"Quantitative Proteomics of rhGH-Doping by Multiplexed Stable Isotope Labeling and MALDI-TOF/TOF Mass Spectrometry"

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Project overview

The detection of doping with recombinant peptide and protein hormones (e.g. human growth hormone -hGH, erythropoietin - Epo) is one of the most challenging analytical problems in doping control. Rapid metabolic degradation paired with a low medical dosing strategy reduces the time window for a successful direct detection by immunological methods in the case of hGH to just a few hours after the last application.

Multiplexed stable isotope labeling of serum or plasma proteins combined with mass spectrometric readout allows the *identification and quantification of thousands of proteins and peptides* and *their individual ratios for the doped versus the non-doped case*. In this project a novel isotope labeling strategy termed *iTRAQ* will be employed on blood samples of athletes who received recombinant human growth hormone over a period of several weeks. In parallel, a second group of athletes who received a placebo pharmaceutical containing no hGH will be compared with the hGH group. The blood serum proteins of each athlete will labeled with an *isotope tag* which has an exclusive *reporter group*. This tag results in a specific reporter peak during mass spectrometric fragmentation. Different isotope tags will be used for both groups permitting relative quantification of the peptides and proteins in each sample. Thus, a *quantitative study of the gene expression at the level of the blood proteome* will be performed. Previous studies employing this technique for disease diagnosis resulted in a panel of several hundreds of differentially expressed peptides and proteins, which were regarded as potential biomarkers for that disease. Hence, we are expecting similar results for the hGH-doping study. These hundreds of biomarkers can be easily monitored within single LC/MS-runs. Thus, this approach holds the potential for resulting in a *fast screening method for hGH-doping being independent of the availability of antibodies* to certain protein isoforms.

Results and Conclusions

Multiplexed stable isotope labelling of plasma proteins combined with mass spectrometric analysis allows the identification and relative quantification (expression levels) of thousands of proteins and peptides for comparing different physiological states (e.g. doped versus non-doped). In this project, an isotope labelling technique termed iTRAQ (isobaric tag for relative and absolute quantitation) was used for blood samples of athletes, who received recombinant human growth hormone over a period of three weeks. A second group of athletes, who received a placebo containing no hGH was compared with the hGH group. Blood plasma proteins of each athlete were labelled in a day-dependant manner via iTRAQ with isotope tags containing characteristic reporter groups. During mass spectrometric fragmentation of the peptides these tags resulted in specific reporter ion peaks (i.e. *m/z* 114, 115, 116, 117). Thus, a quantitative study of the gene expression on the level of the blood plasma proteome was performed. After bioinformatics and statistical analysis differentially expressed proteins were regarded as candidate biomarkers for the hGH-treatment. During the two years of this project following milestones were achieved:

1. Successful conduction of a hGH administration study (8 hGH-treated, 8 placebo-treated athletes; year 1);

2. Successful testing of the obtained samples regarding their validity for the subsequent proteome analysis approach (WADA hGH isoform test; year 1)

3. Establishment of a plasma proteomics workflow including 3D-separation technology (year 1)

4. Analysis of samples of all test persons using the workflow established during the first year (year 2)

5. Bioinformatic analysis of all mass spectrometric raw data in order to identify proteins, obtain relative protein quantities, and identify potential biomarkers for hGH-treatment (year 2)

6. Data analysis using a systems biology approach (year 2)

Conclusion: After rigorous data filtering and statistical analysis, IGF-I and related proteins as well as two new biomarkers for potential long-term detection of hGH abuse were discovered (**Mannose-binding protein C** [MBL2, UniProt B1PN75] and **Dynein-related protein** [UniProt Q99499]). The latter protein remained overexpressed even 14 days after cessation of hGH-treatment.