REVIEW

Omics of endothelial cell dysfunction in sepsis

Jordan C Langston[1], **Michael T Rossi**[2], **Qingliang Yang**[3], **William Ohley**[4], **Edwin Perez**[4], **Laurie E Kilpatrick**[5], **Balabhaskar Prabhakarpandian**[5] and **Mohammad F Kiani**[1,3]

[1]Department of Bioengineering, Temple University, Philadelphia, Pennsylvania, USA
[2]Illumina, San Diego, California, USA
[3]Department of Mechanical Engineering, Temple University, Philadelphia, Pennsylvania, USA
[4]Lewis Katz School of Medicine, Temple University, Philadelphia, Pennsylvania, USA
[5]Center for Inflammation and Lung Research, Department of Microbiology, Immunology and Inflammation, Lewis Katz School of Medicine, Temple University, Philadelphia, Pennsylvania, USA

Correspondence should be addressed to M F Kiani: mkiani@temple.edu

Abstract

During sepsis, defined as life-threatening organ dysfunction due to dysregulated host response to infection, systemic inflammation activates endothelial cells and initiates a multifaceted cascade of pro-inflammatory signaling events, resulting in increased permeability and excessive recruitment of leukocytes. Vascular endothelial cells share many common properties but have organ-specific phenotypes with unique structure and function. Thus, therapies directed against endothelial cell phenotypes are needed to address organ-specific endothelial cell dysfunction. Omics allow for the study of expressed genes, proteins and/or metabolites in biological systems and provide insight on temporal and spatial evolution of signals during normal and diseased conditions. Proteomics quantifies protein expression, identifies protein–protein interactions and can reveal mechanistic changes in endothelial cells that would not be possible to study via reductionist methods alone. In this review, we provide an overview of how sepsis pathophysiology impacts omics with a focus on proteomic analysis of mouse endothelial cells during sepsis/inflammation and its relationship with the more clinically relevant omics of human endothelial cells. We discuss how omics has been used to define septic endotype signatures in different populations with a focus on proteomic analysis in organ-specific microvascular endothelial cells during sepsis or septic-like inflammation. We believe that studies defining septic endotypes based on proteomic expression in endothelial cell phenotypes are urgently needed to complement omic profiling of whole blood and better define sepsis subphenotypes. Lastly, we provide a discussion of how *in silico* modeling can be used to leverage the large volume of omics data to map response pathways in sepsis.

Keywords

- endothelium
- sepsis
- omics
- systems biology
- microphysiological systems

Introduction

Sepsis is a clinical syndrome defined as life-threatening organ dysfunction due to dysregulated host response to infection (1). It is a major health issue with the number of cases ranging from 19 to 50 million per year and is a leading cause of death globally (2). Sepsis can be caused by primary bacterial, fungal or viral infections or secondary infections that can develop following non-infectious insults such as burn or trauma (3). Sepsis is a heterogeneous syndrome

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and diagnosis is complicated due to the broad spectrum of non-specific clinical features (3). In addition, the clinical course is impacted by individual factors relating to infection source, (epi)genetics, comorbidities or demographics (1, 4). Furthermore, there are a multitude of biological signals that play a role in interconnecting pathways, making it difficult to define clinically relevant endpoints besides mortality and to establish a clear understanding of the underlying disease. This wide array of factors determining sepsis onset and response diminishes the likelihood of creating one standard treatment for the heterogeneous cohort of patients. Thus, categorizing sepsis patients into distinct endotype classes should improve the prospects of finding efficacious drugs within each class (5). In sepsis, if organ function is not maintained, organ damage can develop, leading to increased morbidity and mortality (3, 6). Particularly, the microvascular endothelium plays a key role in the development and progression of sepsis (7), but the application of omics, specifically proteomics, towards defining endotypes and unraveling the mechanisms of dysfunction of endothelial cells (ECs) in multiple organs during sepsis is in its infancy. Therapeutic approaches for the treatment of sepsis are supportive, but there are no specific pharmacologic therapies to treat the underlying pathophysiology and maintain endothelial cell function (7).

In the emerging field of omics of sepsis, we believe that this review will provide an initial summary of the literature in the field as a resource and also encourage further studies (8, 9, 10, 11). In particular, as sepsis is a complex process, proteomics provides a quantitative analysis of the protein changes that can help bridge the genotype–phenotype gap (12). Specifically, since proteins are involved in every biological phenomenon, unraveling protein–protein interactions (PPIs) is crucial for identifying pathways contributing to disease (12, 13, 14). In this regard, omics analysis can further our understanding of the subphenotypes of the disease and, in combination with laboratory and clinical variables, suggest future studies with clinical relevance.

In this review, we summarize how omics of various ECs is leveraged to better describe sepsis progression, define sepsis subphenotypes and identify novel therapeutic targets. We not only discuss how genomics has been used to define septic endotype signatures in different populations but also focus on the application of proteomic analysis of organ-specific microvascular ECs during sepsis or septic-like inflammation which has not been reviewed before. Lastly, we provide a brief discussion of how *in silico* modeling can be used to leverage the large volume of omics data for mapping endothelial response pathways in sepsis.

An overview of sepsis, endothelium and omics

Sepsis and the role of the endothelium

The vascular endothelium is a single layer of cells lining the tunica intima (inner layer) of blood vessels (15). The endothelium regulates several physiological functions including vascular tone, permeability and immune response (15). The endothelium of different organs shows heterogeneity in function and morphology, and organ-specific ECs exhibit distinct barrier properties and interactions with immune cells (16). During sepsis, an intense systemic inflammatory response develops in response to pathogen-associated molecular patterns (PAMPs) (7). This systemic inflammation activates a cascade of pro-inflammatory events that results in leukocyte dysregulation and an altered endothelial phenotype, producing increased barrier permeability, coagulation and neutrophil trafficking into critical organs; this results in host tissue damage and multiple organ dysfunction syndrome (MODS) (3, 7, 17). Specifically, neutrophils and ECs engage in crosstalk that leads to neutrophil rolling, adhesion and migration across ECs via a multifactorial process controlled by concurrent chemoattractant-dependent signal, hemodynamic shear forces and adhesive events (18). While neutrophils are crucial to host defense, neutrophil dysregulation has a critical role in the early course of death of ECs through the release of proteases and the formation of neutrophil extracellular traps (NETs) (17, 18). Subsequently, ECs dysfunction induces the activation of the complement and coagulation cascades and disseminated intravascular coagulation (DIC) (19). To date, there are no gold-standard diagnostic measures for sepsis, which complicates hypothesis-driven studies searching for individual biomarkers or therapeutic targets (1). Mechanistic computational modeling based on multi-omic analysis can provide a rational basis for understanding the pathophysiology of sepsis, sepsis phenotypes and design of clinically relevant therapeutics (20).

Omics for understanding disease and developing therapeutics

Since the development of the first genome sequencing method, technologies that further allow the quantification and identification of genes, RNA transcripts, proteins and metabolites (Fig. 1) have been instrumental for understanding disease mechanisms and identifying intervention targets for pathological conditions such as cancer (21).

