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Venous and arterial endothelial proteomics: mining for markers and mechanisms of endothelial diversity

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Abstract

Endothelial cells (ECs) line the inside of arterial and venous blood vessels in a continuous monolayer and have the important function of responding to environmental cues to regulate vascular tone and new blood vessel formation. They also have well-defined roles in supporting tumorigenesis, and alterations in their function lead to cardiovascular disease. Consequently, ECs have been studied extensively as a cellular model of both normal and abnormal physiology. Despite their importance and the increased utility of proteomic tools in medical research, there are relatively few publications on the topic of vascular endothelial proteomics. A thorough search of the literature mined 52 publications focused exclusively on arterial and/or venous endothelial proteomics. These studies mostly relied upon examination of whole-cell lysates from cultured human umbilical vein ECs to investigate *in vitro* effects of various molecules, such as VEGF in the context of altering human umbilical vein EC functions related to angiogenesis. Only a few of these publications focused solely on a proteomic characterization of ECs and our analysis further revealed a lack of published studies incorporating proteomic analysis of freshly isolated ECs from tissues or *in vitro* conditions that mimic *in vivo* variables, such as oxygen tension and shear stress. It is the purpose of this article to account for the diversity of vascular EC proteomic investigations and comment on the issues that have been and should be addressed in future work.

Keywords

artery; endothelial; human umbilical vein endothelial cell; HUVEC; LC-MS/MS; vein

The endothelium is a continuous monolayer of basement membrane secreting adherent cells with a cobblestone appearance that lines the lumen of the vertebrate vasculature. Under normal physiological conditions, the endothelium maintains vascular homeostasis and responds to environmental cues to regulate vascular tone, hemostasis and blood-borne cell adhesion properties that influence local inflammatory and immune responses ^[1].

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Furthermore, the endothelium is an important interface between blood and surrounding tissues in that it determines permeability to cells, solutes, gases and macromolecules. It is also the reservoir of angiogenic potential, where endothelial cells (ECs) proliferate and migrate to form new blood vessels in response to signals derived from local or recruited proangiogenic hematopoietic, epithelial or tumorigenic cells. Together, the endothelium composes one of the largest metabolic organs in the body with important endocrine, paracrine, morphogenic and homeostatic functions [1].

The endothelium is a common dysfunctional denominator among many human vascular diseases including, but not limited to, cardiovascular disease (atherosclerosis), essential hypertension, diabetes and cancer [1]. It is thought that the initiating event in atherogenesis is endothelial injury, which results in inflammation, drawing monocytes into the subendothelial space with subsequent ingestion of modified low-density lipoprotein and differentiation into foam cells. Accumulation of foam cells leads to fatty streak formation, the first morphological lesion in atherosclerosis. Interestingly, lesions appear predominantly in arterial, rather than venous ECs, suggesting some inherent differences in endothelial repair mechanisms between the arterial and venous beds. For decades investigators have detailed specific molecular pathways that regulate the development, maturation and function of endothelium throughout the mammalian organism. Despite this vast knowledge that we have accumulated, it may be the 'tip of the iceberg' as there are many EC subtypes waiting to be explored and characterized at the molecular level.

Vascular endothelium is diverse

There is a basic dichotomy that exists in the form of veins and arteries, which function to remove cellular waste and deliver oxygen and nutrients, respectively. First, because of these distinct differences in function, as well as environmental differences in blood pressure, blood flow, pO₂ and pH, veins and arteries are also distinct at a cellular and molecular level [2]. Second, ECs have functions, morphologies and gene-expression profiles uniquely adapted to meet the needs of their local environment [3,4]. Varying permeability requirements and metabolic demands of the surrounding tissue, among other important parameters, create unique vascular niches to which endothelium must adapt. Third, ECs vary spatially and temporally [5]; even differences in hemodynamic forces between neighboring individual ECs have been observed [6]. It has been argued that this complexity is associated with a healthy endothelium as a dysfunctional endothelium loses this variability [7,8], thus highlighting the importance of characterizing EC diversity at a molecular level. Discovering markers for ECs in various vascular tissue beds would facilitate targeted drug delivery to specific tissues, and proteomic tools are uniquely designed to meet those needs allowing us to envision a future of medicine tailored to individual patient proteomes. A reductionist approach relies heavily upon stochastic discovery and systems biology studies suggest that a holistic, comprehensive approach is necessary to truly understand how individual molecules are acting synergistically to produce a biological effect. Proteomics facilitates this progressive thinking by providing a systematic global discovery approach, bypassing the message 'middle-man' and going directly for the functional molecule.

Proteomics is an extension of genomics, studying the expression, function, identification, interaction and structure of proteins. Although proteins are expressed by genes through the processes of transcription and translation, genomic expression analysis does not necessarily reflect protein levels [9]. Various post-translational modifications occur during or after the translation stage of protein synthesis [10], multiplying the proteome's complexity and adding mechanisms of regulation not reflected in the transcriptome. In addition to providing this extra information, complementary proteomic technologies (gel and liquid chromatography [LC] and mass spectrometry [MS] based) have improved dramatically. Although many

replicates and prefractionation steps are required to attain a truly comprehensive analysis, routine methodologies and instrumentation can now provide a reliable, reproducible and extensive analysis, including protein identification and quantitation. However, surprisingly little is known about the EC proteome, and owing to the inherent difficulties in isolating ECs from different tissues, even less is known regarding the differences in the proteome from ECs in these distinct vascular beds (for an excellent review on this subject see [4]).

