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Glycosylated Notch and Cancer

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Glycosylation is one of the key components influencing several signaling pathways implicated in cell survival and growth. The Notch signaling pathway plays a pivotal role in numerous cell fate specifications during metazoan development. Both Notch and its ligands are repeatedly glycosylated by the addition of sugar moieties, such as O-fucose, O-glucose, or O-xylose, to bring about structural and functional changes. Disruption to glycosylation processes of Notch proteins result in developmental disorders and disease, including cancer. This review summarizes the importance and recent updates on the role of glycosylated Notch proteins in tumorigenesis and tumor metastasis.

Keywords: glycosyltransferases, Notch signaling, tumorigenesis, epidermal growth factor

INTRODUCTION

It has been 60 years since the discovery of a link between the changes in protein glycosylation and oncogenic transformation (1). Numerous studies in cancer biology have supported this finding and underscored the significance of glycosylation in tumorigenesis and metastasis. Numerous proteins, such as mucins, selectins, gonadotrophins, with altered glycosylation have been implicated in tumorigenesis (2, 3). Extensive research has been carried out to understand the glycobiology of the cancerous cell (4, 5). Cancer is a complex disease requiring several accumulated mutations, whose progression additionally depends on tumor cell interactions with the surrounding environment (6). Cell-surface proteins and changes associated with them define the course of cell-to-cell interactions. Altered glycosylated cell-surface proteins are one of the unique features of a cancerous cell (7), and specific glycan changes are critical during tumorigenesis and metastasis (8, 9). Several altered glycans serve as biomarkers to identify malignant cells undergoing epithelial–mesenchymal transition (EMT) and metastasis (9–11). Many essential glycosylated proteins, such as Notch, are altered in malignant cells, and the recent finding of GALNT11 as a new molecular marker in Notch-mediated chronic lymphocyte leukemia (CLL) (12) has increased interest in understanding Notch glycosylation.

The Notch signaling pathway facilitates short-range cell–cell communication to play a central role in proliferation and differentiation during animal development (13, 14). Notch signaling regulates a plethora of genes implicated in various cellular processes, and its signaling activity is extremely sensitive to the Notch receptor levels. Therefore, any slight modulation in Notch activity can perturb the regulation of gene expression and thus promoting several disorders, including cancer. Intriguingly, aberrant Notch signaling activity is highly implicated in several forms of leukemia and solid tumor development (11). T-cell acute lymphoblastic (T-ALL) neoplasm is one of the earliest diseases to be associated with the Notch signaling pathway (15). Membrane-bound Notch proteins (Notch receptor and its ligands) undergo rigorous glycosylation to accomplish its activity. Interestingly, deregulation of the components involved in glycosylating Notch proteins are implicated in Notch-induced tumorigenesis (16–19).
There is extremely robust evidence to suggest that aberrant Notch activity (11, 20, 21) or changes in glycosylation (5, 22, 23) can promote EMT and tumor development, despite the unclear role of glycosylated Notch proteins in relation to tumorigenesis. This review discusses recent advances in our understanding of glycosylation of Notch proteins and the impact of altered Notch glycans in promoting tumorigenesis and metastasis.

**AN OVERVIEW OF NOTCH SIGNALING PATHWAY**

A century ago, flies with Notch-ed wing phenotype led to identification (24) and characterization of evolutionarily conserved Notch signaling pathway (25, 26). The signaling pathway comprising Notch receptor and Delta/Serrate/LAG-2 (DSL) family of ligands play crucial role in determining cell-fate choices in all animals (13, 27). While *Drosophila* has one Notch receptor (28), mammalians have four homologs (Notch1–4) (29) with an extracellular domain (ECD) and an intracellular domain (ICD). Both Notch receptor and DSL ligands show a high degree of structural similarities in the ECD (30).

