Full validation of a high-throughput Immuno-MS PD-assay by LC-MS MRM

Translational Mass Spec

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Many compounds for the treatment of muscle-hypotrophy are currently being developed. Biomarker/PD-readouts are needed for monitoring target-engagement, efficacy, ectr. Myostatin is secreted as a preprotein that is cleaved by BMP-1 family proteases to seperate the 35-40 kDa propeptide from the 25 kDa mature protein. The bioactive form is a disulfide-linked dimer of the mature protein and forms a latent complex with two non-covalently-associated molecules of the propeptide. Amongst other ligands, myostatin binds to the ActRII-receptor. Myostatin is therefore well suited to monitor development of muscle-pathogenesis and/or drug-intervention.

Antibodies have been raised against the active part and potential other isoforms. The combination of immune-enrichment and LC-MS MRM allsow not only the monitoring of active myostatin, but also the preprotein and potential interaction partner. This in turn can provide insights into the regulation of this ligand.

In order to monitor this in a large number of human samples, a high throughput assay was developed, were Myostatin is captured by a biotinylated anti-myostatin-antibody which is immobilized on a streptavidin-coated 96-well plate. After a few washings, signature-peptides from myostatin, the pro-protein and/or potential interaction-partner are directly eluted by tryptic digest in the plate. After enrichment by online-SPE, peptides were separated by an UPLC C-18 17-min gradient. Peptides were fragmented and detected by an AB Sciex 5500 MS.

The assay was then validated according to in-house guidelines, including Intra-assay –and inter-assay accuracy and precision in 4 different sera, matrix-effects, and short and long-term stability. A linear range from 3 – 200 ng/mL was established. After successful validation, samples from healthy volunteers and first patient-samples could be measured. After 9 month, a series of samples was analysed with a very low level of variability between the 2 measurements, thereby showing the robustness of this method.