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Glycans and Glycosaminoglycans in neurobiology: key regulators of neuronal cell function and fate.

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Short running head: Glycans and Neural Function

Abstract

The aim of this study was to examine the roles of L-fucose and the glycosaminoglycans (GAGs) keratan sulphate (KS) and chondroitin sulphate/dermatan sulphate (CS/DS) with selected functional molecules in neural tissues. Cell surface glycans and GAGs have evolved over millions of years to become cellular mediators which regulate fundamental aspects of cellular survival. The glycocalyx, which surrounds all cells, actuates responses to growth factors, cytokines and morphogens at the cellular boundary silencing or activating downstream signalling pathways and gene expression. In this review we have focussed on interactions mediated by L-fucose, KS and CS/DS in the central and peripheral nervous systems. Fucose makes critical contributions in the area of molecular recognition and information transfer in the blood group substances, cytotoxic immunoglobulins, cell-fate mediated Notch-1 interactions, regulation of selectin mediated neutrophil extravasation in innate immunity and CD-34 mediated new blood vessel development and the targeting of neuroprogenitor cells to damaged neural tissue. Fucosylated glycoproteins regulate delivery of synaptic neurotransmitters and neural function. Neural KS-proteoglycans were examined in terms of cellular regulation and their interactive properties with neuroregulatory molecules. The paradoxical properties of CS/DS isomers decorating matrix and transmembrane proteoglycans and the positive and negative regulatory cues they provide to neurons is also discussed.

Abbreviations

AD	Alzheimer's disease
ADAM	a disintegrin and metalloproteinase domain
ADAM-TS	a disintegrin and metalloproteinase domain with thrombospondin motifs
ADCC	antibody dependent cellular cytotoxicity
AGE	advanced glycation end product
Akt	protein-kinase B
ALS	amyotrophic lateral sclerosis
APP	amyloid precursor protein
ATP	adenosine triphosphate
BDNF	brain derived neurotrophic factor
β3GlcNAcT	β1,3-N-acetylglucosaminyltransferase
CS	chondroitin sulphate
CSPG	chondroitin sulphate proteoglycan
CSL	an acronym for CBF-1/RBPJ (recombining binding protein suppressor of hairless)
CNS	central nervous system
DCC	a receptor named *Deleted in Colorectal Cancer*
DRG	dorsal root ganglion
DS	dermatan sulphate
DS	dermatan sulphate proteoglycan
ECM	extracellular matrix
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
FcγRIIIA	activating Fc receptor specific for IgG Fc region expressed by HNK cells and macrophages
FUT	fucosyl transferase
GAG	glycosaminoglycan
GlcNAc6ST	*n*-acetylglucosamine-6-O-sulfotransferase
GSK	glycogen synthase kinase
GTP	guanosine triphosphate
HMBG-1	high-mobility group box-1 protein
HNK	human natural killer
HS	heparan sulphate
HA	hyaluronan
IGD	interglobular domain
IGFBP2	insulin-like growth factor binding protein-2
IgG	immunoglobulin G
KS	keratan sulphate
KSPG	keratan sulphate proteoglycan
KSGal6ST	keratan sulfate galactose 6-O-sulfotransferase
LAD II	leukocyte adhesion deficiency II
LAR	leukocyte common antigen related
LC-MS	liquid chromatography-mass spectroscopy
LRR	leucine rich repeat
MAb	monoclonal antibody
MAPK	mitogen-activated protein kinase
NCAM	neural cell adhesion molecule
NG2	neural/glial antigen 2
NMR	nuclear magnetic resonance
2D MRS	two dimensional magnetic resonance spectroscopy
NG2	neural/glial antigen-2 (CSPG-4)

NGF	neural growth factor
PKA	cAMP dependent protein kinase
POFUT	GDP-fucose protein O-fucosyltransferase 1
POFUT	protein - fucosyl transferase
PG	proteoglycan
PNS	peripheral nervous system
PSGL-1	selectin-P ligand (CD162)
PTPσ	protein tyrosine phosphatase σ
RAGE	receptor for advanced glycation end products
RPTP-σ	receptor-like protein tyrosine phosphatase-σ
SCI	spinal cord injury
SHH	sonic hedge hog
sLeX	sialyl Lewis-X antigen
Trk B	tyrosine receptor kinase B
TGF-β	transforming growth factor-β
TNF-α	tumour necrosis factor-α
TSRs	thrombospondin repeats
SYN	synapsin
RPTP-ζ	receptor protein tyrosine phosphatase-zeta
Wnt	this is a condensation of terms describing the *Winged* and *Int* transcription factor morphogens

1. Introduction

1.1 Aim

This study reviews the roles of selected glycans and glycosaminoglycans (GAGs) which decorate neural glycoproteins and proteoglycans (PGs) and examines how they contribute to neuronal function and repair processes. Due to the complexity of the large number of neural effector molecules and their broad interplay with receptors, ion channels, synaptic and axonal structures in health and disease it has not been possible for this review to provide a comprehensive coverage of all of these aspects. Rather, key interactive molecules have been focussed on and novel aspects of the functional roles of glycans such as L-fucose and GAGs such as keratan sulphate (KS) and chondroitin/dermatan sulphate (CS/DS). The role of heparan sulphate (HS) in neuronal development and function and also pathogenesis (e.g in neurodegenerative conditions such as Alzheimer's disease (AD) is a significant area of glycobiology under intense scientific scrutiny and, as such, is outside the scope of the current review. For this, the reader is referred to a number of recent studies [1-6].

1.2 Analysis of glycan and glycosaminoglycan complexity

While the structural complexity of glycan structures is a daunting subject to investigate [7-10] powerful analytics have been developed to assist in these investigations. These new methodologies include ion-mobility mass spectrometry [11, 12], application of synchtrotron radiation for glycan structural analysis [13], application of high throughput automated N-glycopeptide glycoproteomic identification systems and orbitrap mass spectrometry [14-16], integrated systems glycobiology methodology incorporating glycogenomics, glycoproteomics and glycomics [17], fully automated chip-electrospray mass spectrometric analysis for the determination of CS/DS fine structure[18]. GAG microarrays for the analysis of GAG-protein interactions [19-21] have also been applied to profiling the sulphation patterns of GAGs to determine growth factor interactive sequences [22, 23] and have also identified CS-E tetrasaccharides motifs which act as TNFα antagonists [24]. Development of clickECM cell-derived azide functionalised extracellular matrices (ECMs) [25], photoactivatable and chemoenzymatic glycan labelling tools [26-28], non-invasive two dimensional nuclear magnetic resonance spectroscopy [29], glycoengineering of monoclonal antibodies (MAbs) with improved carbohydrate-protein interactive properties and immune cell targeting capability has improved their efficacy in anti-cancer therapeutics [30]. Multimodal glycosylated conductive polymer biointerfaces suitable for the evaluation of carbohydrate-protein interactions [31] and nanoscale biomatrices for studies on glycocalyx interactions [32] have been developed. Such approaches have been applied to the translation of the 'Sugar Code' into immune and vascular signaling programs with potential therapeutic application [33], such an approach may also provide a better comprehension of the complexities of altered glycodynamics in brain conditions such as Alzheimer's disease, Parkinson's disease, schizophrenia, epilepsy and neural conditions characterised by altered cognitive learning [34].

Analysis of the structural complexity of glycans has been considerably aided with the development of software packages which simplify unambiguous representation of glycans and their structural forms. These include GlycanBuilder [35], KCam[36], GlycResoft, a software package for automated recognition of glycans from liquid chromatography-mass spectrometry (LC-MS) data[37], KEGG Carbohydrate matcher (http://www.genome.jp/ligand/kcam/), SWEET-DB, annotated carbohydrate data collections[38], DrawRINGS, 2D Glycan structure Drawing Tool (http://rings.t.soka.ac.jp/java/DrawRings.html), LINUCS: linear notation for unique description of carbohydrate sequences[39], GLYDE (http://glycomics.ccrc.uga.edu/GLYDE-CT/) [40], EUROCarbDB tools to normalise and convert glycan structures: Glycan builder (http://www.eurocarbdb.org/applications/structure-tools) and analysis of MS spectra : GlycoWorkbench (http://www.eurocarbdb.org/applications/structure-ms-tools). PROCARB is a database of known and modelled carbohydrate binding protein structures with sequence based prediction tools[41]. Establishment of the Consortium for Functional Glycomics (CFG, http://functionalglycomics.org/static/consortium/consortium.html) in 2001 has aided glycan research through the extensive, highly informative reference material readily available on their web-site. Informatics tools are also available for the analysis of GAG structure[42] and conformation [43] and for the determination of interactive GAG sequences [44-49]. Glycomics databases such as EuroCarbDB (http://www.ebi.ac.uk/eurocarb/home.action) and The Functional Glycomics Gateway (http://www.functionalglycomics.org/), Databases of Conformations and NMR Structures of Glycan

Determinants [50] and software for the structural determination of GAGs by mass spectrometry [51], and for automated comparison of low molecular weight heparins from LC/MS data [52] have also been developed [51]. Nuclear magnetic resonance (NMR) spectroscopy has also been applied to the structural analysis of sulphated fucose-CS polymers [53]. Furthermore, novel high sensitivity, low toxicity alkynyl-fucose substrates have been developed for the visualisation of fucose incorporation into glycopolymers, these alkynyl-fucose substrates are incorporated into N-glycans by a wide range of fucosyl transferases[54] enabling their visualisation in cells using biotin-steptavidin Alexa-488 histochemistry and they may be extracted, separated by SDS PAGE and identified by Western blotting [53]. The complexity of glycans surpasses by several magnitudes that of the other major life biomolecules, proteins, lipids and nucleic acids [9, 10, 21, 55, 56] and their analysis has lagged behind due to this complexity however with the improvement in glycan analysis now possible with the methodology outlined above this gap is steadily closing.

