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Background & Objectives

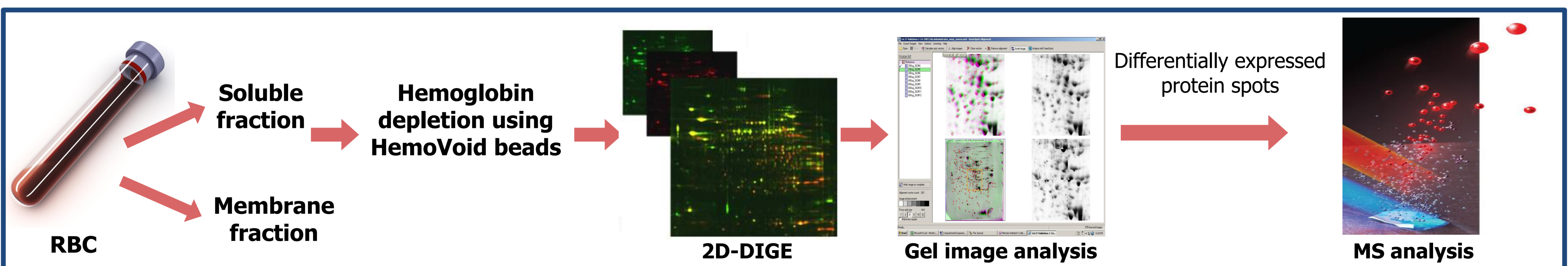
Sickle-cell disease (SCD) is a clinically heterogeneous autosomal recessive monogenic chronic anaemia, characterized by recurrent episodes of severe vaso-occlusion, haemolysis and infection.



Painful crisis are the major SCD clinical manifestation, probably due to significant increase in dense sickled red blood cells (RBC) and consequent decrease in their ability to pass through capillaries. Using 2D-DIGE proteomic strategies we aimed to identify candidate biomarkers, to early predict the transition from steady-state to vaso-occlusive crisis in SCD. This would allow the prompt and specific therapeutic intervention, improving the quality of life of SCD patients.

Material & Methods

RBCs were isolated from peripheral blood of SCD patients (n=6) in steady-state (inter-crisis) or during vaso-occlusive episodes (crisis) and were fractionated into membrane and soluble fractions. The latter was depleted of haemoglobin (Hgb) using HemoVoid™ and labelled with Lumiprobe 3Dye™, followed by 2D-DIGE separation, using 24cm IPG strips (pH 3-10NL) and 12.5% SDS-PAGE. Images were acquired in a Thyphoon Variable Mode Imager and analysed by Progenesis SameSpots software (version 4.5). Differentially expressed spots in crisis *versus* inter-crisis condition (n=31), were excised from Coomassie-stained preparative gel (Fig. 1). Thirteen proteins (Table) were identified by MS analysis with MALDI-TOF/TOF. Pathway analysis was carried out by Ingenuity Pathway Analysis knowledgebase (Fig. 2).



