Brief Notes to lecture Disorders of Glycosylation part II

2. A few numbers illustrating the importance of glycosylation. Genome, proteome, glycome
Glycan synthesis: No template as for protein
3. N- glycans (linkage to amide group of Asn); O-glycans (linkage to OH group of Ser/Thr or hydroxyLys); Larger number of cores structure, larger diversity than N-glycans; cytoplasmic O-GlcNAc: an antagonist to phosphorylation; C-glycans (linkage to Carboxyl of Trp). C-glycan are rare.

**N-Glycosylation**

6. Lysosomal enzymes are glycoproteins. In the ER they receive a Glc3Man9GlcNAc2 that is further processed to Man8. To be targeted to lysosome they need a Mannose phosphate signal.
7. N-acetylglucosamine-l-phosphotransferase is an essential enzyme for the synthesis of a mannose-6-phosphate recognition marker that targets lysosomal enzymes to the lysosome. Defect in lysosomal targeting results in progressive accumulation of glycoprotein and glycolipids.
13. Connective tissue gives shape and structure to tissues in the body and holds them in place.
14. New glycosylation site causes protein misfolding and rapid degradation or the new glycans impair the oligomerisation or formation of signalling complex

**O-Glycosylation**

16. O-Glycans are classified on the basis of he first sugar attached to Ser/Thr; O-GalNAc: most common form of O-glycosylation; ;Tn + mucins; several hundreds of mucins O-glycans structures. Other common type of O-Glycosylation with Large structure diversity: GAGs; attached via tetralinker GalAbeta1-3Galbeta1-3 Galbeta1-4Xyl-Ser; GAGs are long unbranched polysaccharides containing a dissacharide repeat units; O-mannosyl glycans are less common and have been mainly characterised on alpha-dystroglycan; O-Glc and O-Fuc are rare types of Protein glycosylation found on a few proteins such as Notch and Thrombospondin-1
17. Restricted to proteins transiting by the secretory pathway (ER, Golgi, Lysosomal; plasma membrane and secreted proteins). How proteins are recognised by Glycosyltransferases remains unknown.

**O-mannosylation: Congenital muscular dystrophies**

19. Abundant in yeast, Essential for viability.
In human, limited to a few proteins in brain, nerve and skeletal muscle
In brain about 1/3 of O-glycoproteins are O-mannosylated proteins such as alpha-dystroglycan, the best charaterised O-mannosylated protein
20. The dystrophin glycoprotein complex (containing alpha- and beta-dystroglycan) connect the extracellular matrix (ECM) to the cytoskeleton in many tissues. In muscle actin is linked to beta-dystroglycan which spans the cell membrane. The extracellular domain of beta-dystroglycan binds to alpha-dystroglycan, which in turn binds laminin 2 in the ECM. The presence of sialic acid in the most commonly added glycan is determinant for laminin 2 binding.
Interaction necessary for neuron migration and muscular function. Affecting glycosylation of alpha- dystroglycan results in congenital muscular dystrophies, genetic diseases that cause progressive muscle weakness. Not all CMDs are due to impaired O-glycosylation; eg. Duchenne Dystrophy: defect in dystrophin.
21. Co-expression of POMT1 and POMT2 is necessary for the enzymatic activity of O-Mannosyltransferase.
22. Mutations in POMT1 or POMT2 give the same phenotype.
MEB and WWS: In both case the protein is inactive, factors determining severity are unknown

**O-Xylose**

27. Proteoglycans are proteins substituted by GAG, the most abundant heteropolysaccharides in the body; The linkage of GAGs to the protein involves a specific trisaccharide coupled to the protein core through an O-glycosidic bond to a Ser residue in the protein. Some forms of keratan sulfates are linked to the protein core through an N-asparaginyl bond. The protein cores of proteoglycans are rich in S and T residues, which allows multiple GAG attachments. Heparin is highly and uniformly sulfated whereas heparan sulfate is highly sulfated only in defined blocks.

