PROJECT REVIEW

"Prenanalytical and analytical characterization of circulating microRNA-144 for the detection of erythropoiesis-stimulating agent abuse"

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MicroRNAs (miRNAs) are small (19 to 25 nucleotides) noncoding transcripts involved in many cellular and physiological mechanisms such as erythropoiesis. Recently, a new class of miRNAs was found in cell-free body fluids such as serum and plasma. These new class of miRNAs are called "circulating miRNAs". Circulating miRNAs have been found as very stable, specific and sensitive biomarkers. Therefore, they could be altered in a specific manner in doping interventions such as erythropoiesis-stimulating agents (ESA).

First promising results have been obtained from our laboratory related to the utilization of circulating miRNAs to detect ESA abuse.

After microarray profiling and RT-qPCR analysis, an increase of miR-144 was observed up to 27 days after ESA injection. Interestingly, miR-144 has been reported to be essential in erythropoiesis in human and other organisms.

In this project, we plan to investigate more in details the pre-analytical and analytical characterization of the use of miR-144 as long-term biomarker for the detection of ESA. Indeed, we plan to test different biological matrix, extraction methods and the stability in order to facilitate the future utilization of miR-144 as indirect biomarker. Moreover, negative population will be tested to study, more in details, background noise and inter-individual variability of miR-144 concentration in plasma to define threshold limit.

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Results and Conclusion

In this project, different pre-analytical tests have been performed to characterize two selected miRNA. These two miRNAs were potential biomarkers for the detection of ESAs abuse and autologous blood transfusion, respectively.

Extraction protocol was demonstrated to be the most important step in miRNAs analysis. Standard extraction protocol is based on phenol/chloroform extraction followed by silica columns purification. Although very efficient, the use of these toxic solvents should be handled carefully. To prevent the use of phenol/chloroform and to decrease the cost of analysis, different extraction procedures were tested. Since circulating miRNAs are very stable, extraction protocol based on boiling was possible. Comparison with standard method demonstrated that heat-and-shoot protocol was efficient only for minute amount of plasma. In contrast, with a volume of 100 µl standard method is close to 10 fold times better regarding qPCR signal. Plasma matrix is known to contain reverse transcription and PCR enzyme reaction. Since more inhibitors were present in 100 µl of plasma than 12 µl could explain this observation. Thus, boiling is sufficient to counteract inhibitors of enzyme reaction only in small volume.

Minimally invasive test such as fingertip prick test possess some advantages. We observed that heat-and-shoot and standard method could be used to extract circulating miRNAs from digital blood. Digital blood collections are beneficial in clinics but this utility in anti-doping is questioned as some athletes preferred standard blood collection to fingertip prick test because of the pressure applied on fingers when they compete or train. In addition, it is difficult to analyse several

variable in small amount of plasma and thus, combination of biomarkers are not possible.

Circulating miRNAs have been demonstrated to be very stable. From our experiment, one of the miRNAs was unstable after heating sample at 60°C with the 3 pool of plasma tested. In contrast, the other one showed a good stability even in extreme conditions. This observation supported the use of the circulating miRNA as an efficient biomarker to detect autologous blood transfusion