Glycan biodiversity occurred over at least 500 million years of vertebrate and invertebrate evolution and an even longer evolutionary period in bacteria leading to their evolution as mediators of cellular interaction. Glycans occur in the glycocalyx of all cells and they are the first point of contact between that cell and other cells, with that cell and the extracellular matrix or with any invading organism. Thus there were heightened evolutionary pressures on these front-line glycans to develop recognition and effector roles, with this major positive selection stimulus glycans diversified into their present day level of complexity. The glyco-code could therefore be considered a biodiverse IT database which nature has developed over a very significant evolutionary period [57]. Thus many structural permutations were explored and those glycan structures that have persisted to the present day are ones which offer interactive capability with effector molecules in essential physiological processes providing improved survival traits. Deciphering this glyco-code using the sophisticated glycobiological methodology now available is an important research objective and may uncover invaluable insights as to how glycans regulate cells and be of application in repair biology.

2. The complexity of neural tissues

2.1 Cell types in the central and peripheral nervous system.

Neurons and glial cells have a common neuro-epithelial origin in the embryonic nervous system and thus share many structural and molecular characteristics [58, 59]. Neurons and glial cells display unique properties which distinguish these cell types from others. Approximately 10% of all cells in the tissues of the central and peripheral nervous systems (CNS/PNS) are neurons. Accessory cell types also include astrocytes, radial glia, oligodendrocytes, ependymal cells, microglia and microvascular endothelial cells while neural/glial antigen 2 (NG2) positive glia are also considered to be a distinct cell type. Microglia are fundamentally distinct from other brain cell types, being derived from primitive peripheral myeloid progenitors during embryogenesis. Microglia are the resident phagocytic cells of the brain, taking part in immune-mediated defense processes which clear damaged cell debris while other glial cells have roles in the nutrition of the neuron and maintenance of axonal structures [58-61].

The CNS/PNS has an extensive blood supply which services its considerable metabolic demands. Like most cells in the human body, glucose, is also the primary energy source for neurons. The brain is the most energy-demanding organ in the human body and while it may only constitute ~2% of the total mass of the human body it uses 20% of the bodies total energy production [62]. Glucose metabolism is the physiological fuel for brain function and is also required for the generation of ATP and the precursor compounds required in the synthesis of neurotransmitters needed for cell signalling. Brain functions such as thinking, memory, and cognitive learning are intricately interlinked to efficient utilisation of glucose in energy production [63]. However, too much glucose as occurs in type I and II diabetes can also be detrimental to brain function. Type 2 diabetes accelerates brain aging and accelerates functional decline in dementia resulting in significant age dependent cognitive changes in brain function.

While glycans are of particular importance in the provision of the metabolic demands of the CNS/PNS, they also have significant recognition roles in neuronal regulation. Neurons are terminal post-mitotic cells with the ability to communicate precisely and rapidly with other cells in the neural system through long cellular extensions (dendrites) that extend to distant sites in the body. Two features equip neurons with this interactive capability: (i) Neurons have receptive dendrites in the cell body and

289 a transmitting axon at the other end, this arrangement is the structural basis for unidirectional
290 neuronal signaling, (ii) Neurons are electrically and chemically excitable cell types. The neuron cell
291 plasma membrane contains specialized ion channels and receptor proteins that facilitate the regulated
292 flow of specific inorganic ions in and out of the neuron, thereby redistributing charge and creating
293 intracellular electrical micro-currents that alter the voltage across membranes. Such charge changes
294 can produce a wave of depolarization in the form of action potentials along the axon and this is the
295 usual way a signal and neurotransmitter molecules are transmitted from one neuron to another [64].
296 A waxy myelinated sheath surrounding the axon ensures that high conduction velocities are
297 maintained in neurons to optimise their excitatory transmitter properties (Fig 1). Neuro-transmitters
298 are synthesised in the Golgi/endoplasmic reticulum (ER) of the neuronal cell body (soma) and
299 transported by a microtubular system towards the pre-synaptic membrane where they are stored in
300 synaptic vesicles for later co-ordinated delivery into the synaptic gap for transportation to a
301 communicating neuron. Neurons do not use their microtubular assemblies for cell division like other
302 cells, but they use these as internal scaffolding elements for the elongation of axons and dendritic
303 processes. Microtubules act as compression-bearing struts that contribute to the shape of the neuron
304 and also act as directional conduits for the transport of neurotransmitters and organelles from the cell
305 body to the synaptic terminals (Fig 2). Synaptic vesicle membranes contain the fucosylated
306 glycoprotein synaptophysin, which forms pore-like assemblies that provide portals for the entry of
307 Ca2+ ions in and out of these structures. Synapsin is another major fucosylated vesicle associated
308 glycoprotein which interacts with the cytoskeleton tethering synaptic vesicles and co-ordinating their
309 transport to the synaptic gap for eventual synchronised neurotransmitter transmission across the
310 synaptic gap to communicating nerves in the neural network.
311
312 While glial cells are a less excitable cell type than neurons, their membranes nevertheless also
313 contain transporter proteins that facilitate the uptake of ions as well as proteins that remove
314 neurotransmitter molecules from the extracellular space. Thus glial cells act as accessory support cell
315 types to regulate neuronal function and also have roles in the nutrition of neurons and assembly of the
316 myelin sheath. In addition, they undertake running repair processes to ensure the maintenance of
317 neuronal structural integrity (Fig 2). Sophisticated regulatory systems are in place to facilitate neuron-
318 glial cell communication [65-69]. Phosphorylation, ubiquitination, and glycosylation of proteins
319 facilitate weak interactions with multivalent adaptor proteins resulting in the formation of membrane-
320 associated and soluble complexes that mediate information transfer between cells. These systems are
321 dynamic and complex and display remarkable specificity to control signaling pathways and effective
322 communication between neurons and glial cells.

323 It is estimated that there are over 100 distinct types of neurons in humans. These display
324 molecular and cytological bio-diversity displaying different cell body shapes and arrangements of
325 dendritic processes in variable depths of the cerebral cortex. All neurons inherit the same complement
326 of genetic information during development, however each neuron expresses a restricted set of genes
327 in-situ and they produce a restricted range of enzymes, structural, membrane and secretory proteins
328 specifically designed to service their precise environmental needs. While neurons have lost the ability
329 to replicate they, nevertheless, are capable of re-growth after injury provided the resident inhibitory
330 cues are circumvented and they receive appropriate stimulatory cues to promote neuritogenesis.
331 Glycan modified proteoglycans and glycoproteins have important roles to play in this area providing
332 both stimulatory and inhibitory cues which regulate neural repair and regrowth.

333 Astrocytes communicate extensively with neurons, define the margins of functional areas of
334 the brain including gliotic scars and also stabilise its internal environment. The extracellular
335 components the astrocytes lay down (e.g. abakan) form a barrier interfacing with the blood brain
336 barrier to exclude components from entry into brain tissues or the glial scar [70]. Astrocytes provide
337 nutrients to neurons and maintain the integrity of neuronal components replacing old and damaged
338 tissue. Astrocytes modify neuronal signals by secreting glio-transmitters and generating waves of Ca2+
339 action potentials with regulatory properties. Astrocytes also regulate blood flow through extensions
340 which encircle blood vessels and mediate communication with the lining endothelial cells (Fig 2j).
341 Oligodendrocytes assemble the myelin sheath around neurons. Astrocytes also attach to this encircling
342 structure on the neuron which represents a direct line of communication between these two cell types.
343 These astrocyte interconnections dilate and contract blood vessels and influence neuronal signaling in

a dynamic manner to regulate blood flow and neuronal action [71]. Thus the astrocyte is an important coordinative regulator of synaptic function and is believed to have important roles in cognitive learning and memory processes. A single neuron may contain as many as 100,000 synapses and the neuron relies on astrocytes to help control synaptic function through elaborate bidirectional communication between the astrocyte and the neuron. Astrocytes are an underappreciated cell type in neuronal tissues. Astrocytes, like neurons also produce neurotransmitters, generate their own calcium based action potentials and have receptors and ion channels which facilitate constant astrocyte-neuronal communication [72] .

CD34 is an important fucosylated endothelial cell surface molecule containing glycan interactive structures which affect the homing of progenitor cells in microvessels [73, 74]. CD34+ bone marrow haemopoietic stem cells are recruited to sites of brain trauma and differentiate into microglia which participate in neuronal repair processes. ALS, a complex multifactorial progressive degenerative disease with numerous intrinsic and extrinsic factors underlying its etiopathogenesis also displays degenerative vascular pathology underpinned by endothelial cell degeneration [75]. As discussed more fully later in this review, L-Fucose is a component of many *O*-linked and *N*-linked glycan modifications in a number of glycoproteins with important functional roles in many physiological and pathophysiological neural processes[76]. *O*-Fucosylation occurs at consensus sequences on two small cysteine-rich domains in Epidermal growth factor-like (EGF) repeats and Thrombospondin Type 1 Repeats (TSRs) in glycoproteins such as Notch-1, CD-34 and thrombospondin-1 [77]. Mouse Notch-1 contains three *O*-fucosylation sites in EGF repeats 1-5 and thrombospondin-1 has three fucosylation sites in thrombospondin repeats 1-3 [78]. 6-Alkynyl fucose (6AF) is an L-fucose analogue (Fig 4j) which has been developed to facilitate labelling and tracking of these L-fucose motifs in physiological processes [79]. Over 100 proteins are predicted to be *O*-fucosylated on the basis of identified consensus EGF repeat sequences [80]. The Notch receptor family have more predicted *O*-fucosylation sites than any other protein in the recorded databases [81] (Fig 5). Many groups have shown that *O*-fucosylation is essential for Notch's functional properties [80, 82-84]. *O*-fucose also has functional roles in agrin which enables this proteoglycan to cluster acetylcholine receptors in the NMJ [85]. The precise function of *O*-fucose in the vast majority of these proteins however is unknown. Thrombospondins produced by astrocytes have roles in the formation of synapses.

αL-fucose is a terminal or core monosaccharide on *N*-and *O*-linked glycan chains on many glycoproteins (Fig 4d, Fig 5a-g). It also occurs as a capping structure along with sialic acid on the KS-I and KS-II chains of PGs (Fig 4a-c) and in terminal sLeX motifs in glycoproteins (Fig 4f, Fig 5b, Fig 6f-h). KS is heavily substituted with fucose and sialic acid in ALS. The prominent terminal locations of L-fucose points to its role as a molecular recognition site for interacting proteins. Fucose occurs as a terminal sugar linked to a penultimate galactose residue in glycoconjugates or to core GalNAc residues in N-glycans (Fig 5b,f). Fucose can also be directly attached to serine or threonine residues by fucosyl transferases in *O*-linked glycans and can act as an acceptor molecule for the attachment of further saccharides to form small oligosaccharide side chains (Fig 4e).

