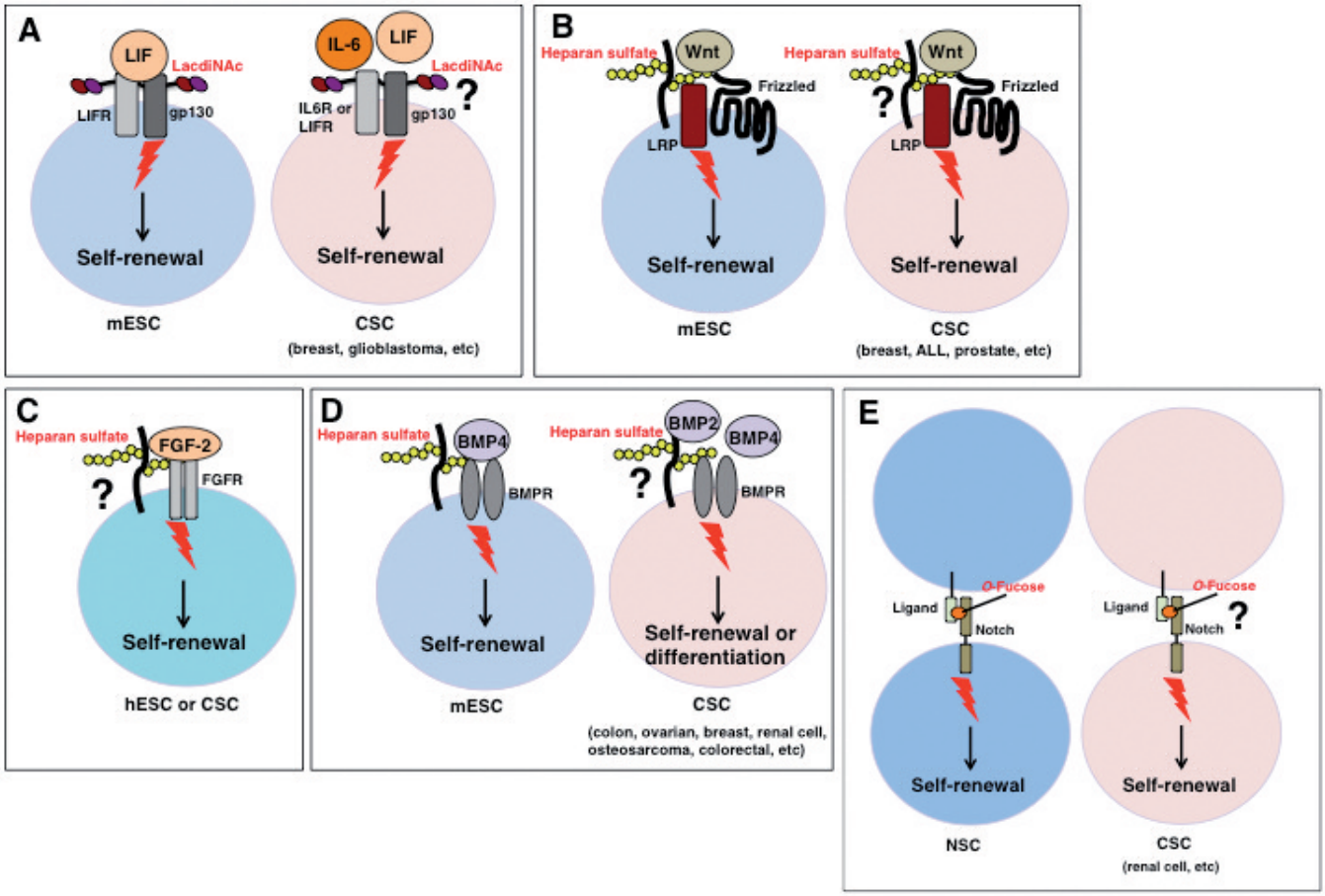

Fig. 2. Functional glycans in signaling pathways required for maintenance of stem cells. **A.** LIF/STAT3 signaling. In mouse ESCs (mESCs), LacdiNAc glycan structures on LIFRβ and gp130 are required for maintenance of self-renewal through LIF/STAT3 signaling. In CSCs, IL-6 and/or LIF/STAT3 signaling are likely involved in maintenance of self-renewal. Similar mechanisms mediated by LacdiNAc might be required for maintenance of naïve-state mESCs. **B.** Wnt/β-catenin signaling. Heparan sulfate (HS) on mESCs contributes to maintenance of self-renewal through regulation of Wnt/β-catenin signaling. Wnt/β-catenin signaling is also involved in the regulation of CSCs, however, the contribution of HS to Wnt/β-catenin signaling in the maintenance of CSC self-renewal is still unknown. **C.** FGF signaling. In human ESCs (hESCs) and CSCs, FGF signaling contributes to self-renewal. However, the involvement of HS proteoglycans in FGF-2 signaling has not been elucidated. **D.** BMP signaling. In mESCs, HS contributes to maintenance of self-renewal through regulation of BMP4 signaling. In CSCs, BMP signaling is associated with CSC properties. However, the relationship between BMP signaling and CSC self-renewal and/or differentiation remains controversial. The regulation of BMP signaling by HS in CSCs has not been clarified yet. **E.** Notch signaling. In adult hippocampal NSCs, *O*-fucosylation of residues on epidermal growth factor-like repeats of Notch contributes to maintenance of self-renewal. In CSCs, Notch signaling is known to contribute to self-renewal. However, the contribution of Notch *O*-fucosylation to the maintenance of CSC self-renewal has not been clarified yet.

1 to FGFR-4) with high ligand specificity (Mohammadi et al., 2005; Ishiwata, 2018). FGF signaling plays various roles in development as well as in adult physiology and pathology (Belov and Mohammadi, 2013). Heparin/HS proteoglycans are required for FGF-2-induced dimerization and transphosphorylation of FGFRs, the key events by which tyrosine kinase receptors initiate downstream signaling (Mohammadi et al., 2005). It is widely known that human ESCs require FGF signaling to sustain self-renewal and the capacity to differentiate into a large number of somatic cell types (Xu et al., 2001). Exogenous addition of heparin contributes to maintain human ESCs in serum-free medium supplemented with FGF-2 (Furue et al., 2008). For maintenance and enrichment of CSCs, suspension cultures are usually supplemented with FGF-2 (Matsuda et al., 2014; Ishiwata et al., 2018). The involvement of heparin/HS proteoglycans in FGF-2 signaling in CSCs has not yet been elucidated, although these glycans are assumed to contribute to maintenance of CSCs by regulating FGF-2 signaling.

BMP signaling

The canonical BMP signaling occurs through type I receptor-mediated phosphorylation of Smad1, Smad5 and Smad8/R-Smad (Miyazono et al., 2000). Two phosphorylated R-Smads form a heterotrimeric complex with a common Smad4 (co-Smad), which translocates into the nucleus and cooperates with other transcription factors to modulate target gene expression (Derynck and Zhang, 2003). In mouse ESCs, BMP-4/Smad signaling contributes to the maintenance of self-renewal and pluripotency (Ying et al., 2003). Genetic studies, which inhibited HS by *EXT1* knockout (Kraushaar et al., 2012) and HS sulfation by knockdown of 3'-phosphoadenosine 5'-phosphosulfate transporters and *N*-deacetylases/*N*-sulfotransferases (Sasaki et al., 2009), revealed that HS contributes to BMP-4/Smad signaling in mouse ESCs. Alterations in the BMP signaling pathway have been observed in numerous types of cancer, in some cases closely associated with CSC properties (Lee et al., 2008). BMP-2 was reported to promote CSC formation in colon and ovarian cancers (McLean et al., 2011; Kim et al., 2015). In breast cancer, BMP-2 also contributes to CSC formation and metastasis via the Rb and CD44 signaling pathways (Huang et al., 2017). In addition, BMP-2 was reported to inhibit CSC tumorigenicity in osteosarcoma and renal cell carcinoma (Wang et al., 2011, 2015). Furthermore, BMP-4 promotes differentiation and apoptosis of colorectal CSCs (Lombardo et al., 2011). Therefore, the relationship between BMP signaling and CSCs remains controversial. The regulation of BMP signaling in CSCs by HS has not been clarified yet.

