Abstract

Glycoproteins are essential for the biological functions of life’s processes and thus today are the focus of a considerable area of research. The functions of glycoproteins are tremendously broad, impossible to adequately summarize, and include cell attachment recognition, homeostasis, transport of molecules, and enzymatic and immunology domains. One of the principal axes of glycoprotein research is gaining a better understanding of the correlation between glycan structure and function. Answering such questions allows solving crucial biological inquiries as the glycoproteins structure is directly correlated to the dynamicity of their life cycle and their environment. Accurate comparison of isoforms and quantification of glycosites is an essential step in this direction. Mass spectrometry has emerged as a powerful analytical technique in the field of glycoprotein characterization. Its sensitivity, high dynamic range, mass accuracy provide both quantitative and sequence/structural information. As part of the 2012 study we explored the use of mass spectrometry and ancillary methodologies to characterize differential glycoforms of human prostate specific antigen (PSA). PSA is used as a tumor marker for prostate cancer with increasing amounts indicating a distinction between normal and cancer or benign prostate hypertrophy. The oligosaccharide on PSA is believed to be biantennary N-linked and it has been observed that prostate cancer tissues and cell line contain more antennas than the benign form. Thus mapping of glycans and their quantification may be very important to determine the distinction between PSA from cancer and PSA related to hypertrophy. We focused our study on using standard peptide based proteomics/glycomics methodologies including LC-MS/MS for peptide sequencing and label-free approaches for differential quantification. An overview of approaches for differential characterization will be presented along with a prospectus on the challenges faced by researchers in this area. We gratefully acknowledge Lee Biosolutions for the gift of PSA for the gPRG2012 Quantitative Glycoprotein Study.