3. Functional roles of the glycosaminoglycan components of brain extracellular and cell associated proteoglycans in neuroregulation

3.1 Neural proteoglycans

ECM proteoglycans (PGs) play important directive roles in the growth of axons and in the navigation, plasticity and regenerative properties of neurons. PGs have paradoxical roles in neuronal growth and repair processes where they can both promote neuronal growth but in other settings can inhibit neural repair [86]. The sulphation positions and charge density of the GAG side chains of PGs can be sources of important signals to the neurons which either inhibit or promote neuronal repair [86]. Thus the CS-A and CS-C chains of lectican PGs such as aggrecan, versican, neurocan and brevican are sources of inhibitory signals and a barrier to neural outgrowth in perineural net formations (Fig 3) which surround areas of axonal damage in glial scar formations [87-90]. CS isomers of higher charge density such as the CS-D and CS-E motifs of phosphacan, bikunin and appican can actually promote neuronal repair processes. Thus, collectively, these CS isomers guide axonal growth and repair with remarkable specificity [91-94]. Another GAG present in some neural PGs is keratan sulphate (KS) and interesting interactive properties are now emerging for this GAG.

3.2 An Emerging Role for KS in the regulation of neuritogenesis

The sulphation status of GAGs is an important functional determinant conveying important molecular recognition and information transfer properties that control cellular behavior [57, 95-98]. GAG sulphation motifs on PGs interact with cytokines, growth factors, chemokines, morphogenetic proteins, and extracellular matrix components modulating signaling pathways which control diverse aspects of cellular behaviour such as proliferation, differentiation, migration and matrix synthesis. After the cornea, neural tissue is the next richest source of KS, however it is a relatively neglected GAG and relatively little is known of its functional properties [99]. When dordal root ganglion (DRG) neurons are cultured on a substratum of CS-PGs, neurite outgrowth is inhibited, correlating with the reduced neural repair evident in glial scar tissue where levels of CS-PGs are elevated [87, 100, 101]. Treatment of DRG neuron cultures with chondroitinase ABC or keratanase results in a recovery of neurite outgrowth and these enzymatic treatments also promote neural repair processes in models of axonal damage [102-104]. KS and CS can both be sources of inhibitory signals in neuritogenesis. Three molecular forms of KS have been identified. KS-I and KS-II are substituted with L-fucose which has recognition roles in *N*- and *O*-linked glycans [99], KS-III is also found in the brain [105]. *O*-fucosylation of the KS chains attached to aggrecan vary along its core protein (Fig 4). The KS-II chains in the KS-rich region contain capping fucose and sialic acid residues but this varies in tissues. These capping structures occur in aggrecan isolated from intervertebral disc and articular cartilage but not in aggrecan isolated from non-weight bearing cartilaginous tissues such as the trachea or nasal cartilage. KS chains interspersed within the CS-2 region of aggrecan are more heavily fucosylated than the KS chains in the KS rich region or the small KS chains found in the G1 and G2 or interglobular domains. These CS-2 KS chains are detected by MAb 3D12H7 [106]. It is not known to what extent brain aggrecan displays such KS modifications, KS chains are however heavily substituted with L-fucose and sialic acid in amyotropic lateral sclerosis (ALS) [99]. The functional significance of these L-fucose and sialic acid substitution patterns on KS has not been determined but it is conceivable that they may modify or sterically impede the interactive properties of KS with neuromodulatory molecules.

Specific KS-PGs (e.g. phosphacan) in the CNS/PNS contain highly charged KS chains and display anti-adhesive properties inhibiting the attachment of neural cells to tenascin-C and laminin and this promotes neuronal outgrowth and axonal repair processes [107, 108]. Other brain KS-PGs (e.g. abakan, PG1000, SV2, claustrin) also contain 5-D-4 positive KS chains which confer interactive properties in neurotransmission, and synaptogenesis [109]. Localization of low and high sulphation phosphacan KS motifs in the Zebra song finch brain are correlated with neural development and cognitive song-learning [110]. Low sulphation KS is diffusely distributed throughout the brain while highly sulphated KS is specifically expressed in the song nuclei centres. GlcNAc-6-O-sulphotranferase (GlcNAc6ST), the enzyme responsible for the biosynthesis of highly sulphated KS is also exclusively associated with the song nuclei. Highly sulphated phosphacan localized to the perisynaptic spaces and dendrites but not the presynapse of the mouse visual cortex has roles in synaptic plasticity [111]. GlcNAc6ST knockout mice express one half of the level of KS of wild type mice. Highly sulphated KS-phosphacan generates T-type Ca2+ channel mediated long-term potentiation of non-deprived eye responses after mononuclear deprivation. E□□□□□□□□□□□□□□□□β3GlcNAcT-7 and GlcNAc6ST-1, TGF-β and FGF-2 in adult mice is elevated in gliotic scars [112]. Fibroblast growth factor 2 (FGF-2) elevates TGF-β1 production by astrocytes and KS expression in gliotic scars which inhibit neural repair. GlcNAc6ST knockout mice display reduced KS expression and enhanced neural regeneration after brain injury [101]. KS-PGs focally upregulated in spinal cord injuries are laid down by reactive microglia, macrophages and oligodendrocyte precursor cells but not by astrocytes [113]. Astrocytes do however produce the KS-PG abakan following injury which defines functional areas and the margins of gliotic scars in the cerebral cortex [114]. Abakan is also associated with malignant astrocytic tumours [115] and glioblastoma [116]. Furthermore, highly sulphated KS levels however are severely reduced in AD with levels reduced to less than 50% of control tissue levels [117].

KS interactions with cell stimulatory molecules regulate tissue homeostasis. KS chains bind insulin-like growth factor binding protein-2 (IGFBP2) [118], Sonic Hedgehog (SHH), FGF1 and FGF2 [119]. KS is a component of neural matrix and cell membrane PGs . KS-I interactions involving highly sulphated KS detected using MAb 5-D-4 have been demonstrated in a microarray of 8268 proteins and

458 custom array of 85 extracellular nerve growth factor protein epitopes [120]. Two hundred and
459 seventeen of the 8268 microarray proteins interacted with KS including 75 kinases, several membrane
460 and secreted proteins, cytoskeletal proteins and a number of nerve function proteins. Surface plasmon
461 resonance confirmed these interactions and allowed the determination of binding their constants. Of
462 the 85 selected ECM nerve-related epitopes, KS bound 40 of these. This included Slit, two Robo's, nine
463 ephrin receptors, eight ephrins, eight semaphorins, and two nerve growth factor receptors. The Slit-
464 Robo cell-signaling pathway is central to axonal guidance, angiogenesis and neurogenesis during spinal
465 development. The slit receptors contain variable numbers of LRR motifs and 7-9 EGF repeat domains
466 which have protein interactive properties. KS interactions in the Robo-Slit cell signaling pathway
467 produces downstream activation of Rho GTPases, actin depolymerisation and cytoskeletal re-
468 organisation. Direct cell-cell interactions between Ephrins and Ephrin protein-tyrosine kinase receptors
469 also regulate a range of important intracellular signaling pathways during development, that control
470 cell migration and are involved in axonal growth cone guidance. The semaphorins, which, exist as both
471 secreted and membrane bound forms, are also involved in axonal growth cone guidance and provide
472 short-range inhibitory signals through interactions with plexin and neuropilin receptors which regulate
473 Rho family GTPases (Fig 8f, g). Such interactions are critical to neural development and neural repair.
474 As seen in Figure 4, substitution of KS-I and II with L-fucose may modulate their interactive properties
475 with the aforementioned receptors. L-Fucose has demonstrated roles in molecular recognition and
476 receptor-ligand interactions involving Notch, selectin-P ligand (PSGL-1) and CD-34 [121-126].
477
478 KS coexists alongside CS chains in brain aggrecan [89, 127] and phosphacan [103, 107, 108,
479 128, 129]. Neurite outgrowth of DRG neurons is inhibited when they are plated on to CS-PGs, and this
480 inhibitory effect is removed by either chondroitinase ABC or keratanase treatment [102, 104].
481 Keratanase treatment promotes functional recovery of spinal cord injury [103]. Developmental
482 changes in KS sulphation patterns are associated with alterations in plasticity and cognitive learning
483 and functional recovery of neural tissues. GlcNAc6ST-1 knock out mice display no gross developmental
484 phenotype, but show changes in the induction of glial scar formation [101], and better axonal growth
485 after both cortical stab wounds and spinal cord injuries [130]. These studies emphasize the importance
486 of highly charged KS chains identified by the KS antibody 5-D-4 in nerve repair processes. The 5-D-4
487 MAb recognizes KS structures containing 6-sulphated Gal and GlcNAc residues. *GlcNAc6ST1* and
488 *KSGal6ST* both contribute to the generation of the 5-D-4 epitope and are essential for 6-sulphation of
489 Gal within KS in the developing and adult brain and induced after injury [131] and in early postnatal
490 brain development. 5-D-4 reactivity is abolished in the KSGal6ST knockout mouse brain. The early
491 phases of ALS are accelerated in GlcNAc6ST-1(-/-) mice where CNS KS is also ablated [132]. KS
492 produced by M2 microglia suppress the early phases of ALS, microglia produce KS heavily modified
493 with fucose and sialic acid. GlcNAc6ST1(-/-) mice display a complete absence of microglial KS but
494 increased phagocytosis of amyloid-β protein and reduced levels of cerebral amyloid deposition [133].
495 Inhibition of KS biosynthesis by targeting GlcNAc6ST1 thus represents a therapeutic target in AD.
496 Functional roles for KS have been suggested in spinal cord development in GlcNAc6ST1 knockout mice
497 where KS binds to Shh and acts as a morphogen regulating murine embryonic spinal development
498 [134]. KS interactions in late phase *Shh* signaling acts as a morphogenetic switch regulating the
499 generation of oligodendrocyte progenitor cells from motor neurons [134]. The KS-PG, phosphacan also
500 acts as a developmental molecular switch which regulates neuronal development. KS chains inhibit
501 neuronal attachment but promote outgrowth activity, an effect reversible by keratanase treatment
502 [135].
503
504 Other lines of evidence demonstrate key roles for KS in development and repair/remodeling in other
505 tissues. For example, KS may be chondroprotective in inflammatory arthritis models [136]. Murine
506 aggrecan has a truncated core protein devoid of a KS rich region thus KS levels are low in murine knee
507 joints. Intraperitoneal administration of KS ameliorated IL-1 induced GAG release and protected
508 cartilage from arthritic changes in GlcNAc6ST1 (-/-) mice. Furthermore, GlcNAc6ST1 activity is
509 significantly reduced in macular corneal dystrophy resulting in the occurrence of low- or non-sulfated
510 KS and corneal opacity [137].
511
512 *3.3 CS/DS and their cell and matrix regulatory roles in neural tissues*
513 CS is the most abundant GAG in the human body and is *O*-sulphated at the 2, 4 and C6 positions [55].
514 GlcA may also be epimerised to α L-IdoA in the related GAG, DS, leading to structural diversity in CS/DS

