PROJECT REVIEW

“Autologous blood transfusion detection through erythrocyte membrane proteome changes after blood storage”

J. Segura, Z. Nikolovski, D. Andreu, R. Ventura (Fundacio IMIM, Barcelona, Spain)**, E. Borras, M. Rodriguez** (Pompeu Fabra University, Barcelona, Spain)

Recently, autologous blood transfusion is one of biggest challenges for abuse detection. For reasons of convenience and safety, autologous transfusions, in which the cells are the athlete's own, are reportedly far more common than homologous transfusions, in which the cells belong to someone else with compatible blood. So far there is no adequate and unequivocal method to confirm illicit boosting of an athlete's red blood-cell via transfusion of one’s own blood.

Presently our institution is running the WADA sponsored project “Overall approach for blood transfusion detection (autologous/homologous)”. As a complementary approach the present project aims to add another alternative to the possible ways to demonstrate autologous blood transfusion developing tests, based on comparison of red blood cell membrane proteomes of fresh red blood cells and stored red blood cells for different times, at standard storage conditions.

Several studies employing different strategies describe in details normal human erythrocyte proteome, while some studies have postulated changes in erythrocyte proteome after red blood cell storage (Anniss et al.; Manojkumar et al.) This project is presented with an idea to identify detectable changes in erythrocyte membrane proteome before and after standard storage procedures in order they can be used as a methods to identify autologous blood transfusion.

For such purpose we will apply proteomics methods such as 2D electrophoresis, 2D-DIGE, mass spectrometry of identified proteins. The project is presented as a preliminary step of one year in order to test the hypothesis. If, as expected, differential proteome appears as an actual possibility next step would be to develop adequate tests which would employ flow cytometry and proper markers in order to demonstrate existence of different populations of erythrocytes in the blood of the same subject, which would confirm anterior autologous transfusion.

“Autologous blood transfusion detection through erythrocyte membrane proteome changes after blood storage”

J. Segura, Z. Nikolovski, D. Andreu, R. Ventura (Fundacio IMIM, Barcelona, Spain)**, E. Borras, M. Rodriguez** (Pompeu Fabra University, Barcelona, Spain)

Results and Conclusions

In the present project we employed two complementary proteomic strategies in order to get an insight to the changes occurring at the membranes of the RBC over prolonged storage time. 2D gel electrophoresis as a strategy of protein separation has its drawbacks such a difficulties to separate efficiently extremely basic, acidic, or hydrophobic proteins. So, it was necessary to complement it with isotope tagging for relative and absolute quantitation (iTRAQ) methodology, which is peptide oriented and helps to achieve satisfactory resolution of the changes occurred. Not a single method is sufficient to follow all the changes happening. From the application of each one of the methodologies, several important proteins appear as serious candidates for further targeting.

However, within the same 2D spot it is possible to identify several proteins with different degree of certainty, even though that experimentally some of them may be discarded taking into account molecular mass, sequence coverage, % or score number and other indicators. When it was possible we compared also graphs of corresponding spots (increase or decrease of spots volume ratio) and ratios of iTRAQ results when corresponding identified proteins appeared in both of them.

According to the results from our studies and also available results of other authors we propose to group observed findings such as:

- Transmembrane proteins
- Cytoskeletal proteins
- Other proteins
- Other investigator’s findings related to our studies