PROJECT REVIEW

"Sensitivity and Specificity of a Gene doping test detecting transgenic DNA on a single molecule level in peripheral blood probes"

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Our proposed project aims at completing the hitherto establishment of our novel direct detection technique for "gene doping" in its primary meaning, i.e. doping by genetically manipulating the human body. In gene therapy trials, aiming at curing diseases, polymerase chain reaction (PCR) is routinely used for monitoring plasma and serum levels of infectious transgenic DNA (tDNA) that often is undetectable within a few hours following gene therapy. In white blood cells gene therapists succeeded to show persistence of non-infectious tDNA for at least 3 months, following many different gene transfer protocols to muscle or other solid tissue of humans (and monkeys). However, this had been achieved under the advantage that the tDNA sequence to be discriminated from the naturally occurring genomic DNA (gDNA) of blood cells has been known exactly.

Supported by the WADA research grant 06B7PS we establish a novel technology which enables detection of tDNA on a single molecule level even if the distinct "misused" tDNA sequence is not known to us. As a big advantage, our testing procedure only requires conventional blood collection from athletes under ordinary field conditions. As a first step, our patent pending single copy PCR technology (PCT/EP2007/003385) is now established in the laboratory detecting the candidate genes EPO, GDF8 (Myostatin), GH1 (incl. GH2, CSH1, CSH2, CSHL1, CSH1/CSH2), IGF-2, PPARD, PPP3CA, VEGF-A, and VEGF-D. We already succeeded in sensing one single genetically manipulated cell (tDNA containing) among 100.000 normal blood cells even if the probe was stored at room temperature for 3 h following blood collection.

Our follow-up WADA project now focusses on the following important topics:
_ further improvement of our "first generation" technical protocol;
_ extension and updating of our list of gene doping candidates;
_ verification of specificity and sensitivity for all established gene doping candidates in vivo.

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Results and Conclusions

In this project we have been able to demonstrate for the first time that direct and long-term detection of gene doping is possible with conventional whole blood samples. Detection protocols using a specifically designed ultra-sensitive detection protocol for transgenic DNA (spiPCR) have been developed for EPO, GH1, HIF1a, VEGFA, VEGFD, IGF1 (including MGF), and FST (Beiter et al. 2010). To make our detection strategy amenable for routine testing, we implemented a robust sample preparation and processing protocol that allows cost-efficient analysis of small human blood volumes (200 µl) with high specificity and reproducibility. The practicability and reliability of our detection strategy was validated by a screening approach including 327 blood samples taken from professional and recreational athletes under field conditions.

Sensitivity of our detection strategy was verified in a mouse model, giving positive signals from minute amounts (20 µl) of blood samples for up to 56 days following intramuscular gene transfer. We employed adenovirus (rAd) and adeno-associated virus (rAAV) as the most likely candidate vector systems to be misused for gene doping. The sensitivity of the methodology was tested in a blinded fashion in mice following prototypic gene transfer with the adenoviral vector rAd-VEGFD and with the adeno-associated viral vector rAAV-VEGFA. Detectability of transgene VEGF elements was tested before and until 8 weeks post- transduction. Both, *VEGFA* and *VEGFD* elements could be efficiently detected in all transduced animals for several weeks and for *VEGFA* as long as 56 days.

From a practical point of view, our testing procedures for gene transfer with EPO, GH1, HIF1a, VEGFA, VEGFD, IGF1 (including MGF), and FST using our spiPCR are safe and principally applicable for gene doping analysis in our laboratory setting.