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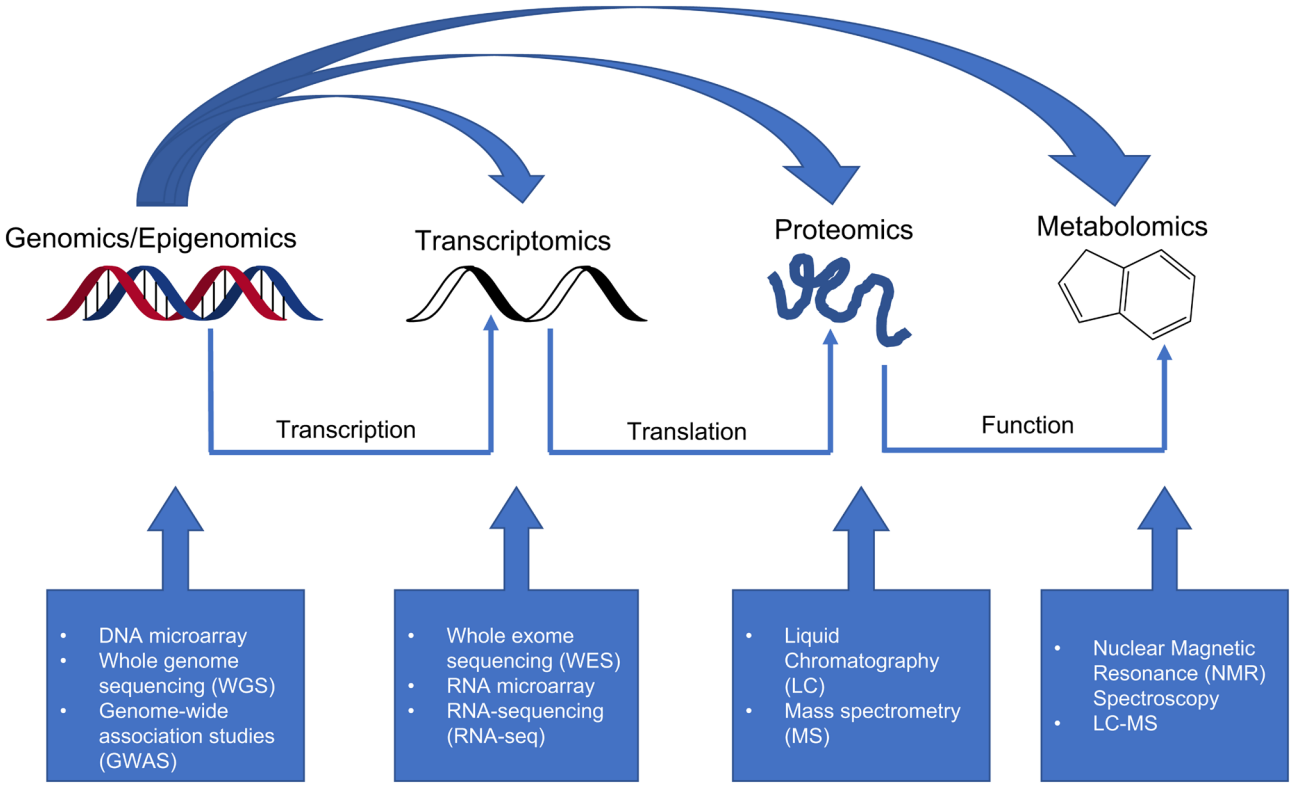

Figure 1
The four major omic components along with associated high-throughput techniques used in each. Metabolomics, which is the systemic study of metabolite byproducts from enzymatic reactions, best represents the biological system's phenotype. Additionally, the signaling cascade from proteomics to metabolomics can also be characterized as 'function' since this omics component describes how cellular state leads to functional phenotype.

The field of systems biology represents a leap forward from reductionist methods by providing the ability to quantify the entire state of a biological system in the context of the four major classes of biomolecules (DNA, RNA, protein, metabolites) (14). Genomics focuses on whole-genome sequencing, while transcriptomics focuses on RNA-sequencing (RNA-seq) and analyzing differential RNA transcript expression patterns (14). Single-cell RNA-seq (scRNA-seq) is an emerging technology that captures differential transcript expression from individual cells. This technique permits the evaluation of biological events at a greater resolution compared to performing bulk RNA-seq (22). Thus, incorporating scRNA-seq in studying endothelial cell heterogeneity during sepsis would be beneficial in characterizing organ-specific omic expression patterns. Proteomics quantifies differentially expressed proteins (DEPs) in a biological sample, while metabolomics analyzes metabolites within a cell (23). In proteomics and metabolomics, liquid chromatography (LC) methodologies separate complex mixtures based on size, resin affinity or charge; mass spectrometry (MS) ionizes and fragments protein mixtures into peptides and nuclear magnetic spectroscopy (NMR) is used to determine molecular structure (14). Additionally, newer mass spectrometry technologies help capture the heterogeneity of cell response during sepsis by measuring low-abundance proteins in samples, improving detection of peptides and increasing sensitivity over traditional 2-D-LC proteomic assays (24). Once fragmented, protein databases are utilized to determine the targeted protein(s) of interest (23). Omics can help with molecular sub-typing of specific diseases and tailoring of treatment strategies for different patient groups by analyzing large amounts of data to characterize biomolecule expression (25), enabling the development of next-generation therapeutics for complex, poorly characterized diseases such as sepsis. Omics provide tools for the characterization of biomolecule expression in a tempo-spatial manner, thus allowing us to quantify the dynamics of pathway signaling during disease progression (25, 26). Furthermore, omics can generate hypothesis-driven experiments and identify pathways and biomolecules from samples *a priori* which can then be tested in experimental models to investigate the role of the identified biomolecules in signaling pathways (27).

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Omics in septic research and endothelial dysfunction

Omics in sepsis research

Given the dynamic nature of sepsis, omic analysis, combined with clinical input regarding the stage of the disease, can be used to characterize pathologically relevant biomarkers (4). For example, omics can be particularly useful in sepsis research for discovering (a) biomarkers to differentiate between infectious and non-infectious sources, (b) prognostic biomarkers, (c) biomarkers that aid in sepsis therapy and (d) biomarkers to predict individual patient response to therapy (28). Discovering these synergistic combinations of biomarkers is of high interest, given the fact that no single biomarker is sensitive and specific enough to capture the entirety of an individual's septic condition (29). A recent study found 60 biomarkers that were able to distinguish between sepsis and systemic inflammatory response syndrome (SIRS), but only 7 of these contain sufficient data for further evaluation (30). One of them is PCT, which is the only FDA-approved sepsis biomarker; the other six are presepsin, CRP, IL6, sTREM1, LBP and CD64 (30, 31). PTX-3 is another biomarker that has been studied in septic shock (32). Limitations of these biomarkers include: low diagnostic and prognostic accuracy when used alone, lack of studies directly comparing one over another, variability of concentration during early or late-stage sepsis and lack of standardized diagnostic cut-off values (29, 30). Comprehensive reviews of sepsis biomarkers can be found elsewhere (29, 30).

Several studies have proposed classification systems that stratify sepsis patients into unique endotypes based on genomic data and/or modeling approaches (5, 11, 33, 34, 35). The success in stratifying septic patients into endotypes and in associating these features with clinical outcomes illustrates the clinical relevance of the heterogeneous aspects of sepsis. The papers serve as blueprints for precision medicine to reconsider therapeutic approaches on a patient-by-patient basis depending on individual omic profiling. While research has elucidated clinical signs of these endotypes in septic patients, more investigation on the underlying biomolecules and pathways of disease is needed to establish the physiological basis for these endotypes (25). This is where the integration of systems biology and omics plays a major role. Since sepsis affects multiple cellular compartments and organs in an entropic manner, omics can capture patient-specific biomolecule expression in biological systems and, in combination with computational methods, decipher how underlying biological networks are dysregulated (36). This will then permit sub-typing of patients according to common clinical features (25, 26) and characterize the underlying endotype. Table 1 shows a summary of different genomic and modeling studies that have stratified patients into sepsis endotypes with selected differential gene expression and corresponding outcomes.

