"Targeting recombinant EPO by LC-MS analysis of intact glycopeptides"

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Project Summary:

In this project, we want to detect recombinant EPO in plasma samples by liquid chromatography - mass spectrometry through the analysis of intact glycosylated peptides.

We hypothesize that with the new fragmentation techniques such as EThcD, high energy HCD and laser photodissociation, present in the latest orbitrap MS analyzers, it is feasible to detect EPO in body fluids after thorough purification protocols. We aim thus to develop a purification protocol that after a specific EPO immunocapture from plasma samples, it extracts and fractionates glycopeptides based on their disctinct glycan structures through different LC resins. After comprehensive offline fractionation, then we will quantify these intact glycopeptides through parallel reaction monitoring (PRM) acquisition. We will independently monitor each different type of recombinant EPO through trypsin or AspN proteotypic peptides, characterized by different glycan structures that will elute and fragment distinctively.

Results and Conclusions:

Electrophoretic methods provide the sensitivity for distinguishing recombinant and endogenous EPO in human body fluid samples. However, the detection of EPO in biological samples containing both endogenous and recombinant EPO remains challenging. Mass spectrometry (MS) has emerged as a powerful tool to efficiently provide a direct view of glycoprotein profiles and it is useful for assessing differences in natural and recombinant EPO variants.

In this research project, we demonstrated the potential of native MS analysis at the intact protein level for the determination of complex glycosylation profiles of EPO. We demonstrated the feasibility of using intact glycopeptide profiles for characterizing EPO produced in CHO cells and HEK293 cells, respectively. We generated a large list of potential targets for distinguishing recombinant and endogenous EPO in human body fluid samples. The selection criteria will include the feasibility of lowering the limit of detection compatible with human sample analysis, and selection of non- overlapping glycoforms for specific and sensitive EPO detection.

We propose an integrative approach combining native MS analysis at the intact EPO protein level with intact EPO-glycopeptide MS analysis to facilitate comprehensive characterization of EPO produced in different cell systems. Integration of data from these two approaches will confirm the feasibility of EPO-glycopeptide characterization, and ultimately facilitate the selection of potential mass spectrometry-based biomarkers for doping control.