Label-free Proteomic Analysis in Bone Marrow Mesenchymal Stromal Cells from patients with Myelodysplastic Syndrome revealed New Insights into the Leukemic Evolution

Teresa de Souza Fernandez [1], Renata Binato[1], Luciana Pizzatti[2], Gabriela Lemos[1], Viviane Lamim Lovatel[1], Luis Fernando Bouzas[1], Eliana Abdelhay[1]

1. Divisão de Laboratórios do CEMO, Instituto Nacional de Câncer, Rio de Janeiro, Brazil.
2. Laboratório de Biologia Molecular e Proteômica do Sangue / Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.

INTRODUCTION

Myelodysplastic syndrome (MDS) comprises a heterogeneous group of clonal bone marrow disorders with increased risk of evolution to acute myeloid leukemia (AML). The key component of the microenvironment is bone marrow mesenchymal stromal cells (BM-MSCs). The bone marrow microenvironment has been indicated as an important contributor to MDS pathogenesis. However, the molecular and cytogenetic alterations in BM-MSCs involved in MDS pathogenesis remain unclear.

OBJECTIVE

Investigate proteins, processes and signaling pathways in BM-MSCs using high-resolution label-free proteomic approach and analyze cytogenetically BM-MSCs from MDS patients to characterize alterations involved in the evolution from MDS to AML.

MATERIAL AND METHODS

Bone marrow samples were obtained from 25 MDS patients and 20 healthy donors (HD). Mononuclear cells were cultured in DMEN-low glicose and FBS. The MSCs were collected after 3 passage for protein extraction and cytogenetic analyses. The proteomic analysis was performed using the label-free strategy. The peptides generated were identified by qualitative and quantitative bidimensional chromatography nanoUPLC and nanoESI-MSE experiments performed in the Synapt HDMS mass spectrometry. In silico analysis was conducted in MetaCore data base (Figure 1).

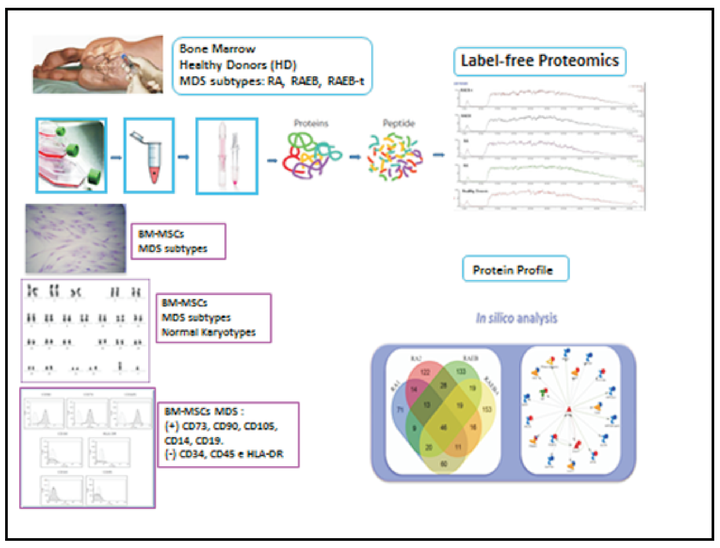

Figure 1: Methodological approaches

RESULTS

BM-MSCs from MDS patients had no clonal chromosomal abnormalities. Venn diagram analyses showed 46 proteins in BM-MSCs-MDS subtypes, suggesting a common molecular signature for BM-MSCs-MDS compared with BM-MSCs-HD (Figure 2). The primary biological processes altered were cytoskeletal modeling, cell cycle and immune response (Figure 3). c-MYC is a possible key protein, acting as an up-regulator associated with common and exclusive proteins at initial and advanced MDS stages (Figure 4).

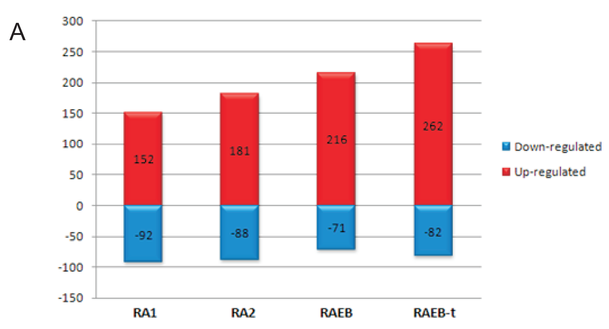

Figure 2: Differential expression analysis. (A) Differential expression analysis obtained with ExpressionE showing proteins with increased and decreased expression in BM-MSCs-MDS patients compared with BM-MSCs-HDs. (B) Venn diagram of the differentially expressed proteins showing the overlapping and unique differentially expressed proteins in the BM-MSCs from the MDS subtypes.

