"Epigenetic profiling to detect doping abuse"

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Project overview

Drug abuse and physical activity result in a variety of adaptation processes of the organism down to the molecular level. Because direct detection of drugs in doping analysis is getting more and more complex, long term monitoring of physiological parameters is a promising supportive strategy in the fight against doping. A new field for the identification of biomarkers is epigenetics. Epigenetics is defined as the heritable change in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence. It refers to functionally relevant modifications to the genome that do not involve a change in the nucleotide sequence such as DNA methylation and histone deacetylation. Evidence is accumulating that drugs, nutrition, but also physical activity result in the modulation of the epigenome. Identification of relevant mechanisms has resulted in pharmaceutical strategies. It's very likely that such drugs will be abused in doping.

Knowledge of specific epigenetic modulations as the result of abusing drugs can serve as biomarkers for an indirect doping detection. In the proposed project experts from the center of preventative doping research of the german sports university and from the Division Epigenomics and Cancer Risk Factors of the German Cancer Research Center want to combine their skills to identify a signature of genes differentially methylated by doping abuse. Therefore data from animal experiments, training studies in humans and a field study with bodybuilders abusing anabolic steroids will be combined. In white blood cells (WBCs) and muscle tissue from the animal study and WBCs and urine from the human studies genome wide methylation patterns will be monitored by methyl-CpG immunoprecipitation (MCIp) of methylated DNA followed by next generation sequencing (NGS). In a second step methylation of identified differentially methylated regions will then quantified in a high-throughput manner by Maldi-TOF Mass spectroscopy.

Results and Conclusions

Endocrine active agents have been shown to influence the epigenome. The kinetics, extent and persistence of such changes are however not thoroughly established and need to be investigated in well controlled studies both in animal models as well as in human pilot studies.

The aim of our research project was to identify a methylation profile specific for doping by genome-wide methylation profiling. Based on this profile we intended to develop a methylation signature that might be useful as high

throughput biomarker for drug abuse when measured by mass spectrometry-based quantitative methylation analyses in blood cells.

In a human pilot study performed with healthy control subjects (C), natural (non-doping) body builders (BB), and body builders abusing anabolic substances (ABB) DNA of 31 subjects participating in the pilot study was available for 450k analyses. When combining all the information gathered on DNA methylation in blood derived DNA, we conclude that its potential use as a biomarker for the detection of drug abuse is hampered by several limitations and confounding factors. First we could only detect few and small significant methylation differences. The detected methylation differences between groups are small, in the range of 5-10%. We analyzed >450.000 CpG sites, but only about 0.1% of the sites (around 500) passed our selection cutoff criteria: Significant differences between groups with $p<0.05$, methylation differences >5%, standard deviation below 15%. Of these, most were significant only between two of the analyzed groups, but not between all three groups. Second the genome of individuals differs. Although we removed all sites affected by SNPs during our bioinformatic analysis, some sites might still overlap with less frequent or not annotated SNPs. Even one of our most discriminating site cg21365902 carries a SNP with a very low minor allele frequency of 0.06. Since the frequency of SNPs differs between races, the ethnic background and the variability of the study group also contributes to whether a site might be a good biomarker or not. Third, DNA methylation is cell type specific. Since the blood cell composition of the study subjects differed, we cannot exclude that the methylation differences between the groups are merely due to differences in blood cell composition. The fact that we had genome wide methylation data allowed us to correct for the blood cell composition. However, these genome-wide analyses are time and cost-intensive. Overall, detection of drug abuse by measuring DNA methylation from blood samples appears to be prone to artifacts and is affected by several confounding factors.

In order to be able to understand the relevance of DNA methylation changes identified in blood cells, we also performed an animal study with male Wistar rats (rat training study). Half of the animals were treated with methandienone (5 mg/kg/bw/d) once at the age of 91 days to mimick “doping”. In addition, half or the rats underwent a training program for 6 weeks. The animals were allocated to four groups, a control group (C), a training-only group (T), a “doping”-only group (D) and a group with combined training and doping (TD). DNA from muscle tissue and blood cells were available for methylation profiling. Methylation analyses in the muscle tissue provide interesting new insights into potential epigenetic gene regulation related to doping. However, as outlined in the human study, ideally methylation levels should be measured in all cell types composing a tissue, as changes in cell type composition cannot be excluded. So far, the rat genome is less characterized than the human or mouse genome, and further epigenomic information not available. To confirm the results, it might be preferable to perform a study in mice instead of rats, or to analyze human biopsies. In PBMCs obtained from the rat training study we identified only few significant methylation changes that passed our selection criteria. This

demonstrates that in comparison to muscle tissue, the number of training or MD induced methylation changes is even smaller.

We conclude from these evaluations that the analysis of blood cells only provides very limited information, and methylation profiling of target tissues such as muscle should be preferred.