Vascular endothelial proteomics

To provide a comprehensive review, we carried out a thorough search of the literature in four separate databases using the following Boolean parameters and keywords: proteomics AND (arterial OR venous) AND endothelial. Each keyword included its synonyms, such as MS, proteome, proteomic, proteomics, 'MS/MS' and MSMS for proteomics. We found 496, 214, 213 and 363 entries in the Scopus, PubMed, Ovid and EBSCO databases, respectively. Of those entries, 551 were unique among all four databases, and by manually checking each entry, it was determined that there were only 52 publications relevant to arterial or venous EC proteomics [11–63]. We noted that there was a gradual increase in the number of publications per year from three in 2003 up to 11 in 2009 (Figure 1A). Since the application of LC-MS/MS for proteomics, many protein analytical strategies have developed, ranging from gel-based to gel-free and from peptide mass fingerprinting (PMF) to MS/MS [64]. Most of these 52 studies (88%) were gel-based, meaning they relied upon 1D or 2D electrophoresis (1- or 2-DE) (Figure 1B) for protein separation and analysis, a trend that continued through 2009. The majority of publications also cited the use of MS/MS over PMF techniques, such as MALDI-TOF, indicating that most of the protein identifications are of higher confidence, and there appears to be a trend toward increased use of MS/MS (Figure 1C).

Owing to the ease of availability of pre-mortem human tissue in the form of the umbilical cord and the larger size of the vein, human umbilical vein ECs (HUVECs) have become a predominant source of human ECs for cell culture. Indeed, 42 out of the 52 papers utilized cultured HUVECs as an EC source. This is reflected in the pie charts shown in Figure 2, which describe the categorization of these studies by species, organ and vessel. The majority of studies did not focus on protein profiling, rather they utilized the HUVEC model in particular to investigate a variety of treatment agents in different biological contexts (Figure 3A).

While the treatment agents were diverse, as indicated in the 'other' slice of the pie chart (Figure 3A), it is interesting that there were multiple vascular EC proteomic investigations using a single test agent. For example, two studies investigated mechanisms whereby sphingosine 1-phosphate (S1P), a bioactive phospholipid growth factor, enhances endothelial barrier integrity [11,36]. Guo *et al.* found that myristoylated alanine-rich protein kinase C substrate (MARCKS) and MARCKS-related protein are recruited to membrane rafts following S1P stimulation and that by silencing expression of these two genes using small interfering RNA, S1P signaling probably occurs via these proteins [36]. Another group published two proteomic studies using HUVECs as a model to better understand the dual positive effects of ouabain, an endogenous Na^+/K^+ ATPase inhibitor also used to treat congestive heart failure, on EC growth and apoptosis. In 2007, ouabain was used at a low-dose to induce EC proliferation and a proteomic approach enabled the discovery of new targets and mechanisms of ouabain action [34]. In 2008, a higher dose of ouabain was administered to induce EC apoptosis, and a number of differentially expressed proteins were identified that may explain alterations in cell behavior induced by higher concentrations of ouabain. In particular, ouabain downregulated heat-shock protein 60, a key anti-apoptotic protein, by threefold in the cultured HUVECs [25]. Other groups utilized the HUVEC model

to investigate various EC functions involved in angiogenesis, an important process in embryonic vascular development, wound healing, tissue regeneration and tumorigenesis. VEGF is a critical regulator of angiogenesis; however, its mechanism of action on vascular ECs is still incompletely understood. To that end, several groups sought to shed light on this problem by determining the proteins that are differentially expressed or phosphorylated following VEGF stimulation of cultured HUVEC [16,30,35,48,56]. Pawlowska *et al.* reported that alterations in a number of structural proteins, including T-plastin, vimentin, α -tubulin, actin and myosin may explain some of the morphological changes that occur during EC migration [48]. Kim *et al.* sought to discover more of the downstream targets of VEGF signaling by incorporating anti-phosphotyrosine western blotting, 2-DE and MS to identify putative VEGF-regulated pathways. They identified ten protein spots that were phosphorylated in response to VEGF, including several proteins related to actin polymerization possibly involved in capillary tubule formation [30].