Notch signaling involves receptor activation, Notch ICD (NICD) generation, and target stimulation (Figure 1A). Nascent Notch protein is initially glycosylated in the ER and Golgi apparatus (17, 31–33). In mammals, it is proteolytically cleaved by Furin at site 1 (S1) (this cleavage does not occur in *Drosophila*) (34). Following this, the mature Notch receptor heterodimer, comprising the ECD and transmembrane-ICD, gets tethered to the cell surface of the signal-receiving cell. Notch receptor interaction with membrane-bound ligands such as Delta/Serrate/LAG-2 (DSL) family proteins in the signal-sending cell initiates two successive proteolytic cleavages at site 2 (S2) and site 3 (S3) mediated by a disintegrin and metalloprotease (ADAM) and presenilin/γ-secretase complex, respectively. The NICD is then released, which translocates into the nucleus and binds to CSL (CBF1/SuH/LAG-1) transcriptional regulators to activate target genes (14, 35). The events that lead to the release of Notch ICD (and Notch activation) rely on Notch ECD shedding. Evidences indicate presence of a non-canonical mode of signaling without ligand–receptor interaction to release NICD [Figure 1A (36, 37)]. Therefore, factors that influence shedding of the Notch ECD (either positively or negatively) can directly modulate Notch activity. Numerous extra- and intracellular modulators involving glycosylation, and trafficking machineries maintain the cellular pool of Notch in a context-specific manner.

Notch protein exerts its biological functions by both canonical (ligand-dependent) and non-canonical (ligand-independent) signaling modes (Figure 1A). Contradicting earlier reports that ligand-dependent Notch activity alone is indispensable for the positive regulation of Notch signaling, recent work suggests that ligand-independent Notch signaling also plays a crucial positive role in regulating Notch activity (36, 38). Namely, changes in trafficking can lead to ligand-independent signaling (Figure 1A). Defects in any one of the two signaling modes can lead to tumorigenesis and tumor progression. Strikingly, glycosylation is one such process that modulates ligand–receptor binding and trafficking activities [Figure 1B (39, 40)]. In the following section of this review, we discuss the impact of glycosylation on Notch and its ligands to accomplish its biological function.

**GLYCOXYLATION OF NOTCH PROTEINS**

Glycosylation is an enzymatic reaction that mediates a chemical linkage of mono- or polysaccharides (glycans) onto other saccharides, proteins, or lipids occurring in Golgi apparatus and endoplasmic reticulum (ER). Nascent Notch proteins enroute the secretory pathway (41) to undergo a rigorous glycosylation on their ECD with 29–36 epidermal growth factors (EGF)-like repeats to emerge as a mature receptor and get localized on the cell surface (42). Predominantly, Notch receptor undergoes O-glycosylation at serine/threonine residues (31), and to a lesser extent, N-glycosylation on AsnXSer/Thr residues of EGF repeats (17).

The EGF repeats are modified by O-fucose, O-glucose, O-GlcNAc, and O-xylose (Figure 2). This short EGF repeat has six conserved Cys residues that form three disulphide bridges, wherein O-fucosylation at C5–X–X–X–S/T–G–X–X–C6 by EGF-specific O-GlcNAc-transferase 1 (O-PucT-1) (encoded by Oft1 gene in *Drosophila* and Pofut1 in mammals) (39, 43) and elongated by Fringe, an N-acetylgalcosaminyl transferase. Fucosylation is one of the prevalent glycosylation types on Notch proteins. Fringe is essential to promote Notch/Delta-binding, in preference to Notch/Serrate, whose interaction is inhibited by this modification (44, 45). Similarly, O-glycosylation (C6–X–S–X–P–A–C3) is mediated by O-glucosyltransferase, Rumi in *Drosophila* or POGLUT1 in mammals (40, 46–48), and elongated by Shams, a xylosyltransferase. In humans, xylosyltransferase (GXYLT)1 and (GXYLT)2, that add first and second xylose residues to Notch EGF repeats, have been identified (49, 50). Although Rumi is not required for the ligand-binding activity of Notch, it has been suggested that it functions to promote extracellular cleavage. In contrast to the glucose residues, xylosylation negatively regulates Notch signaling. A unique non-nucleocytoplasmic O-GlcNAc is reported to occur on the consensus sequence of Notch C5–X–X–G–X–S/T–G–X–X–C6 by EGF-specific O-GlcNAc-transferase (EOGT) in *Drosophila* and Eogt1 in mammals that mediate extracellular matrix interactions (51–54). The specific role of O-GlcNAc modifications on Notch activity is still not clear (Figure 2C).

Recently, the presence of mucin-type-O-GalNAc glycans on the Notch ECD and N-glycans have been reported (19). The last four amino acids in the spacer between EGF repeats cooperates with calcium-ion binding and plays an important role in enhancing the rigidity and stability of EGF repeats (55). Recent research suggests that a strong crosstalk exists between glycosylating machinery and calcium modulating chaperones in regulating Notch activity.

