Vascular Ehlers-Danlos syndrome dermal fibroblasts' transcriptome: pathomechanisms and targetable molecules

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BACKGROUND. Vascular Ehlers-Danlos syndrome (vEDS) is a rare connective tissue disorder caused by dominant mutations in the *COL3A1* gene, which encodes type III collagen (COLLIII). COLLIII is primarily expressed in blood vessels and hollow organs, and its reduction leads to fragility of soft connective tissues with arterial and organ ruptures. Currently, there are no specific targeted therapies available for vEDS patients. Although abnormal protein folding due to disruption of the COLLIII triple helical structure is implicated in the disease, the underlying molecular pathology is not well understood. To shed light on altered gene expression profile and associated pathomechanisms, a comprehensive transcriptome analysis was performed on a large cohort of dermal fibroblasts from vEDS patients and healthy donors using RNA- and miRNA-seq.

STUDY DESIGN AND METHODOLOGY. *Human subjects.* All enrolled vEDS patients were clinically evaluated according to the 2017 International classification of EDS at the specialized outpatient clinic for EDS and related connective tissue disorders of the University Hospital Spedali Civili of Brescia. Patients were molecularly characterized for a pathogenic dominant negative *COL3A1* variant in our lab. Signed written informed consent for study participation and skin biopsy was obtained from the 18 recruited vEDS adults and 36 healthy donors according to Italian bioethics laws. *Targeted whole-transcriptome and miRNome.* Dermal fibroblast cell cultures from patients and healthy donors were established in our lab under standardized experimental conditions. To ensure the accuracy and reproducibility of the data, a biological replicate was prepared for each patient and control sample. Quantity and quality control of purified total RNA samples and sequencing libraries was achieved with the QIAxcel system. RNA-seq libraries were performed with the Ion Ampliseq Transcriptome Human Gene Expression panel, which is designed to profile 18,574 protein-coding RNAs (mRNAs) and 2,228 non-coding RNAs (ncRNAs, such as lncRNAs); miRNA-seq libraries were prepared with the QIAseq miRNA library kit. Sequencing was achieved using the Ion S5 Platform. Differential expression (DE) analysis were carried out both with the Transcriptome Analysis Console (mRNAs, ncRNAs) and DESeq2 R package (miRNAs) by applying an FDR-adjusted *p*-value ≤ 0.05 and a fold-change threshold of ±1.5. *Biological interpretation of RNA-seq data.* Gene Ontology (GO) and pathway analyses were performed by querying DAVID database and using the Cytoscape software with the ClueGO and CluePedia plugins. Potential interactions among miRNAs, lncRNAs, and DEGs were obtained from miRNet 2.0.

RESULTS. *RNA-seq findings.* We identified in vEDS *vs* control cells a total of 3,189 DEGs, 3,067 of which were mRNAs and 122 ncRNAs. As shown in the Volcano plot (Figure 1), 2,618 of these DEGs were downregulated and 571 showed an increased expression. Principal component analysis showed the presence of two distinct clusters that clearly separate healthy donors from vEDS patients (Figure 2). *Identification of biological networks.* Significantly enriched biological networks are mainly associated with cellular response to stress, mRNA processing, translation, membrane trafficking including retrograde Golgi-endoplasmic reticulum transport and related to proteostasis and cellular catabolic processes such as ubiquitin-mediated proteolysis, endocytosis, and autophagy (Figure 3). *miRNome profiling.* We identified 137 DE-miRNAs in patient *vs* control cells (71 downregulated and 66 upregulated). Our initial focus was on miR-29a-3p and miR-29b-3p due to their role in regulating critical cellular processes such as remodeling of extracellular matrix (ECM) in the vascular network, autophagy, and apoptosis. To further investigate the role of these miRNAs, we searched for predicted miRNA targets among the 3,189 DEGs. GO analysis revealed that both miRNAs may alter fundamental biological functions like gene expression and translation regulation, and ECM organization. KEGG pathway analysis revealed that miR-29a and miR-29b may impact several biological processes, such as autophagy, cellular senescence, RNA degradation, and affect different signal transduction pathways including mTOR and FoxO signaling pathways (Figure 4A,B). *Target prediction analysis.* Target prediction and regulatory networks analyses suggested potential interactions among miR-29a and miR-29b, lncRNAs (e.g., NEAT1), and many target genes linked to ECM organization, protein degradation pathway, mTOR signaling, and autophagic response (Figure 5A,B).

