PROJECT REVIEW

"Microarray Detection Methods for GH and IGF-1"

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The use of gene transfer methods for athletic enhancement is inevitable. To prepare for such eventuality, it will be necessary to develop more efficient and more effective methods for detection of the foreign genetic information and/or the vector used to deliver the transgene. Current screening methods rely partly on the existence of detectable differences between the functional gene products of endogenous genes and the foreign or exogenously administered proteins. For instance, the detection of erythropoietin administration relies on structural differences between endogenous EPO and the administered drug.

However, those committed to either drug- or gene-based doping will certainly eventually have available those forms of drugs that are indistinguishable from the endogenous human form or gene transfer vectors that express gene products completely identical to the endogenous function. In that case, current approaches to detection of doping will become ineffective. We propose to take advantage of powerful and broad new methods for gene expression screening to detect secondary changes in gene expression that result from systemic or local administration of genes most likely to be subjects for gene-based doping attempts – erythropoietin (EPO), growth hormone (GH) and insulin-like growth factor (IGF-1).

The hypothesis underlying this proposal is that administration of these extremely and widely bioactive agents or the genes expressing them will be associated with reproducible and detectable secondary changes in gene expression in many affected tissues, including peripheral blood. Erythropoietin is very likely to produce changes in gene expression of nucleated cell lineages in the peripheral blood and therefore detectable in simple blood samples. In addition, exposure to the powerful growth effects of GH or IGF-1 administered locally may also produce effects on the pituitary GH-GHRH-IGF-1 axis and also be detectable in peripheral blood.

It is the purpose of this proposal to examine the patterns of gene expression in cells from peripheral blood of mice exposed to GH, IGF-1 and EPO and to gene transfer vectors expressing them and to identify associated changes in gene expression through global microarray techniques.

MICROARRAY DETECTION METHODS FOR GH AND IGF-1

Results and Conclusions

The goal of these studies has been the use of molecular genetic tools of transcriptional profiling and proteomic analysis to identify molecular "signatures" of exposure to doping substances. The underlying principle is the hypothesis that exposure to growth factors and to many other kinds of doping agents will be accompanied by systemic changes in gene expression and in the proteomes if tissues readily accessible for testing. As an introduction to that concept, we have concentrated on the demonstration of genetic and proteomic changes in a number of tissues both in vulture and in vivo after exposure to one of the important potential doping agents – IGF-1. In our in in vitro studies of muscle IGF-treated stem cells, we have identified acute changes in gene expression of several hundred genes, with far more genes being up-regulated than down-regulated. Most impressively, we have found uniform reproducible up-regulation of the genes that regulate cholesterol and steroid biogenesis and of genes fatty acid biosynthesis. If any of these changes come to be documented in vivo, combinations of these changes are candidates for assays for exposure to IGF-1.

Under the same conditions, we have used 2-dimensional gel/mass spectroscopic proteomic methods to establish a large data set of proteins that show altered levels acutely after IGF-exposure.

The in vivo studies in skeletal muscle in IGF-treated mice have also identified altered expression of several hundred genes, but, in contrast to the in vitro results with muscle stem cells, more genes are down-regulated than up-regulated in vivo in skeletal muscle, including a number of genes that specify several muscle growth factors.

The most important conclusion that we come to as a result of these studies is that the identification of methods useful for doping detection and screening will require a much higher degree of data sharing and comparison among the WADA-sponsored studies undertaking such genetic studies than is currently occurring. These disparate studies are using many different experimental designs, research platforms and analytical tools to identify genes and proteins perturbed by drug exposure, and it seems highly likely that these disparate studies have identified many common or inter-related functions but that those results are not being identified by current analytical methods. Toward that end, our laboratory has proposed a pilot study with WADA to test the feasibility of establishing a core bioinformatics facility to collect, collate and analyze data from all relevant WADA-supported laboratories to greatly enhance the likelihood of identifying authentic and diagnostic molecular and metabolic changes that can be used in the fight against sport doping.

Presentations

a. WADA Stockholm Symposium in December 2005

b. ICRAV Congress (International Congress of Raving Analysts and Veterinarians, Tokyo 2006.
c. Hasting Center working Group on Sport Doping, Garrison, New York, 2005, 2006.
d. Research Seminar, Dept. Pediatric, UCSD, 2006.
e. WADA Genetics Panel, La Jolla, Oct. 2006.
f. Pan American Medical Congress 2007, Rio de Janeiro, 2007.
g. Whitehill Bioethics Research Seminar, UCSD, 2007.