**NOTCH-INDUCED TUMORIGENESIS**

Recent glycomic studies suggest that common glycosylation changes associated with tumor development are fucosylation, sialylation, O-glycan truncation, and O- and N-linked branching (8), and most of these changes are frequently associated with...
Notch proteins. Notch pathway cooperates with other mutations or deregulation in other oncogenic or tumor-suppressive genes of other signaling pathways, polarity regulators, and endocytic compartments to potentiate tumor progression (56–58).

Aberrant Notch activity has distinct roles in the development of several solid and hematopoietic tumors, and it has been shown to have either oncogenic or tumor-suppressive roles in a context-specific manner (21). In hematopoietic cancers, for example, T cell active lymphoblastic leukemia (T-ALL), Notch has an oncogenic role (59, 60), while in acute myeloid leukemia (AML), it has a tumor-suppressive role (61, 62). However, in solid tumors, such as hepatocellular carcinoma (HCC) (63) and medulloblastoma...
FIGURE 2 | Glycosylation sites present on EGF repeats of the Notch extracellular domain. Consensus sites (A) and glycosyltransferases (B) of O-fucosylation, O-glycosylation, and O-GlcNAcylation that occur on an EGF repeat of NECD. (C) Table displaying a partial list of the glycosyltransferases that modify the Notch receptor.
Notch may have either an oncogenic or tumor-suppressive role depending on context (65, 66). It has been suggested that the switch between canonical and non-canonical Notch signaling can have a tumor-suppressive role, as demonstrated with Notch1 in the mouse (67). Not only does the glycosylation process of the Notch receptor aid in ligand-dependent signaling activity, but also reports suggest that changes in glycosylation events may lead to ligand-independent Notch activation (37). Most importantly changes in glycosylation could switch from one mode of signaling to the other, leading to deregulated Notch activity.

Ligand-Dependent Notch-Mediated Tumorigenesis

Notch protein undergoes extensive post-translational modification (68). To a larger extent, the ligand-dependent Notch signaling pathway requires glycosylation of the Notch ECD for signaling activation. It has been demonstrated that altered carbohydrate structure can play a very significant role in modulating ligand-binding activity (31, 69). Glycan modifications on EGF repeats of the Notch receptor indicate that the EGF8 repeat is required for Serrate-specific binding (70), while EGF12 is specifically required for Fringe function to inhibit Serrate and promote Delta-binding (16, 71), which mediates positive regulation on Notch signaling (Figure 2). Biochemical studies suggest that O-fucose addition on EGF14 leads to either dysregulated receptor–ligand activation or truncation effects. Fusocyltransferase, which modulates the receptor–ligand interaction, is an important epitope on the EGF repeat (72–74) and impairment to this process is implicated with various forms of malignancies. Reports indicate that aberrant ligand-dependent Notch activity is highly associated with development of HCC, T-cell leukemia, and breast cancer (23). Interestingly, in humans, upregulation and aberrant gene expression of OFut1 (55, 81) and Rumi (46, 82) are proposed to bind to calcium ions to enhance rigidity and also aid in modulating the thermodynamics of the protein. It has been shown that calcium binds to the EGF12 repeat (55, 83). Calcium binding on EGF repeats is a highly conserved phenomenon assigning a crucial role to the structure of the protein (84). Calcium binding occurs on certain amino acids on a short linker sequence, N–N–x–N–C1 (where N can be D/E/Q/N, x-any amino acid, and C1 is the first conserved Cys of the EGF) between two EGF repeats (55, 83). From our knowledge of how calcium binding can affect EGF repeats, it has been proposed that depending on the rigidity and flexibility provided, Fringe might facilitate interactions with elongated glycans or inhibit interactions with neighboring regions. Studies indicate that calcium depletion dissociates and activates heterodimeric Notch receptors (85). Reports suggest that crosstalk exists between calcium levels and Notch activity during tumorigenesis. In line with this, it has been demonstrated that calcium/calmodulin-dependent kinase II (CaMKII) regulates Notch1 activity in prostate carcinoma development (86).

Factors that influence Notch heterodimerization can have a significant impact on receptor–ligand interactions. Interestingly, OFut1 (55, 81) and Rumi (46, 82) are proposed to bind to calcium ions to enhance rigidity and also aid in modulating the thermodynamics of the protein. It has been shown that calcium binds to the EGF12 repeat (55, 83). Calcium binding on EGF repeats is a highly conserved phenomenon assigning a crucial role to the structure of the protein (84). Calcium binding occurs on certain amino acids on a short linker sequence, N–N–x–N–C1 (where N can be D/E/Q/N, x-any amino acid, and C1 is the first conserved Cys of the EGF) between two EGF repeats (55, 83). From our knowledge of how calcium binding can affect EGF repeats, it has been proposed that depending on the rigidity and flexibility provided, Fringe might facilitate interactions with elongated glycans or inhibit interactions with neighboring regions. Studies indicate that calcium depletion dissociates and activates heterodimeric Notch receptors (85). Reports suggest that crosstalk exists between calcium levels and Notch activity during tumorigenesis. In line with this, it has been demonstrated that calcium/calmodulin-dependent kinase II (CaMKII) regulates Notch1 activity in prostate carcinoma development (86).

