**"Exploring the potentials of transcriptomic and novel micro RNA screening approaches for the indirect detection of gene doping"**

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## Project aims

Many of the different substances and procedures that have the potential to be abused for doping, finally act in the same way within the athlete's body by activating just a few key pathways relevant for mediating performance enhancing effects. This common feature may eventually lead to common and more or less permanent alterations on the molecular level within the athlete's blood. Finding just a single specific signature for doping would therefore serve as an indirect prove for many different procedures and substances that could have been abused.

In this project we will exploit the possibility to detect molecular signatures of gene doping based on the level of proteins (proteome), RNAs (transcriptome) or the non-coding, regulatory microRNA that are modified upon modifications of the pathways induced by IGF1 and HIF1a, the former improving muscle properties and the latter increasing oxygen delivery and aerobic capacity. For this purpose we will in first place assess data on the variability of gene expression signatures found in the blood cells of athletes under various physiological conditions.

In second place we will then look for molecular signatures of doping in two mouse models using gene transfer with HIF1a and with IGF1 as a procedure that is currently undetectable. Along with our search for signatures specific for these doping procedures we will try to show long-term, direct detection using a novel PCR procedure which we have recently developed for WADA.

All data obtained in this project will be supplied to the WADA Informatics Facility for extensive cross study comparison in order to assess the specificity of potential molecular signatures for the detection of doping.

## Results and Conclusions

The project consisted out of three main parts. Part 1 was an in vivo study in cyclists looking for changes in the transcriptome in response to training in normoxia and in normobaric hypoxia simulating an altitude training session. Part 2 was investigating in an in vitro study which candidate genes could be assessed on mRNA or miRNA level in order to detect gene expression changes in response to hypoxia or Insulin like growth Factor 1 (IGF1). Part 3 was an in vivo study in mice that underwent gene transfer with IGF1 as a potential gene doping scenario and the feasibility of both indirect detection and direct detection were investigated.

Results and general conclusions

In study one we revealed that a typical endurance training session at 75 % of the individual anaerobic threshold under hypoxic and under normoxic conditions only induced some minor alterations in gene expression. 16 candidate genes showed a more than two-fold alteration in gene expression compared to resting conditions. The changes in gene expression between training in normoxic or in hypoxic settings were for most parts non-significant. Gene expression analysis revealed ankyrin repeat domain 37 (Ankrd37) as the only candidate.

Subsequent in vitro testing and bioinformatics analysis in study part 2 confirmed Ankrd37 as a hypoxia sensitive candidate gene. Additionally, we revealed a list of miRNAs that are potential candidates for indirect detection of hypoxia or proliferative effects induced by IGF1. However, critical factors are a striking cell type specificity of these effects and a limited magnitude of gene expression alteration of candidates that show response in vivo and in vitro. High inter- and intra-individual differences that can be assumed to exist in typical settings in elite sports may therefore limit the applicability of our findings for indirect detection of the alteration of the HIF1□□pathway.

With this regards the results of our third study following AAV1, AAV2 and AAV9 mediated gene transfer of IGF1 to mouse muscle in vivo were much more promising. Gene transfer of IGF1 to muscle cells evoked a strong proliferative effect and along with this the miRNA profile of all investigated candidates was severely down regulated. We then established a digital droplet PCR (ddPCR) based transgene detection approach using a priming strategy previously described by our working group that specifically amplifies sequences devoid of intronic DNA. We showed that ddPCR was able to directly detect the transgene following AAV9 mediated IGF1 gene transfer in the peripheral blood taken from the living animals for as much as 33 days following gene transfer.

Two of our findings are primarily of interest for the development of a doping test. First the highly proliferative effect of IGF1 that was highly associated with a general down regulation of miRNAs and second the ability to directly detect gene transfer on the level of the transgenic DNA.

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