Notch signaling

The Notch signaling pathway is a highly conserved molecular cell signaling pathway that plays roles in proliferation, stem cell maintenance, cell fate specification, differentiation and homeostasis of multicellular organisms and regulates angiogenesis (Liu et al., 2010; Zhou et al., 2013). In adult hippocampal neural stem cells (NSCs), Lunatic fringe (*Lfng*), which modulates Notch activity by modifying *O*-fucose residues on epidermal growth factor-like repeats of Notch, is selectively expressed and contributes to maintenance of NSCs (Semerci et al., 2017). In *Drosophila* intestinal stem cells (ISCs), *O*-fucosylation of Notch is implicated in ISC commitment and is dispensable for terminal cell fate choices (Perdigoto et al., 2011). Increasing evidence suggests that Notch signaling may promote proliferation, survival, self-renewal, differentiation, angiogenesis and migration of CSCs in several malignant cancers (Kraushaar et al., 2012; McAuliffe et al., 2012; Yan et al., 2014). In renal cell carcinoma CSCs, blockage of Notch1 or Notch2 using pharmacological inhibitors leads to loss of stemness features (Xiao et al., 2017). However, the contribution of glycans to Notch signaling in the maintenance of CSCs still has to be clarified.

Sphere formation and glycans

Sphere-forming cancer cells can be induced in suspension cultures with serum-free medium containing epidermal growth factor and FGF-2. Sphere formation is particularly useful to enrich potential CSC subpopulations. Sphere-forming cultures were first developed in 1996 using NSCs (Reynolds and Weiss, 1996). Neurosphere-forming cells (Fig. 3A) maintained NSC phenotype with the ability to undergo multilineage differentiation even after serial passage. It has been demonstrated that several glycans contribute to maintenance of NSC self-renewal and pluripotency in sphere formation (Yagi and Kato, 2017) (Fig. 3B). Sirko et al. reported that CS glycosaminoglycans are selectively required for neurosphere formation, proliferation and self-renewal of NSCs in response to FGF-2 (Sirko et al., 2010). NSCs derived from GD3-synthase knockout mice could not be maintained in *in vitro* sphere-forming cultures (Wang and Yu, 2013), indicating that GD3 contributes to maintenance of NSCs. Enhancing *N*-glycan branching on the NSC surface in sphere-forming cultures with GlcNAc decreases neurogenesis and increases astrogenesis upon differentiation (Yale et al., 2018), indicating a contribution of *N*-glycans to NSC differentiation. On the other hand, the contribution of glycans such as CS to CSC sphere formation has not been demonstrated (Fig. 3C,D). GD2-positive CSCs in breast cancer have high sphere-forming ability (Battula et al., 2012). In these cells, reduction of GD2 by suppression of GD3 synthase hampers sphere formation, indicating that GD2 contributes to maintain CSC properties. Another report suggested that GD3 also contributes to sphere formation in breast cancer (Liang et al., 2013). Che et al. reported

that the LacdiNAc glycan on EGFR contributes to maintain stemness of colon CSCs in sphere-forming cultures (Che et al., 2014). In pancreatic cancer, sphere formation induced an increase in fucosylated glycans (Terao et al., 2015), although the functional role of this increase is still unknown.

Epithelial-mesenchymal transition (EMT) and glycans

EMT is a highly conserved cellular process that transforms epithelial cells into mesenchymal cells. In cancer, EMT (also exhibited by CSCs) is relevant to the acquisition and maintenance of stem cell-like characteristics. This relationship between EMT and CSCs might have many implications in tumor progression (De Craene and Berx, 2013). EMT is an important step in the promotion of tumor metastasis, whereby epithelial cells lose cell polarity and cell-cell adhesion ability and acquire migratory and invasive properties to gain mesenchymal phenotype (Thiery, 2002; Chaffer and Weinberg, 2011; Chen et al., 2017). CSCs undergoing EMT are resistant to anti-cancer therapies and thus cause cancer recurrence. In addition, CSCs that undergo EMT migrate into adjacent stromal tissues, enter the blood or lymph vessels and reach target organs (Pang et al., 2016). The contribution of glycans to EMT has been demonstrated in several reports (Li et al., 2016) (Fig. 4A,B). Xu et al. reported that TGF-β1-induced EMT dramatically down-regulated GnT-III expression and bisected *N*-glycans, whereas overexpression of GnT-III downregulated EMT through prolonged E-cadherin turnover on the cell surface (Xu et al., 2012). Increased expression of β-galactoside α2,6-