As shown in Table 1, a selective number of genes were used to characterize the endotypes of interest in sepsis in several different population/demographic groups. The fact that different genes were identified across studies may in part be based on the type of study (retrospective vs prospective), time of patient recruitment (months vs years), if recruitment occurred before or after the sepsis-3 definition (1), study population (children vs adults), demographics (country of origin, race) and time of assay. Wong *et al.* performed genome-wide expression profiling using whole-blood-derived RNA from 98 children with septic shock (33). Three subclasses were established via unsupervised hierarchical clustering: subclasses A, B and C. Subclass A had the highest mortality (36%), illness severity and degree of organ failure. Also, subclass A had repressed genes in immunity (44 genes including *LAT* and *TRAT1*) and zinc biology which help to maintain homeostasis (181 genes including *ZnT*), and thus this cohort exhibited lower adaptive immunity and increased mortality than other subclasses (33). Additional pathway hits corresponding with these repressed genes included B-cell and glucocorticoid signaling which further confirms that the subclass A cohort did not have the immune-related genes expressed during sepsis (33). These initial findings support the efforts to stratify patients into various endotypes based on differential omic expression in sepsis. In another study conducted within the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) project (34), a clinical trial investigating sepsis endotypes in ICUs, eight genes were identified which, in specific combinations, could be used to systematically classify patients into the MARS 1, 2, 3 or 4 endotype. The MARS 1 group showed decreased innate and adaptive gene expression, MARS 2 exhibited increased cell motility and cytokine pathway expression, MARS 3 demonstrated increased adaptive immune gene expression and the MARS 4 group had increased *IL6*, *NFkB* and interferon gene expression (34). This is of particular importance since having a large, complex omic signature that is sensitive enough to correctly classify different patients into various endotypes is impractical in the clinic and thus having a smaller signature would enable additional studies to evaluate its relevance in sepsis pathophysiology and predict treatment responses on a larger scale (37). The comparison between these studies is further complicated

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Table 1 Examples of genomic and modeling studies to classify septic human patients into various endotypes. Gene definitions can be found in Supplementary Table 1.

Endotype classification	Endotype outcome	Genes		Study population	Reference
		Upregulated	Downregulated		
A, B, C				Prospective study; 98 children with septic shock were recruited; Males were prevalent in 2 of 3 endotypes	(33)
Subclass A group	Increased organ failure, highest mortality		44 key adaptive immune genes (i.e. T/B-cell related such as *KAT2B, SOS1, JAK2, GK, TAF1, PTPRC, MA3K7* etc.) in subclass A compared to B and C. 181 key zinc biology-related genes (i.e. *ZnT/SLC, etc*) downregulated in subclass A compared to B and C		
Subclasses B and C groups	Decreased mortality				
SRS 1, 2				Prospective study; Total of 371 adult patients with sepsis due to pneumonia were recruited; Males were prevalent in all cohorts	(11)
SRS1 group	Higher mortality and T-cell exhaustion	*IRAK3*, *TOLLIP*, *CBL*, *PAG1*, *HIF1A*, *EPAS1*, *IL18RAP*, *CCR1*, *LDHA*, *GAPDH*	*LAT, CD247, HLA family, CIITA, RFX5, CCR3, MTOR, SIRT1, CD247*		
SRS2 group	Increased cell response to infection, low mortality	HLA family class II, T-cell and B-cell complexes			
MARS 1–4				Prospective observational study; Total of 787 adult patients with sepsis due to pneumonia were recruited; Majority of patients recruited were Caucasian males	(34)
MARS 1 group	Highest 28-day mortality, decreased immune gene expression	*BPGM, TAP2*			
MARS 2 group	Increased cytokine pathway expression	*GADD45A, PCGF5*			
MARS 3 group	Increased adaptive immunity expression, lowest 28-day mortality	*AHNAK* nucleoprotein, *PDCD10*			

(*Continued*)

Table 1 Continued.

Endotype classification	Endotype outcome	Genes		Study population	Reference
		Upregulated	Downregulated		
MARS 4 group	Increased interferon gene expression	*IFIT5, GLTSCR2/NOP53/NOL5A*			
Inflammopathic, adaptive and coagulopathic				Retrospective study; Total of 23 bacterial sepsis/inflammation datasets (12 in children, 11 in adults) were analyzed; Majority of patients in the cohorts were males from first-world nations	(35)
Inflammopathic group	Highest mortality and innate immunity expression	*ARG1, LCN2, LTF, OLFM4*	*HLA-DMB*		
Adaptive group	Lowest mortality and increased adaptive immunity expression	*YKT6, PDE4B, TWISTNB/POLR1F, BTN2A2*	*GADD45A, CD24, S100A12, STX1A*		
Coagulopathic group	High mortality and coagulopathy	*KCNMB4, CRISP2, HTRA1, PPL*	*RHBDF2, ZCCHC4, YKT6, DDX6*		
Alpha, beta, gamma, delta					(5)
α group	Less organ dysfunction, normal blood tests and lowest mortality	*IL10*	D-*dimer, IL6, IL8, TNFα,* Procalcitonin, C-reactive protein		
β group	Chronic illness and renal dysfunction	*IGFBP7, COL4, TIMP2*	*IL10, IL66,* procalcitonin, *SELE, PAI1*		
γ group	Increased inflammation and fever	*IL6, KIM1/HAVCR1,* procalcitonin, *PAI1, ICAM1, SELE*			
δ group	High coagulation and hypotension and the highest mortality	*IL10, IL6, IL8,* procalcitonin, *TNFα, COL4,* D-*dimer, PAI1, VCAM1,* TAT complex			

MARS, Molecular Diagnosis and Risk Stratification of Sepsis; SRS, sepsis response signature.

by the fact that these patterns characterize differences in gene expression at different times. For example, in many studies outlined in Table 1, data are collected and profiled within the first 24–48 h of hospital admission; however, another study indicated that 50% of patients can change from one endotype to another within the first 5 days of hospital admission (4). Thus, tracking of omic expression in a time-dependent manner is important.

Although each study in Table 1 utilizes unique methods to categorize sepsis patients into their own endotype groups, there are commonalities across different endotypes groups which can be utilized to promote future therapeutic research. Many studies have performed genomic profiling of leukocytes or mononuclear cells (33, 38, 39, 40, 41, 42, 43); however, only a few studies focus on grouping patients into different endotypes. Focusing on endotype-dependent studies is critical for developing appropriate therapeutic intervention, since one needs to identify which pathway is critical to target in a particular patient, a goal of precision medicine. Among endotypes, low mortality groups (SRS 2 (11), MARS 3 (34), adaptive (35), α (5)) shared the common characterization of increased adaptive immune signaling, but high mortality groups (subclass A (33), SRS 1 (11), MARS 1 (34), Inflammopathic (35), δ (5)) were not as uniformly characterized by immune status and had repressed immune function. Certain groups were characterized by hyperinflammation (inflammopathic (35), δ (5)), and others were linked to immunosuppression (subclass A (33), SRS 1 (11), MARS 1 (34)). Furthermore, emerging studies are beginning to evaluate different endotype signatures across populations (e.g. evaluating SRS endotype signatures in pediatric patients) to investigate their performance with respect to mortality (44). Additional knowledge from the combination of endotypes can verify common biological targets between populations leading to an endotype, as described in other diseases such as acute respiratory distress

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syndrome (ARDS) (45). However, there is currently a lack of standards by which common endotypes can be identified in different studies.

