A novel view to varicose veins pathogenesis: Proteomic analysis

R. Srinanthalogen MD[1], M. Urbonavicius, stud.pharm[2], G.Urbonaviciene MD, PhD[3],
J. Cicenas, PhD[4], M.Valius PhD[5], S. Urbonavicius, MD, PhD[1]

[1]Dept. of Vascular Surgery, Cardiovascular research unit, Viborg, Hospitalsenhed Midt, Denmark
[2]Dept. Of Pharmacology, University of Copenhagen, Denmark
[3]Dept. of Internal Medicine, Cardiovascular research unit, Silkeborg, Hospitalsenhed Midt, Denmark
[4]Swiss Institute of Bioinformatics, University of Basel, Switzerland
[5]Proteomic Center, Institute of Biochemistry, Vilnius University, Lithuania

Introduction

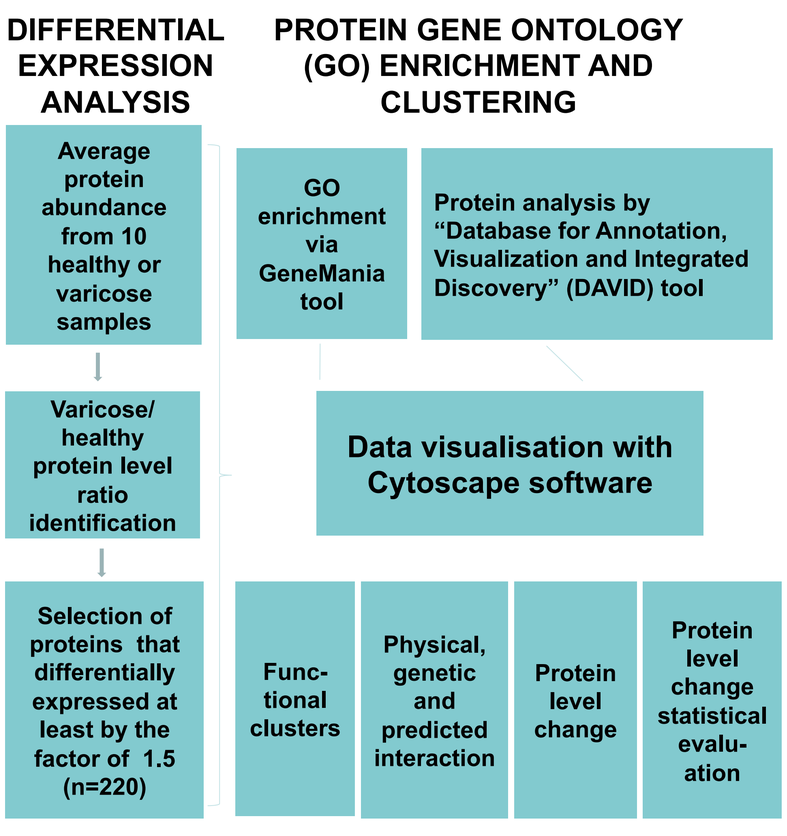
The advent of proteomics techniques allows large-scale studies of gene expression at protein level. Although morphological and anatomical studies indicate that venous wall weakening and subendothelial fibrosis characterize varicose veins, the pathogenesis of varicose veins remains poorly understood.

The aim of this study is to obtain protein expression profiles in patients with varicose veins. Finally, the identification of possible biomarkers may open possibilities for pharmacological inhibition of disease progression.

Methods

Varicose saphenous veins removed during phlebectomy and normal saphenous veins obtained during vascular surgery were collected for proteomics analysis. The same layers of venous wall from varicose and non-varicose veins were incubated, and the proteins released were analyzed by ion mobility spectrometry (IMS-MS) with Synapt G2. Peptides from each sample were separated into 6 SCX fractions, each fraction was analysed three times by RP-LC directly coupled to Synapt G2 HDMS.

Fig.1. Protein identification workflow



Results

Proteomic analysis of the human vein revealed totally 1389 proteins. 220 proteins demonstrated significant differences in their quantity (more than 1.5 fold) between the two types of venous tissue ($p<0.05$). Among the most differentially expressed proteins 10 were found significantly decreased in the varicose vein tissue, and only two increased (1 Table). CXXC-type zinc finger protein was more permanent (38-fold down regulated). This protein is known as receptor for vascular endothelial growth factor. All differentially expressed proteins and their pathways, coexpression and physical interactions were analyzed in GeneMANIA and AmiGO databases.