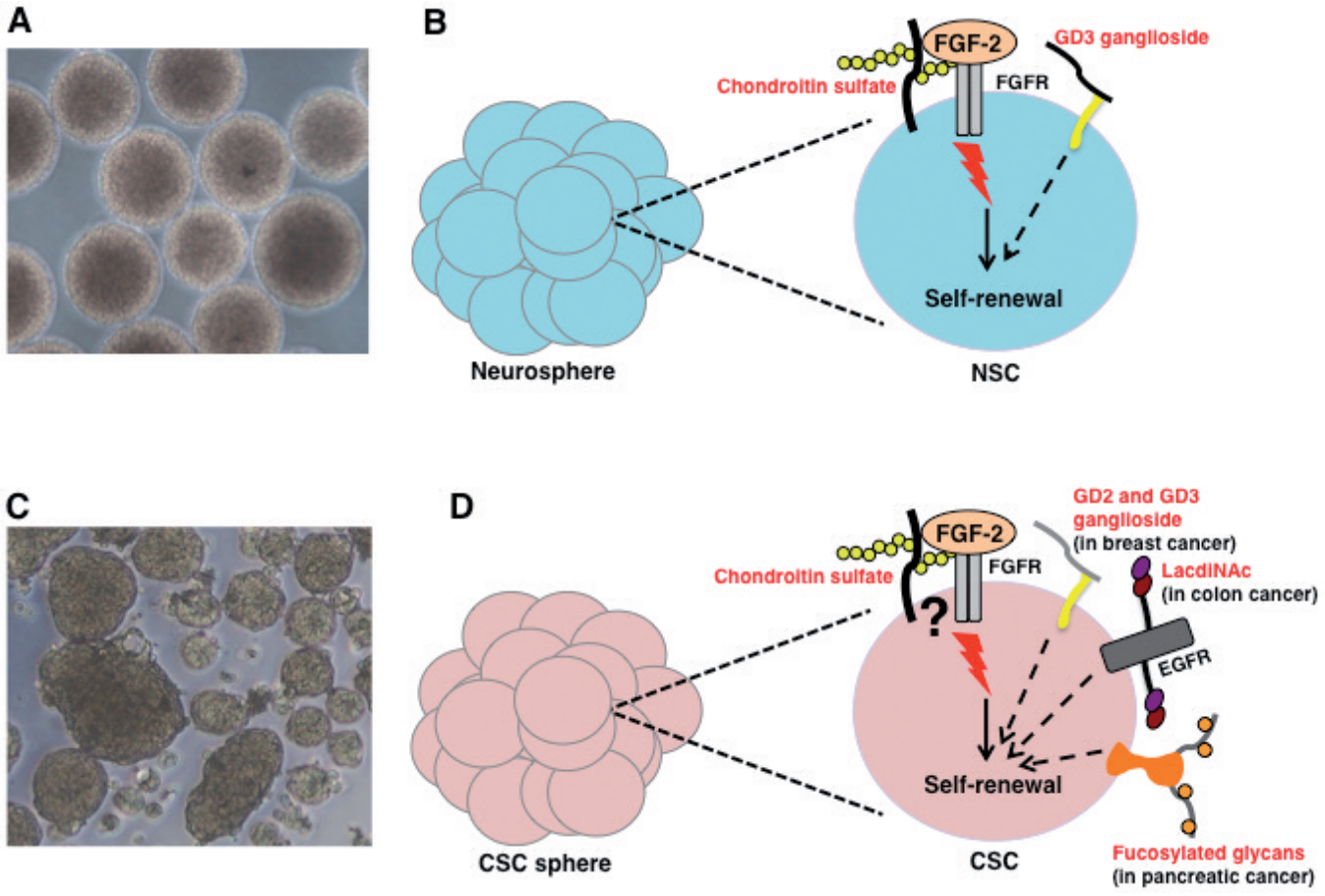

Fig. 3. Functional glycans in sphere formation. **A.** Representative photo of spheres in human induced pluripotent stem cell-derived NSCs cultured on an ultra-low attachment plate (KH, unpublished data). **B.** In NSCs, glycans contribute to maintenance of self-renewal and sphere formation. Chondroitin sulfate glycosaminoglycans contribute to neurosphere formation and self-renewal of NSCs in response to FGF-2. Although the molecular mechanisms are unknown, GD3 is likely to contribute to maintenance of NSCs. **C.** Representative photo of spheres in human colon adenocarcinoma HT29 cells cultured on an ultra-low attachment plate (NS, unpublished data). **D.** The contribution of glycans to CSC sphere formation has not been demonstrated. In breast CSCs, although the detailed molecular mechanisms are still unknown, GD2 and GD3 are likely to contribute to maintenance of self-renewal. The LacdiNAc glycan on EGFR contributes to maintain stemness of colon CSCs in sphere-forming cultures, but the molecular mechanisms have not been elucidated. In pancreatic cancer, sphere-forming cells show an increase in fucosylated glycans, but the functional roles of this increase are still unknown.

sialyltranferase 1, which catalyzes the terminal α2,6-sialylation of *N*-glycans, contributed to TGF-β1-induced EMT in MDA-MB-231 human breast cancer cells through a non-Smad signaling pathway (Lu et al., 2014). Overexpression of MUC1 *O*-glycans positively induced EMT in renal carcinoma cells by promoting the interaction with β-catenin to activate the transcription factor Snail (Gnemmi et al., 2014). It has been reported that Gg4 (asialo-GM1 ganglioside) is reduced in EMT and has a suppressive effect on TGF-β1-induced EMT (Guan et al., 2009). In addition, inhibition of GD3 synthase, which leads to reduction of the CSC marker GD2, attenuated TGF-β1-induced EMT in breast cancer cells (Sarkar et al., 2015).

EMT is also important for generating multiple tissues during organism development (Thiery et al., 2009). In most ESCs, EMT might occur during differentiation. The following processes are involved in the differentiation of human ESCs on adherent cultures: the conversion from E-cadherin to N-cadherin, increased vimentin expression, increase in EMT markers such as Snail and Slug, increased gelatinase activity and increased cellular motility (Eastham et al., 2007). Furthermore, EMT occurred in the embryoid body derived from human ESCs or human induced pluripotent stem cells (Chan et al., 2012). In mouse ESCs, E-cadherin interacts with key components of the naïve stemness pathway. Therefore, depletion of E-cadherin converts naïve mouse ESCs into primed epiblast-like stem cells (Pieters and van Roy, 2014), suggesting a contribution of EMT in mouse ESC differentiation. Thus, the occurrence of EMT in ESCs and embryoid bodies reflects the EMT observed during gastrulation in development. The involvement of glycans in the EMT of ESCs still has to be characterized. However, we expect that glycans, which regulate EMT-related signaling molecules such as TGF-β, Wnt and Notch, might also be involved in the EMT of ESCs similarly to CSCs (Fig. 4C).