Cell culture conditions reveal endothelial adaptation

As the endothelium is particularly adaptive and flexible, its environment will influence its phenotype. This is especially true in EC culture where typical conditions do not mimic their *in vivo* surroundings in terms of shear stress and various parameters, such as oxygen tension, may not be physiologically significant. Turbulent shear stress, leading to endothelial dysfunction is thought to be a causal factor behind atheroma formation and cardiovascular disease. However, there are also normal levels of laminar shear flow that influence regulation of EC proliferation, differentiation, remodeling and nitric oxide release that determines vascular tone. Most EC culture occurs in static conditions, which do not mimic *in vivo* physiology and possibly increase phenotypic drift; however, several groups have investigated the EC proteome in cells under physiological levels of shear stress, ranging from 10 to 30 dynes/cm² [13,17,21,32]. In 2007, Wang *et al.* exposed bovine aortic ECs to shear stress for varying lengths of time (10 min, 3 or 6 h). At each time point they discovered hundreds of differentially expressed proteins related to shear stress signaling pathways, including integrins, G-protein-coupled receptors, glutamate receptors, phosphoinositol 3-kinase/serine–threonine kinase Akt (PI3K/AKT), apoptosis, Notch and cAMP-mediated signaling pathways. This novel study provided new insights into potential regulatory mechanisms of the response of EC to shear stress and ‘footprints’ for further studies on the signaling mechanisms induced by shear stress, while simultaneously providing evidence that static cultured EC are not the same as those EC cultured under shear stress [32]. In 2009, Huang *et al.* published an elegant study identifying over 100 proteins modified by *S*-nitrosylation, a post-translational modification forming from nitric oxide that may alter protein function and signaling [17]. A total of 12 proteins, including tropomyosin and vimentin, displayed a significant increase in *S*-nitrosylation in response to EC shear stress *in vitro*.

Culturing cells in room air oxygen (20%) is much higher than tissue oxygen tension for the majority of the vasculature, which has been reported to range from 3 to 13% [65,66]. Indeed, culturing ECs at varying oxygen pressures has been shown to affect cellular functions, such as proliferation, chemotaxis and tubulogenesis [67]. Whether it is due to phenotypic drift or actually an active adaptation process, it is clear that ECs *in vitro* differ from their *in vivo* counterparts and that seems to increase as the cells continue to divide. Several proteomic studies were carried out looking at differences between early and late passage ECs, largely for the purposes of investigating mechanisms behind replicative senescence [45,52,62]. Chang and colleagues discovered a loss of cytokeratin 7 in senescent HUVECs, which they noted could contribute to the observed enlarged and flattened morphology by destabilizing the cytoskeleton. They also compared early and late passage ECs with ECs immortalized by ectopic expression of the catalytic subunit of human telomerase. They found many common

differentially expressed proteins between the senescent and immortalized cells compared with the early passage ECs, which could be attributed to culturing rather than senescence [52]. Durr *et al.* identified 450 proteins in luminal EC plasma membranes from rat lungs and cultured rat lung microvascular ECs using multidimensional protein identification technology (MudPIT) [68]. They found that 41% of proteins expressed *in vivo* were not detected *in vitro* despite having at least 95% confidence of analytical completeness (i.e., identification of all identifiable proteins present in the sample), thus giving support to the idea that *in vitro* culture studies may not be as relevant to *in vivo* physiology as previously hoped. Moreover, it should be noted that HUVECs may not even be a relevant model for endothelium in general as they are unique among veins in that they carry oxygenated blood and possess their unique histology compared with regular veins. They concluded by advising large-scale mapping of ECs *in vivo* because of the dominant effect the tissue microenvironment may play in EC physiology. Therefore, to date, the accumulated data suggest that studies should be performed with freshly isolated ECs whenever possible; unfortunately, only one of the 52 studies, Bianchi *et al.*, utilized freshly isolated ECs for their analysis [41].

Proteomic characterization of endothelium

Only two publications comparing venous and arterial ECs using a proteomic approach were identified among the 52 papers selected, and these were among 12 reports that involved no treatment (Figure 3A). Bianchi and colleagues compared bovine arterial (thoracic aorta) and venous (inferior vena cava) ECs with lymphatic (thoracic duct) ECs and reported that protein profiles of venous EC were more similar to the lymphatic than the arterial EC [41]. Of the 64 proteins identified, some potentially important differences between artery and vein ECs were noted and three bovine EC 2-DE reference maps for the various vascular sources were highlighted. In particular, the authors noted the presence of PDZ and LIM domain protein 1, as well as finding that tumor protein D52 was not identified in venous ECs, and annexin II was more than sixfold higher in arterial ECs. Furthermore, carbonic anhydrase II, peroxiredoxin II and LMNA protein were at least twofold higher in venous than arterial endothelium. Several of the cytoskeletal proteins were identified as pleiotropic in the EC from all the samples, partially explaining the adaptive nature of the endothelium. Nguyen *et al.* published a similar report in 2010 using cultured ECs from smaller vessels of the bovine mesentery harvested at the twentieth passage. They identified 39 differentially expressed proteins between all three (arterial, venous and lymphatic) EC types and also reported that lymphatic and arterial EC were the most divergent in terms of their protein profiles [61]. However, there was little overlap with Bianchi's reported list of proteins. The differences could be due to the fact that Bianchi used freshly isolated cells, or simply because they isolated cells from different anatomical sources. As the authors astutely noted, the differences in heat-shock protein and peroxiredoxin levels between cell types may reflect differences in the way they responded to the hyperoxic stress of room air culture conditions.