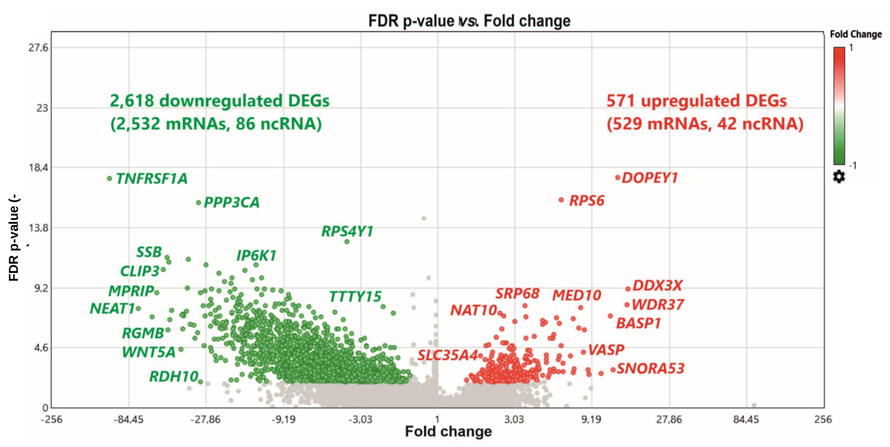

Figure 1. Differential gene expression analysis. Volcano plot displays the distribution of the 2,618 downregulated (green) and the 571 upregulated (red) DEGs. The plots represent expression values as fold change (x-axis) plotted against the -log10 FDR-adjusted p-value (y-axis).

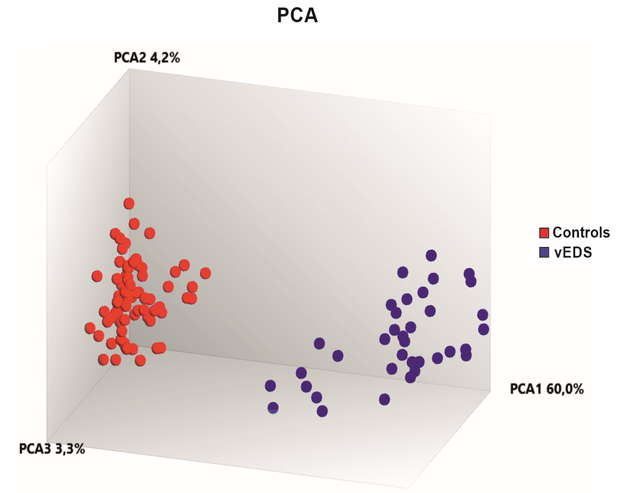

Figure 2. Principal component analysis shows a clear clustering of the 72 control (red dots) and the 36 vEDS (blue dots) samples.