with over one thousand different pentasaccharide combinations possible [55]. The large number of structural permutations possible with CS/DS facilitates interactions with a diverse repertoire of cytokines, chemokines, morphogens and growth factors with regulatory properties in tissue development and ECM remodelling [55, 138-142]. CS also occurs as a number of isoforms including the high charge density CS-D and CS-E and lesser charged CS-A, CS-B and CS-C [98]. CS-D and CS-E are enriched in the brain transmembrane PGs phosphacan, syndecan-1, syndecan-4, NG2 proteoglycan/CSPG4, neuroglycan-C/CSPG7, and ECM PGs appican (β-APP) and bikunin [143-145]. CS-A, B, C are abundant in the brain hyalectan proteoglycan family consisting of brevican, neurocan, versican and aggrecan. The CS-D and CS-E motifs embedded within the CS-A side chains of β-APP, bikunin and phosphacan convey neuroregulatory properties [108, 145]. While CS-D and CS-E can promote neural repair the same cannot be said of the CS-A, B, C side-chains of neural net PGs layed down in the gliotic scar. Perineural nets [146] have been immunolocalised in rat brain tissues using the MAb 1-B-5 to a non-sulphated aggrecan stub epitope generated by chondroitinase ABC. 1-B-5 reactivity is displayed in extensive extracellular distributions encompassing a large group of neurons (Fig 3 a, b) as well as pericellularly surrounding single or small numbers of neurons (Fig 3c, d) [147]. Formation of glial scars, seals the injury but also creates a barrier to axonal regrowth. The scar centre is highly inflammatory and populated by NG2+ glia, astrocytes seal the border of the scar but in so doing entrap axons attempting to regrow within the scar, thus activated astrocytes and ECM components laid down in the scar contribute to regenerative failure[148]. The NG2 positive glia are a progenitor cell type for oligodendrocytes which participate in neural remodelling and repair processes whereas astrocytes define the boundary of the gliotic scar and do not participate in its repair. PGs in neural tissues thus have paradoxical modes of action, CS-PGs, of the lectican family hinder axonal regrowth while the transmembrane CS-PG (NG2/CSPG4) and phosphacan, upon shedding from the cell by ADAM 10 (a disintegrin and metalloproteinase containing protein 10), promote axonal re-growth and production of synaptic adhesion molecules, promoting synaptic signaling, plasticity and functional recovery. The positive contribution of CSPG4 to neural repair processes is confirmed from knockout studies of NG2/CSPG4 mice which display aggravated tissue loss, inflammation and neurologic deficits after traumatic brain injury. Progranulin, a functional ligand of Notch and Eph2a acts in concert with NG2/CSPG4 to overcome neuronal inflammation and structural recovery of damaged neuronal tissue. Progranulin is upregulated after spinal contusion in mice [149]. Progranulin is produced by neurons and glia and has roles in inflammation and wound repair [150, 151]. Progranulin is proteolytically processed into peptide fragments (granulins) during tissue remodelling and these display different biological activity to the native molecule. Progranulin has trophic properties while the granulins act as inflammatory mediators and contribute to neuroinflammation, dementia and development of AD [151-153]. Neuronal expression of α9β1 integrin, trkB, and protein tyrosine phosphatase σ (PTPσ) , which are receptors for tenascin-C, brain derived neurotrophic factor (BDNF) and CSPGs respectively, have also been shown to significantly enhance regeneration of injured axons[154-157]. Thus with the correct expression of these cell surface receptors, growing axons can respond to appropriate guidance cues in their extracellular micro-environments by regulating their intracellular signaling pathways to modify growth cone behaviour and promote intrinsic repair [154, 156, 157]. Neuronal regeneration has been induced by transgenic integrin expression [158], lentiviral trk-B induced Erk activation [159] or by modulation of PTPσ expression [157]. PTPσ and the related leukocyte common antigen-related (LAR) and Nogo receptors 1 and 3 (NgR), bind the inhibitory glycosylated side chains of CSPGs and regulate synaptic structure and neuroplasticity [160, 161].

As already noted, progranulin expressed in mature neurons and microglia, has protective roles in neurogenerative disorders [162-164] and plays a central role in the regulation of neural inflammation, enhancing neuronal survival and stimulating neurite outgrowth activity. Progranulin achieves this through modulation of glycogen synthase kinase (GSK)-3β. Inhibition of GSK-3β has received interest as a therapeutic target in the treatment of traumatic brain injury and is neuroprotective, promoting functional recovery after intracerebral hemorrhagic stroke [165]. GSK-3β inhibitors rescue cognitive impairment in AD, Fragile X syndrome, Down syndrome, Parkinson's disease and spinocerebellar ataxia type 1 [166]. Levels of phosphorylated tau protein are elevated following traumatic brain injury and may contribute to pathological structural changes in the CNS [167]. Misfolded amyloid-β-peptides and hyperphosphorylated tau protein accumulation is a hallmark of AD [168]. Caspase-3 regulates tau phosphorylation in AD, is mediated by the GSK-3β pathway and

involves cleavage of protein-kinase B (Akt) by Caspase-3 [168]. Progranulin thus has significant roles in the promotion of neural repair processes following traumatic brain injury and it acts in concert with CSPG4 to promote these. The interaction of progranulin with neural PGs and neural receptors in specific regions of traumatic brain injury is mediated by GAGs attached to PGs in the traumatised area oversulphated CS isomers play a significant role in such binding interactions. This is consistent with progranulins interactive properties with the HS-PG, perlecan [169]. Oversulphated DS also displays neuritogenic activity in hippocampal neurons [170]. Novel CS/DS-GAGs identified in shark fin cartilage can bind neurotrophic factors and these also display neurite outgrowth promoting activity. CS-octasaccharides have been isolated from shark cartilage containing CS-D hexasaccharide sequences with neurite outgrowth promoting activity [171]. Novel oversulphated CS-E tetrasaccharides have also been isolated from squid cartilage [172] with neuroregulatory activity [173]. CS-E containing CS tetrasaccharides have been synthesized and demonstrated to have potent FGF-2 binding properties but their neurite outgrowth stimulatory profiles have not been determined [174] despite an earlier study which demonstrated this activity in a CS-tetrasaccharide [175]. Neurite outgrowths by hippocampal neurons are stimulated by CS-E tetrasaccharide, desulphated CS-E tetrasaccharide is inactive as is a CS-E disaccharide (Fig 7). CS-A and CS-C inhibit neural outgrowth activity thus collectively CS isomers can both promote and inhibit neural repair.

3.4 Contributions from other GAG types in neuroregulation and neural repair processes

As already noted, CSPGs in glial scars prevent neurite outgrowth in-vitro and nerve regeneration in-vivo[176]. Astrocytes stimulated with IL-1β do not upregulate any of their CSPG genes suggesting that these are not the only reactive glial scar proteoglycans. Rat cortical astrocytes produce more HS than CS in culture and these highly charged GAGs are more effective at stimulating nerve growth factor (NGF) signaling in PC12 cells. Furthermore, the heparin binding domain of laminin also promotes neurite outgrowth along with NGF [177] thus HS proteoglycans also contribute to neuritogenic events. Furthermore, domain V of perlecan delays the onset of glial scarring in rat models by down-regulating neurocan and phosphacan expression and upregulating NGF activity[178]. The balance between CS and HSPG levels can therefore either inhibit or stimulate neurite outgrowth and nerve regeneration. The laminin-like LG3 fragment of perlecan is not associated with glial scarring, mice deficient in NG2/CSPG4 have reduced glial scarring and are more permissive to axonal regrowth[148]. These animals have a similar phenotype to progranulin deficient mice[148]. Progranulin is neuroprotective [179] and binds to the C-terminal LG1 and LG2 repeats of perlecan domain V [180]. The C-terminal region of perlecan also binds CSPG4 [181] and has neuroprotective and pro-angiogenic properties in a rat ischemic model thus also contributes to neural repair processes [182]. Thus while CS GAGs are a major focus in this review any potential synergism or antagonistic effects with other GAG types also need to be considered in a holistic approach to better understand neural repair processes.

3.5 SHH ,HS, CS, and KS interactions model tissue patterning and neural development.

Hedgehog (HH) proteins are highly conserved morphogenetic signaling molecules with fundamental roles to play in vertebrate and invertebrate embryonic development [183-186]. The HH signaling pathway plays key roles during embryonic development and remains active in adults. The GAG chains of cell surface PGs shape HH gradients and signal transduction [119, 134, 187, 188]. Three HHs have been identified in mammals, Sonic, Indian, and Desert hedgehog, these are typically expressed in the nervous system, cartilage and testis respectively. SHH is synthesized as a 45-kDa precursor protein which undergoes autocatalytic cleavage to a 20-kDa N-terminal fragment (residues 24–197 in the human gene sequence) responsible for all known hedgehog biological activity. This is membrane-associated through a palmitic acid attachment at its N-terminus [189] and cholesterol at its C -terminus [190-192]. Patched (Ptc), a 12 span transmembrane protein SHH receptor acts as a negative regulator of SHH signaling. SHH is interactive with glypican and CS GAG isomers and these are responsible for the production of SHH gradients which are a driving force during tissue morphogenesis. Surface plasmon resonance studies have demonstrated that corneal KS has interactive properties with SHH [119]. KS regulates the switch from motor neuron to oligodendrocyte generation during development of the spinal cord [134]. Glypican and CS participate in SHH mediated cell signaling [187] regulating tissue patterning and development of the neural system. SHH cell signaling is important in foetal and postnatal brain development and regulates the proliferation of early cerebral cortex progenitor and oligodendroglial lineage cells, expansion of their numbers is critical in the development of the neocortex [183, 185, 193, 194]. SHH guides axonal development during neurogenesis, cellular

responses in early brain injury and following demyelination [195]. SHH may represent a therapeutic target to focus on in neurological disorders [196]. Co-ordinated SHH and Wnt mediated cell signaling regulates cranial nerve development [197]. SHH has roles in the differentiation of oligodendrocytes [198] and in glial neural cell communication during brain development which provides neuroprotection [186] and neuroplasticity . Neurons diversify astrocytes in the adult brain through SHH signaling [199]. SHH is a regulator of extracellular glutamate levels in epilepsy and modulates the release of gliotransmitters from cultured cerebellar astrocytes [200, 201].