Several groups offered proteomic characterization of ECs. In 2003, Bruneel *et al.* used low-level protein loading (20–30 µg) with 2-DE, MALDI-TOF MS and LC-MS/MS to characterize the HUVEC proteome [60]. In addition to providing 2-DE maps of HUVECs, they also identified 53 proteins of suspected endothelial origin including cytoskeletal, cellular motility, apoptosis, senescence, and coagulation-related proteins. In 2006, González-Cabrero *et al.* published a chapter detailing methods of EC proteome characterization. They provided a 2-DE map of the endothelial proteome detecting 600 protein spots using 4.0–7.0 pH range IPG strips [38].

The majority of the 52 publications discussed here have utilized whole-cell lysates; however, several groups reported investigations of subcellular compartments, such as cell

membrane, secreted caveolae, lipid rafts and microparticle subproteomes (Figure 3B), and several of these groups were interested in characterizing the proteomes. For example, in 2005, Scheurer *et al.* described a new method involving isolation of membrane proteins through biotinylation and subsequent creation of 2D peptide maps (chromatographic fraction and *m/z* value acquired by MALDI-TOF) for relative quantitation. They identified 71 proteins, 41% of which were classified as type I membrane proteins such as PECAM-1, integrins, VE-cadherin, integral membrane proteins, such as monocarboxylate transporter and sodium channel protein type I α -subunit, and membrane associated proteins, such as caveolin-1 and α -1 catenin. Also in 2005, Banfi *et al.* isolated EC-derived microparticles following stimulation with TNF- α and identified over 70 proteins, demonstrating a procoagulant effect of the microparticles on ECs [54]. In 2009, Tunica *et al.* characterized the HUVEC secretome using free-flow electrophoresis for sample complexity reduction. They identified 374 proteins, and according to an unsupervised pathway analysis, the top four canonical pathways represented were members of the coagulation system, IGF-1 signaling, complement system and leukocyte extravasation signaling.

Expert commentary

The endothelium is one of the largest organs in the human body and plays a central role in both cancer and cardiovascular disease. Nevertheless, relatively little is known regarding its proteome, the molecular network responsible for its function. ECs have many diverse subtypes that are largely unexplored and vary, both spatially and temporally, in human or even model organisms. Given the need for tissue-specific drug targets, proteomic approaches are invaluable in meeting those needs. As tools of proteomics continue to improve towards a more complete analysis identifying lower abundant proteins, they should continue to be applied to unraveling mechanisms of endothelial function and dysfunction. The rate-limiting step, however, will be developing methods to isolate pure populations of ECs from the many diverse tissues in the human body. As markers and mechanisms are discovered, medical therapies will follow with the ultimate goal being tailored personalized medicine.

Five-year view

Our understanding of the formation, maintenance and repair of the vascular endothelium is improving with better strategies and techniques. As proteomic tools continue to improve, it will be important to apply these tools to areas of endothelial biology. However, a current limitation to the widespread use of proteomics technologies is the relative high cost of the procedure compared with the limited funding available for this type of 'discovery' research. It is important to note that proteomics approaches often lead to novel insights and new opportunities for mechanistic evaluation. Thus, it is hoped that some new funding sources for discovery research may permit more broad use of the latest proteomic applications by all scientific fields. We anticipate an increased use of MS/MS-based tools, not only for identification but also for quantitation of EC proteins as the field moves away from gel-based approaches. As the need for tissue-specific drug targets increases, so will our need for protein profiling in various endothelial subtypes. However, the rate-limiting step in these analyses will be isolating ECs from these specialized tissue beds. As sensitivity of MS instrumentation improves and less starting material is required, techniques such as laser capture microscopy (with or without fixation) and micro-manipulative mechanical dissection will be of greater use. In the next 5 years, we should expect to see a continued use of HUVECs as an endothelial model for the investigation of various molecules, although use of tissue culture conditions that more recapitulate tissue microenvironments with respect to extracellular matrix composition, oxygen tension, nutrients and shear stress are inevitable to better model *in vivo* vertebrate physiology. Proteomic prefractionation strategies, such as

