

PROJECT REVIEW

“Application of Microarray Technology for the Detection of Changes in Gene Expression after Doping with Recombinant Human Growth Hormone”

G. Gmeiner, C. Nohammer, C. Reichel, R. Pichler (ARC Seibersdorf Research, Seibersdorf, Austria)

The present project aims to introduce 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). Microarray technology will be used to search for changes in leukocyte gene expression which are directly related to the application of human growth hormone (hgh). Knowledge generated along this research project and the introduction of microarray technology into doping analysis should open novel approaches and strategies for future detection of doping substances.

In the first phase of the project cell lines of specific leukocyte subsets (THP-1/monocyte, IM-9/T lymphocyte, H9/B lymphocyte), PBMCs (peripheral blood mononuclear cells) from healthy donors, respectively will be treated in vitro with hgh to identify candidate genes influenced by hgh-application. Whole genome cDNA microarrays as well as oligonucleotide microarrays including leukocyte-relevant genes will be used to characterize potential hgh-specific genes by comparing gene expression profiles of treated and untreated cells.

To find additional hgh-candidate genes, suppression subtractive hybridization (SSH) technology will be applied on hgh-treated PBMCs and on those cultured leukocyte subsets which showed a response to hgh before during in vitro studies. 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/leukocyte relevant arrays.

To establish a selective “hgh-chip” the newly designed oligonucleotide probes for the hgh-candidate genes will be printed on a microarray. The “hgh-chip” will then be thoroughly evaluated by performing numerous gene expression experiments on hgh-treated and untreated leukocyte subsets, cultured PBMCs as well as PBMCs obtained from hgh-patients.

During the proposed project new, nucleic acid-based analytical techniques will be used to gain insight into specific effects of hgh on gene expression of leukocytes. Experience and expertise gained along this project should be of great importance for novel strategies esp. in fields of doping analysis, where so far no satisfying analytical test exists.

Application of Microarray Technology for the Detection of Changes in Gene Expression after Doping with Recombinant Human Growth Hormone

Results and Conclusions

The aim was to establish a selective human Growth Hormone (hgh) microarray which can be used in hgh doping control by detecting the specific effects of hgh administration rather than the presence of the hormone itself as its very short half-life complicates direct detection. To this purpose cell lines of specific leukocyte subsets (THP-1/ monocyte, IM-9/ B- lymphocyte, H9/ T-lymphocyte) and PBMCs (peripheral blood mononuclear cells) from healthy donors, respectively were treated in vitro with hgh to identify candidate hgh-sensitive genes. Results obtained from gene expression profiling utilising whole genome arrays were in accordance with published data. Additionally a range of novel hgh sensitive genes could be discovered. Hgh treatment caused anabolic effects mainly by diversion of energy to protein synthesis. More genes were found to be up-regulated than down-regulated after hgh administration. Responses of the cell subtypes were highly different. The T lymphocyte model cell line H9 was the most responsive. Genes clustering in the categories fatty acid beta oxidation, cell adhesion, DNA replication and polyamine biosynthesis were up-regulated indicating increased lipolysis, cell attachment, proliferation and growth. Genes for non-apoptotic cell death and regulation of osmotic pressure were down-regulated. The B lymphocyte cell line RA-1 showed gene upregulation in the categories opioid receptor, oxidoreductase and GMP-reductase activity. Genes of groups similar to those detected as repressed in H9 cells were also down-regulated in the RA-1 cell line along with genes responsible for muscle development. THP-1 cells, a model system for monocytes, were least sensitive to the application of hgh due to the lack of insulin-like growth factor 1 (IGF-1) production. At doping relevant hgh concentrations effects on THP-1 cells were observed only after a 30 min incubation when hgh was still present. Several collagen types, fatty acid metabolism genes and superoxide dismutase 1 expression were up-regulated. After 180 min hgh incubation, only a high hgh dosage led to over-expression of genes indicating increased cell proliferation, hormone and androgen catabolism and cell cycle checkpoint control. In PBMCs hgh administration led to increased cell maintenance and anti-apoptotic activity. Cell proliferation, C21-steroid hormone biosynthesis, insulin receptor signalling pathways and protein amino acid phosphorylation and glycosylation were activated.