Conclusion

In this review, we compared functional cell surface

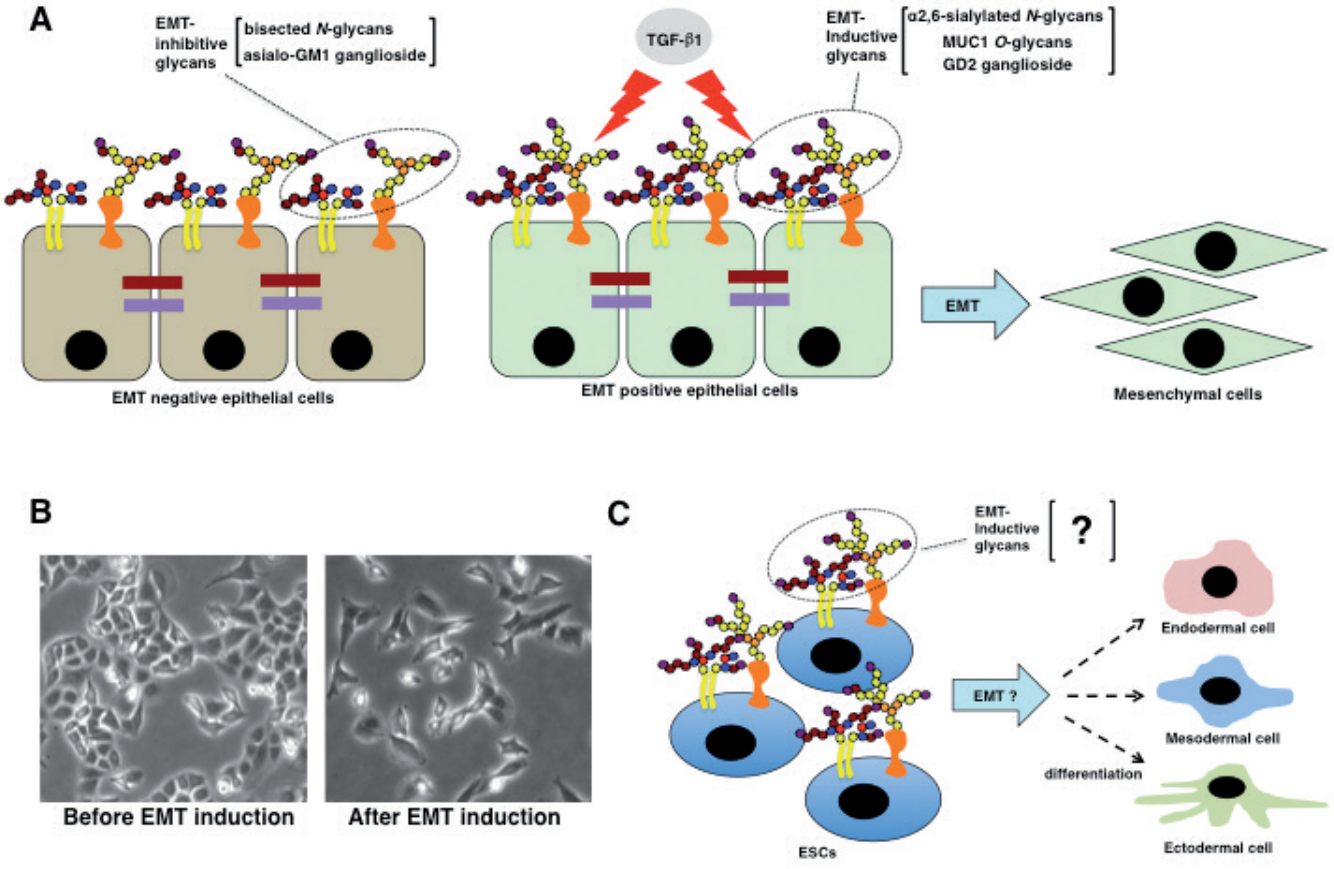

Fig. 4. Functional glycans in EMT. **A.** Glycans contribute to the EMT of cancer cells. EMT-positive epithelial cells, in which TGF-β1-inducible and/or TGF-β1-responsive EMT-inductive glycans are expressed, can differentiate into mesenchymal cells after TGF-β1 stimulation. In contrast, EMT-negative epithelial cells, in which TGF-β1-inducible and/or TGF-β1-non-responsive EMT-inhibitive glycans are expressed, are unable to differentiate into mesenchymal cells. **B.** Representative images of pancreatic cancer cells (PANC-1) before and after EMT induction by TGF-β1 (NS, unpublished data). **C.** In ESCs, EMT likely occurs during differentiation into three germ layers. The glycans involved in EMT of ESCs are still unknown.

glycans involved in cell signaling in CSCs and normal stem cells. To date, a number of CSC markers containing glycans have been identified, however, CSC-specific glycans and their functional roles have not been well-clarified compared to normal stem cells. Both CSCs and normal stem cells share various stemness markers and utilize similar molecular mechanisms to drive self-renewal and similar signaling pathways to induce their differentiation. Future experiments shall aim to compare glycans between CSCs (purified using markers, sphere cultures and/or side population fractionation) and non-CSCs using analysis methods such as lectin microarray. To develop early detection methods and therapeutic strategies for CSCs, future efforts will focus on the identification of CSC-specific glycans and the clarification of their functional roles.

Acknowledgements. This work was supported by JSPS KAKENHI, Grant Numbers 16K10613 and 16K08263 (Grant-in-Aid for Scientific Research (C)), by the Pancreas Research Foundation of Japan and by the Ichiro Kanehara Foundation for the Promotion of Medical Sciences and Medical Care.

References

Aigner S., Sthoeger Z.M., Fogel M., Weber E., Zarn J., Ruppert M., Zeller Y., Vestweber D., Stahel R., Sammar M. and Altevogt P. (1997). Cd24, a mucin-type glycoprotein, is a ligand for p-selectin on human tumor cells. Blood 89, 3385-3395.

Al-Hajj M., Wicha M.S., Benito-Hernandez A., Morrison S.J. and Clarke M.F. (2003). Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. USA 100, 3983-3988.

Battula V.L., Shi Y., Evans K.W., Wang R.Y., Spaeth E.L., Jacamo R.O., Guerra R., Sahin A.A., Marini F.C., Hortobagyi G., Mani S.A. and Andreeff M. (2012). Ganglioside GD2 identifies breast cancer stem cells and promotes tumorigenesis. J. Clin. Invest. 122, 2066-2078.

Belov A.A. and Mohammadi M. (2013). Molecular mechanisms of fibroblast growth factor signaling in physiology and pathology. Cold Spring Harb. Perspect. Biol. 5, a015958.

Bisson I. and Prowse D.M. (2009). Wnt signaling regulates self-renewal and differentiation of prostate cancer cells with stem cell characteristics. Cell Res. 19, 683-697.

Boiko A.D., Razorenova O.V., van de Rijn M., Swetter S.M., Johnson D.L., Ly D.P., Butler P.D., Yang G.P., Joshua B., Kaplan M.J., Longaker M.T. and Weissman I.L. (2010). Human melanoma-initiating cells express neural crest nerve growth factor receptor cd271. Nature 466, 133-137.

Bonnet D. and Dick J.E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat. Med. 3, 730-737.

Bussolati B., Bruno S., Grange C., Ferrando U. and Camussi G. (2008). Identification of a tumor-initiating stem cell population in human renal carcinomas. FASEB J. 22, 3696-3705.

Chaffer C.L. and Weinberg R.A. (2011). A perspective on cancer cell metastasis. Science 331, 1559-1564.

Chan D.N., Azghadi S.F., Feng J. and Lowry W.E. (2012). PTK7 marks the first human developmental emt in vitro. PLoS One 7, e50432.

Che M.I., Huang J., Hung J.S., Lin Y.C., Huang M.J., Lai H.S., Hsu W.M., Liang J.T. and Huang M.C. (2014). Beta1, 4-n-acetylgalactosaminyltransferase iii modulates cancer stemness through egfr signaling pathway in colon cancer cells. Oncotarget 5, 3673-3684.

Chen T., You Y., Jiang H. and Wang Z.Z. (2017). Epithelial-mesenchymal transition (EMT): A biological process in the development, stem cell differentiation, and tumorigenesis. J. Cell. Physiol. 232, 3261-3272.

Cheung S.K., Chuang P.K., Huang H.W., Hwang-Verslues W.W., Cho C.H., Yang W.B., Shen C.N., Hsiao M., Hsu T.L., Chang C.F. and Wong C.H. (2016). Stage-specific embryonic antigen-3 (ssea-3) and beta3galt5 are cancer specific and significant markers for breast cancer stem cells. Proc. Natl. Acad. Sci. USA 113, 960-965.

Clarke M.F., Dick J.E., Dirks P.B., Eaves C.J., Jamieson C.H., Jones D.L., Visvader J., Weissman I.L. and Wahl G.M. (2006). Cancer stem cells--perspectives on current status and future directions: Aacr workshop on cancer stem cells. Cancer Res. 66, 9339-9344.

Clevers H. and Nusse R. (2012). Wnt/beta-catenin signaling and disease. Cell 149, 1192-1205.

Clevers H., Loh K.M. and Nusse R. (2014). Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science 346, 1248012.

Collins A.T., Berry P.A., Hyde C., Stower M.J. and Maitland N.J. (2005). Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res. 65, 10946-10951.

Cox C.V., Martin H.M., Kearns P.R., Virgo P., Evely R.S. and Blair A. (2007). Characterization of a progenitor cell population in childhood t-cell acute lymphoblastic leukemia. Blood 109, 674-682.

Curley M.D., Therrien V.A., Cummings C.L., Sergent P.A., Koulouris C.R., Friel A.M., Roberts D.J., Seiden M.V., Scadden D.T., Rueda B.R. and Foster R. (2009). CD133 expression defines a tumor initiating cell population in primary human ovarian cancer. Stem Cells 27, 2875-2883.

Davis S., Aldrich T.H., Stahl N., Pan L., Taga T., Kishimoto T., Ip N.Y. and Yancopoulos G.D. (1993). LIFR beta and gp130 as heterodimerizing signal transducers of the tripartite cntf receptor. Science 260, 1805-1808.

De Craene B. and Berx G. (2013). Regulatory networks defining emt during cancer initiation and progression. Nat. Rev. Cancer 13, 97-110.

Derynck R. and Zhang Y.E. (2003). Smad-dependent and smad-independent pathways in TGF-beta family signalling. Nature 425, 577-584.

Du L., Wang H., He L., Zhang J., Ni B., Wang X., Jin H., Cahuzac N., Mehrpour M., Lu Y. and Chen Q. (2008). CD44 is of functional importance for colorectal cancer stem cells. Clin. Cancer Res. 14, 6751-6760.

