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Towards Immunoglobulomics - IgG, IgA and IgM Fc Profiling by Light Chain Affinity Capturing by Nano-LC-MS

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Summary

The constant domain (Fc) of antibodies is responsible for the binding to effector cells and thereby triggering downstream immune responses. However, antibodies exhibit post-translational modifications (PTMs) with glycosylation as one of the most important PTM which can modulate these immune responses. Until now endogenous antibodies and their PTMs are characterized using bottom-up approaches resulting in a loss of information on the combination of multiple PTMs. Due to new developments in mass spectrometry, intact or middle-up protein analysis are increasingly applied for glycoform characterization, since it provides comprehensive structural information compared to bottom-up analysis. In middle-up analysis, antibodies are cleaved via specific proteases into two subunits, Fc and variable domain (Fab). So far, only IgG has been studied using middle-up approaches. Therefore, endogenous IgGs are captured by FcXL beads which bind to the Fc of IgG, followed by a hinge-region cleavage by IdeS and elution of the Fc subunits under acidic conditions. In this project, we have developed a middle-up analysis platform for sequential Fc profiling of IgG, IgA and IgM. To capture different antibody isotypes a light chain affinity capturing, using a mixture of kappa and lambda light chain beads, was established. After capturing, the Fc/2 subunits of IgG, IgM and IgA1 were sequentially released by specific proteases directly providing the Fc portions and eliminating any elution step. The Fc/2 subunits of each isotype were individually analyzed by nano-RPLC-MS. Comparing the light chain capturing and traditional FcXL approach no bias for IgG Fc/2 profiles could be observed. For IgM and IgA Fc/2 subunits very complex glycosylation profiles containing multiple N-glycosylation sites were detected which were annotated by integrating the middle-up and site-specific bottom-up information. Next to glycosylation, other modifications such a C-terminal tyrosine truncation were observed. In addition to Fc/2 subunits, the corresponding joining chains from IgA and IgM were detected and different glycoforms could be annotated. The proposed method was applied to characterize three independent donors resulting in different profiles, therefore illustrating the potential of the approach to study antibody Fc/2 changes.