Results

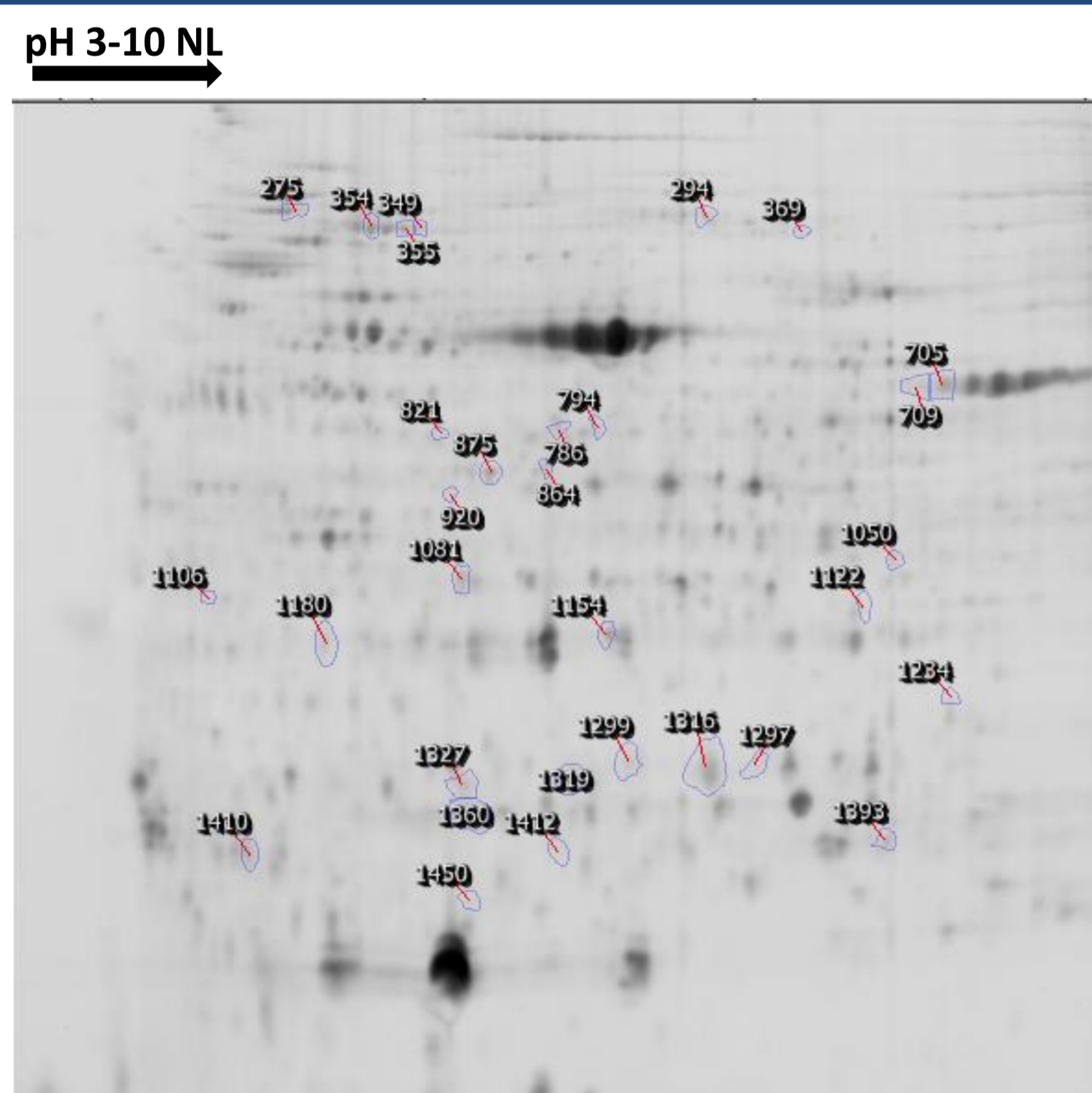


Fig. 1 2D-DIGE gel of RBC soluble fraction, with 31 protein spots differentially expressed between crisis *versus* inter-crisis condition.

Spot ID	Protein name	Access n.
705 & 709	Catalase	CATA_HUMAN
1122	Aflatoxin B1 aldehyde reductase member 2	ARK72_HUMAN
1316	Glutathione S-transferase	GSTO1_HUMAN
1050	Aspartate aminotransferase, cytoplasmic	AATC_HUMAN
1299 & 1319	Serotransferrin	TRFE_HUMAN
1327 & 1081	Peroxiredoxin-2	PRDX2_HUMAN
1180	Eukaryotic translation initiation factor 2 subunit 1	IF2A_HUMAN
875	Glutathione synthetase	GSHB_HUMAN
821	T-complex protein 1 subunit beta	TCPQ_HUMAN
369	Delta-aminolevulinic acid dehydratase	HEM2_HUMAN