Though biomarkers can provide valuable insight to guide therapeutic decisions and enhance patient management by preventing, for example, unnecessary antibiotic therapy (29) and commonalities between omic studies can be further validated experimentally, it is highly improbable that a universal endotype signature for sepsis can be developed due to the heterogeneity of the disease (37). It is therefore important to understand different endotypes in the disease to allow the development of tailored therapeutics. It is important to note that the overall goal of endotyping is to unravel molecular subtypes of a disease, and it should not be used as a definitive prognostic tool (44). An international effort to form a standardized consensus on omic profiling procedures would be beneficial (37). Additionally, it would be useful to further validate the omic expression changes in each endotype across populations in time-lapse, multi-institutional prospective studies and plan endotype studies that employ the current definition of sepsis (1), since many omic studies in Table 1 were conducted using the former consensus definition (33, 46, 47, 48).

Omics of microvascular endothelium in sepsis/inflammation

Microvascular ECs play a central role in neutrophil–endothelial crosstalk, and excessive neutrophil migration leads to edema, shock and MODS (7, 17). Since sepsis progresses rapidly, and there are no standard diagnostic procedures to determine a patient's clinical condition between admission and first course of 'treatment' (1), omics would be beneficial in determining how ECs of vital organs are impacted in the early phase of sepsis (7, 17, 27). Understanding organ-specific omics of ECs should be of high importance due not only to its role in maintaining homeostasis and immunity but also for how its dysfunction can lead to organ failure (7, 17, 28). Furthermore, characterizing differential omic expression patterns of ECs phenotypes will help us better understand how each vascular bed responds to inflammatory insults and what gene ontologies (GO) and signaling pathways are unique to each bed or common across beds. A summary of the genomic, sepsis/inflammatory studies performed in mice is presented in Table 2. Though there have been concerns about whether results from mice can translate to human trials, data from mouse models are still needed to help understand the pathology of sepsis (49). Mouse models are also critical for establishing the response of ECs phenotypes to inflammatory stimuli for the evaluation of genetic (e.g. knock-in or knock-out) or pharmacological effects in a living system, since these studies cannot be done in patients. In this section, we discuss genomic studies, followed by proteomic studies of ECs in 'Proteomics of ECs and in *silico* modeling of omics'. To our knowledge, there are no published metabolomic or epigenomic studies of mouse microvasculature ECs challenged with an inflammatory insult.

Organ-specific ECs have been stimulated with exogenous substances (e.g. LPS, bacteria) to induce septic-like conditions over different time points (e.g. 6 or 24 h) (50, 51, 52, 53) to identify unique genes, pathways or GO differentially expressed in various organs using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and GO database. Overall, most of the KEGG signaling pathways, gene families (e.g. *Cxcl, Tnfa, Sele, Selp*) and GO overexpressed in the ECs beds correlate with the activation of the innate immune system (e.g. TLR signaling), leukocytes (e.g. leukocyte migration) and coagulation (54). These findings are consistent with our understanding that sepsis causes dysregulated host response to infection, leading to activation of ECs and immunity pathways (7, 17).

Additional organ-specific pathways based on endothelial-specific gene expression have also been reported (54). For example, upregulation of adipose tissue-specific ECs genes (e.g. *Car3*, *Csf2rb*) drives osteoclast differentiation, kidney-specific ECs genes (e.g. *Dram1*, *Dkk2*) aid in endocytosis, cardiac-specific ECs genes (e.g. *Kcna5*, *Myadm*) drive axon guidance and brain-specific ECs genes (e.g. *Edn3*, *Foxf2*) help maintain ErbB signaling (54). To date, most omic endothelial-based sepsis/inflammatory studies have focused on the lung, liver and brain showing that a number of unique as well as common pathways and genes are associated with these different ECs (50, 51, 52, 53, 54). Even though common pathways are expressed among all ECs, differential gene expression still occurs. For example, Wnt signaling is a common pathway among ECs, but brain ECs show higher expression of Nkd1 or Fzd6 while liver ECs express *Apc* or *Ep300* (54). Though this initial study (54) was not done under inflammatory stimuli, these findings can provide an organ-specific understanding of the signaling mechanisms to examine during sepsis. Additionally, pathway changes in adipose tissue, mammary or adrenal glands or skeletal muscle ECs during normal or disease conditions have not been systematically studied and warrant further investigation. Furthermore, omics studies investigating intra-organ endothelial heterogeneity

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Table 2 Summary of genomic studies done in mouse microvascular endothelial cells investigating differential gene expression after inflammatory/septic-like stimulation. Gene definitions can be found in Supplementary Table 2.

Reference	Methodology	Region	KEGG pathway hits		GO pathway hits		Genes	
			Upregulated	Downregulated	Upregulated	Downregulated	Upregulated	Downregulated
(54)	Embryonic stem cells were differentiated into organ-specific ECs							
		AT	Osteoclast differentiation, MAPK signaling, metabolism				*Csf2rb, Cldn15, Acox1, da, Papss2*	
		Brain	ErbB signaling, RPAR signaling, MAPK signaling				*Slco1c1, Slco1a4, Slc22a8, Mfsd2a, Stra6*	
		Diaphragm	Toxoplasmosis, RIG-I-like signaling, apoptosis				*Gm12216, Adamts4, Sphk1, Prkcc*	
		heart	Focal adhesion, axon guidance signaling, ECM–receptor interaction				*Ccnd1*, *Ctnnbip1, Plcb4, Myadm, Slc28a2*	
		Kidney	Endocytosis, hematopoietic cell lineage, calcium signaling					
		Liver	TGF-B signaling, complement and coagulation, hematopoietic cell lineage				*Clec4g, Fcgr2b, Stab2, Mrc1, Plxnc1, Apc, Lrp6, Ep300*	
		Lung	Neuroactive ligand-receptor interaction, WnT signaling, metabolism				*Tmem100, Scn7a, Adrb1, Daam1, Nkd1*	
		MG	JAK-STAT, NOD-like receptor signaling, MAPK signaling				*Emp1 Atf4, Dusp5, Gadd45b, Myc, Relb*	

(*Continued*)

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Table 2 Continued.

Reference	Methodology	Region	KEGG pathway hits		GO pathway hits		Genes	
			Upregulated	Downregulated	Upregulated	Downregulated	Upregulated	Downregulated
		Pancreas	Adherens junction, focal adhesion, MAPK signaling				*Fgfr1, Ntf3, Pla2g1, Cele2a, Prss2*	
		SM	JAK-STAT, TLR signaling, metabolism				*Csf1, Cxcl, Cxcl10, Cxcl2, Osmr*	
		Trachea	Gap junction, NOD-like receptor signaling, TLR signaling				*Rgs16, Col13a1, Stc1, Psd, Nr4a2, Bhihe40*	
(50)	Cultured brain, lung and heart ECs were stimulated with LPS for 6 and 24 h							
		Brain						
		6 h			Leukocyte migration, response to LPS		*Ccl11, Timp1, Tnfa, Il1a , I1b, Sele, Selp*	
		24h			Response to chemokine, cell chemotaxis, leukocyte/neutrophil migration		*24 hours: Ccl3, Timp1, Ccl11, Selp, Sele*	
		Heart						
		6 h			Cell chemotaxis, leukocyte migration		*Ccl3, Sele, Selp, Cxcl55, Cxcl1, Il6, Cxcl3*	
		24 h			Leukocyte migration, neutrophil/leukocyte chemotaxis, response to chemokine		*Cxcl5, Cxcl3, Selp, Sele, Acod1*	
		Lung						
		6 h			Acute inflammatory response, cell chemotaxis		*Cxcl1, Cxcl9, I1r2, Casp6, Il10, Ly96*	
		24 h			Cell chemotaxis, leukocyte/neutrophil chemotaxis, leukocyte migration		*Mmp8, Il10, Acod1, Cxcl9*	

(*Continued*)

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Table 2 Continued.