MudPIT, free-flow electrophoresis and subcellular fractionation, should be applied toward global characterization, revealing lower abundant proteins that could likely explain functional and structural differences at the cellular, tissue and organ level.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1. Aird, WC. Endothelial Biomedicine. Cambridge University Press; Cambridge, UK: 2007.
2. Lin FJ, Tsai MJ, Tsai SY. Artery and vein formation: a tug of war between different forces. *EMBO Rep.* 2007; 8(10):920–924. [PubMed: 17906673]
3. Chi JT, Chang HY, Haraldsen G, et al. Endothelial cell diversity revealed by global expression profiling. *Proc Natl Acad Sci USA.* 2003; 100(19):10623–10628. [PubMed: 12963823]
4. Carver, LA.; Schnitzer, JE. Proteomic mapping of endothelium and vascular targeting *in vivo*. In: William, C.; Aird, MD., editors. Endothelial Biomedicine. Cambridge University Press; Cambridge, UK: 2007. p. 881–897.
5. Aird WC. Spatial and temporal dynamics of the endothelium. *J Thromb Haemost.* 2005; 3(7):1392–1406. [PubMed: 15892866]
6. Davies PF, Zilberberg J, Helmke BP. Spatial microstimuli in endothelial mechanosignaling. *Circ Res.* 2003; 92(4):359–370. [PubMed: 12623874]
7. Aird WC. Endothelial cell dynamics and complexity theory. *Crit Care Med.* 2002; 30(5 Suppl):S180–S185. [PubMed: 12004233]
8. Goldberger AL, Amaral LA, Hausdorff JM, Ivanov P, Peng CK, Stanley HE. Fractal dynamics in physiology: alterations with disease and aging. *Proc Natl Acad Sci USA.* 2002; 99(Suppl 1):2466–2472. [PubMed: 11875196]
9. Maier T, Guell M, Serrano L. Correlation of mRNA and protein in complex biological samples. *FEBS Lett.* 2009; 583(24):3966–3973. [PubMed: 19850042]
10. Rai AJ, Zhang Z, Rosenzweig J, et al. Proteomic approaches to tumor marker discovery. *Arch Pathol Lab Med.* 2002; 126(12):1518–1526. [PubMed: 12456215]
11. Zhao J, Singleton PA, Brown ME, Dudek SM, Garcia JGN. Phosphotyrosine protein dynamics in cell membrane rafts of sphingosine-1-phosphate-stimulated human endothelium: role in barrier enhancement. *Cell Signal.* 2009; 21(12):1945–1960. [PubMed: 19755153]
12. Wu H-L, Li Y-H, Lin Y-H, et al. Salvianolic acid B protects human endothelial cells from oxidative stress damage: a possible protective role of glucose-regulated protein 78 induction. *Cardiovasc Res.* 2009; 81(1):148–158. [PubMed: 18815184]
13. Wang XL, Fu A, Spiro C, Lee HC. Proteomic analysis of vascular endothelial cells-effects of laminar shear stress and high glucose. *J Proteomics Bioinform.* 2009; 2:445. [PubMed: 20148124]
- 14•. Tunica DG, Yin X, Sidibe A, et al. Proteomic analysis of the secretome of human umbilical vein endothelial cells using a combination of free-flow electrophoresis and nanoflow LC-MS/MS. *Proteomics.* 2009; 9(21):4991–4996. Demonstrates excellent usage of proteomic tools for complexity reduction and identifies hundreds of secreted endothelial proteins. [PubMed: 19810032]
15. Rudic B, Song H, Breedijk A, et al. Hypothermic preservation up-regulates calpain expression and increases ubiquitination in cultured vascular endothelial cells: influence of dopamine pretreatment. *J Surg Res.* 2009; 160(2):325–332. [PubMed: 19375722]
16. Jorge I, Navarro P, Martínez-Acedo P, et al. Statistical model to analyze quantitative proteomics data obtained by $^{18}\text{O}/^{16}\text{O}$ labeling and linear ion trap mass spectrometry: application to the study of vascular endothelial growth factor-induced angiogenesis in endothelial cells. *Mol Cell Proteomics.* 2009; 8(5):1130–1149. [PubMed: 19181660]