Table : Differentially expressed Protein spots identified by MALDITOFTOF

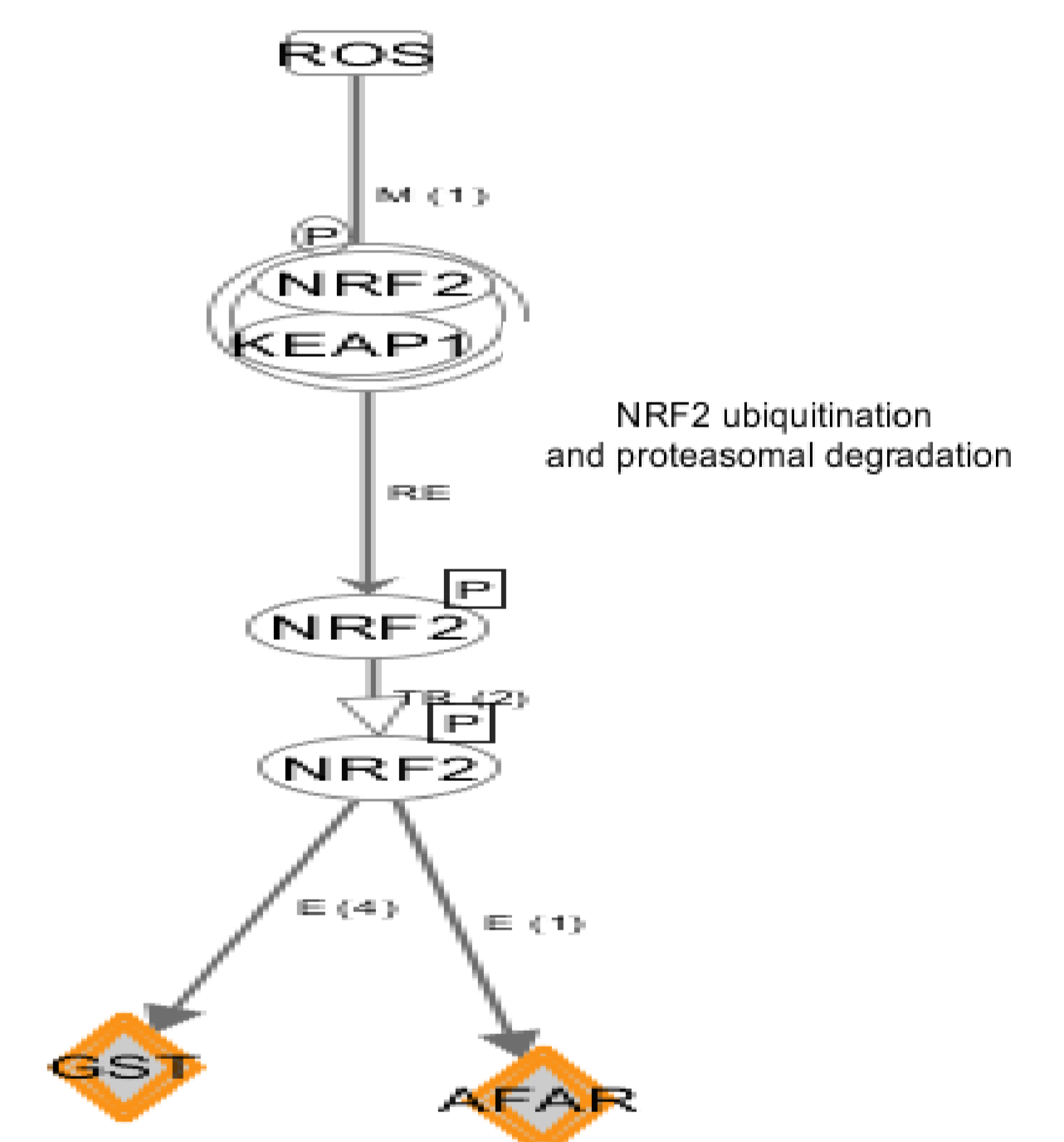


Fig. 2 Ingenuity Pathway Analysis knowledgebase.

Conclusion

The most significant network retrieved was associated with NRF2 [Nuclear factor (erythroid-derived 2)-like 2] antioxidant response pathway, that once activated in crisis episodes, maybe important for oxidative homeostasis in erythrocytes and for the prevention of haemolytic anaemia in these patients. The heme pathway enzyme delta-aminolevulinic acid dehydratase, involved in oxidative stress and metal intoxication, was down-regulated during crisis condition. The complete identification and validation of differentially expressed proteins, will provide better understanding of vaso-occlusion episodes in SCD, that ultimately can be translated into newly effective diagnosis/prognosis tools.

References

- Pasini EM, Lutz HU, Mann M and Thomas AW, Red blood cell (RBC) membrane proteomics – Part I: Proteomics and RBC physiology, *J Proteomics* 2010, 73:403-20.
Roux-Dalvai *et al.* Extensive analysis of the cytoplasmic proteome of human erythrocytes using the peptide ligand library technology and advanced mass spectrometry. *Mol Cell Proteomics* 2008; 7:2254-69.
Alexandre BM, Charro N, Blonder J, Lopes C, Azevedo P, Bugalho de Almeida A, Chan KC, Prieto DA, Issaq H, Veenstra TD, Penque D, Profiling the erythrocyte membrane proteome isolated from patients diagnosed with chronic obstructive pulmonary disease. *J Proteomics*. 2012 Dec 5;76 Spec No.:259-69
N Charro, Hood BL, Faria D, Pacheco P, Azevedo P, Lopes C, de Almeida AB, Couto FM, Conrads TP, Penque D. Serum proteomics signature of cystic fibrosis patients: a complementary 2-DE and LC-MS/MS approach. *J Proteomics*. 2011 Jan 1;74(1):110-26.

Acknowledgements

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