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Abstract

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“Growth Hormone elicits Specific Effects on Blood Cell Gene Expression levels”

DNA microarray technology was utilised for the identification of potential marker genes for the abuse of human growth hormone in athletes. Gene expression levels of distinct blood cell populations were interrogated by whole genome microarrays covering 19.000 human genes, ESTs respectively. Cell cultures of three leukocyte subsets were established. Peripheral blood mononuclear cells were isolated from donated blood and short term cultivated. Cells were treated with recombinant human growth hormone at doping-relevant levels of 2 µg/mL and 20 µg/mL, lysed and total RNA was isolated. mRNA was specifically primed and under the incorporation of fluorescently labelled nucleotides reversely transcribed to cDNA. cDNA from growth hormone stimulated cells was labelled with one fluorescent dye whereas cDNA from unstimulated cells with another. Both cDNAs were hybridised to microarrays to display patterns of differential gene expression. Growth hormone treatment of these cell cultures demonstrated anabolic effects mainly by diversion of energy to protein synthesis. More genes were up-regulated than down-regulated. Responses of the cell types were highly different due to cell differentiation; still effects were in-line with known symptoms of growth hormone over-dosage such as increased lean tissue mass and fluid retention together with reduced body fat. 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 over-expressed indicating increased lipolysis, cell attachment, proliferation and growth. Genes for non-apoptotic cell death and regulation of osmotic pressure were under-expressed. 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 growth hormone due to the lack of IGF-1 production. Effects on THP-1 cells lasted only while growth hormone was still present in the solution after 30 min. Several collagen types, fatty acid metabolism genes and superoxide dismutase 1 expression were up-regulated. After 180 min, only a high growth hormone dosage led to over-expression of genes indicating increased cell proliferation, hormone and androgen catabolism and cell cycle checkpoint control. In peripheral blood mononuclear cells growth hormone 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. The results of this study are in accordance with published data; furthermore new growth hormone sensitive genes were discovered by this novel approach. If the concept of detection of growth hormone abuse by means of gene expression also proves to be applicable to an in vivo situation still has to be thoroughly investigated.

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