3.6 CS interactions modulate neural cell behaviour.

CS is a prominent CNS GAG and occurs in a number of isomeric forms with differing degrees of sulphation and interactive properties [19, 20, 22, 202-204]. CS microarrays have proved useful in the assessment of CS-protein interactions [19, 20, 22] and has detected neurostimulatory and inhibitory CS species as well as a tumour necrosis factor α (TNFα) antagonist [24, 175]. Interactions of neurons with CS/DS promotes cellular survival [205]. The CS glycan chains of PGs interact with a diverse collection of proteins in the CNS to promote neural growth, proliferation, differentiation and long term survival. Some CS isoforms provide chemorepulsive nerve guidance cues which regulate axonal development and repair processes following traumatic injury. CSPGs inhibit the growth cone by interaction of CS chains with laminin, collagen and cell surface integrins. Receptor type protein tyrosine phosphatase-σ (RPTP-σ) also acts as a neural CS receptor [161] while RPTP-ζ interacts with the NCAM resulting in an inhibition of neural cell adhesion and growth (Fig 8c, d). The ecto-domain of RPTP-ζ is enzymatically released from the cell surface by ADAMS 10 generating the soluble phosphacan which can promote neural outgrowth and repair processes. Highly charged CS isomer side chains such as CS-E on proteoglycans bind FGFs and present these to FGF receptors (FGFRs) to promote cell signaling, neural growth and differentiation (Fig 8e). Interaction of the attractive guidance protein Semaphorin 5A with CS converts this to a repulsive guidance protein (Fig 8h). Semaphorin 3A is a cell membrane bound and secreted short range repulsive inhibitor guidance protein which interacts with CS-E in lectican perineural net formations to inhibit nerve regrowth. This effect is mediated by interaction with neuropilin-1 and neuroplexin neural receptors (Fig 8f). Plexin acts as a signal transduction molecule along with transmembrane neuropilin co-receptors in the neuropilin-plexin receptor complex (Fig 8f).

Eph receptors and ephrins display broad spatial and temporal expression patterns throughout the nervous system [206, 207]. During early development, these interactions contribute to neurogenesis (reviewed in [208]) and differentiation [208, 209]. Eph-ephrin signaling influences the functions of Rho GTPase proteins, which in turn regulate the actin cytoskeleton influencing neuronal migration during development. Eph-ephrin signalling can generate both attractive and repulsive interactions and can positively support neurogenesis, axonal guidance and neural repair [208, 209]. EphA2 receptor tyrosine kinase is a functional cell surface receptor for the secreted glycoprotein progranulin. Fourteen Ephrin receptors have so far been identified. Ephrin-Eph receptor cell signaling regulates cellular morphology and proliferation influencing the adhesive properties of cells during cellular migration in embryonic development, vasculogenesis and angiogenesis and has roles to play in axonal guidance, and synaptogenesis. Progranulin also promotes angiogenesis through the Ephrin receptors and upregulation of vascular endothelial growth factor (VEGF) production to modulate neuroinflammation [151, 210]. Phosphorylation of EphA2 by progranulin leads to tyrosine phosphorylation of other tyrosine kinases such as EphA4, EphB2, and EGFR through extensive cross talk (Fig 9b) among receptor tyrosine kinases [211]. Progranulin promotes the activation of the mitogen-activated protein kinase (MAPK) and Akt signaling pathways. Progranulin is secreted as a dimer containing up to 14 granulin modules per dimer which are available for protein–protein interaction. This may enable the dimer to bridge several receptors on a cell and serve as a multi-receptor signaling complex explaining the cross-talk when progranulin binds to EphA2 (Fig 9b).

The guidance of axonal development is a complex highly integrated process dependent on a myriad of inhibitory and stimulatory effector ECM proteins. Perineural net formations with hyaluronic acid (HA), tenascin-R and lectican PGs in gliotic scars are prominent stabilizing and protective structures which minimize further damage to neural tissues and protect neural cell populations in the scar from oxidative stress. Myelin-associated glycoprotein, Nogo, and the semaphorins all provide inhibitory cues over axonal development. CS and KS interact in a sulphation-dependent manner with a number of axonal guidance proteins, including slit2, netrin1, ephrinA1, ephrinA5, and semaphorin 5B

[22]. Netrin-1 modulates axonal growth direction and speed and directs F-actin reorganization, essential for mammalian neural development. The best characterized netrin-1 receptor, *Deleted in Colorectal Cancer* (DCC), is localized to growth cones, but is also observed in neuronal cell bodies [212]. Netrin-1 attracts and repels distinct motor axon populations, according to the spatio-temporal expression of Netrin receptors [213] in neural tissues. The guidance cues provided by Netrin-1 are influenced by its interactive properties with ECM PGs, a theme recapitulated by most of the axonal guidance promoter proteins. These represent complex interplays between multiple components which regulate spatio-temporal neural growth [100, 214]. Netrin-1 can also synergize with ephrin receptors to regulate axonal formation [213]. A greater understanding of these axonal guidance cues would be insightful in therapeutic strategies aimed at producing guided nerve regeneration [215-219].

CSPG4 promotes neural repair processes through upregulation of epidermal growth factor receptor (EGFR) expression [220, 221] and interaction with progranulin [148, 222, 223]. Progranulin is upregulated after spinal contusion [149]. CSPG4 is highly expressed by macrophages, microglial cells, tumour, perivascular and oligodendrocytes involved in cell adhesion and migration [224-228]. CSPG4 is upregulated in glioblastoma, astrocytoma and a number of other human tumours [221, 229, 230]. Activated microglial cells form synapses with neurons to participate in neural repair [224] and re-organisation of the gliotic scar and improve neural outgrowth [148, 231, 232]. Following traumatic injury to the brain, the cells in the impacted area upregulate aggrecan, versican, brevican, neurocan in HA-macroaggregate perineural net structures stabilised by link protein and tenascin-R providing protection from oxidative stress and further mechanical injury. Astrocytes seal the margins of these gliotic scars by upregulating the brain matrix proteoglycan abakan. These perineural nets inhibit nerve outgrowth. Chondroitinase ABC selectively depolymerises the CS side chains of the lectican PGs improving neural recovery in the gliotic scar [233] and improves spinal cord repair [102, 234-237]. Chondroitinase C also significantly improves repair of peripheral nervous tissue but appears to have a more specific mode of action [238].

ADAMTS-4 (a disintegrin and metalloproteinase with thrombospondin motifs 4) is localised in regions of the spinal cord undergoing spontaneous repair and specifically targeting of the lectican PGs in the scar tissue [239, 240]. KSPGs are similarly up-regulated in glial scars and inhibit axonal repair [101, 113, 114, 135, 241, 242]. Mutant mice deficient in the enzyme GlcNAc6ST-1 show improved functional recovery following spinal cord injury [130]. Therapeutic use of keratanase also improves axonal repair [103]. The KSPG phosphacan is also upregulated in scar tissues where it promotes mossy fiber outgrowth and nerve regeneration [107]. Chondroitin-6-sulphate upregulated in scar tissue is reported to have nerve regenerative potential.

3.7 RAGE in the brain

Receptor for advanced glycation endproducts (RAGE) is a receptor which binds advanced glycation end products (AGEs) and CS in brain tissues (Fig 10). RAGE acts as a receptor for oversulphated CS isomers such as CS-E [243, 244]. AGEs modulate amyloidogenic precursor protein (APP) processing and Tau protein phosphorylation regulating AD development [245]. AGEs in glioblastoma have a modulatory role over tumour development [246]. RAGE mediates amyloid β accumulation in a mouse model of AD by regulation of β- and γ-secretase activity [247]. Targeted inhibition of RAGE reduces amyloid-β influx across the blood-brain barrier and improves cognitive deficits in mice [248]. High-mobility group box-1 protein (HMGB-1) and β-amyloid oligomers promote neuronal differentiation of adult hippocampal neural progenitors via RAGE and the NF□B pathway [249] sustaining neurogenesis counteracting the hostile AD brain microenvironment [6]. This promotes survival of vulnerable brain cell populations [249, 250]. AGEs impair NLRP3 inflammasome-mediated innate immune responses in macrophages and modulates neuroinflammation through the NF□B pathway [251].