CXXC-type zinc finger protein, nucleoporin SEH1 and Glyceraldehyde 3-phosphate dehydrogenase GAPDH2 were proved by Western Blotting (WB) analysis (Fig.3).

Fig. 2. Statistical significance of the change of protein level

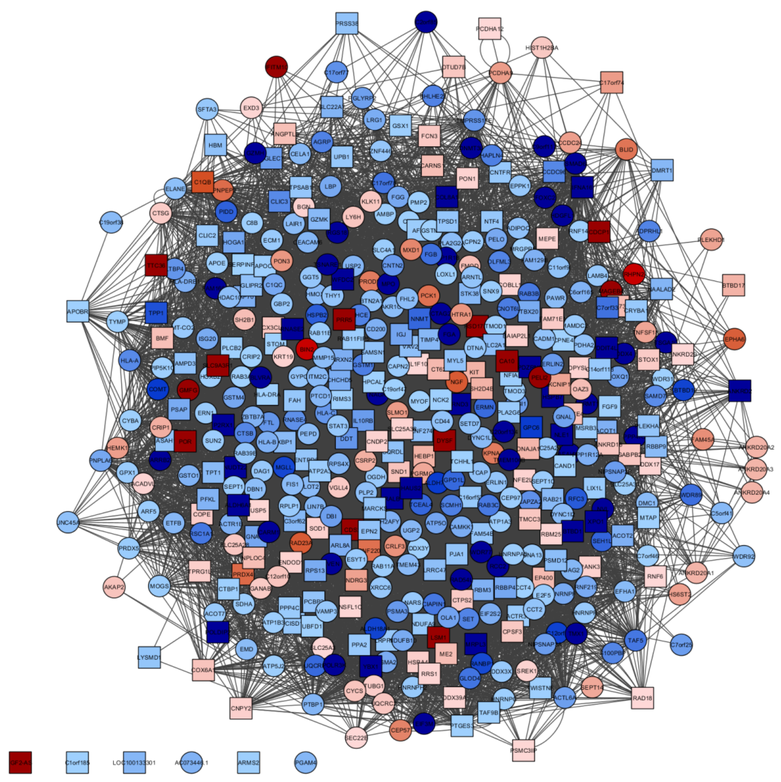
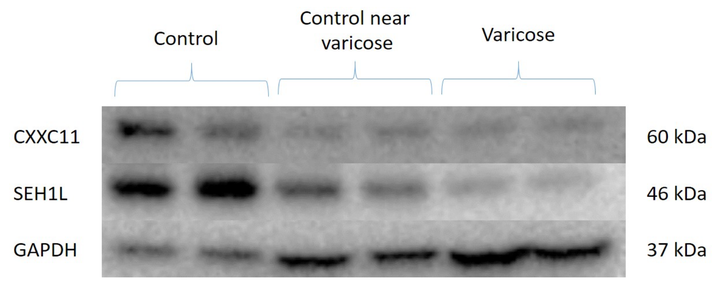


Table 1. Identified proteins

Proteins				
Description	T-test (p)	Anova (p)	Fold	Protein regulation
Keratin, type I cytoskeletal	0,05	0,00	1,47	up
Glyceraldehyde 3-phosphate dehydrogenase	0,03	0,00	2,74	up
CXXC-type zinc finger protein	0,05	0,01	37,68	down
Nucleoporin SEH1	0,04	0,00	3,46	down
Olfactomedin-like protein 3	0,05	0,00	2,80	down
HLA class II histocompatibility antigen	0,03	0,00	2,26	down
Emerin	0,03	0,00	2,15	down
Beta-centractin	0,05	0,01	2,07	down
Transmembrane protein 43	0,04	0,00	1,84	down
Myelin regulatory factor	0,04	0,00	1,72	down
Erlin-1	0,02	0,00	1,63	down
Cell surface glycoprotein	0,04	0,00	1,60	down

Fig. 3. Western blotting analysis of some proteins



Conclusion

This study provides novel insights into the biochemical mechanisms of this disease and provides a basis for further studies.

Our proteomics discovery approach suggests that altered connective tissue proteins and increased proteolytic enzyme activity appear to be central to the pathophysiology of varicose veins. Abnormalities in vein wall architecture probably precede the development of valvular incompetence and overt varicosities.

Larger studies are required to confirm the potential and clinical role of the identified proteins.