Project Review

"Application of microarray technology for the detection of changes in gene expression after doping with recombinant hGH – part 2"

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The present project aims to establish microarray technology as a new analytical tool into the field of doping control analysis to gain insight in specific effects of recombinant human growth hormone (hGH) on blood cells (leukocytes). The obtained information shall be finally used for the development of a selective "hGHmicroarray", applicable for hGH-doping control in the near future.
Experience and expertise gained along this project will be of general importance and interest for developing similar assays for other doping substances, where so far also no satisfying analytical test exists.
Preceding the current project a feasibility study was already done on cell lines of specific leukocyte subsets (THP-1/monocyte, H9/T lymphocyte, RA-1/B lymphocyte), PBMCs (peripheral blood mononuclear cells) from healthy donors, respectively which had been treated in vitro with hGH. Comparing gene expression profiles of treated and untreated cells on genome wide microarrays, numerous genes could be identified that showed a specific response to hGH treatment.
In the current project time series gene expression studies will be performed on PBMCs obtained from athletes receiving a 3 weeks' hGH-treatment in comparison to healthy controls. To find additional hGH-doping candidate genes, suppression subtractive hybridization (SSH) technology will be applied on PBMCs from hGH-doped athletes and non-doped individuals. After confirming SSH-genes to be differentially expressed in a series of microarray experiments, oligonucleotide probes will be designed for both SSH-genes and the candidate genes identified along gene expression profiling using whole genome arrays. To establish a selective "hGH-microarray" the newly designed oligonucleotide probes for the hGH-candidate genes will be printed on a microarray. The "hGH-microarray" will then be thoroughly evaluated by performing numerous gene expression experiments on PBMCS from hgh-treated and untreated individuals. Results from microarray studies on the hGH-microarray will be spot check-like validated with microarray-independent methods such as quantitative PCR (Taqman).

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Results and Conclusions

Testing for capture antibody specificity using various recombinant doping-relevant antigens we found that for the same capture antibodies cross reactivity with other antigens and for some antibodies antigen affinity was rather modest leading to detection limits which were not suited for detection of doping relevant substances in serum/plasma or urine.

In the case of hGH detection, on which we were focusing in this project, we were not able to detect any reliable changes in hGH levels in samples which had been treated with hGH compared to placebo-treated controls even when testing with 5 different types of hGH antibodies on the microarray. The inability of detecting changes in hGH is most likely related to the detection limit (caused by rather low affinity of the capture antibodies used) and to the fact that matrices such as serum and plasma contain interfering substances which obviously hinder antigen detection. The latter could be clearly seen in experiments where hGH antigen was spiked in PBS compared to undiluted serum or plasma or in 1:10 diluted serum or plasma samples. Whereas spiked hGH antigen could be detected in PBS and the diluted serum/plasma samples, it was not possible to detect an increase in hGH in undiluted plasma or serum samples.