Eastham A.M., Spencer H., Soncin F., Ritson S., Merry C.L., Stern P.L. and Ward C.M. (2007). Epithelial-mesenchymal transition events during human embryonic stem cell differentiation. Cancer Res. 67, 11254-11262.

Elsaba T.M., Martinez-Pomares L., Robins A.R., Crook S., Seth R., Jackson D., McCart A., Silver A.R., Tomlinson I.P. and Ilyas M. (2010). The stem cell marker CD133 associates with enhanced colony formation and cell motility in colorectal cancer. PLoS One 5, e10714.

Eramo A., Lotti F., Sette G., Pilozzi E., Biffoni M., Di Virgilio A., Conticello C., Ruco L., Peschle C. and De Maria R. (2008). Identification and expansion of the tumorigenic lung cancer stem cell

population. Cell Death Differ. 15, 504-514.

Fang D., Nguyen T.K., Leishear K., Finko R., Kulp A.N., Hotz S., Van Belle P.A., Xu X., Elder D.E. and Herlyn M. (2005). A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res. 65, 9328-9337.

Fukusumi T., Ishii H., Konno M., Yasui T., Nakahara S., Takenaka Y., Yamamoto Y., Nishikawa S., Kano Y., Ogawa H., Hasegawa S., Hamabe A., Haraguchi N., Doki Y., Mori M. and Inohara H. (2014). CD10 as a novel marker of therapeutic resistance and cancer stem cells in head and neck squamous cell carcinoma. Br. J. Cancer 111, 506-514.

Furue M.K., Na J., Jackson J.P., Okamoto T., Jones M., Baker D., Hata R., Moore H.D., Sato J.D. and Andrews P.W. (2008). Heparin promotes the growth of human embryonic stem cells in a defined serum-free medium. Proc. Natl. Acad. Sci. USA 105, 13409-13414.

Gassenmaier M., Chen D., Buchner A., Henkel L., Schiemann M., Mack B., Schendel D.J., Zimmermann W. and Pohla H. (2013). CXC chemokine receptor 4 is essential for maintenance of renal cell carcinoma-initiating cells and predicts metastasis. Stem Cells 31, 1467-1476.

Ghosh N. and Matsui W. (2009). Cancer stem cells in multiple myeloma. Cancer Lett. 277, 1-7.

Gnemmi V., Bouillez A., Gaudelot K., Hemon B., Ringot B., Pottier N., Glowacki F., Villers A., Vindrieux D., Cauffiez C., Van Seuningen I., Bernard D., Leroy X., Aubert S. and Perrais M. (2014). MUC1 drives epithelial-mesenchymal transition in renal carcinoma through wnt/beta-catenin pathway and interaction with snail promoter. Cancer Lett. 346, 225-236.

Guan F., Handa K. and Hakomori S.I. (2009). Specific glycosphingolipids mediate epithelial-to-mesenchymal transition of human and mouse epithelial cell lines. Proc. Natl. Acad. Sci. USA 106, 7461-7466.

Haraguchi N., Ishii H., Mimori K., Tanaka F., Ohkuma M., Kim H.M., Akita H., Takiuchi D., Hatano H., Nagano H., Barnard G.F., Doki Y. and Mori M. (2010). CD13 is a therapeutic target in human liver cancer stem cells. J. Clin. Invest. 120, 3326-3339.

Hoffmeyer K., Raggioli A., Rudloff S., Anton R., Hierholzer A., Del Valle I., Hein K., Vogt R. and Kemler R. (2012). Wnt/beta-catenin signaling regulates telomerase in stem cells and cancer cells. Science 336, 1549-1554.

Holland J.D., Klaus A., Garratt A.N. and Birchmeier W. (2013). Wnt signaling in stem and cancer stem cells. Curr. Opin. Cell Biol. 25, 254-264.

Hu J., Li G., Zhang P., Zhuang X. and Hu G. (2017). A CD44v(+) subpopulation of breast cancer stem-like cells with enhanced lung metastasis capacity. Cell Death Dis. 8, e2679.

Huang P., Chen A., He W., Li Z., Zhang G., Liu Z., Liu G., Liu X., He S., Xiao G., Huang F., Stenvang J., Brunner N., Hong A. and Wang J. (2017). BMP-2 induces emt and breast cancer stemness through RB and CD44. Cell Death Dis. 3, 17039.

International Stem Cell I., Adewumi O., Aflatoonian B., Ahrlund-Richter L., Amit M., Andrews P.W., Beighton G., Bello P.A., Benvenisty N., Berry L.S., Bevan S., Blum B., Brooking J., Chen K.G., Choo A.B., Churchill G.A., Corbel M., Damjanov I., Draper J.S., Dvorak P., Emanuelsson K., Fleck R.A., Ford A., Gertow K., Gertsenstein M., Gokhale P.J., Hamilton R.S., Hampl A., Healy L.E., Hovatta O., Hyllner J., Imreh M.P., Itskovitz-Eldor J., Jackson J., Johnson J.L., Jones M., Kee K., King B.L., Knowles B.B., Lako M., Lebrin F., Mallon B.S., Manning D., Mayshar Y., McKay R.D., Michalska A.E., Mikkola M., Mileikovsky M., Minger S.L., Moore H.D., Mummery C.L., Nagy A., Nakatsuji N., O'Brien C.M., Oh S.K., Olsson C., Otonkoski T., Park K.Y., Passier R., Patel H., Patel M., Pedersen R., Pera M.F., Piekarczyk M.S., Pera R.A., Reubinoff B.E., Robins A.J., Rossant J., Rugg-Gunn P., Schulz T.C., Semb H., Sherrer E.S., Siemen H., Stacey G.N., Stojkovic M., Suemori H., Szatkiewicz J., Turetsky T., Tuuri T., van den Brink S., Vintersten K., Vuoristo S., Ward D., Weaver T.A., Young L.A. and Zhang W. (2007). Characterization of human embryonic stem cell lines by the international stem cell initiative. Nat. Biotechnol. 25, 803-816.

Ishiwata T. (2016). Cancer stem cells and epithelial-mesenchymal transition: Novel therapeutic targets for cancer. Pathol. Int. 66, 601-608.

Ishiwata T. (2018). Role of fibroblast growth factor receptor-2 splicing in normal and cancer cells. Front. Biosci. 23, 626-639.

Ishiwata T., Matsuda Y., Yoshimura H., Sasaki N., Ishiwata S., Ishikawa N., Takubo K., Arai T. and Aida J. (2018). Pancreatic cancer stem cells: Features and detection methods. Pathol. Oncol. Res. 24, 797-805.

Itakura Y., Kuno A., Toyoda M., Umezawa A. and Hirabayashi J. (2013). Podocalyxin-targeting comparative glycan profiling reveals difference between human embryonic stem cells and embryonal carcinoma cells. J. Glycomics lipid. S5.

Itoh N. and Ornitz D.M. (2004). Evolution of the FGF and FGFR gene families. Trends Genet. 20, 563-569.

Jang J.W., Song Y., Kim S.H., Kim J.S., Kim K.M., Choi E.K., Kim J. and Seo H.R. (2017). CD133 confers cancer stem-like cell properties by stabilizing egfr-akt signaling in hepatocellular carcinoma. Cancer Lett. 389, 1-10.

Jiang Y., He Y., Li H., Li H.N., Zhang L., Hu W., Sun Y.M., Chen F.L. and Jin X.M. (2012). Expressions of putative cancer stem cell markers ABCB1, ABCG2, and CD133 are correlated with the degree of differentiation of gastric cancer. Gastric Cancer 15, 440-450.

