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POSTER ABSTRACTS

113.SICKLE CELL DISEASE, SICKLE CELL TRAIT AND OTHER HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIAS: BASIC AND TRANSLATIONAL

The Proteomic Signature of Extracellular Vesicles from Sickle Cell Anemia Patients Provides Insights into Their Possible Role in the Pathophysiology of the Disease

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Sickle cell anemia (SCA) is marked by hemolysis and vaso-occlusion, which contribute to various other complications observed in the disease. Extracellular vesicles (EVs), released by activated cells, serve as carriers of various markers, proteins, and RNA derived from their parent cells. These structures have the ability to transfer their cargo to the recipient cells, modulating their activity. Despite EVs involvement in physiological processes, there is growing evidence of their role in certain blood diseases, making them a subject of extensive research in recent years.

In sickle cell disease (SCD), EVs have been previously associated with hemolysis markers, leg ulcers, and endothelial cell activation and several other authors have emphasized the significant role of EVs in its pathophysiology. However, despite these advances, the precise protein content of these EVs, which may hold the key to unraveling their involvement in disease complications, remains largely unexplored.

Here, we aimed to characterize the proteomic profile of EVs from patients with SCA. We conducted a comparative analysis, comparing the differentially abundant proteins in the SCA group to those in the control group. Additionally, we focused on analyzing the distinct protein profiles that were uniquely present in the SCA group studied.

Nine healthy volunteers (HC) and nineteen patients with SCA were included in the study, with three receiving no treatment, twelve undergoing hydroxyurea (HU) therapy, and four on HU+Crizanlizumab (CRIZ) combination treatment. EVs were isolated from plasma using a combination of differential centrifugation and size exclusion chromatography techniques, and their quantification was performed using nano-tracking analysis. The protein profile of EVs was assessed through mass spectrometry after sample digestion and desalination in a developed protocol for EVs research. The obtained data were analyzed using MaxQuant and Perseus software.

To determine differentially abundant proteins between the HC and SCA groups, statistical tests such as ANOVA followed by Benjamini-Hochberg correction were applied, considering a significance level of $p<0.05$. Additionally, gene ontology analysis was conducted to identify the relevant biological processes (FDR <0,05) associated with the differentially abundant proteins. Firstly, we identified 41 unique proteins in the SCA group. The relevant biological processes associated with these proteins are primarily related to erythroid differentiation, inflammation, cellular response to oxidative stress, and the upregulation of signaling pathways mediated by TNF and other cytokines. These findings align with the characteristic pathophysiology of SCA.

The proteins found in higher concentrations in EVs of SCA patients compared to HC group ($p<0,05$) were mainly related to endocytosis and exocytosis, inflammation, immune response and coagulation process. Moreover, we observed higher levels of hemoglobin chains in SCA EVs. The delta chain was increased in the SCA group with no treatment in relation to the HC group and the SCA group with HU. Beta and alpha chains were also found in increased levels in the SCA group without HU compared to the HC group, SCA with HU and SCA with HU+CRIZ. Additionally, both transferrin (TF) and the transferrin receptor (TFRC-CD71) were increased in all three subgroups of SCA patients compared to the HC group.

The identification of various types of hemoglobin in these EVs holds significant importance. This observation aligns with the potential role of these structures in promoting the generation of reactive oxygen species (ROS), activating endothelial

cells, and reducing nitric oxide levels in individuals with SCA. Such findings may provide valuable insights into the underlying mechanisms contributing to the pathophysiology of SCA and its associated complications.
Moreover, our data shows higher levels of Tenascin-C in the EVs of SCA patients (all subgroups) ($p<0,05$), a protein related to tissue damage and inflammation that has not yet been described in the pathophysiology of the disease.
In summary, we access, for the first time, the protein content of EVs from patients with SCA. The data described here are novel and could help to elucidate the putative functions of EVs in the context of SCA providing valuable insights for future investigations and potential therapeutic strategies.

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