Ligand-Independent Notch-Mediated Tumorigenesis

In recent years, several reports indicate that ligand-independent Notch signaling is implicated in tumorigenesis. Several endocytic components have been associated with Notch in promoting tumor progression. In spite of this, regulatory mechanisms that initiate ligand-independent Notch signaling activity remain elusive. It is highly logical to think that such events are triggered during early stages of nascent Notch protein production in the Golgi compartment. Glycosylation is not only indispensable for protein folding and protein activity, but it has an unprecedented role in intracellular transport/localization and degradation/half-life of the protein.

OFut1/Pofut1 has both enzymatic fucosyl activity and fusocyl-independent chaperone activity on Notch proteins (39, 87). In addition to its usual role as O-fucosyltransferase, OFut1 has been implicated in maintaining the Notch pool by recycling cell-surface Notch through endosomes and on to lysosomes in a fusocyl-dependent manner (39). Similarly, another study has provided evidence of the involvement of OFut1 and fusocylation in localizing Notch to the sub-apical complex/adherens junction of epithelial cells by dynamin dependent transcytosis (88). These interesting data prompt further investigation into the possible mechanisms of the process. Fringe activity follows the OFut1 reaction on specific EGF repeats of Notch. There is evidence indicating possible glycosylation events on other sites of EGF repeats too. Therefore, Fringe activity on different EGF repeats of Notch proteins, or yet to be identified glycosylation activity, might promote cleavage of Notch that inhibits the localization of processed Notch protein to the plasma membrane, retaining it in the intracellular compartment (Figure 1B). Deregulated function of Fringe or glycosyltransferase like OFut1 might possibly lead to aberrant Notch activity. Rumi activity has been demonstrated to be required for ligand-independent Notch activation caused by deletion of LNR repeats (47, 48). Mutations in the
heterodimerization domain on the EGF repeats may impair S2 cleavage of Notch leading to either ligand-independent activation or ligand-mediated hypersensitivity. A recent report has shown a cooperation of Ofut1 chaperone activity and Rumi in Notch transport (40). In the absence of ligands, preliminary results demonstrating glycosylation-mediated Notch trafficking defects are yet to be linked to tumorigenesis.

**PERSPECTIVE**

Studies to date, in most contexts, demonstrate that the addition of O-glucose positively regulates Notch signaling, while updates from Shams/GXYLT suggest that the addition of O-xylose residues downregulates Notch activity in a context-specific manner. It is proposed that this regulation, by changing the distribution of forms and length of sugar residues, offers a novel paradigm to modulate Notch signaling (48). Report suggests that the addition of Xylose to isolated Ser or Thr residues initiates Glycosaminoglycans (GAG) synthesis (89). As several studies from them, the addition of O-xylose residues to protein N-linked glycans (89) suggests that the addition of O-glucose positively regulates Notch signaling, while updates to date, in most contexts, demonstrate that the addition of O-xylose residues to protein N-linked glycans (89). As several studies from them, the addition of O-glucose positively regulates Notch signaling, while updates to date, in most contexts, demonstrate that the addition of O-xylose residues to protein N-linked glycans (89).