Jin L., Lee E.M., Ramshaw H.S., Busfield S.J., Peoppl A.G., Wilkinson L., Guthridge M.A., Thomas D., Barry E.F., Boyd A., Gearing D.P., Vairo G., Lopez A.F., Dick J.E. and Lock R.B. (2009). Monoclonal antibody-mediated targeting of CD123, IL-3 receptor alpha chain, eliminates human acute myeloid leukemic stem cells. Cell Stem Cell 5, 31-42.

Kamohara H., Sakamoto K., Ishiko T., Mita S., Masuda Y., Abe T. and Ogawa M. (1994). Human carcinoma cell lines produce biologically active leukemia inhibitory factor (LIF). Res. Commun. Mol. Pathol. Pharmacol. 85, 131-140.

Kawaguchi-Ihara N., Murohashi I., Nara N. and Tohda S. (2008). Promotion of the self-renewal capacity of human acute leukemia cells by WNT3a. Anticancer Res. 28, 2701-2704.

Kim B.R., Oh S.C., Lee D.H., Kim J.L., Lee S.Y., Kang M.H., Lee S.I., Kang S., Joung S.Y. and Min B.W. (2015). BMP-2 induces motility and invasiveness by promoting colon cancer stemness through STAT3 activation. Tumour Biol. 36, 9475-9486.

Kraushaar D.C., Rai S., Condac E., Nairn A., Zhang S., Yamaguchi Y., Moremen K., Dalton S. and Wang L. (2012). Heparan sulfate facilitates FGF and BMP signaling to drive mesoderm differentiation of mouse embryonic stem cells. J. Biol. Chem. 287, 22691-22700.

Lee J., Son M.J., Woolard K., Donin N.M., Li A., Cheng C.H., Kotliarova S., Kotliarov Y., Walling J., Ahn S., Kim M., Totonchy M., Cusack T., Ene C., Ma H., Su Q., Zenklusen J.C., Zhang W., Maric D. and Fine H.A. (2008). Epigenetic-mediated dysfunction of the bone morphogenetic protein pathway inhibits differentiation of

glioblastoma-initiating cells. Cancer Cell 13, 69-80.

Li C., Heidt D.G., Dalerba P., Burant C.F., Zhang L., Adsay V., Wicha M., Clarke M.F. and Simeone D.M. (2007). Identification of pancreatic cancer stem cells. Cancer Res. 67, 1030-1037.

Li X., Wang X., Tan Z., Chen S. and Guan F. (2016). Role of glycans in cancer cells undergoing epithelial-mesenchymal transition. Front. Oncol. 6, 33.

Liang Y.J., Ding. Y., Levery. S.B., Lobaton. M., Handa. K., and Hakomori. S.I. (2013). Differential expression profiles of glycosphingolipids in human breast cancer stem cells vs. cancer non-stem cells. Proc. Natl. Acad. Sci. USA 110, 4968-4973.

Lind T., Tufaro F., McCormick C., Lindahl U. and Lidholt K. (1998). The putative tumor suppressors ext1 and ext2 are glycosyltransferases required for the biosynthesis of heparan sulfate. J. Biol. Chem. 273, 26265-26268.

Liu C., Luo Y., Liu X., Lu P. and Zhao Z. (2012). Clinical implications of CD44+/CD24- tumor cell ratio in breast cancer. Cancer Biother. Radiopharm. 27, 324-328.

Liu J., Sato C., Cerletti M. and Wagers A. (2010). Notch signaling in the regulation of stem cell self-renewal and differentiation. Curr. Top. Dev. Biol. 92, 367-409.

Liu Y., Ren S., Xie L., Cui C., Xing Y., Liu C., Cao B., Yang F., Li Y., Chen X., Wei Y., Lu H. and Jiang J. (2015). Mutation of N-linked glycosylation at ASN548 in CD133 decreases its ability to promote hepatoma cell growth. Oncotarget 6, 20650-20660.

Lombardo Y., Scopelliti A., Cammareri P., Todaro M., Iovino F., Ricci-Vitiani L., Gulotta G., Dieli F., de Maria R. and Stassi G. (2011). Bone morphogenetic protein 4 induces differentiation of colorectal cancer stem cells and increases their response to chemotherapy in mice. Gastroenterology 140, 297-309.

Lu J., Isaji T., Im S., Fukuda T., Hashii N., Takakura D., Kawasaki N. and Gu J. (2014). Beta-galactoside alpha2,6-sialyltranferase 1 promotes transforming growth factor-beta-mediated epithelial-mesenchymal transition. J. Biol. Chem. 289, 34627-34641.

Lubeseder-Martellato C., Hidalgo-Sastre A., Hartmann C., Alexandrow K., Kamyabi-Moghaddam Z., Sipos B., Wirth M., Neff F., Reichert M., Heid I., Schneider G., Braren R., Schmid R.M. and Siveke J.T. (2016). Membranous CD24 drives the epithelial phenotype of pancreatic cancer. Oncotarget 7, 49156-49168.

Luo L., Zeng J., Liang B., Zhao Z., Sun L., Cao D., Yang J. and Shen K. (2011). Ovarian cancer cells with the CD117 phenotype are highly tumorigenic and are related to chemotherapy outcome. Exp. Mol. Pathol. 91, 596-602.

Marotta L.L., Almendro V., Marusyk A., Shipitsin M., Schemme J., Walker S.R., Bloushtain-Qimron N., Kim J.J., Choudhury S.A., Maruyama R., Wu Z., Gonen M., Mulvey L.A., Bessarabova M.O., Huh S.J., Silver S.J., Kim S.Y., Park S.Y., Lee H.E., Anderson K.S., Richardson A.L., Nikolskaya T., Nikolsky Y., Liu X.S., Root D.E., Hahn W.C., Frank D.A. and Polyak K. (2011). The JAK2/STAT3 signaling pathway is required for growth of CD44(+)CD24(-) stem cell-like breast cancer cells in human tumors. J. Clin. Invest. 121, 2723-2735.

Matsuda T., Nakamura T., Nakao K., Arai T., Katsuki M., Heike T. and Yokota T. (1999). STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. EMBO J. 18, 4261-4269.

Matsuda Y., Yoshimura H., Ueda J., Naito Z., Korc M. and Ishiwata T. (2014). Nestin delineates pancreatic cancer stem cells in metastatic foci of NOD/SHI-scid IL2Rgamma(null) (NOG) mice. Am. J. Pathol. 184, 674-685.

McAuliffe S.M., Morgan S.L., Wyant G.A., Tran L.T., Muto K.W., Chen Y.S., Chin K.T., Partridge J.C., Poole B.B., Cheng K.H., Daggett J., Jr., Cullen K., Kantoff E., Hasselbatt K., Berkowitz J., Muto M.G., Berkowitz R.S., Aster J.C., Matulonis U.A. and Dinulescu D.M. (2012). Targeting notch, a key pathway for ovarian cancer stem cells, sensitizes tumors to platinum therapy. Proc. Natl. Acad. Sci. USA 109, E2939-2948.

McLean K., Gong Y., Choi Y., Deng N., Yang K., Bai S., Cabrera L., Keller E., McCauley L., Cho K.R. and Buckanovich R.J. (2011). Human ovarian carcinoma-associated mesenchymal stem cells regulate cancer stem cells and tumorigenesis via altered bmp production. J. Clin. Invest. 121, 3206-3219.

Merrill B.J. (2012). Wnt pathway regulation of embryonic stem cell self-renewal. Cold Spring Harb. Perspect. Biol. 4, a007971.

