"Pilot study: Evaluating the feasibility of erythropoiesis specific mRNA profiling for ESA doping detection in red blood cells"

Dr. P. Simon, Dr. D. Moser, Dr. E. Neuberger (Johannes Gutenberg University, Germany)

Project Summary

The detectability of erythropoiesis stimulating agent (ESA) abuse is a major challenge on the way to a doping free sport. Despite existing tests, the detection of ESA abuse has proven to be very difficult. According to the large number of novel ESAs that are marketed around the world, or are in preclinical or clinical trials it seems to be virtually impossible to develop direct tests for each substance. To face this problem, indirect detection strategies have been suggested. During the last decade, molecular biological techniques enabled screening for the human transcriptome.

However, the ´transcriptomic´ approach is still facing a number of problems before robust biomarker can be detected. The problems include biological- and technical bias that are accompanied with the choice of target RNA, sample type and sample processing. Here, we suggest for the first time to utilize micro RNA (miRNA) extracted from mature red blood cells.

Since erythrocytes do not contain nuclei, gene – environment interactions that typically hamper establishing gene signatures for doping are excluded and will not affect miRNA levels. This stability against physiological bias or other factors along with their long life-span makes the erythrocytes the ultimate cell type for addressing the question of whether gene signatures for ESA doping can be found. With this pilot study we want to evaluate the feasibility of erythropoiesis specific miRNA profiling for ESA doping detection in red blood cells.

Results and Conclusions:

This study intended to examine recombinant human EPO (rhEPO) related effects on microRNA (miRNA) profile of red blood cells (RBCs), to discover markers which are indicative for altered erythropoiesis in induced by rhEPO. RBCs, as a homogenous sample material, were chosen to avoid typical bias that can hamper reliable miRNA marker detection, such as shifts in blood cell composition and cell lysis.

A double blind placebo controlled cross over study, including 14 healthy male participants, who received microdoses of recombinant human erythropoietin (rhEPO) was conducted at the University of Glasgow, supervised by Prof. Pitsiladis. To discover potential novel biomarkers, which are indicative for rhEPO doping with microdoses, highly purified red blood cell (RBC) samples from 7 subjects before and after 6 weeks of rhEPO administration were analyzed using Next Generation Sequencing (NGS).

As a result of 6 weeks rhEPO administration mean haematocrit (Hct) and haemoglobin concentration values increased significantly ($p<0.01$) from baseline values (42,57 ± 1,71 to 47,41 ± 2,97 (%) and 14.77 ± 0.61 to 16.36 ± 1,12 (g/dl), respectively). Concomitantly, the percentage of reticulocyte remained unchanged.

Using the Illumina NextSeq500 sequencing system (Illumina, San Diego, CA, USA) for small RNAs, RBC miRNA profiles of 7 subjects were discovered before and after 6 weeks of microdoses rhEPO administration. 13 out of 1,202 discovered mature miRNAs were significantly dysregulated ($P<0.05$, $FDR<0.05$). The most dysregulated miRNA, has-miR-150-5p is known to be down-regulated in RBCs and erythropoietic progenitors cells of polycythemia vera (PV) patients who suffer from disordered erythropoiesis. This information is concordant with our findings that has-miR-150-5p is down-regulated in healthy subjects who received rhEPO. Presumably, microdoses of rhEPO dysregulate the tightly controlled process of erythropoiesis and can be reflected by screening the RBCs miRNA transcriptome. This makes RBC specific miRNAs valuable biomarker candidates, which could be used in an additive way to generate a predictive marker for rhEPO doping, independent of changes in reticulocyte counts.