17. Huang B, Chen SC, Wang DL. Shear flow increases S-nitrosylation of proteins in endothelial cells. *Cardiovasc Res*. 2009; 83(3):536–546. [PubMed: 19447776]
18. Bussone G, Dib H, Dimitrov JD, et al. Identification of target antigens of self-reactive IgG in intravenous immunoglobulin preparations. *Proteomics*. 2009; 9(8):2253–2262. [PubMed: 19296548]
19. Bossi F, Rizzi L, Bulla R, et al. C7 is expressed on endothelial cells as a trap for the assembling terminal complement complex and may exert anti-inflammatory function. *Blood*. 2009; 113(15):3640–3648. [PubMed: 19179470]
20. Bieler S, Meiners S, Stangl V, Pohl T, Stangl K. Comprehensive proteomic and transcriptomic analysis reveals early induction of a protective anti-oxidative stress response by low-dose proteasome inhibition. *Proteomics*. 2009; 9(12):3257–3267. [PubMed: 19562799]
21. Asif AR, Oellerich M, Armstrong VW, Hecker M, Cattaruzza M. T-786C polymorphism of the *nos-3* gene and the endothelial cell response to fluid shear stress – a proteome analysis. *J Proteome Res*. 2009; 8(6):3161–3168. [PubMed: 19320461]
22. Zoeller JJ, Iozzo RV. Proteomic profiling of endorepellin angiostatic activity on human endothelial cells. *Proteome Sci*. 2008; 6:7. [PubMed: 18269764]
23. Servettaz A, Guilpain P, Camoin L, et al. Identification of target antigens of antiendothelial cell antibodies in healthy individuals: a proteomic approach. *Proteomics*. 2008; 8(5):1000–1008. [PubMed: 18240136]
24. Qiu J, Gao HQ, Liang Y, Yu H, Zhou RH. Comparative proteomics analysis reveals role of heat shock protein 60 in digoxin-induced toxicity in human endothelial cells. *Biochim Biophys Acta*. 2008; 1784(11):1857–1864. [PubMed: 18692161]
25. Qiu J, Gao HQ, Li BY, Shen L. Proteomics investigation of protein expression changes in ouabain induced apoptosis in human umbilical vein endothelial cells. *J Cell Biochem*. 2008; 104(3):1054–1064. [PubMed: 18247327]
26. Gustin C, Delaive E, Dieu M, Calay D, Raes M. Upregulation of pentraxin-3 in human endothelial cells after lysophosphatidic acid exposure. *Arterioscler Thromb Vasc Biol*. 2008; 28(3):491–497. [PubMed: 18162608]
27. Evans IM, Britton G, Zachary IC. Vascular endothelial growth factor induces heat shock protein (HSP) 27 serine 82 phosphorylation and endothelial tubulogenesis via protein kinase D and independent of p38 kinase. *Cell Signal*. 2008; 20(7):1375–1384. [PubMed: 18440775]
28. Eguchi R, Naitou H, Kunimasa K, et al. Proteomic analysis of hypoxia-induced tube breakdown of an *in vitro* capillary model composed of HUVECs: potential role of p38-regulated reduction of HSP27. *Proteomics*. 2008; 8(14):2897–2906. [PubMed: 18655027]
29. Young MK, Seo J, Yung HK, et al. Systemic analysis of tyrosine phosphorylated proteins in angiopoietin-1 induced signaling pathway of endothelial cells. *J Proteome Res*. 2007; 6(8):3278–3290. [PubMed: 17595127]
30. Young MK, Eun JS, Seo J, Kim HJ, Lee KJ. Proteomic analysis of tyrosine phosphorylations in vascular endothelial growth factor- and reactive oxygen species-mediated signaling pathway. *J Proteome Res*. 2007; 6(2):593–601. [PubMed: 17269716]
31. Wu J, Liu W, Sousa E, et al. Proteomic identification of endothelial proteins isolated in situ from atherosclerotic aorta via systemic perfusion. *J Proteome Res*. 2007; 6(12):4728–4736. [PubMed: 17997517]
32. Wang XL, Fu A, Raghavakaimal S, Lee HC. Proteomic analysis of vascular endothelial cells in response to laminar shear stress. *Proteomics*. 2007; 7(4):588–596. Reports results of a thorough proteomic analysis identifying hundreds of differentially expressed proteins in response to shear stress. [PubMed: 17309104]
33. Wadham C, Parker A, Wang L, Xia P. High glucose attenuates protein S-nitrosylation in endothelial cells: role of oxidative stress. *Diabetes*. 2007; 56(11):2715–2721. [PubMed: 17704302]
34. Qiu J, Gao HQ, Zhou RH, et al. Proteomics analysis of the proliferative effect of low-dose ouabain on human endothelial cells. *Biol Pharmaceut Bull*. 2007; 30(2):247–253.