Reference	Methodology	Region	KEGG pathway hits		GO pathway hits		Genes	
			Upregulated	Downregulated	Upregulated	Downregulated	Upregulated	Downregulated
(51)	Mice were injected with LPS for 4 h prior to isolation of heart, brain liver and lung ECs	Kidney, brain, liver, lung, heart			Leukocyte migration, response to lipopolysaccharide, response to bacterium			
		Brain					*Sele, Selp, Icam1*	*Cdh5, Ctnna1, Cldn5, Thb, Vwf, Jam2*
		Heart					*Sele, Vcam1, Selp, Icam1, Serpine1*	*Cdh5, Ctnnb1, Ocln, Plau, Thbd*
		Liver					*Sele, Selp, Serpine1*	*Cdh5, Ctnnd11, Jam2, Jam3, Cldn5, Plat, Tfp1*
		Lung					*Sele, Vcam1, Selp, Serpine1*	
(52)	Mice were injected with LPS for 3 h prior to isolation of adrenal ECs	Adrenal			Innate immune response, inflammatory response, cellular response to LPS and *Il1* activation	Activation of MAPK activity, Rho protein signal transduction, protein phosphorylation	*Csf3, Ccl2, Cxcl10, Selp, Sele, Vcam1, Pdgf, Il6, Ifit2, Ifit3*	*Rapsn, Dsc2, Zc4gz, Kcbn1, Pcdh12, Kcnb1, Shh, Dapk2*
(53)	Mice were injected with influenza infection for 6 h prior to isolation of lung ECs	Lung			Blood vessel development, positive regulation of cell motility, sprouting angiogenesis		*Gpihbp1, Ifi47, Plvap, Sox17, Atf3, Nrp1, Nusap1, Birc5, Cdk1, Top2a, Kitl, Kdr, Atf3, Cd34*	
(55)	Cultured mouse brain ECs were stimulated with avian *E. coli* for 1–6 h	Brain	Ribosome, legionellosis, TNF signaling, HIF-1 signaling	Biosynthesis of amino acids, glycolysis		Nuclear part, intracellular part, intracellular organelle, cellular macromolecule metabolic process	*Nectin3, Cxcl1, Cxcl2, Cxcl16, Ccl2, Ccrl2, C3ar1, Actb, Tlr4, Tlr13*	*Cdh5, Cdh24, Pcdh1, Pcdhgc3, Nectin1, Nectin2, Actn1*

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are needed to understand how inflammatory stimuli impact different ECs of the same organ. These urgently needed studies will help in generating hypotheses for the validation of experiments in experimental models to enhance our understanding of how sepsis impacts ECs in various organs and to identify druggable targets. Most studies in Table 2 report genes that are upregulated, but all use either KEGG and/or GO to find the biological processes or signaling pathways these genes play a role in. However, only two report KEGG and/or GO hits that are downregulated (52, 55). More studies reporting what processes and pathways are downregulated are necessary to obtain a comprehensive understanding of how the functionality and structural properties of organ-specific ECs are altered in response to sepsis.

Proteomics of ECs and *in-silico* modeling of omics

Proteomics of mouse ECs

A summary of proteomic organ-specific ECs studies in mice under septic/inflammatory conditions is outlined in Table 3. Most studies report up- and downregulated proteins and use either KEGG or GO to find the biological processes or signaling pathways in which these proteins play a role. If KEGG or GO hits were not reported, protein lists were submitted to these databases, and the hits are reported in Table 3. None of these studies report KEGG pathways and/or GO hits that are downregulated. Consistent with genomic studies, proteins expressed correlate with the upregulation of KEGG pathways and GO hits related to coagulation, cell adhesion and immune response (56, 57, 58, 59, 60, 61, 62). Thus, proteins correspond with gene expression to determine final cellular pathways and biological processes overexpressed in septic-like conditions. Interestingly, studies have also shown that COVID-19, which has been described as a form of viral sepsis, significantly affects the endothelium (63). For example, the proposed KEGG COVID-19 pathway is shown to play a role in endothelial dysfunction (56), thus implicating the endothelium as a potential target for COVID-19 therapeutics. Though there have been emerging studies on the omics of COVID pathogenesis and progression (64), there are currently no omic studies on endothelial dysfunction in COVID-19.

Many studies discussed in Tables 2 and 3 do point to the molecular players and pathways already known in sepsis that produce a cellular phenotype. Nevertheless, differential omic analysis can provide insights for the design of future studies based on shared and unique proteins across organ-specific ECs. While certain pathways are highly upregulated among ECs (e.g. Wnt signaling), there are also organ-specific upregulated pathways such as axon guidance in cardiac ECs or endocytosis in kidney ECs (54). Additionally, organ-specific ECs express cell surface proteins, and thus the variety of genes and proteins shown in Tables 2 and 3 could be classified as potential therapeutic targets to preserve the vasculature of the tissue and prevent downstream damage such as edema and MODS, which are hallmarks of sepsis damage to the endothelium (54). However, it should be noted that all of these studies were performed in mouse ECs, and further validation of these findings must be complemented with experimental models such as microphysiological systems (MPS) using human cells that recapitulate the 3-D geometry and physiologically relevant flow conditions of the microvasculature (18, 65).

Other than causing differential regulation of coagulation, cell adhesion and immune response proteins in ECs, sepsis has been shown to affect the glycocalyx (a gel-like layer composed of proteoglycans coating ECs) and vascular smooth muscle cells (VSMCs) (7, 58). The synthesis of new peptides in response to sepsis related to lipid transport (e.g. Apo family), immunity or oxidative stress (C7) is all downregulated in the glycocalyx (58). Thus, designing studies investigating potential proteins that shed from the glycocalyx during sepsis would be beneficial. While proteomic studies in mouse microvascular ECs provide a better understanding of the basic mechanisms of sepsis, *in vitro* proteomic studies using human ECs, specifically with ECs exposed to physiological and abnormal shear flow conditions (66), have been done providing potential relevance to clinical studies.

Proteomics of human ECs

ECs under shear stress convert mechanical stimuli into intracellular signals that affect cellular functions under both normal and diseased conditions. However, traditionally, proteomic expression patterns of ECs have been studied under static conditions, mostly in human umbilical vein ECs (HUVECs) (67, 68). For example, IL1B and IL13 are two cytokine proteins that are released from ECs during inflammation, thus inflammatory and cell adhesion proteins (e.g. RIPK22, SERPINB2, VCAM1) were upregulated in HUVECs (67, 68). Most proteins involved in molecular functions in ECs such as enzyme regulation and metabolic regulation of the cytoskeleton (e.g. cystatin-SN and profilin-1) are upregulated in inflammation (69). While HUVECs are well-established and easy to use *in vitro* models for studying ECs function, for the most part, they are

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Table 3 Summary of proteomic studies in mouse microvascular endothelial cells investigating differential protein expression after inflammatory/septic-like stimulation. Protein definitions can be found in Supplementary Table 3.