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**REFERENCES**

1. Ladenson RP, Schwartz SO, Ivy AC. Incidence of the blood groups and the secretor factor in patients with pernicious anemia and stomach carcinoma. *Am J Med Sci* (1949) 217:194–4. doi:10.1097/00000441-194902000-00011
2. Kobata A, Amano J. Altered glycosylation of proteins produced by malignant cells, and application for the diagnosis and immunotherapy of tumors. *Immunol Cell Biol* (2005) 83:429–39. doi:10.1111/j.1440-1711.2005.01351.x
3. Brockhausen I. Mucin-type O-glycans in human colon and breast cancer: glycodynamics and functions. *EMBO Rep* (2006) 7:599–604. doi:10.1038/sj.embor.7400705
4. Kolb AC, Andergassen U, Jeschke U. The role of glycosylation in breast cancer metastasis and cancer control. *Front Oncol* (2015) 5:219. doi:10.3389/fonc.2015.00219
5. Stowell SR, Ju T, Cummings RD. Protein glycosylation in cancer. *Annu Rev Pathol* (2015) 10:473–510. doi:10.1146/annurev-pathol-022414-040438
6. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) 144:646–74. doi:10.1016/j.cell.2011.02.013
7. Häuselmann I, Borsig L. Altered tumor-cell glycosylation promotes metastasis. *Front Oncol* (2014) 4:28. doi:10.3389/fonc.2014.00028
8. Christiansen MN, Chik J, Lee L, Anugraham M, Abrams JL, Packer NH. Cell surface protein glycosylation in cancer. *Proteomics* (2014) 14:525–46. doi:10.1002/pmic.201300387
9. Freire-de-Lima L. Sweet and sour: the impact of differential glycosylation in cancer cells undergoing epithelial-mesenchymal transition. *Front Oncol* (2014) 4:59. doi:10.3389/fonc.2014.00059
10. Tuccillo FM, de Laurentiis A, Palmieri C, Fiume G, Bonelli P, Borrelli A, et al. Aberrant glycosylation as biomarker for cancer: focus on CD43. *Biomed Res Int* (2014) 2014:742831. doi:10.1155/2014/742831
11. Nitzachristiots P, Lim JS, Sage J, Aifantis I. From fly wings to targeted cancer therapies: a centennial for notch signaling. *Cancer Cell* (2014) 25:318–34. doi:10.1016/j.ccr.2014.02.018
12. Libisch MG, Casás M, Chiribao M, Moreno P, Cayota A, Osnaga E, et al. GALNT11 as a new molecular marker in chronic lymphocytic leukemia. *Gene* (2014) 533:270–9. doi:10.1016/j.gene.2013.09.052
13. Artavanis-Tsakonas S. Notch signaling: cell fate control and signal integration in development. *Science* (1999) 284:770–6. doi:10.1126/science.284.5415.770
14. Bray SJ. Notch signalling: a simple pathway becomes complex. *Nat Rev Mol Cell Biol* (2006) 7:678–89. doi:10.1038/nrm1900
15. Ellisen LW, Bird J, West DC, Soreng AL, Reynolds TC, Smith SD, et al. TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* (1991) 66:64–9. doi:10.1016/0092-8674(91)10011-B
16. Haines N, Irvine KD. Glycosylation regulates notch signalling. *Nat Rev Mol Cell Biol* (2003) 4:786–97. doi:10.1038/nrm1228
17. Stanley P. Regulation of notch signaling by glycosylation. *Curr Opin Struct Biol* (2007) 17:530–5. doi:10.1016/j.sbi.2007.09.007
18. Takeuchi H, Haltiwanger RS. Role of glycosylation of notch in development. *Semin Cell Dev Biol* (2010) 21:638–45. doi:10.1016/j.semcdb.2010.03.003
19. Takeuchi H, Haltiwanger RS. Significance of glycosylation in notch signalling. *Biochim Biophys Acta* (2014) 1843:235–42. doi:10.1016/j.bbamcr.2014.05.115
O-Fucose monosaccharide of notch has a temperature-sensitive fringe modulates notch-delta interactions. J Biol Chem (2000) 275:13084–9. doi:10.1074/jbc.275.17.13084

23. Rana NA, Haltiwanger RS. Fringe benefits: functional and structural impacts of O-glycosylation on the extracellular domain of Notch receptors. Struct Biol (2007) 134:354–65. doi:10.1016/j.sbb.2006.02.002

24. Hori K, Sen A, Artavanis-Tsakonas S. Notch signaling at a glance. Curr Opin Struct Biol (2004) 14:91–5. doi:10.1016/j.sbb.2004.09.012

25. Djiane A, Krejci A, Bernard F, Fexova S, Katherine Millen K, Bray SJ. Dissecting the mechanisms of notch induced hyperplasia. EMBO J (2013) 32:60–71. doi:10.1038/emboj.2012.326

26. Patel PH, Edgar BA. Tissue design: how Drosophila tumors remodel their neighborhood. Semin Cell Dev Biol (2014) 28:86–95. doi:10.1016/j.semcdb.2013.03.012

27. Kannan S, Sutphin RM, Hall MG, Golfman LS, Fang W, Nolo RM, et al. Activating mutations of NOTCH1 in human T cell lymphoma. Cancer Res (2004) 64:3019–27. doi:10.1158/0008-5472.CAN-03-2868