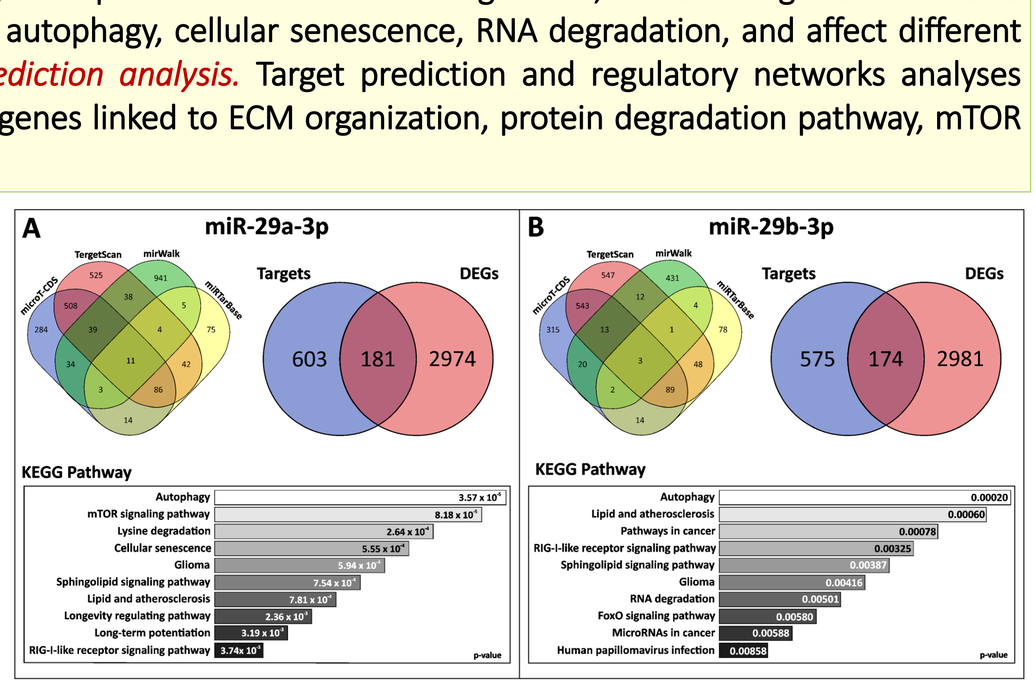

Figure 4. Target prediction analysis for miR-29a-3p (A) and miR-29b-3p (B) by querying the microT-CDS, TargetScan, miRwalk, and miRTarBase databases. The predicted targets for both miRNAs were compared with the 3,189 DEGs, identifying 181 and 174 targets for miR-29a-3p and miR-29b-3p, respectively.

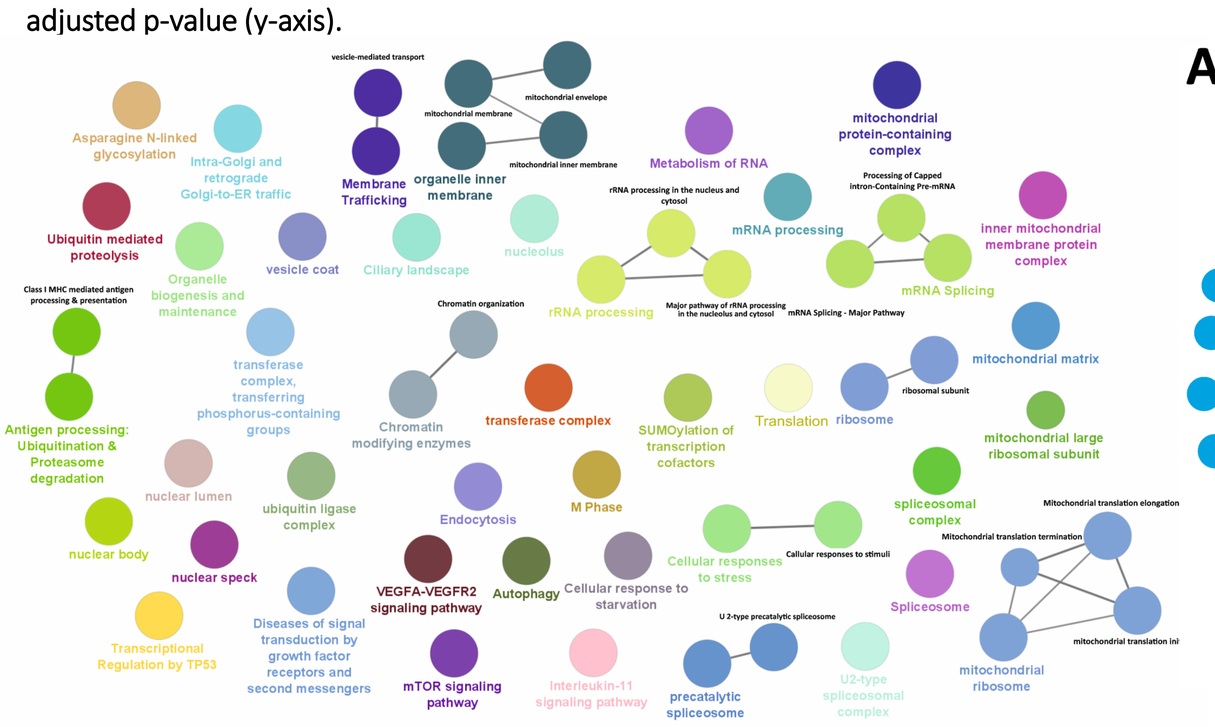

Figure 3. Enriched biological networks obtained with the full list of DEGs identified in vEDS *vs* control cells. Functionally grouped clusters integrating DAVID, WikiPathways, KEGG and Reactome biological databases with terms as nodes linked based on their κ score level. Only enriched terms with a *p*-value ≤ 0.01 were selected.