Reference	Methodology	Region	KEGG pathway hits		GO pathway hits		Proteins	
			Upregulated	Downregulated	Upregulated	Downregulated	Upregulated	Downregulated
(56)	MRSA was injected in mice for 24 h before kidney, liver, heart, brain and white adipose tissue ECs isolation							
		Liver	Cell adhesion molecules, *Staphylococcus aureus* infection		Leukocyte proliferation, cell–cell adhesion, positive regulation of cell death		SAA1, VCAM1, CXCL9, SAA1	HPGD, APOE, MUP3
		Brain			Cell–cell adhesion, neuron migration, axonogenesis		24 h: HP, ITIH4, CFB, CP, HPX	
		Kidney	COVID-19, ECM-receptor interaction		Cell–cell adhesion, negative regulation of growth, cell morphogenesis		SAA1, SAA2, HP, FGB, ITIH3	RBP4, HDLBP
		Heart	COVID-19, PPAR signaling, hypertrophic cardiomyopathy		Skeletal tissue development, muscle contraction		SAA2, VCAM1, HP, ORM2, PDK4	TTR, C8, VCAN
		WAT			Collagen degradation, response to peptide hormone			LRPAP1, VCAN
(57)	Mice were injected with oleic acid for 6 h before lung ECs isolation	Lung	Complement and coagulation, ECM–receptor interaction, phagocytosis, amoebiasis		Immune system response, defense response, response to external stimulus		C1QA, C4BP, FGA, FGG, C4B, SERPINA6, C3, CFB	COL5A1, GNAI1, ATP8, MLST8, POSTN, TH, ST3GAL1, POLR2M
(58)	Mice were injected with LPS over 48 h before isolation of vascular beds							

(*Continued*)

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Table 3 Continued.

Reference	Methodology	Region	KEGG pathway hits		GO pathway hits		Proteins	
			Upregulated	Downregulated	Upregulated	Downregulated	Upregulated	Downregulated
		Endothelial secretome	Metabolic pathways, endocytosis, biosynthesis of antibiotics, complement and coagulation, viral carcinogenesis, cell adhesion					
		EC					SAA1, HX and HPX	CLU, AZGP1, C6, CFD, TLN1, GSN, F10
		Glycocalyx						APOB, C3, CFH, TLN1, C7, SPP2
		Vascular smooth muscle						GC, F12, C8, APOA4
(59)	Mice received a dose of cardiac radiation at 8 or 16 Gy before isolation of heart ECs							
		8 Gy	EIF2 signaling, remodeling of epithelial adherens junction		Inflammatory response, cell assembly and organization, DNA repair/replication			
		Heart					ICAM2, ITGB3, HSPA12b, THBS1, TUBA1A, TUBA4A, MCAM	VWF, ICAM1, LAMB, DLAT, NCL, VCP, FH1, HIST1HE, LMNB2
		16 Gy	EIF2 signaling, actin cytoskeleton signaling		Energy production, cell–cell signaling, cell movement			
		Heart					CDH13, GDI2, LC25A4, DYNC1H1, CLTC	ACADM, ACTB, CALD1, DES, ECI1, MSN, PRKCDBP, TPM1
(60)	Mice were irradiated with a dose of 10 Gy at the thorax prior to the isolation of lung ECs	Lung	Metabolic pathways, endocytosis, pathways in cancer, PI3k–Akt signaling, cGas/STING-pathway				GBP2, ISG15, H2D1, SERPINB2, B2M, CASP7	FADS1, GBE1, DNPH1, MLYCD, FAM120C, ALDOC

(*Continued*)

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Table 3 Continued.

Reference	Methodology	Region	KEGG pathway hits		GO pathway hits		Proteins	
			Upregulated	Downregulated	Upregulated	Downregulated	Upregulated	Downregulated
(61)	Bile duct ligation was performed in mice prior to isolation of CD31-pulmonary cells		Secretion of chemokine and cytokine, Regulation of cell adhesion and migration, Complement and Coagulation		Secretion of chemokine and cytokine, Regulation of cell adhesion and migration, Complement and Coagulation		Lung: SERPINB1A, ANXA1, S100A9	
(62)	Cultured mouse brain microvascular ECs were infected with herpes simplex virus for 24 h	Brain	NF-kB signaling, IL-17 signaling, NOD-like receptor signaling, TNF signaling, cell adhesion		Defense response, immune system response, response to biotic stimulus		VCAM1, JAMA, PDI1, CJUN, CCL2, CCL5, CXCR5, CCL2	TPST1, SNRPC, COBL, MMP15, CD9, BRD3, LRIG1

unsuitable models for sepsis research, since they come from large vessels of the umbilical cord which are not considered early targets of sepsis. Also, HUVECs do not recapitulate the 3-D, morphological and functional microenvironment of organ-specific microvascular ECs (70). A study investigating the effect of a bacterial strain on human brain microvascular ECs was performed (71). Exposure to bacterial strains can disrupt the blood–brain barrier (BBB) and increase the likelihood of toxins to enter the brain resulting in sepsis-associated encephalopathy (SAE) (6, 72). Studies such as these, given the significant level of heterogeneity in ECs from different organs, and additional studies of omic analysis of phenotypes of ECs under flow conditions directly affected by inflammation are urgently needed (70).

Pioneering shear flow-based studies by McCormick in HUVECs and Chen in human aortic ECs in the early 2000s highlighted gene expression changes under flow conditions in a time-dependent manner during 6 or 24 h of flow (73, 74). Endothelial survival genes involved in angiogenesis or matrix remodeling (e.g. *TIE2, FLK1*) were upregulated during long-term shear exposure which help maintain an anti-inflammatory phenotype, while genes which switch an endothelial phenotype from anti- to pro-inflammatory such as *MYD88* and *CD30* were downregulated during flow (73, 74). Following these initial studies, a number of other investigators have reported similar gene expression trends under laminar or abnormal shear flow (75, 76, 77).

Particularly, proteomic studies of ECs under shear flow, again in HUVECs, have identified proteins corresponding with the underlying genes. Proteomic analysis of the secretome, defined as proteins secreted from cells, following exposure of ECs to shear stress, could determine if plasma proteins are altered in flow-dependent vascular diseases (66). Over 100 proteins were identified to be secreted under control, laminar or abnormal shear stress conditions (66). Those identified under laminar (e.g. PTHR and LTBP4) or abnormal shear flow (e.g. endothelin-1 and insulin-like growth factor II) conditions are again proteins involved in conferring an anti- or pro-inflammatory ECs phenotype and thus correspond to endothelial genes identified under similar conditions (66). Thus, anti-inflammatory genes and proteins are upregulated by exposure to laminar flow, while pro-inflammatory proteins contributing to vascular inflammation (e.g. DKK1 and Endothelin-1) are expressed by exposure to abnormal shear flow (78, 79). Specifically, during sepsis, the endothelium becomes dysfunctional due to abnormal shear stress, resulting in decreased oxidation in ECs and increased coagulation, among other outcomes (80). Pathogen-associated molecular patterns (PAMPS) and pro-inflammatory cytokines initiate

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sepsis leading to the breakdown of ECs–ECs contact, ECs exhibiting a procoagulant phenotype and the shedding of the glycocalyx; these events could lead to hemorheological defects (increased red blood cell aggregation and viscosity) and subsequent reduced arterial pressure, hypotension and abnormal shear stress (80). One of the first proteins found to be impacted by shear stress in vascular inflammation was forkhead box P (a gatekeeper of vascular inflammation) (81) which is downregulated by Kruppel-like factor 2 (a protein involved in adipogenesis, inflammation and T-cell viability) and, in turn, is suppressed by abnormal shear stress (82); this same mechanism applies to sepsis (83). Low shear or no shear stress decreases the activation of Prospero homeobox 1 (a protein-regulating cell fate) and forkhead box C2 (a protein involved in mesenchymal tissue development) and thus decreases thrombomodulin (a glycoprotein that controls coagulation) and endothelial protein C receptor (another protein that regulates coagulation) expression, causing pro-inflammation and leukocyte adhesion/migration (80). Overall, in sepsis, abnormal shear stress significantly impacts endothelial function and glycocalyx shedding and contributes to MODS and death. Thus, additional studies investigating the detrimental effects of differential shear stress on ECs proteomic expression are needed to provide further insight on how sepsis progresses and causes tissue damage.