35. Katanasaka Y, Asai T, Naitou H, Ohashi N, Oku N. Proteomic characterization of angiogenic endothelial cells stimulated with cancer cell-conditioned medium. *Biol Pharmaceut Bull.* 2007; 30(12):2300–2307.
36. Guo Y, Singleton PA, Rowshan A, et al. Quantitative proteomics analysis of human endothelial cell membrane rafts: evidence of MARCKS and MRP regulation in the sphingosine 1-phosphate-induced barrier enhancement. *Mol Cell Proteomics.* 2007; 6(4):689–696. Provides an excellent model for the use of proteomics combining tandem mass spectrometry and subcellular fractionation followed by investigation of the hypotheses generated. [PubMed: 17210631]
37. Guilpain P, Servettaz A, Tamby MC, et al. A combined SDS-PAGE and proteomics approach to identify target autoantigens in healthy individuals and patients with autoimmune diseases. *Ann NY Acad Sci.* 2007; 1109:538–549. [PubMed: 17785343]
38. González-Cabrero J, Pozo M, Durán MC, de Nicolás R, Egido J, Vivanco F. The proteome of endothelial cells. *Methods Mol Biol.* 2007; 357:181–198. [PubMed: 17172688]
39. Chen CY, Lee CM, Hsu HC, Yang CY, Chow LP, Lee YT. Proteomic approach to study the effects of various oxidatively modified low-density lipoprotein on regulation of protein expression in human umbilical vein endothelial cell. *Life Sci.* 2007; 80(26):2469–2480. [PubMed: 17509619]
40. Bouyssie D, Gonzalez de Peredo A, Mouton E, et al. Mascot file parsing and quantification (MFPaQ), a new software to parse, validate, and quantify proteomics data generated by ICAT and SILAC mass spectrometric analyses: application to the proteomics study of membrane proteins from primary human endothelial cells. *Mol Cell Proteomics.* 2007; 6(9):1621–1637. [PubMed: 17533220]
41. Bianchi L, Lorenzoni P, Bini L, et al. Protein expression profiles of *Bos taurus* blood and lymphatic vessel endothelial cells. *Proteomics.* 2007; 7(10):1600–1614. The sole proteomic report using freshly isolated tissues to demonstrate differences between artery and vein endothelial cells. [PubMed: 17486557]
42. Wang T, Chen Z, Wang X, Shyy JY, Zhu Y. Cholesterol loading increases the translocation of ATP synthase beta chain into membrane caveolae in vascular endothelial cells. *Biochim Biophys Acta.* 2006; 1761(10):1182–1190. [PubMed: 16996794]
43. Sprenger RR, Fontijn RD, van Marle J, Pannekoek H, Horrevoets AJG. Spatial segregation of transport and signalling functions between human endothelial caveolae and lipid raft proteomes. *Biochem J.* 2006; 400(3):401–410. [PubMed: 16886909]
44. Ma ZC, Gao Y, Wang J, Zhang XM, Wang SQ. Proteomic analysis effects of ginsenoside Rg1 on human umbilical vein endothelial cells stimulated by tumor necrosis factor- α . *Life Sci.* 2006; 79(2):175–181. [PubMed: 16487547]
45. Eman MR, Regan-Klapisz E, Pinkse MW, et al. Protein expression dynamics during replicative senescence of endothelial cells studied by 2-D differences in-gel electrophoresis. *Electrophoresis.* 2006; 27(8):1669–1682. [PubMed: 16609940]
46. Cai H, Gehrig P, Scott TM, Zimmermann R, Schlapbach R, Zisch AH. MnSOD marks cord blood late outgrowth endothelial cells and accompanies robust resistance to oxidative stress. *Biochem Biophys Res Comm.* 2006; 350(2):364–369. [PubMed: 17010941]
47. Scheurer SB, Rybak JN, Roesli C, et al. Identification and relative quantification of membrane proteins by surface biotinylation and two-dimensional peptide mapping. *Proteomics.* 2005; 5(11):2718–2728. [PubMed: 15986331]
48. Pawlowska Z, Baranska P, Jerczynska H, Koziolkiewicz W, Cierniewski CS. Heat shock proteins and other components of cellular machinery for protein synthesis are up-regulated in vascular endothelial cell growth factor-activated human endothelial cells. *Proteomics.* 2005; 5(5):1217–1227. [PubMed: 15761953]
49. Lamé MW, Jones AD, Wilson DW, Segall HJ. Monocrotaline pyrrole targets proteins with and without cysteine residues in the cytosol and membranes of human pulmonary artery endothelial cells. *Proteomics.* 2005; 5(17):4398–4413. [PubMed: 16222722]
50. Karsan A, Pollet I, Yu LR, et al. Quantitative proteomic analysis of sotrasterol sulfate-stimulated primary human endothelial cells. *Mol Cell Proteomics.* 2005; 4(2):191–204. [PubMed: 15611527]

51. Chen XL, He XH, Zhang Y, et al. Effect of chronic enhanced external counterpulsation on arterial endothelial cells of porcine with hypercholesteremia. *Di 1 Jun Yi Da Xue Xue Bao*. 2005; 25(12): 1491–1493. [PubMed: 16354613]
52. Chang MWF, Grillari J, Mayrhofer C, et al. Comparison of early passage, senescent and hTERT immortalized endothelial cells. *Exp Cell Res*. 2005; 309(1):121–136. [PubMed: 15964568]
53. Bruneel A, Labas V, Mailloux A, et al. Proteomics of human umbilical vein endothelial cells applied to etoposide-induced apoptosis. *Proteomics*. 2005; 5(15):3876–3884. [PubMed: 16130169]
54. Banfi C, Brioschi M, Wait R, et al. Proteome of endothelial cell-derived procoagulant microparticles. *Proteomics*. 2005; 5(17):4443–4455. [PubMed: 16220532]
55. Yu M, Chen DM, Hu G, Wang H. Proteomic response analysis of endothelial cells of human coronary artery to stimulation with carbachol. *Acta Pharmacologica Sinica*. 2004; 25(9):1124–1130. [PubMed: 15339386]
56. Traxler E, Bayer E, Stöckl J, Mohr T, Lenz C, Gerner C. Towards a standardized human proteome database: quantitative proteome profiling of living cells. *Proteomics*. 2004; 4(5):1314–1323. [PubMed: 15188398]
57. Sprenger RR, Speijer D, Back JW, de Koster CG, Pannekoek H, Horrevoets AJ. Comparative proteomics of human endothelial cell caveolae and rafts using two-dimensional gel electrophoresis and mass spectrometry. *Electrophoresis*. 2004; 25(1):156–172. [PubMed: 14730580]
58. Scheurer SB, Rybak JN, Rösli C, Neri D, Elia G. Modulation of gene expression by hypoxia in human umbilical cord vein endothelial cells: a transcriptomic and proteomic study. *Proteomics*. 2004; 4(6):1737–1760. [PubMed: 15174142]
59. Franzen B, Duvefelt K, Jonsson C, et al. Gene and protein expression profiling of human cerebral endothelial cells activated with tumor necrosis factor- α . *Brain Res Mol Brain Res*. 2003; 115(2): 130–146. [PubMed: 12877984]
60. Bruneel A, Labas V, Mailloux A, et al. Proteomic study of human umbilical vein endothelial cells in culture. *Proteomics*. 2003; 3(5):714–723. [PubMed: 12748950]
- 61•. Nguyen VP, Hanna G, Rodrigues N, et al. Differential proteomic analysis of lymphatic, venous, and arterial endothelial cells extracted from bovine mesenteric vessels. *Proteomics*. 2010; 10(8): 1658–1672. Compares cultured artery and vein endothelial cells and compares results with those by Bianchi et al ^[41]. [PubMed: 20186751]
62. Kamino H, Hiratsuka M, Toda T, et al. Searching for genes involved in arteriosclerosis: proteomic analysis of cultured human umbilical vein endothelial cells undergoing replicative senescence. *Cell Struct Funct*. 2003; 28(6):495–503. [PubMed: 15004419]
63. Li A, Li H, Jin G, Xiu R. A proteomic study on cell cycle progression of endothelium exposed to tumor conditioned medium and the possible role of cyclin 29(3–4), D₁/E. *Clin Hemorheol Microcirc*. 2003;383–390. [PubMed: 14724365]
64. Lambert JP, Ethier M, Smith JC, Figeys D. Proteomics: from gel based to gel free. *Anal Chem*. 2005; 77(12):3771–3787. [PubMed: 15952756]
65. Fehrer C, Brunauer R, Laschober G, et al. Reduced oxygen tension attenuates differentiation capacity of human mesenchymal stem cells and prolongs their lifespan. *Aging Cell*. 2007; 6(6): 745–757. [PubMed: 17925003]
66. Steurer J, Hoffmann U, Dur P, Russi E, Vetter W. Changes in arterial and transcutaneous oxygen and carbon dioxide tensions during and after voluntary hyperventilation. *Respiration*. 1997; 64(3): 200–205. [PubMed: 9154671]
67. Decaris ML, Lee CI, Yoder MC, Tarantal AF, Leach JK. Influence of the oxygen microenvironment on the proangiogenic potential of human endothelial colony forming cells. *Angiogenesis*. 2009; 12(4):303–311. [PubMed: 19544080]
- 68•. Durr E, Yu J, Krasinska KM, et al. Direct proteomic mapping of the lung microvascular endothelial cell surface *in vivo* and in cell culture. *Nat Biotechnol*. 2004; 22(8):985–992. Reports the results of a comprehensive proteomic study identifying 450 luminal plasma membrane proteins and demonstrates important differences in cells isolated *in vivo* compared with those cultured *in vitro*. [PubMed: 15258593]

