OPTIMIZING THE DISCOVERY OF PREDICTORS OF VASO-OCCLUSION IN SICKLE-CELL DISEASE BY PROTEOMICS

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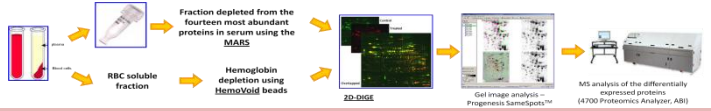
INTRODUCTION & OBJECTIVES

Painful crises are the major sickle-cell disease (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 (see figure below), we aimed to discover novel SCD prognosis biomarkers as early predictors of the transition from steady-state to vaso-occlusive crises thus, allowing a prompt and specific therapeutic intervention.



METHODS

Plasma and RBC from peripheral blood of SCD (SS) patients in steady-state or undergoing a vaso-occlusive episode (n=12). Plasma was depleted of the 14 most abundant proteins using the MARS-14 and RBC were membrane/soluble fractionated, the later was haemoglobin depleted using HemoVoid™

Samples were labeled with Lumiproble 3Dye™ 2D DIGE kit and separated by 2DE (pH 4-7 and 3-10, respectively). The 2D-images were acquired in a Thyphoon Imager and analysed by Progenesis SameSpots. Differentially expressed spots were identified by MALDI-TOF/TOF MS. Functional characterization was achieved by different bioinformatic resources (IPA, GO, PIKE).

RESULTS

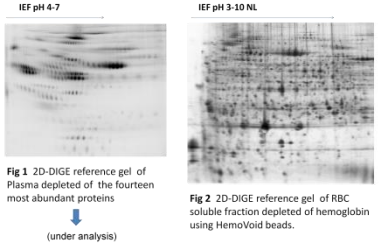


Fig 1 2D-DIGE reference gel of Plasma depleted of the fourteen most abundant proteins (under analysis)

Fig 2 2D-DIGE reference gel of RBC soluble fraction depleted of hemoglobin using HemoVoid beads.

In RBC-soluble fraction 2DIGE maps, more than 900 spots *per* gel were resolved and a total of 226 spots differentially expressed spots were recognized, corresponding a 134 proteins by MS analysis (see figures 1- 5).

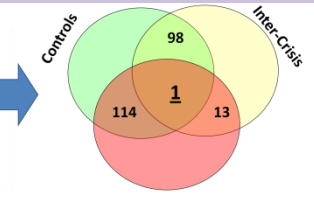


Fig 3 Venn diagram depicting the differentially expressed spots in pair wise .comparisons.

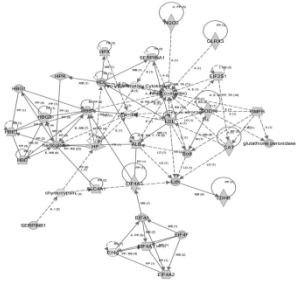


Fig 4 A Functional network associated with hematological disease and cell death retrieved by IPA.

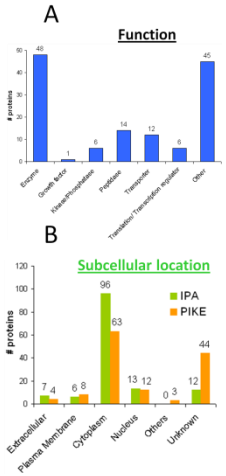


Fig 5 Function (**A**) and Subcellular location (**B**) of proteins by IPA and IPA and PIKE, respectively.

References:

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Conclusion

Molecular functions and associated pathways revealed enrichment in proteins involved in haematological disease, cytoskeleton rearrangements, signal transduction or response to reactive oxygen species, altered mechanisms with implications in SCD vaso-occlusive episodes. Further complete characterization and validation of differently expressed proteins in both RBC and plasma may constitute a specific biosignature of steady-state to crisis transition in SCD.