4. Roles for L-Fucose in Neuro□□□□□□□□□□□□Processes□

4.1 O- and N-linked fucosylated proteins in neural tissues

While neuronal mitochondria utilize glucose as an obligate primary energy resource in the tricarboxylic acid glycolytic pathway to generate energy neurons are also responsive to sugars other than glucose as cell regulatory agents. Positive selection pressure over at least 500 million years of vertebrate evolution has resulted in sugars which have evolved molecular recognition and information

transfer properties equipping them as cellular mediators serving as critical determinants of protein folding, trafficking, and stability . Glycans are abundant in the brain and are involved in various neural functions including learning and memory, brain development, and spinal cord injury [102, 252-254]. The precise molecular mechanisms whereby glycans influence these processes is not well understood but it is clear that synaptic transfer of information between neurons occurs through glycoprotein mediated interactions. L-fucose exists as a terminal residue on *N*- or *O*-linked glycoproteins attached to the C-3 and C-6 position of N-acetylglucosamine or the C-2 position of galactose (Fig 4). The fucose α1-2 galactose (Fuc α1-2Gal) linkage has been implicated in cognitive processes such as learning and memory. Non-invasive two dimensional magnetic resonance spectroscopy (2D MRS) has identified six Fuc α1-2 Gal sugars in brain tissue. 2D MRS offers an unprecedented insight into the molecular mechanisms by which fucosylated sugars contribute to neuronal processes and how they alter during development, ageing and disease [29]. Fucose is an unusual sugar in that it exists as a 6-deoxy αL-galactopyranose configuration and is a prominent functional component of neural tissues such as synaptic membranes [29]. Addition of 2-deoxy D-galactose, an L-fucose analog to hippocampal neuronal cultures potently inhibits neural outgrowth activity whereas 3-deoxy D-galactose is inactive, moreover addition of D-galactose to 2-deoxy-D-galactose treated cultures results in the functional recovery of normal neuron growth characteristics. Fuc α 1-2 Gal is a non-reducing terminal component of many glycans [29] and is implicated in neurite outgrowth, synaptogenesis, neuronal development, learning, and memory [27, 28, 255, 256]. Treatment of animals with 2-deoxy-D-galactose, disrupts the formation of Fuc α 1-2Gal linkages, and causes reversible amnesia [257] interfering with the maintenance of long-term potentiation in an electrophysiological model of learning and memory [258]. Furthermore loss of 1, 6-fucosyl transferase activity also decreases hippocampal long term potentiation [259].

αL-fucose is a terminal or core monosaccharide on *N*-and *O*-linked glycan chains on many glycoproteins (Fig 4d, Fig 5a-g). It also occurs as a capping structure along with sialic acid on the KS-I and KS-II chains of PGs (Fig 4a-c) and in terminal sLeX motifs in glycoproteins (Fig 4f, Fig 5b, Fig 6f-h). KS is heavily substituted with fucose and sialic acid in ALS. The prominent terminal locations of L-fucose points to its role as a molecular recognition site for interacting proteins. Fucose occurs as a terminal sugar linked to a penultimate galactose residue in glycoconjugates or to core GalNAc residues in N-glycans (Fig 5b,f). Fucose can also be directly attached to serine or threonine residues by fucosyl transferases in *O*-linked glycans and can act as an acceptor molecule for the attachment of further saccharides to form small oligosaccharide side chains (Fig 4e).

4.1.1 L-Fucose as a functional component of blood group substances and Immunoglobulins

Fucose also occurs as terminal Fuc α1-2 Gal terminal saccharides in small glycolipids attached to red blood cells identifying the A, B, O blood group antigens (Fig 6a). Over 95% of circulatory human IgG antibodies also contain a fucose (core-fucose) residue attached to the first GalNAc in the glycosylation site of their Fc region. The majority of other plasma proteins are not substituted with fucose in this manner. Fucosylation dramatically reduces IgG binding to FcγRIIIA, an activating Fc receptor specific for IgG Fc region expressed by immune human natural killer (HNK) cells and macrophages [260-262]. FcγRIIIA initiates antibody dependent cellular cytotoxicity (ADCC) by HNK cells and phagocytosis of antigens by macrophages. This core fucose attenuates potentially harmful ADCC activity. Conversely, ADCC induced by non-fucosylated IgG improves the efficacy of therapeutic anticancer antibodies. IgG lacking the core-fucose is over 100 times more effective in initiating ADCC than the fucosylated version (Fig 6b-h).

4.1.2 Synapsin and Synaptophysin

The synapsins are fucosylated proteins [256] which regulate the release of synaptic vesicles to coordinate release of neurotransmitters within the synaptic vesicles at the synaptic gap [263]. They do so by tethering the vesicles to cytoskeletal components to prevent the diffusion of vesicles to the synaptic membrane preventing the un-coordinated release of neurotransmitters at the synaptic gap [264]. During the transmission of an action potential down the neuron from the cell body the synapsins are phosphorylated by cAMP dependent protein kinase (PKA). This releases the synaptic vesicles to the pre-synaptic membrane [265] which depolarizes in response to the action potential allowing the synaptic vesicle to fuse with the synaptic membrane and release the enclosed neurotransmitters into the synaptic gap and these are transported across to the post synaptic

membrane of a communicating neuron [266]. This results in the transmission of neural signals along the neural network. There are three synapsin proteins and each occur as two isoforms. Synapsin 1a is implicated in bipolar disorder and schizophrenia [267]. The synapsin Ia/Ib isoforms are the most highly expressed hippocampal pre-synaptic vesicle associated phosphoproteins and are implicated in thought formation and cognitive learning [268-270]. Synapsin is a major neuronal fucosylated glycoprotein [256, 271, 272]. The synapsin family consists of 3 major isoforms encoded by 3 genes SYN1, SYN2, SYN3. Each gene occurs as two alternatively spliced forms leading to a total of six isoforms. Mice lacking synapsin I, II, III are prone to seizures and display learning difficulties and in humans is associated with bipolar disorder and Schizophrenia[34, 267].

4.1.3 Fucosylated Glycoproteins and Proteoglycans

Fucosylated glycoproteins and PGs have prominent roles in many physiological and pathological processes including leucocyte adhesion, host-microbe interactions, neuronal development and neural protection[273-275]. Fucosylated glycolipids on erythrocytes form the ABO blood group antigens[276]. However, aberrantly fucosylated glycoconjugates are also found in cancer, inflammation and neoplastic processes [276-279]. The fucosylated sialyl Lewis-X, and sialyl Lewis-Y antigens are prominently upregulated in some cancers and associated with tumour progression. Deficient levels of fucose occur in impaired leucocyte interactions with the vascular epithelium in immunodeficiency. Leukocyte adhesion deficiency II (LAD II) is a rare congenital disease caused by a defect in fucosylation of glycoconjugates such as P-selectin glycoprotein ligand-1 (PSGL-1) (Fig 5a) which normally facilitate leucocyte binding to the selectins on the epithelium during inflammation [280]. This interaction facilitates leucocyte rolling and their extravasation through blood vessels to tissue sites to combat infection. Leukocyte adhesion deficiency type II (LAD II)-patients show severe mental and growth retardation indicating an additional essential role for fucose in brain growth and development [281]. The P-selectin PSGL-1 ligand is a dimeric mucin-like 120-kDa glycoprotein located on leukocyte surfaces that binds to P-, E- and L-selectin and promotes leucocyte adhesion to the endothelium facilitating leucocyte rolling during inflammation. PGSL-1 is heavily fucosylated as part of the branched Lewis X antigen *O*-glycan structure (NeuAcα2$\rightarrow$3Galβ1$\rightarrow$4[Fuca1$\rightarrow$3]GlcNAcβ1$\rightarrow$R)[125] (Fig 5a). High affinity cell adhesion interactions also require the presence of three tyrosine sulfate residues located near the Lewis-X antigen structure at the N-terminus of PSGL-1 [125, 282, 283].

4.1.4 Functional role of L-Fucose in Notch Signaling

O-fucosylation is essential for the functional properties of Notch [124, 284, 285], a transmembrane receptor that co-ordinates a number of cell-fate decisions in neural development and in neuron-glial cell interactions which determine neuritogenesis, neuronal migration and differentiation[286] (Fig 5g). Fucose knockout causes developmental defects in mice and abnormal vasculogenisis, somitogenisis and neurogenesis [124], Notch is an important mediator in all of these processes. Notch-1 is a member of a family of transmembrane glycoprotein receptors which contains a large number of extracellular epidermal growth factor repeats. These are heavily substituted with fucose providing the extracellular Notch domain with important interactive properties (Fig 5g). Ligand binding to the extracellular domain of Notch-1 by Delta, Jagged or Serrate ligands induces proteolytic cleavage of Notch and the cleaved intracellular domain enters the nucleus to modify gene functions. Upon ligand binding with Notch, ADAM 10 cleaves the extracellular domain and this continues to interact with the ligand in solution. The intracellular portion of Notch is then cleaved by γ-secretase and it is transported to the nucleus where it regulates gene expression through the transcription factor CSL, an acronym for CBF-1/RBPJ (recombining binding protein suppressor of hairless). CSL acts as a co-repressor negatively regulating Notch signaling to control cell fate decisions[121, 287] in developmental contexts. Notch is widely expressed in many cell types and has fundamental roles in development.

Fucose occurs on structurally diverse *N*- and *O*-linked glycans through the action of over a dozen fucosyl biosynthetic enzymes. Fucosyl transferase 1 (FUT1) and FUT2 attach fucose to galactose in Fuc α1-2 Gal containing glycans. FUT3 attaches Fuc via α 1-3 and α 1-4 linkages to Gal and GlcNAc residues in glycan chains. FUT4-7 form exclusively α 1-3 linked fucose residues in glycans. FUT8 and FUT9 generate Fuc α1-6 GlcNAc linkages, FUT8 attaches these to core asparagine residues in N-glycans whereas FUT9 attaches these to the GlcNAc units of polylactosamine chains. FUT10 and FUT11 are

putative fucosyltransferases catalyzing the generation of α 1-3 linked Fuc in glycans. POFUT1 and POFUT2 are *O*-fucosyltransferases which attach Fuc directly to serine and threonine residues in the modular EGF and thrombospondin repeats of glycoproteins.

Although a relatively minor sugar, its strategic positioning on key functional glycoproteins points to fucose having a significant role to play in neural pathobiology. As already indicated *O*-fucosylation is essential for the activity of Notch (Fig 5g) and has significant roles to play in leucocyte PSGL-1 P-selectin mediated interactions (Fig 5a) with the endothelium in neuro-inflammation [286]. CD-34 is heavily substituted with both *N*- and *O*- linked fucosylated oligosaccharides in microvascular progenitor cells (Fig 5e). The mode of action of L-Fuc in Notch has been suggested to be due to induction of conformational changes in the epidermal growth factor (EGF) repeat domains or in the Notch ligands. Notch signaling is essential for the maintenance of neural progenitors and regulates cell-fate decisions in neuronal and glial cells to modulate neuronal differentiation and migration [288, 289]. Deletion of *POFUT1* is embryonic lethal causing developmental defects in vasculogenesis, somitogenesis and neurogenesis similar to those obtained when Notch receptors are deleted. This reinforces the importance of L-Fuc as a mediator in combination with Notch in neuronal development.