Key issues

- The endothelium is a diverse, multisystem regulating tissue with important roles in vascular homeostasis and pathophysiology.
- There are relatively few publications focusing on the topic of vascular endothelial proteomics: only 52 publications regarding arterial and/or venous endothelial proteomics have been identified in a recent search.
- Most of these studies utilized human umbilical vein endothelial cells (ECs) as a model to discover novel mechanisms of action for various growth factors or drugs.
- Endothelial cell culture conditions typically do not include use of variable oxygen tension and shear flow conditions similar to the resident *in vivo* environment resulting in increased phenotypic drift or adaptation and decreased relevance and thus, changes in shear stress and passage in cell culture alter the endothelial proteome of isolated primary cells.
- A surprising paucity of proteomic information is available from freshly isolated arterial and venous ECs and less is known of the tissue specific changes in the EC proteome displayed among different tissues and organs.

Figure 1. Vascular endothelial cell proteomics over time and techniques used

The graphs indicate how the 52 publications reviewed here are distributed over (A) time and by technique in terms of separation method used, for example, (B) gel-based and by (C) the mass spectrometry technique used for protein identification.

1-DE: 1D electrophoresis; 2-D DIGE: 2D difference in-gel electrophoresis;

2-DE: 2D electrophoresis.

Figure 2. Vascular endothelial cell proteomic publications by species, organ and vessel

Of the 52 vascular endothelial cell proteomic publications discussed in this article, the majority utilized human umbilical venous endothelial cells, which is reflected in these charts that describe the distribution of publications in terms of (A) species, (B) organ and (C) vessel.

Figure 3. Vascular endothelial cell proteomic investigations

(A) Analysis of vascular endothelial cell publications reveals several common areas of investigation as determined by the treatment agent used (e.g., protein, drug, condition). 'Other' includes carbachol, cholesterol, COMP-Ang1, atherosclerosis, cytokines (TNF- α , IFN- γ and lymphotoxin α/β), digoxin, dopamine, endorepellin, etoposide, high-glucose, IgG, low-density lipoprotein, L-NAME, lysophosphatidic acid, MG132 (proteasome inhibitor), monocrotaline, and SB203580 (p38 kinase inhibitor), salvianolic acid B (Sal B) and sokotrasterol sulfate. (B) Whole cell lysates account for the majority of vascular endothelial cell publications although there have been several subcellular investigations. 'Other' includes *in situ* biotinylation of vascular endothelial cells resulting in a membrane-rich fraction, cell fractionation (removal of nuclei and cytoskeletal components leaving the plasma membrane, Golgi, endoplasmic reticulum, mitochondria, lysosomes, and all other membrane-bound vesicles), microparticles and supernatant S-100 fraction (cytoplasmic protein fraction enriched in organelles, except nuclei).