In silico modeling of omics

The large volume of data obtained in omic studies is inherently complex and requires special computational tools for mapping endothelial response pathways, understanding the evolution of inflammatory signaling during sepsis, identifying druggable targets and predicting how different therapeutics may impact the progression of inflammatory signaling. Thus, in addition to experimental approaches, *in silico* modeling can generate testable hypotheses, and simulations can provide new, non-intuitive knowledge on complex systems (20). These models can accelerate the process of discovering novel therapeutic candidates (20) and have been used to investigate how ECs interact with a pathological microenvironment or respond to stimuli. For example, *in silico* modeling has been used to examine how ECs interact with the tumor microenvironment in angiogenesis (84). Other *in silico* models study ECs interacting with other cell types such as hepatocytes (85) or responding to shear stress (86). More recent studies have used organ-specific ECs in pathway models to predict therapeutic targets for specific pathologies, such as diseases in the brain (87). Despite these models providing further insight on how ECs are regulated under various conditions, they have not been applied to investigate the dysfunction of ECs in sepsis which is urgently needed.

In systems biology, several different methods of *in silico* modeling are implemented including agent models, equation models and network models (20). Specifically, network models, based on Boolean logic, are constructed through the integration of the interactome (e.g. protein–protein interaction data) and omics data and do not require *a priori* quantitative knowledge of biological reactions, which is difficult to achieve (36, 88). Network models are initially constructed by submitting a gene/protein list to a database that maps the entities onto a global PPI model to illustrate their physical interaction or functional association with other entities based on statistical parameters (e.g. confidence scores) (36). Figure 2 is a general workflow of biological network construction and the application of network algorithms.

PPIs are constructed from large-scale experiments or computational predictions and maintained in databases such as BioGRID or STRING (36). Additionally, there are tissue-specific PPI (such as GIANT and TISPIN), and since sepsis affects multiple tissues, the incorporation of omics in multiple tissue-specific *in silico* models for the comparison of differential disease-associated signaling would be beneficial (36). Once an *in silico* network model is generated, graph theory analyses are performed to characterize topological features of the network such as network diameter or node degree (36). Pathway databases such as KEGG can be used in the early stages of modeling to examine signaling pathways in omics data to evaluate the relationships between the data and disease pathways (36). There are numerous strategies to construct and validate *in silico* network models that are discussed elsewhere (88). Overall, *in silico* models have the potential to model drug–protein or protein–protein interactions for a specific pathology, and it is critical to develop such models to decipher omic changes in ECs during sepsis to determine how endothelial inflammatory signaling evolves within and between tissues affected in sepsis.

Conclusions

In this review, we discuss the importance of omics, specifically proteomics, profiling of microvascular endothelial cells and leukocytes under septic and/or inflammatory conditions in humans and mice, respectively. In sepsis, microvascular ECs are key targets leading to edema, capillary leak syndrome and MODS

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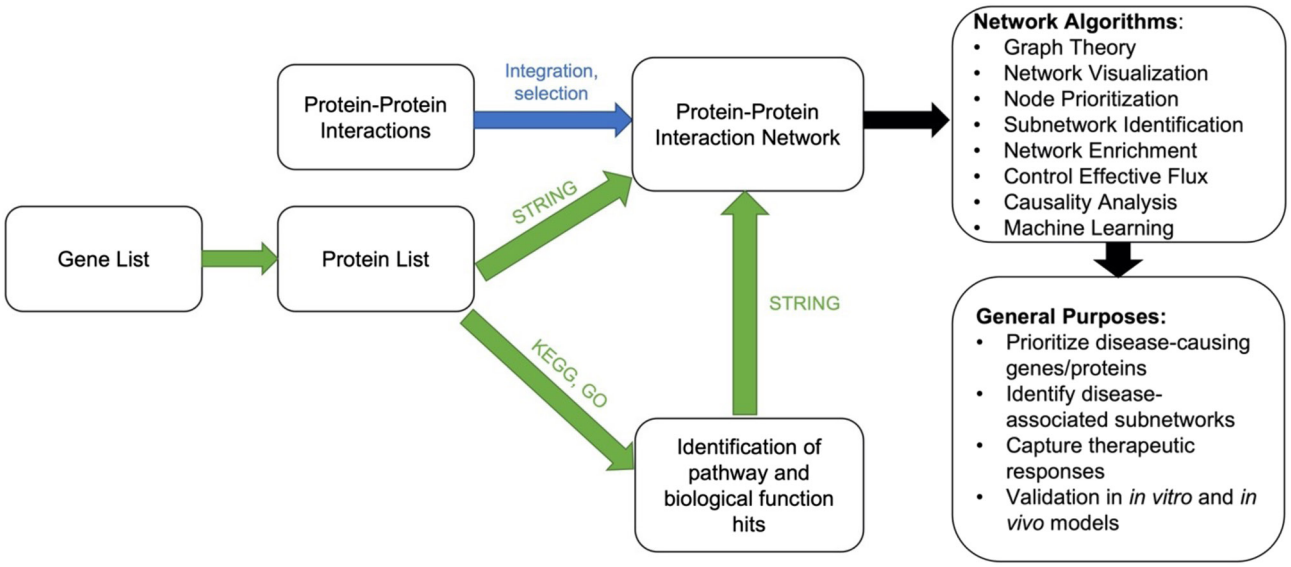

Figure 2
General framework of biological network construction for *in silico* modeling. The blue arrow and text correspond to the construction of biological networks, the green arrows and text correspond to the mapping of omics data onto biological networks and the black arrows correspond to network analyses.

(3, 7, 17). Since endothelial cells are early targets of sepsis (7), research focused on creating a systems biology, mechanistic understanding of microvascular endothelial cell dysfunction in sepsis, especially across organs damaged early on such as the lungs, liver and kidneys, is critical. A key area where future research should be directed is profiling the proteome of microvascular endothelial cells in microphysiological systems using cultured organ-specific human microvascular endothelial cells subjected to shear flow to identify differentially expressed proteins (DEPs) and protein–protein interactions that contribute to a disease subphenotype or endotype. Thus, incorporating physiologically relevant flow conditions and organ-specific primary endothelial cells would provide more realistic microenvironments to help identify how organ-specific endothelial proteomes are altered in response to shear and aid in the discovery of therapeutics targeting endothelial cells. Omics can not only characterize the underlying molecular mechanisms of diseases by identifying and quantifying all the biomolecular interactions in a biological system but also categorize patients into endotypes based on their omic expression patterns (5, 11, 33, 34, 35). Using bioinformatic tools to identify differentially expressed cellular pathways or GO in ECs could answer questions such as where genes or proteins are expressed in a system during different pathologies and their corresponding molecular functions (50, 51, 52, 53, 54). While genomics can identify causal variants that contribute to disease, complementary experimental and validation studies are often required and needed to identify and characterize the functionality of the variant(s) in disease progression within a heterogenous population. Proteomics, in particular, can yield novel insight since proteins are involved in every biological phenomenon and thus unraveling the complex protein–protein interactions (PPIs) in a cell can identify DEPs between disease and control groups (36). In addition, one needs to consider that proteins *in vivo* are subjected to post-translational modifications during and/or after synthesis (89), and thus future research should focus on identifying these modifications, their potential role in disease and how these modifications can be targeted for therapy. Another critical area of research is the application of *in silico* network modeling incorporating proteomics data to screen and test therapeutics for a disease in a realistic model prior to *in vitro* and *in vivo* experimentation (36, 90). Evaluating the effect of a therapeutic on protein(s) or protein complex(es) *in silico,* especially if the protein complex plays a role in multiple pathways or processes leading to disease, will generate novel hypotheses that (a) would be tested and validated experimentally, (b) complement and refine experimental testing and protocol and (c) potentially reduce the number of animal models needed for experimental studies. *In silico* models that enable pharmacological intervention of a target (e.g. *in silico* simulation of knock-out or pharmacological alteration of a biological pathway) would be beneficial

