Plasma and red blood cell proteome in sickle-cell disease

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INTRODUCTION

Sickle-cell disease (SCD) is a clinically heterogeneous autosomal recessive monogenic chronic anaemia characterized by recurrent episodes of severe vaso-occlusion, haemolysis and infection. Painful crises are the major SCD clinical manifestation probably due to significant increase in dense red blood cells (RBC) and reduction of their ability to pass through capillaries. Using proteomic strategies, we aim to discover novel and better SCD prognosis biomarkers as early predictors of the transition from steady-state to crisis namely vaso-occlusive episodes, thus, allowing a prompt and specific therapeutic intervention.

MATERIAL & METHODS

Plasma and RBC were isolated from peripheral blood of SCD (SS) patients in steady-state or undergoing a vaso-occlusive episode (n=12). Plasma samples were depleted of the 14 most abundant proteins using the MARS^{-14} and RBC were fractionated into membrane and soluble fractions, the latter being depleted of haemoglobin using Hemovoid^{TM}. Depleted plasma and soluble RBC samples were labelled with alternative fluorescent dyes (Lumiproble 3Dye^{TM} 2D DIGE kit) and separated by 2DE using 24cm IPG strips (pH 4-7 and 3-10NL, respectively). After 2nd dimension, images were acquired in a Typhoon Variable Mode Imager and analysed by Progenesis SameSpots software. Differentially expressed spots were excised from Coomassie-stained preparative gels and proteins identified by MALDI-TOF/TOF MS.

RESULTS & CONCLUSION

Prior to use, the efficiency of Lumiproble fluorescent dyes was assessed in comparison with GE Healthcare and Ramidus Dye systems. The Lumiproble Dye system showed better results with less overlay between the emission spectra of these dyes when compared with the others. The 2D-maps analysis showed differentially expressed spots across the different groups/samples in the study. The proteins identified by MS were annotated according to molecular function and associated pathways to disclose altered mechanisms related with SCD. The complete characterization and validation of these differently expressed proteins are in progress and may constitute a specific biosignature of steady-state to crisis transition in SCD.

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