Miyazono K., ten Dijke P. and Heldin C.H. (2000). TGF-beta signaling by Smad proteins. Adv. Immunol. 75, 115-157.

Mohammadi M., Olsen S.K. and Ibrahimi O.A. (2005). Structural basis for fibroblast growth factor receptor activation. Cytokine Growth Factor Rev. 16, 107-137.

Monzani E., Facchetti F., Galmozzi E., Corsini E., Benetti A., Cavazzin C., Gritti A., Piccinini A., Porro D., Santinami M., Invernici G., Parati E., Alessandri G. and La Porta C.A. (2007). Melanoma contains cd133 and abcg2 positive cells with enhanced tumourigenic potential. Eur. J. Cancer 43, 935-946.

Muramatsu T. and Muramatsu H. (2004). Carbohydrate antigens expressed on stem cells and early embryonic cells. Glycoconj. J. 21, 41-45.

Nishida H., Yamazaki H., Yamada T., Iwata S., Dang N.H., Inukai T., Sugita K., Ikeda Y. and Morimoto C. (2009). CD9 correlates with cancer stem cell potentials in human b-acute lymphoblastic leukemia cells. Biochem. Biophys. Res. Commun.382, 57-62.

O'Brien C.A., Pollett A., Gallinger S. and Dick J.E. (2007). A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 445, 106-110.

Orian-Rousseau V. (2010). CD44, a therapeutic target for metastasising tumours. Eur. J. Cancer 46, 1271-1277.

Pang R., Law W.L., Chu A.C., Poon J.T., Lam C.S., Chow A.K., Ng L., Cheung L.W., Lan X.R., Lan H.Y., Tan V.P., Yau T.C., Poon R.T. and Wong B.C. (2010). A subpopulation of CD26+ cancer stem cells with metastatic capacity in human colorectal cancer. Cell Stem Cell 6, 603-615.

Pang M.F., Georgoudaki A.M., Lambut L., Johansson J., Tabor V., Hagikura K., Jin Y., Jansson M., Alexander J.S., Nelson C.M., Jakobsson L., Betsholtz C., Sund M., Karlsson M.C. and Fuxe J. (2016). TGF-beta1-induced emt promotes targeted migration of breast cancer cells through the lymphatic system by the activation of ccr7/ccl21-mediated chemotaxis. Oncogene 35, 748-760.

Penuelas S., Anido J., Prieto-Sanchez R.M., Folch G., Barba I., Cuartas I., Garcia-Dorado D., Poca M.A., Sahuquillo J., Baselga J. and Seoane J. (2009). TGF-beta increases glioma-initiating cell self-renewal through the induction of lif in human glioblastoma. Cancer Cell 15, 315-327.

Perdigoto C.N., Schweisguth F. and Bardin A.J. (2011). Distinct levels of notch activity for commitment and terminal differentiation of stem cells in the adult fly intestine. Development 138, 4585-4595.

Pieters T. and van Roy F. (2014). Role of cell-cell adhesion complexes in embryonic stem cell biology. J. Cell Sci. 127, 2603-2613.

Reynolds B.A. and Weiss S. (1996). Clonal and population analyses

demonstrate that an EGF-responsive mammalian embryonic cns precursor is a stem cell. Dev. Biol. 175, 1-13.

Ricci-Vitiani L., Lombardi D.G., Pilozzi E., Biffoni M., Todaro M., Peschle C. and De Maria R. (2007). Identification and expansion of human colon-cancer-initiating cells. Nature 445, 111-115.

Sarkar T.R., Battula V.L., Werden S.J., Vijay G.V., Ramirez-Pena E.Q., Taube J.H., Chang J.T., Miura N., Porter W., Sphyris N., Andreeff M. and Mani S.A. (2015). GD3 synthase regulates epithelial-mesenchymal transition and metastasis in breast cancer. Oncogene 34, 2958-2967.

Sasaki N., Okishio K., Ui-Tei K., Saigo K., Kinoshita-Toyoda A., Toyoda H., Nishimura T., Suda Y., Hayasaka M., Hanaoka K., Hitoshi S., Ikenaka K. and Nishihara S. (2008). Heparan sulfate regulates self-renewal and pluripotency of embryonic stem cells. J. Biol. Chem. 283, 3594-3606.

Sasaki N., Hirano T., Ichimiya T., Wakao M., Hirano K., Kinoshita-Toyoda A., Toyoda H., Suda Y. and Nishihara S. (2009). The 3'-phosphoadenosine 5'-phosphosulfate transporters, PAPST1 and 2, contribute to the maintenance and differentiation of mouse embryonic stem cells. PLoS One 4, e8262.

Sasaki N., Shinomi M., Hirano K., Ui-Tei K. and Nishihara S. (2011). LacdiNAc (GalNAcbeta1-4GlcNAc) contributes to self-renewal of mouse embryonic stem cells by regulating leukemia inhibitory factor/STAT3 signaling. Stem Cells 29, 641-650.

Schatton T., Murphy G.F., Frank N.Y., Yamaura K., Waaga-Gasser A.M., Gasser M., Zhan Q., Jordan S., Duncan L.M., Weishaupt C., Fuhlbrigge R.C., Kupper T.S., Sayegh M.H. and Frank M.H. (2008). Identification of cells initiating human melanomas. Nature 451, 345-349.

Semerci F., Choi W.T.-S., Bajic A., Thakkar A., Encinas J.M., Depreux F., Segil N., Groves A.K. and Maletic-Savatic M. (2017). Lunatic fringe-mediated notch signaling regulates adult hippocampal neural stem cell maintenance. Elife 6, e24660.

Shi C., Tian R., Wang M., Wang X., Jiang J., Zhang Z., Li X., He Z., Gong W. and Qin R. (2010). CD44+ CD133+ population exhibits cancer stem cell-like characteristics in human gallbladder carcinoma. Cancer Biol. Ther. 10, 1182-1190.

Shu Q., Wong K.K., Su J.M., Adesina A.M., Yu L.T., Tsang Y.T., Antalffy B.C., Baxter P., Perlaky L., Yang J., Dauser R.C., Chintagumpala M., Blaney S.M., Lau C.C. and Li X.N. (2008). Direct orthotopic transplantation of fresh surgical specimen preserves CD133+ tumor cells in clinically relevant mouse models of medulloblastoma and glioma. Stem Cells 26, 1414-1424.

Singh A. and Settleman J. (2010). EMT, cancer stem cells and drug resistance: An emerging axis of evil in the war on cancer. Oncogene 29, 4741-4751.

Sirko S., von Holst A., Weber A., Wizenmann A., Theocharidis U., Gotz M. and Faissner A. (2010). Chondroitin sulfates are required for fibroblast growth factor-2-dependent proliferation and maintenance in neural stem cells and for epidermal growth factor-dependent migration of their progeny. Stem Cells 28, 775-787.

Son M.J., Woolard K., Nam D.H., Lee J. and Fine H.A. (2009). SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. Cell stem cell 4, 440-452.

Takaishi S., Okumura T., Tu S., Wang S.S., Shibata W., Vigneshwaran R., Gordon S.A., Shimada Y. and Wang T.C. (2009). Identification of gastric cancer stem cells using the cell surface marker CD44. Stem Cells 27, 1006-1020.

Tang C., Lee A.S., Volkmer J.P., Sahoo D., Nag D., Mosley A.R., Inlay M.A., Ardehali R., Chavez S.L., Pera R.R., Behr B., Wu J.C., Weissman I.L. and Drukker M. (2011). An antibody against ssea-5 glycan on human pluripotent stem cells enables removal of teratoma-forming cells. Nat. Biotechnol. 29, 829-834.