Hyaluronic is unique among the GAGs in that it does not contain any sulfate and is not found covalently attached to proteins as a proteoglycan. It is, however, a component of non-covalently formed complexes with proteoglycans in the ECM. Hyaluronic acid polymers are very large (with molecular weights of 100,000 - 10,000,000) and can displace a large volume of water. This property makes them excellent lubricators and shock absorbers.

28. GAGs are highly negatively charged molecules, with extended conformation that imparts high viscosity to the solution. GAGs are located primarily on the surface of cells or in the extracellular matrix (ECM). Along with the high viscosity of GAGs comes low compressibility, which makes these molecules ideal for a lubricating fluid in the joints. At the same time, their rigidity provides structural integrity to cells and provides passageways between cells, allowing for cell migration. Hyaluronic acid polymers are very large (with molecular weights of 100,000 - 10,000,000) and can displace a large volume of water. This property makes them excellent lubricators and shock absorbers.

29. Xylose added in late ER or early Golgi by a soluble Xylosyltransferase. All other transferases are membrane bound: Core synthesised by specific enzymes: eg.B4GalT7; CS and DS are synthesised if GalNAc is added first whereas HS is synthesised if GlcNAc is added first. Elongation by sequential addition of monosaccharide. Sulfate is added in the same time by various sulfotransferases that uses PAPS (phosphoadenosinylphosphosulfate) as activated donor. Sulfation also seems to regulate the chain length of GAGs.

31. Ehlers Danlos
The connective tissues support many different body parts, including the skin, muscles and ligaments. Affect collagen.

34. In the case of diastrophic dwarfism, the brain is fine, but the skeleton is deformed, and the risk of death from respiratory failure is high in infancy. Persons with diastrophic dwarfism who survive early childhood, however, are likely to enjoy a normal life span.

37. Keratan sulfate affected; Dermatan sulfate and chondroitin are larger and more sulfated.
Hypothesis: Regulation of chain length of GAG by sulfation

39. Mucins contain numerous carbohydrate chains attached to the polypeptide; Core distinguished by the second sugar added and the linkage to GalNAc
42. In addition to mucins, GalNAc linked to Ser/Thr is found in the Tn antigen. Tn antigen can be converted to core 1 T antigen by core 1 Gal. All core structures can be further extended to form complex O-glycans.

**GPI and GSL**

45. Gangliosides is composed of ceramide linked by a glycosidic bond to an oligosaccharide chain containing hexose and sialic acid. First discovered in brain by the German Ernst Klenk. 10-12% of the total lipid content (20-25% of the outer layer) of neuronal membranes. Sphingosine is usually the main sphingoid base, accompanied by the C20 analogue in gangliosides of the central nervous system mainly. Stearic acid (18:0) can be 80 to 90% of the fatty acid constituents, accompanied by small amounts of 16:0, 20:0 and 22:0.

46. Involve sequential activities of sialyltransferases and glycosyltransferases as illustrated. The required enzymes are bound to the membranes of the Golgi apparatus, in a sequence that corresponds to the order of addition of the various carbohydrate components. The sialyltransferase that catalyses the synthesis of the relatively simple ganglioside GM3 is located in the cis-region of the Golgi, while those that catalyse the terminal steps of ganglioside synthesis are located in the distal or trans-Golgi region. At least five distinct sialyltransferases are known to operate, each using CMP-SA to transfer the sialic acid residue to the oligosaccharide chain. Finally, the gangliosides are transferred to the plasma membrane by a transport system involving vesicle formation.

47. As the brain develops, there is an increase in the content of gangliosides and in their degree of sialylation. The main gangliosides of human brain are GM1, GD1a, GD1b and GT1, while GM3 is found mainly in the extraneural tissues. Gangliosides are believed to be functional ligands for myelin stability and the control of nerve regeneration by binding to a specific myelin-associated glycoprotein. Cell-type specific antigens that control growth and differentiation of cells, and to have an important role in the interactions between cells; They act as receptors of interferon, epidermal growth factor, nerve growth factor and insulin and in this way may regulate cell signalling.