28. Fan X, Mikolaenko I, Elhassan I, Ni X, Wang Y, Ball D, et al. Notch1 and notch2 have opposite effects on embryonal brain tumor growth. Cancer Res (2004) 64:9787–95. doi:10.1158/0008-5472.CAN-04-1444

29. Fujimoto A, Totoki Y, Abe T, Borovych KA, Hossoda F, Nguyen HH, et al. Whole-genome sequencing of liver cancers identifies etiological influences
on mutation patterns and recurrent mutations in chromatin regulators. Nat Genet (2012) 44:760–4. doi:10.1038/ng.2291
66. Guichard C, Amao-Ado G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IJ, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet (2012) 44:694–8. doi:10.1038/ng.2256
67. Blanpain C, William E, Lowry WE, Pasolli HA, Fuchs E. Canonical notch signaling functions as a commitment switch in the epidermal lineage. Genes Dev (2006) 20:3022–35. doi:10.1101/gad.147760
68. Fortini ME. Notch signaling: the core pathway and its posttranslational regulation. Dev Cell (2009) 16:633–47. doi:10.1016/j.devcel.2009.03.010
69. Chen J, Moloney DJ, Stanley P. Fringe modulation of jagged1-induced notch signaling requires the action of beta 4galactosyltransferase-1. Proc Natl Acad Sci U S A (2001) 98:13716–21. doi:10.1073/pnas.241398098
70. Yamamoto S, Charng W, Rana NA, Kalkuda S, Jaiswal M, Bayat V, et al. A mutation in EGF repeat-8 of notch discriminates between serrate/jagged and delta family ligands. Science (2012) 338:1229–32. doi:10.1126/science.1228745
71. Lei L, Xu A, Panin VM, Irvine KD. An O-fucose site in the ligand binding domain inhibits notch activation. Development (2003) 130:6411–21. doi:10.1242/dev.00883
72. Dear AE, Medcalf RL. The urokinase-type-plasminogen-activator receptor (CD87) is a pleiotropic molecule. Biochem Biophys Res Commun (1998) 252:185–93. doi:10.1006/bbrc.1998.520185.x
73. Wang S, Sdrulla AD, diSibio G, Bush G, Nofziger D, Hicks C, et al. Notch receptor activation inhibits oligodendrocyte differentiation. Neuron (1998) 21:63–75. doi:10.1016/S0896-6771(00)80515-2
74. Wang Y, Shao L, Shi S, Harris RJ, Spellman MW, Stanley P, et al. Modification of epidermal growth factor-like repeats with O-fucose. Molecular cloning and expression of a novel GDP-fucose protein O-fucosyltransferase 1. J Biol Chem (2001) 276:40338–45. doi:10.1074/jbc.M107849200
75. Blomme B, Van Steenkiste C, Callewaert N, Van Vlierberghe H. Alteration and expression of a novel GDP-fucose protein O-fucosyltransferase. J Biol Chem (2005) 280:10556–62. doi:10.1074/jbc.M505569200
76. Sasaki N, Sasamura T, Ishikawa HO, Kanai M, Ueda R, Saigo K, et al. Polarized exocytosis and transcytosis of notch during its apical localization in Drosophila epithelial cells. Genes Cells (2007) 12:89–103. doi:10.1111/j.1365-2443.2007.01037.x
77. Esko JD, Kimata K, Lindahl U. Proteoglycans and sulfated glycosaminoglycans. 2nd ed. In: Varki A, Cummings RD, Esko JD, et al., editors. Essentials of Glycobiology (Chaps. 16), Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press (2009). p. 1–20.
78. Ahrifi N, Gialeli C, Nikitovic D, Tsegemidis T, Karoussou E, Theocharis AD, et al. Glycosaminoglycans: key players in cancer cell biology and treatment. FEBS J (2012) 279:1177–97. doi:10.1111/j.1742-4658.2012.08529.x
79. Belting M. Glycosaminoglycans in cancer treatment. Thromb Res (2014) 133:S95–101. doi:10.1016/S0049-3848(14)50016-3
80. Nagarajan U, Pakkiriswami S, Pillai AB. Sugar tags and tumorigenesis. Front Cell Dev Biol (2015) 3:69. doi:10.3389/fcell.2015.00069
81. Andersson ER, Lendahl U. Therapeutic modulation of notch signalling – are we there yet? Nat Rev Drug Discov (2014) 13:357–78. doi:10.1038/nrd4252

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