4.1.5 Roles for L-Fucose in CD-34.

Another cell surface protein with cell adhesion and cell regulatory properties in the CNS/PNS is CD-34 (Fig 5e). CD34 is a heavily fucosylated type I transmembrane sialoprotein, that can be phosphorylated by a number of kinases including PKC and Tyrosine kinase. The CD-34 proteins are a family of sialomucin transmembrane adhesion proteins. CD-34 is expressed in early haematopoietic and vascular tissues and lymph node epithelium. CD-34 interacts with L-selectin expressed by T cells in the lymph node epithelium. Podocalyxin and endoglycan are related to CD-34 and also facilitate cell attachment and cell migration during microvessel development in neural tissues [126]. Terminal fucosylation of these PGs confer unique functional properties in a variety of biological settings. Fucose is an essential component of the carbohydrate ligands for the selectin family of cell adhesion receptors [290, 291]. E-, P-, and L-selectin are C-type lectin proteins expressed by platelets (P-selectin), endothelial cells (E- and P-selectin), and leukocytes (L-selectin). Selectins bind to oligosaccharides decorating specific cell surface and secreted proteins expressed by leukocytes (E- and P-selectin ligands) and high endothelial venules (L-selectin ligands). Interaction between selectins and their ligands enable the rolling of leukocytes on the endothelium, and is an essential requirement for leukocyte extravasation. The carbohydrate selectin ligands are fucosylated structures related to the sialyl Lewis-X antigen, an α 1,3-fucosylated glycan structure also known as stage-specific embryonic antigen-1 (SSEA-1) and CD15 expressed during early embryogenesis [292].

4.1.6 L-Fucose as a component of Lewis-X-Antigen

Lewis^x epitopes are present in multiple areas of the developmental embryonic brain[293-296], controlled by FUT 9 expression, an enzyme which is regulated by the transcription factor Pax 6 [297]. The functional role of Lewis^x in the developing brain has yet to be determined, but its dynamic expression patterns during embryogenesis suggests it may have roles in aspects of molecular recognition which support the assembly of neural structures [273, 298]. The Lewis^x epitope, an α 1,3-fucosylated glycan also known as the stage-specific embryonic antigen-1 (SSEA-1) and CD15, is expressed during early embryogenesis [292]. Exposure of pre-implantation mouse embryos at the morula developmental stage to Lewis^x oligosaccharides causes decompaction apparently through disruptive multimeric interactions affecting cell-cell adhesion in early embryos [299, 300]. Oligosaccharides containing L-fucose form part of a recognition signal in sperm–egg attachment in mammals [301]. At the endometrial surface, adaptations are also required to accommodate the implanting embryo [302, 303]. These adaptations at the materno-fetal boundary are highly species-specific. Fucose containing carbohydrate structures in the embryonic–maternal interface have important molecular recognition roles to play which define the maternofetal glyco-code. Localization of fucose oligosaccharides at a surface or interface is important in predicting functional roles in cell recognition. Each mammalian species has its own characteristic materno-fetal glyco-code. This glycotype permits interbreeding between compatible species like the horse and donkey which have almost identical patterns of placental glycosylation, whereas the camel has a totally different placental glycosylation signature and cannot interbreed with either the horse or donkey [304]. Specific fucosylated glycoconjugates vary in abundance during the receptive and non-receptive phases of

implantation [305] and are altered in the infertile endometrium [306]. By analogy with selectin mediated intercellular adhesive interactions during extravasation of leucocytes in the innate immune response a similar process may occur at implantation between endometrial sialyl Lewis^x and trophectodermal selectins [307-309]. Overexpression of FUT 7 in a mouse implantation model promotes embryo adhesion and implantation [310, 311]. Thus fucose oligosaccharides may serve molecular recognition roles both in the fertilization and implantation stages of reproduction.

Defucosylation of the EGF domain from urokinase-type plasminogen activator abolishes its mitogenic activity despite having no effect on its binding properties at the cell surface , thus *O*-fucosylation can modulate ligand-receptor interactions necessary for productive signal transduction outcomes. *O*-fucose residues are also present on EGF domains of the mammalian Notch receptors, a family of transmembrane cell-fate determining signaling proteins during somite formation, neurogenesis, angiogenesis, and lymphoid development. Ligand-induced Notch signaling events are impaired in a fucose-deficient cell line but can be restored by correction of the fucosylation defect, indicating that *O*-fucosylation of Notch affects its interactions with ligands and is in line with functional roles for L-fucose in molecular recognition [312, 313]. Such *O*-fucosylation effects are not limited to EGF domains, as glucose-extended fucose modifications (Glc-Fuc-O -Ser/Thr) have been demonstrated in three thrombospondin type 1 repeats on thrombospondin-1 [78] and it remains to be determined if the activity of additional protein modules can also be modified by *O*-fucosylation. Fucosylated glycans impact on the pathogenesis of several human diseases. Expression of A and B blood group antigens (Fig 8a) is lost in many tumours accompanied by increases in H and Lewis-Y expression associated with poor clinical prognosis [314, 315]. Up-regulation of sialyl Lewis-X and sialyl Lewis-a occurs in many cancers associated with advanced tumor grade and poor prognosis. Increased expression of fucosylated serum immunoglobulins (Fig 6f-h) is evident in juvenile and adult rheumatoid arthritis [260, 261] its contribution to the pathogenesis of inflammatory arthritis is not known or whether this is a secondary effect due to an upregulation in fucosylation driven by an autoimmune response. Elevated fucosylation of mucins has also been observed in cystic fibrosis, accompanied by a decrease in sialylation [278]. Fucosylation impacts on leukocyte recruitment, selectin-selectin ligand interactions and the development of numerous pathological processes, including atherosclerosis, reperfusion injury following ischemic events, inflammatory skin diseases, and asthma [316]. A reduction in the density of cell surface fucosylated glycans in patients with LAD II (also known as congenital disorder of glycosylation) [280] results in recurrent infections due to defective selectin ligand biosynthesis and an impairment in the innate immune response. Mental retardation and skeletal abnormalities are also prominent features in LAD II, but it is not known if these are due directly to fucose-dependent processes, such as *O*-fucosylation of Notch receptors or are due to Lewis-X mediated interactions in the embryonic brain.

4.2 Fucosylated Chondroitin Sulphate as a Therapeutic Agent

Investigations over the last 25 years [317-319] on fucosyated CS (Fuc-CS) isolated from the holothurian echinoderm marine sea cucumber (*Holothuroidea* class) has identified a family of molecules with great therapeutic potential in a number of physiological processes (Fig 4h). The Fuc-CSs consist of a core structure consisting of CS-E and CS-A disaccharides with CS-A constituting 10-50% of the disaccharides. Sulphated fucose side chains are attached through *O*-3 to GlcA of the core structure. These fucose residues can be monosulphated or disulphated at the 2,4 or 3,4 positions (Fig 4g, h), side chains and disulphated GlcA in the core structure have also been detected adding to the structural complexity and charge density of the Fuc-CSs. Native Fuc-CS preparations have been isolated up to 64 kDa in size and for some therapeutic applications these have been depolymerized to smaller 3-12 kDa forms. The branched sulphated fucose side chains are important features of the Fuc CSs [320, 321]. Synthetic branched 2,4-di-*O*-sulphated fucosylated CS glycoclusters have been prepared in order to reproduce this structural feature, these display anti-coagulant properties and specific inhibition of intrinsic coagulation pathways [322]. Furthermore, the activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin times (TT) of these polymers can be fine-tuned for specific therapeutic applications [322]. The structural features of the Fuc-CSs have been investigated using high-resolution Fourier transform ion cyclotron resonance mass spectrometry [323] and to chemical and NMR spectroscopic structural investigations [324, 325]. In NMR and molecular dynamic simulations, the Fuc-CS repeat unit adopted a similar conformation to the Lewis-X blood group determinant [326, 327]. This structure accommodates the localization of several sulphate

groups in close proximity to one another and these form large negative patches which are distributed along the helical CS backbone of Fuc-CS [326, 327]. Native Fuc-CS preparations display anti-coagulant [323, 328-331] anti-angiogenic [329], anti-inflammatory, blood lipid lowering properties [323, 328-333], and stimulate haemostasis [334], promote neurite outgrowth [335], anti-cancer [336] and potent HIV-1 gp 120 protein binding properties which inhibit HIV replication by preventing viral entry into cells [337]. While neurite outgrowth promoting properties have been noted for native Fuc-CS preparations, a synthetic Fucose-CS trisaccharide (Fig 4j) has been shown to be more potent than CS-E tetrasaccharides (Fig 7) for the outgrowth of DRG hippocampal neurons in monolayer culture. The molecular recognition properties of specific glycan structures can therefore be employed in therapeutic interactions of physiological importance and undoubtedly when further information becomes available on these structures will also be employed in improved applications in repair biology.

5. Conclusions

Fucose, CS and KS have evolved properties of molecular recognition and information transfer which equips the proteoglycans and glycoproteins they are attached to with properties as cellular mediators controlling cellular behaviour in a number of physiological processes and in neural development and repair. A greater understanding of this evolved glycocode and how it regulates cells may allow a greater understanding of physiological and repair processes and how these might be manipulated in order to improve therapeutic interventions developed in response to altered glycodynamics in neural disorders. These are expected to improve repair responses in cognitively impaired brain tissues.

Legends to Figures

Figure 1.

Morphological features of cultured neurons. Fluorescent images of cultured neural iPSCs (a) and neuroblastoma cells (b) and IPSCs stimulated with nerve growth factor (c). In (a) cell nuclei were stained with Hoechst 33258 DNA stain, axons with anti-tubulin Alexa 488 and dendrites and synapses with anti-F actin phalloidin Alexa 568. In (b) cultured mouse neuroblastoma cells were stimulated with retinoic acid to induce differentiation. Nuclei (i.e. DNA) are stained yellow; microtubules (anti-tubulin antibody) are cyan; f-actin (fluorescent phalloidin) is purple. The image was pseudo- coloured, individual channels were initially recorded with the regular red/green/blue fluorophors (i.e. Alexa 488 and 568 and DAPI) then pseudo coloured as shown. Images a, b, c supplied courtesy of Torsten Wittmann, PhD, Dept of Cell and Tissue Biology, University of California, San Francisco, USA. Indirect fluorescent immunolocalisation of paraformaldehyde fixed rat hippocampal neurons with anti-synapsin-1/2 (red, Alexa 488) raised to a synthetic peptide corresponding to amino acids 2 to 28 from rat Synapsin-1 (UniProt Id: P09951)[cat # 106-006] and mouse anti-microtubule associated protein (MAP-2, green, FITC) [cat# 188-011], nuclei stained with DAPI (blue) (d). Image d supplied courtesy of Synaptic Systems.

