PROJECT REVIEW

"Development of a Prototype Blood-Based Test for Endogenous Erythropoietin Activity Based on Transcriptional Profiling"

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We are developing a test for exogenous erythropoietin (epo) activity based on transcriptional profiling. This test would work for any method of Epo abuse, including gene doping.

Epo, a hormone used to treat anaemia by stimulating red blood cell maturation, is frequently used by endurance athletes to boost their aerobic capacity. Treating anaemia with recombinant Epo is costly and gene therapy (in which the Epo gene is inserted into cells and stimulated to produce the hormone) is a promising alternative. Gene therapy has the potential to revolutionize medicine, but unfortunately many experts believe that illicit application of this technology to performance enhancement is inevitable and that Epo will likely be in the vanguard of this threat.

Epo produced by a gene injected into athletes to augment normal production would be identical to the natural product and undetectable by current testing methods; however, the biological pathway of which Epo is part (the response to hypoxia) is complex and involves the coordinate regulation of genes in a variety of distinct, but related pathways. Selectively stimulating only the Epo pathway would not have the same system-wide effects as the natural stimulation of entire hypoxia pathway. Distinguishing between the augmented and the natural response would be possible by comparing transcriptional profiles, which represent the total complement of RNA transcripts (the intermediates that transmit instructions from the genes to the body) in the tissue.

In our project, Serial Analysis Gene Expression is being used to compare the transcriptional profile of the hypoxia response to that of foreign Epo activity. We hypothesize that comparing these profiles will identify differentially expressed genes that can be used to develop a diagnostic test. We will identify these genes in a mouse model, locate and characterize their human counterparts, and incorporate the latter into blood-based test for exogenous Epo expression.

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Results and Conclusions

qPCR analysis of potential candidate genes (based on differential expression in the mouse in response to hypoxia or EPO) showed that the variation for the most promising candidate genes exceeded the variation between treatments. As the mice are inbred, sex and age matched, and kept in controlled environments, there is too much variation in the candidate genes identified by SAGE analysis of blood for them to be reliable, predictable and consistent markers for Erythropoietin (EPO) use in humans. In the absence of strong candidate genes for follow-up, analysis in humans was not initiated.

Given the results of the experiments, differential gene expression in blood cells following red-cell expansion is not a promising method of detecting EPO (or EPO gene) use. As this proposed test was an indirect measure (i.e. measured the effect of EPO rather than EPO itself, it was necessary that the results be: 1) quantitatively and significantly distinguishable from hypoxia induced erythropoiesis and 2) be highly invariant between individuals. I did not find highly differentially expressed genes that could clearly different between EPO and hypoxia and, although promising candidates were found based on un-linking the pathways (i.e. identifying genes expressing unilaterally that that should be co-regulated), none of the genes tested showed sufficient consistency in expression to be reliable biomarkers.