Terao N., Takamatsu S., Minehira T., Sobajima T., Nakayama K., Kamada Y. and Miyoshi E. (2015). Fucosylation is a common glycosylation type in pancreatic cancer stem cell-like phenotypes. World J. Gastroenterol. 21, 3876-3887.

Terris B., Cavard C. and Perret C. (2010). EpCAM, a new marker for cancer stem cells in hepatocellular carcinoma. J. Hepatol. 52, 280-281.

Thiery J.P. (2002). Epithelial-mesenchymal transitions in tumour progression. Nat. Rev. Cancer 2, 442-454.

Thiery J.P., Acloque H., Huang R.Y. and Nieto M.A. (2009). Epithelial-mesenchymal transitions in development and disease. Cell 139, 871-890.

Todaro M., Gaggianesi M., Catalano V., Benfante A., Iovino F., Biffoni M., Apuzzo T., Sperduti I., Volpe S., Cocorullo G., Gulotta G., Dieli F., De Maria R. and Stassi G. (2014). CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. Cell Stem Cell 14, 342-356.

van Rhenen A., Feller N., Kelder A., Westra A.H., Rombouts E., Zweegman S., van der Pol M.A., Waisfisz Q., Ossenkoppele G.J. and Schuurhuis G.J. (2005). High stem cell frequency in acute myeloid leukemia at diagnosis predicts high minimal residual disease and poor survival. Clin. Cancer Res. 11, 6520-6527.

Varki A. (2017). Biological roles of glycans. Glycobiology 27, 3-49.

Varki A. and Lowe J.B. (2009). Biological roles of glycans. In: Essentials of glycobiology. Varki A., Cummings R.D., Esko J.D., Freeze H.H., Stanley P., Bertozzi C.R., Hart G.W. and Etzler M.E. (eds). Cold Spring Harbor Laboratory Press, The Consortium of Glycobiology Editors, La Jolla, California. Cold Spring Harbor (NY).

Varki A., Cummings R.D., Esko J.D., Stanley P., Hart G.W., Aebi M., Darvill A.G., Kinoshita T., Packer N.H., Prestegard J.H., Schnaar R.L. and Seeberger P.H. (2017). Essentials of Glycobiology. 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Visvader J.E. and Lindeman G.J. (2008). Cancer stem cells in solid tumours: Accumulating evidence and unresolved questions. Nat. Rev. Cancer 8, 755-768.

Wang J. and Yu R.K. (2013). Interaction of ganglioside GD3 with an EGF receptor sustains the self-renewal ability of mouse neural stem cells in vitro. Proc. Natl. Acad. Sci. USA 110, 19137-19142.

Wang L., Park P., Zhang H., La Marca F., Claeson A., Valdivia J. and Lin C.Y. (2011). BMP-2 inhibits the tumorigenicity of cancer stem cells in human osteosarcoma os99-1 cell line. Cancer Biol. Ther. 11, 457-463.

Wang L., Park P., La Marca F., Than K.D. and Lin C.Y. (2015). BMP-2 inhibits tumor-initiating ability in human renal cancer stem cells and induces bone formation. J. Cancer Res. Clin. Oncol. 141, 1013-1024.

Wilson R.J., Thomas C.D., Fox R., Roy D.B. and Kunin W.E. (2004). Spatial patterns in species distributions reveal biodiversity change. Nature 432, 393-396.

Xiao W., Gao Z., Duan Y., Yuan W. and Ke Y. (2017). Notch signaling plays a crucial role in cancer stem-like cells maintaining stemness and mediating chemotaxis in renal cell carcinoma. J. Exp. Clin. Cancer Res. 36, 41.

Xu C., Inokuma M.S., Denham J., Golds K., Kundu P., Gold J.D. and Carpenter M.K. (2001). Feeder-free growth of undifferentiated

human embryonic stem cells. Nat. Biotechnol. 19, 971-974.

Xu Q., Isaji T., Lu Y., Gu W., Kondo M., Fukuda T., Du Y. and Gu J. (2012). Roles of N-acetylglucosaminyltransferase III in epithelial-to-mesenchymal transition induced by transforming growth factor beta1 (TGF-beta1) in epithelial cell lines. J. Biol. Chem. 287, 16563-16574.

Yagi H. and Kato K. (2017). Functional roles of glycoconjugates in the maintenance of stemness and differentiation process of neural stem cells. Glycoconj. J. 34, 757-763.

Yale A.R., Nourse J.L., Lee K.R., Ahmed S.N., Arulmoli J., Jiang A.Y.L., McDonnell L.P., Botten G.A., Lee A.P., Monuki E.S., Demetriou M. and Flanagan L.A. (2018). Cell surface N-glycans influence electrophysiological properties and fate potential of neural stem cells. Stem Cell Reports 11, 869-882.

Yamazaki H., Nishida H., Iwata S., Dang N.H. and Morimoto C. (2009). CD90 and CD110 correlate with cancer stem cell potentials in human t-acute lymphoblastic leukemia cells. Biochem. Biophys. Res. Commun. 383, 172-177.

Yan B., Liu L., Zhao Y., Xiu L.J., Sun D.Z., Liu X., Lu Y., Shi J., Zhang Y.C., Li Y.J., Wang X.W., Zhou Y.Q., Feng S.H., Lv C., Wei P.K. and Qin Z.F. (2014). Xiaotan sanjie decoction attenuates tumor angiogenesis by manipulating notch-1-regulated proliferation of gastric cancer stem-like cells. World J. Gastroenterol. 20, 13105-13118.

Yang Z.F., Ngai P., Ho D.W., Yu W.C., Ng M.N., Lau C.K., Li M.L., Tam K.H., Lam C.T., Poon R.T. and Fan S.T. (2008). Identification of local and circulating cancer stem cells in human liver cancer. Hepatology 47, 919-928.

Yang L., Tang H., Kong Y., Xie X., Chen J., Song C., Liu X., Ye F., Li N., Wang N. and Xie X. (2015). LGR5 promotes breast cancer progression and maintains stem-like cells through activation of wnt/beta-catenin signaling. Stem Cells 33, 2913-2924.

Ying Q.L., Nichols J., Chambers I. and Smith A. (2003). BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell 115, 281-292.

Zhang W.C., Shyh-Chang N., Yang H., Rai A., Umashankar S., Ma S., Soh B.S., Sun L.L., Tai B.C., Nga M.E., Bhakoo K.K., Jayapal S.R., Nichane M., Yu Q., Ahmed D.A., Tan C., Sing W.P., Tam J., Thirugananam A., Noghabi M.S., Pang Y.H., Ang H.S., Mitchell W., Robson P., Kaldis P., Soo R.A., Swarup S., Lim E.H. and Lim B. (2012). Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. Cell 148, 259-272.

Zhou B.B., Zhang H., Damelin M., Geles K.G., Grindley J.C. and Dirks P.B. (2009). Tumour-initiating cells: Challenges and opportunities for anticancer drug discovery. Nat. Rev. Drug Dis. 8, 806-823.

Zhou W., Wang G. and Guo S. (2013). Regulation of angiogenesis via notch signaling in breast cancer and cancer stem cells. Biochim. Biophys. Acta 1836, 304-320.

Zhu Z., Hao X., Yan M., Yao M., Ge C., Gu J. and Li J. (2010). Cancer stem/progenitor cells are highly enriched in CD133+CD44+ population in hepatocellular carcinoma. Int. J. Cancer 126, 2067-2078.

Accepted April 26, 2019