Figure 2.

Neural structural organisation and synaptic neurotransmitter transmission. Artistic rendition of a synapse with progressive stages of transmission of neurotransmitters across the synaptic gap to a communicating neuron (a-c). Diagrammatic representation of SV2A proteoglycan intercalated in the plasma membrane of a synaptic vesicle and the Ca2+ /neurotransmitter (GABA) smart gel complex formed by interaction with the KS side chains of SV2A which forms a neurotransmitter transport delivery system (d). Pseudo coloured TEM of a synapse (X 50,000) courtesy Science PhotoLibrary. Michondria (purple), synaptic vesicles (red), synaptic gap (pink) (e). Diagrammatic depiction of a synaptic bouton with 1. microtubular transport system which transports the neurotransmitters generated in the neural cell body. 2. mitochondria and 3. synaptic vesicle accumulation in the synaptic terminal and 4.the post synaptic neurotransmitter receptors and voltage gated ion-channels on a communicating neuron which deliver neurotransmitters such as GABA (as shown) (f). Higher power view of the boxed area in (f) showing details of the depolarisation of the synaptic membrane and merging of synaptic vesicle plasma membrane and delivery of Ca2+ and neurotransmitters across the synaptic cleft to neurotransmitter receptors and voltage gated ion channels in a communicating neuron (g). Details of synaptic vesicles adjacent to the synaptic membrane of the synaptic cleft viewed by TEM (h). Plates a-c from Shutterstock. Plate d modified from [338] and f-h modified from Becker, W., Hardin, J., Bertoni, G., Kleinsmith, L. (2012). Becker's World of the Cell (8th ed.). Boston, MA: Benjamin Cummings. Source: http://www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-13/13_16.jpg A neuron and surrounding glial cells. (i) H & E stained section of neural tissue depicting a central large neuron body with multiple prominent dendritic processes surrounded by numerous small glial cells. Image obtained from Pinterest. Cartoon depicting the co-ordinated interplay between neurons and blood vessels mediated by astrocytic interconnections (j).

Figure 3.

Immunolocalisation of aggrecan in perineural nets using MAb 1-B-5 following chondroitinase ABC digestion of rat brain (a), rat dorsal root ganglion (b) or in isolated neurons (c, d). Images courtesy of Caterson archive, BioImaging Unit, University of Cardiff.

Figure 4.

Fucosylation of KS-I (a) and KS-II from the KS rich region of aggrecan (b) and KS-II chains located within the CS2 region of the aggrecan core protein (c) which are detected by MAb 3D12H7. Fucose modified glycan structures. The structural diversity of the L- fucose containing glycan chains of *O*-linked glycans of the mucin family (d-f), small glycan chains where L-fucose is linked directly to *O*-serine residues of the glycoprotein or proteoglycan core protein and which acts as an acceptor molecule for subsequent additions of additional saccharides (e) and the 6-sulphated Lewis-X epitope which has a widespread distribution in glycoproteins (f).Fucosylated chondroitin sulphates (Fuc-CS) of therapeutic potential (g). Representative structure of a native Fuc-CS [327] isolated from sea cucumber (g) The structure shown is that specified for a Fuc-CS displaying interactive properties with P-and L-selectin which prevented

selectin mediated extravasation of neutrophils in inflammation. The fucose side chains of Fuc-CS display some structural variation as shown in the proportion of mono- and disulphated fucose side chains (h). Structure of the CS-A and CS-E disaccharide core structures (i). The CS-A content of the core varies between 10%-50%. Structure of a fucosyl-CS trisaccharide (j) which displays potent neurite outgrowth promoting activity[335].

Figure 5.

O-fucosylated PSGL-1, CD-34 and Notch-1. Schematic depiction of the structural organization of PSGL-1 of leucocytes which facilitates binding to P-selectin in the endothelium (a). PSGL-1 is heavily substituted with O- linked L-fucose oligosaccharides containing the sialyl Lewis X epitope (b). A terminal sialyl LeX epitope is associated with sulphated tyrosine residues which are important for the interactive properties of PSGL-1 with P-selectin. Fucose modifications on branched glycan structures in PSGL-1 (b). The structural diversity of the L-fucose containing glycan chains in *N*-linked glycans displaying tri-antennary, tetra-antennary, high mannose, bi antennary glycan chains and those bearing the N-linked 6-sulphated Lewis-X epitope.

O- and *N*-fucosylation of CD-34. Diagrammatic representation of the CD-34 cell surface receptor that is widely expressed by a number of microvascular cells in brain development (c-f). N- and O- linked 6-sulphated Lewis-X motifs are prominent interactive components of CD-34 (c, e, f) these are shown distributed along the core protein of a model of the transmembrane CD-34 molecule (d).The key contribution of *O*-fucosylation to Notch functional organization (g). Structural representation of the Notch-1 receptor modular structural organization and the glycosylation of its epidermal growth factor (EGF) repeat units which conveys its interactive properties with a number of ligands (h). The EGF repeats are particularly heavily substituted with L-fucose containing glycans as shown, while the other glycosylations have a regulatory role to play over the interactive properties mediated by L-fucose glycans. Blood vessels can be visualized in human brain tissues using CD-34 immunolocalisation, 5 week post conception spinal tissue (i). Abbreviations HB , hind brain; OT, otic nerve; SC, spinal cord. Image obtained by Open Access from llgård K, Dziegielewska KM, Holst CB, Habgood MD, Saunders NR. Brain barriers and functional interfaces with sequential appearance of ABC efflux transporters during human development. Sci Rep. 2017 ;7(1):11603.

Figure 6.

Fucosylated blood group antigens and immunoglobulins. Fucose containing glycan chains attached to red blood cells which identify the A, B, O blood types (a). Glycan chains attached to serum immunoglobulins (b) which determine ADCC toxicity which some IgGs elicit (c-e) and an inflammatory response (f-h)

Figure 7 .

Neurostimulation by glycans and glycosaminoglycans. The stimulatory effect of CS-E saccharides on cultured hippocampal neurons. Immuno-fluorescent localisation of neurons cultured ± CS-E saccharides using anti-tau FITC antibodies (a-d). Control (a), CS-E disaccharide (b), unsulphated CS-E tetrasaccharide (c), d. CS-E tetrasaccharide (d), Scale bars =45 μm. The minimum size of CS-E required for neuronal stimulation was a CS-E tetrasaccharide, the non sulphated CS-E tetra-saccharide and CS-E disaccharide were both non-stimulatory. This contrasts with CS-A and CS-C which inhibit neural outgrowth. L-fucose and related sugars also have stimulatory properties of on cultured hippocampal neurons (e-h). L-fucose (6-deoxy L-galactose), D-galactose and deoxy D-galactose saccharides all influence the morphology of cultured hippocampal neurons. Immunofluorescent detection of dendrite outgrowth from cultured hippocampal neurons using FITC labeled anti-tau antibodies. Control, L-fucose (e), 2-deoxy L-galactose (f); 2-deoxy L-galactose + D-galactose (g); 3 deoxy L-galactose (h). 2-deoxy D-galactose has an inhibitory effect on dendrite outgrowths. The structures of these sugars are shown in (i)-(m). The structure of an L-Fuc analogue, 6 alkynyl Fuc which is used in the labeling and tracking of O-Fucosylation in glycoproteins is also shown (n) [79]. Images a-h reprinted (adapted) with permission from from Murrey HE, Hsieh-Wilson LC. The chemical neurobiology of carbohydrates. Chem Rev. 2008 ;108(5):1708-31,and Tulley SE, Mabon CI, Gama CI, Tsai SM, Liu X and Hsieh-Wilson LC. J am Chem Soc 2004; 126, 7736-7737 copyright (2004 and 2008) American Chemical Society.

Figure 8.

Diagrammatic depiction of interactive structures on the surface of neurons and the contributions of CS-proteoglycans to neural growth arising from interactions with cell surface integrins, laminins and pericellular collagen and fibronectin fibres and hyaluronan(a) and RPTP-σ (b)which acts as a CS-receptor (1). Neural outgrowth arising from the interaction of RPTP-ζ with NCAM (neural cell adhesion molecule) (c,d) (2). Cellular proliferative and cell survival effects stimulated by delivery of fibroblast growth factors to their receptors (FGFR)(e) (3). Chemorepulsive cues generated by interaction of CS-proteoglycans in perineural net formations with semaphorin 3A which is normally an attractive guidance cue. This inhibitory signal is generated by interactions of Sem3A with neuropilin-1 and neuroplexin (f,g). Semaphorin 5A also interacts with cell surface CS-proteoglycans generating a signal which inhibits nerve outgrowth (h). Figure modified from L Djerbal, H Lortat-Jacob, JCF Kwok Chondroitin sulfates and their binding molecules in the central nervous system Glycoconj J. 2017; 34(3): 363–37. Open Access.

Figure 9.

Diagrammatic illustration of the interaction of progranulin dimer with CSPG4 (a) and Eph A2 showing the resulting phosphorylation of Eph A2 and cross-talk with adjacent receptors leading to their activation. Figure available under a Creative Commons License (Attribution–Noncommercial–Share Alike 3.0 Unported license), http://creativecommons.org/licenses/by-nc-sa/3.0/). Bateman A. Progranulin and the receptor tyrosine kinase EphA2, partners in crime? J Cell Biol. 2016;215(5):603-605.

Figure 10.

Schematic depiction of the structural organization of transmembrane RAGE showing its extracellular, transmembrane and cytoplasmic portions and the glycan interaction region which acts as a receptor for AGEs and highly charged CS isomers such as CS-E. Diagram reproduced from Hegab Z, Gibbons S, Neyses L, Mamas MA. Role of advanced glycation end products in cardiovascular disease. World J Cardiol. 2012;4(4):90-102. Under Open Access creative commons attribution Non Commercial (CC BY-NC4.0) licence with permission of Bashideng Publishing Group, CA, USA.

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