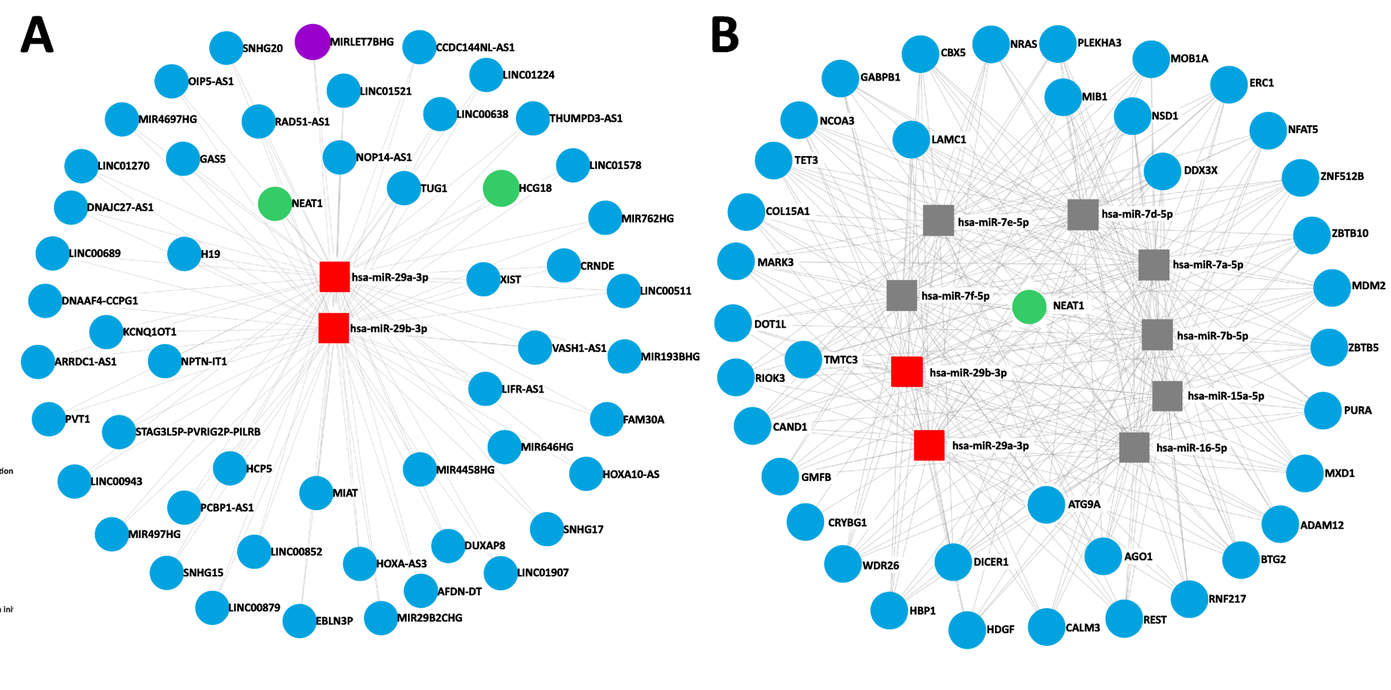

Figure 5. Construction of a miRNA-mRNA-lncRNA interaction network. In silico prediction of co-regulatory lncRNA-miRNA networks involving miR-29a-3p and miR-29b-3p (A). Potential regulatory network involving DE-miRNAs-DEGs-DE-lncRNAs (B). Red squares indicate upregulation of miR-29a-3p and miR-29b-3p, green circles represent downregulation of lncRNAs *NEAT1* and *HCG18*, purple circle indicates upregulation of lncRNA *MIRLET7BHG*, and grey squares represent other DE-miRNAs.

CONCLUSIONS. Our research offers new evidence that defects in the *COL3A1* gene lead to disturbances in the crosstalk and interactions between different components of the proteostasis machinery, compromise protein degradation pathways to overcome endoplasmic reticulum stress, and interfere with the autophagic flux. The impairment of these essential homeostatic and cellular quality control processes that are necessary for preserving healthy cellular function appears to significantly contribute to the molecular pathogenesis of vEDS. We also provide the first detailed miRNAs expression profile in patient cells, demonstrating that several aberrantly expressed miRNAs can negatively impact critical cellular functions involved in disease pathophysiology, such as proteostasis, autophagy, mTOR signaling, and ECM organization. The current transcriptome data significantly advance our knowledge of the molecular pathophysiology underlying vEDS by shedding light on several pathomolecular mechanisms and promising areas for future research aimed at developing therapeutic options.

This research was supported by Fondazione Telethon and "Associazione con Giacomo contro vEDS" - Grant ID: GSA21F001, Seed Grant Fall 2021 vEDS - to Nicola Chiarelli. The main results of this study are recently submitted to the BBA – Molecular Basis of Disease for publication.