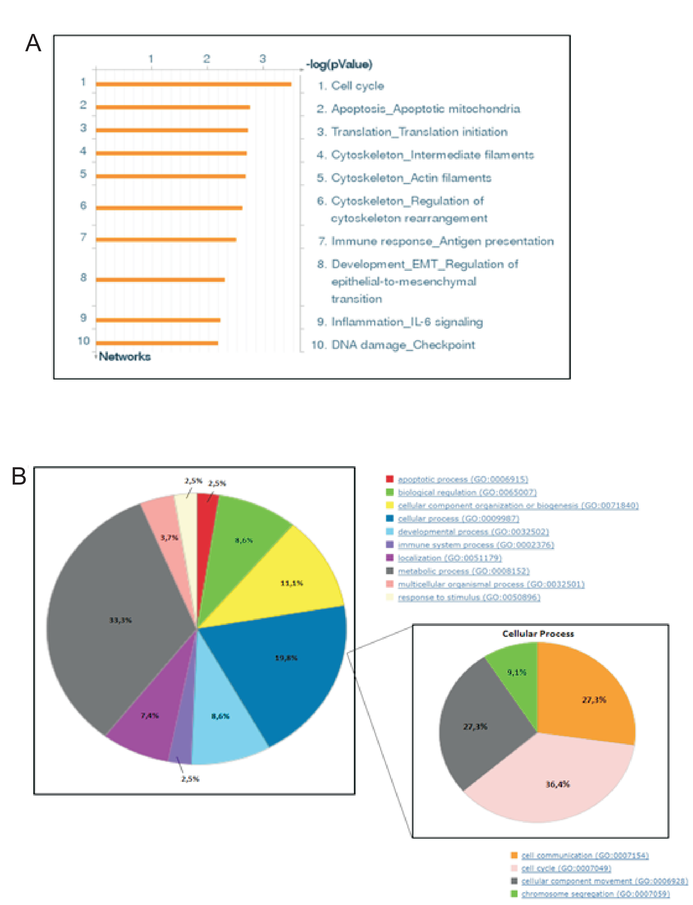

Figure 3: (A) Process Networks from 46 differentially expressed proteins common to the BM-MSC-MDS subtypes: The top ten scored process networks from the Metacore™ in silico analysis of the 46 common proteins revealed by Venn analysis. **(B) Protein Biological Processes:** Gene Ontology analysis of all 46 proteins using the PANTHER online tool.

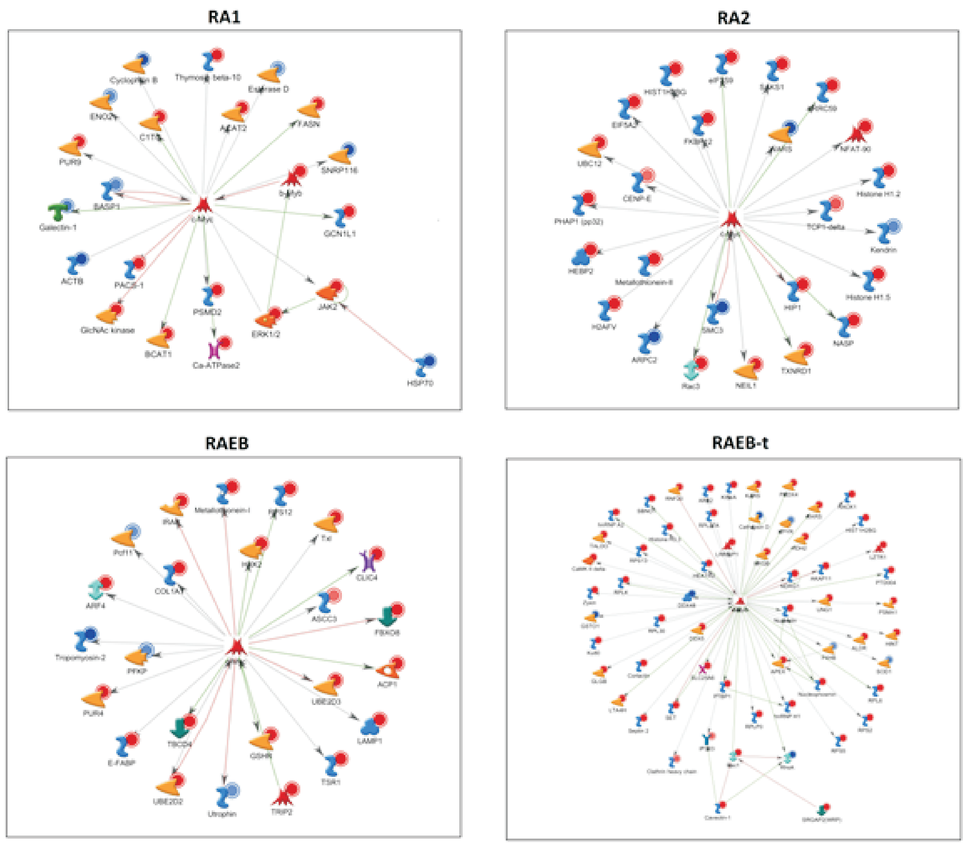

Figure 4: The interactomes of the exclusively expressed proteins were analyzed in the BM-MSCs of the MDS subtypes. c-MYC might function as a potential up-regulator in BM-MSCs during the initial stage (RA) and advanced stages (RAEB and RAEB-t) of MDS. A larger number of proteins regulated by c-MYC are identified as the disease progresses, which is characteristic of the complexity of MDS biology. RA: refractory anemia; RAEB: refractory anemia with excess of blasts; RAEB-t: refractory anemia with excess of blasts in transformation

CONCLUSION

We conducted the first study using label-free proteomic analysis to characterize alterations in BM-MSCs in MDS pathogenesis. The primary biological processes altered in BM-MSCs were cytoskeletal modeling, cell cycle, immune response. The number of proteins that may be regulated by c-MYC increased as disease progressed. Our results suggest that BM-MSCs play an important role in MDS pathogenesis.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA