Bio-Lubrication

Structural Investigation of Lubricin and its Glycosylation

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I. Ali, L., Kenny, D.T., Hayes, C.A., and Karlsson, N.G. (2012) **Structural Identification of *O*-Linked Oligosaccharides Using Exoglycosidases and MSn Together with UniCarb-DB fragment Spectra Comparison**. *Metabolites*. 2(4): 648-666.

II. Ali, L., Jin, C., and Karlsson, N.G. (2012) **Glycoproteomics of Lubricin-Implication of Important Biological Glyco- and Peptide-Epitopes in Synovial Fluid**, **In Rheumatoid Arthritis –Etiology Consequences and Co-Morbidities**. *Intech*. 131-150

III. Flowers, S.A., Ali, L., Lane, C.S., Olin, M., and Karlsson, N.G. (2013) **Selected reaction monitoring to differentiate and relatively quantitate isomers of sulfated and unsulfated core 1 *O*-glycans from salivary MUC7 protein in rheumatoid arthritis**. *Mol. Cell. Proteomics*. 12: 921-931

IV. Ali, L., Flowers, S.A., Jin, C., Bennet, E.P., Ekwall, A.K., and Karlsson, N.G. (2014) **The *O*-glycomap of Lubricin, a Novel Mucin Responsible for Joint Lubrication, Identified by Site-Specific Glycopeptide Analysis**. *Mol. Cell. Proteomics*. Accepted

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The sliding articular cartilage surfaces of the human diarthrodial joints are surrounded by biolubricating synovial fluid (SF), creating a perfect low friction biological biobearing structure with excellent lubrication and wear resistance, even during motion. Lubrication is predominately provided by surface adhered biomolecules including phospholipids, hyaluronic acid and synovial lubricin. Inflammation, such as arthritis and injury, changes the joint assembly resulting in detachment of essential surface molecules, increasing friction and wear of the sliding articular cartilage. Changes in the composition and distribution of these surface molecules is suggested to aggravate the disease. The lubricative, heavily glycosylated mucin-like synovial glycoprotein, lubricin, has previously been observed to contain glycosylation changes related to rheumatoid arthritis and osteoarthritis. Therefore, a structural investigation of lubricin and its glycosylation was initiated in order to better understand the biolubricating ability of lubricin and its pathological involvement in arthritis diseases. An investigation was undertaken to better understand the nature of the dominant glycan structure, sialic acid. Sialidase specific for α2-3 linked sialic acid and subsequent UniCarb-DB fragment spectra comparison of the resultant structure indicated the exclusive 3-linked nature of core 2 sialylation. However, core 1 structures had both 3 and 6 linked sialylation. In arthritis, lubricin has been shown to degrade as identified by its fragments in the synovial fluid. This may open up a new possibility for identification of disease specific biomarker. The mass spectrometric glycopeptide analysis showed that lubricin contains an extended serine, threonine and proline (STP) rich domain composed of imperfect tandem repeats (EPAPTTPK), the target for *O*-glycosylation. The *N*-acetylgalactosaminyltransferase (*GALNTs*) expression analysis of the fibroblast-like synoviocytes showed high expression of the less understood *GALNT5* and *15* in addition to the ubiquitously expressed *GALNT1* and *2*. This indicated that lubricin required a unique combination of transferase genes for its glycosylation.

Overall, this study revealed that negatively charged sialic acid in the mucin-like domain makes the lubricin molecule amphoteric in nature, as the arginine and lysine rich protein core is positively charged. The number of glycosylation sites and sialylation were shown to be essential for this amphoteric nature and may be important for its function as an amphoteric biolubricator.

Keywords: Lubricin, mass spectrometry, EPAPTTPK, GALNTs, biolubricator

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