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(91). Additionally, models that can simulate therapeutic responses over time in multiple biological compartments will provide an even more physiologically relevant tool for drug screening and evaluation. Using *in silico* modeling to repurpose existing therapeutics for treating other diseases (92) is an emerging area of research interest, and its potential for screening therapeutics to target endothelial cells for treating sepsis should be further investigated. Further application of the emerging field of omics for treating sepsis is needed in studies comparing biomarkers used alone or in combination in a time-dependent manner to evaluate their impact on disease progression. A major hurdle for implementing omics in medical practice is a lack of consensus on standardization of methodologies in the scientific community (4). Validation in multicenter, diverse cohorts is urgently needed to effectively test these omic models in clinical trials before translation, and further investigation can assess their utility and cost effectiveness (4). Furthermore, since sepsis progresses rapidly, the quick turn-around time from omic testing to results in healthcare, which is necessary for effective, tailored therapy, has not been achieved yet. Addressing these issues will allow for the translation of omics from the bench to the bedside and, coupled with advances in *in silico* modeling, establishment of scientific and clinical standards to utilize the potential of omic analyses for clinical treatment of sepsis. Overall, this will significantly advance the goal of precision medicine for delivering the right therapeutic to the right patient at the right time.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/VAB-22-0003.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

J C L, M T R, W O, E P and Q Y prepared the manuscript. M F K, L E K and B P developed the ideas and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

References

1 Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, *et al.* The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016 **315** 801–810. (https://doi.org/10.1001/jama.2016.0287)

2 Chiu C & Legrand M. Epidemiology of sepsis and septic shock. *Current Opinion in Anaesthesiology* 2021 **34** 71–76 (https://doi.org/10.1097/ACO.0000000000000958)

3 Cao Z & Robinson RAS. The role of proteomics in understanding biological mechanisms of sepsis. *Proteomics: Clinical Applications* 2014 **8** 35–52. (https://doi.org/10.1002/prca.201300101)

4 Leligdowicz A & Matthay MA. Heterogeneity in sepsis: new biological evidence with clinical applications. *Critical Care* 2019 **23** 80. (https://doi.org/10.1186/s13054-019-2372-2)

5 Seymour CW, Kennedy JN, Wang S, Chang CH, Elliott CF, Xu ZY, Berry S, Clermont G, Cooper G, Gomez H, *et al.* Derivation, validation, and potential treatment implications of novel clinical phenotypes for sepsis. *JAMA* 2019 **321** 2003–2017. (https://doi.org/10.1001/jama.2019.5791)

6 Deutschman CS & Tracey KJ. Sepsis: current dogma and new perspectives. *Immunity* 2014 **40** 463–475. (https://doi.org/10.1016/j.immuni.2014.04.001)

7 Joffre J, Hellman J, Ince C & Ait-Oufella H. Endothelial responses in sepsis. *American Journal of Respiratory and Critical Care Medicine* 2020 **202** 361–370. (https://doi.org/10.1164/rccm.201910-1911TR)

8 Borren N, Plichta D, Joshi A, Bonilla G, Sadreyev R, Vlamakis H, Xavier R & Ananthakrishnan A. Multi-omics profiling in patients with quiescent inflammatory bowel disease identifies biomarkers predicting relapse. *Journal of Crohn's and Colitis* 2020 **14** S069–S070. (https://doi.org/10.1093/ecco-jcc/jjz203.069)

9 Anchang CG, Xu C, Raimondo MG, Atreya R, Maier A, Schett G, Zaburdaev V, Rauber S & Ramming A. The potential of OMICs technologies for the treatment of immune-mediated inflammatory diseases. *International Journal of Molecular Sciences* 2021 **22** 7506. (https://doi.org/10.3390/ijms22147506)

10 Hasson D, Goldstein SL & Standage SW. The application of omic technologies to research in sepsis-associated acute kidney injury. *Pediatric Nephrology* 2021 **36** 1075–1086. (https://doi.org/10.1007/s00467-020-04557-9)

11 Davenport EE, Burnham KL, Radhakrishnan J, Humburg P, Hutton P, Mills TC, Rautanen A, Gordon AC, Garrard C, Hill AV, *et al.* Genomic landscape of the individual host response and outcomes in sepsis: a prospective cohort study. *Lancet: Respiratory Medicine* 2016 **4** 259–271. (https://doi.org/10.1016/S2213-2600(16)00046-1)

12 Hasin Y, Seldin M & Lusis A. Multi-omics approaches to disease. *Genome Biology* 2017 **18** 83. (https://doi.org/10.1186/s13059-017-1215-1)

13 Veenstra TD. Systems biology and multi-omics. *Proteomics* 2021 **21** e2000306. (https://doi.org/10.1002/pmic.202000306)

14 Veenstra TD. Omics in systems biology: current progress and future outlook. *Proteomics* 2021 **21** e2000235. (https://doi.org/10.1002/pmic.202000235)

15 Cahill PA & Redmond EM. Vascular endothelium – gatekeeper of vessel health. *Atherosclerosis* 2016 **248** 97–109. (https://doi.org/10.1016/j.atherosclerosis.2016.03.007)

16 Aird WC. Endothelial cell heterogeneity. *Cold Spring Harbor Perspectives in Medicine* 2012 **2** a006429. (https://doi.org/10.1101/cshperspect.a006429)

17 Hattori Y, Hattori K, Suzuki T & Matsuda N. Recent advances in the pathophysiology and molecular basis of sepsis-associated organ dysfunction: novel therapeutic implications and challenges. *Pharmacology and Therapeutics* 2017 **177** 56–66. (https://doi.org/10.1016/j.pharmthera.2017.02.040)

18 Soroush F, Zhang T, King DJ, Tang Y, Deosarkar S, Prabhakarpandian B, Kilpatrick LE & Kiani MF. A novel microfluidic assay reveals a key

role for protein kinase C delta in regulating human neutrophil-endothelium interaction. *Journal of Leukocyte Biology* 2016 **100** 1027–1035. (https://doi.org/10.1189/jlb.3MA0216-087R)
19 Gentile LF, Cuenca AG, Efron PA, Ang D, Bihorac A, McKinley BA, Moldawer LL & Moore FA. Persistent inflammation and immunosuppression: a common syndrome and new horizon for surgical intensive care. *Journal of Trauma and Acute Care Surgery* 2012 **72** 1491–1501. (https://doi.org/10.1097/TA.0b013e318256e000)
20 An G, Nieman G & Vodovotz Y. Computational and systems biology in trauma and sepsis: current state and future perspectives. *International Journal of Burns and Trauma* 2012 **2** 1–10.
21 Lee D, Park Y & Kim S. Towards multi-omics characterization of tumor heterogeneity: a comprehensive review of statistical and machine learning approaches. *Briefings in Bioinformatics* 2021 **22** bbaa188. (https://doi.org/10.1093/bib/bbaa188)
22 Olsen TK & Baryawno N. Introduction to single-cell RNA sequencing. *Current Protocols in Molecular Biology* 2018 **122** e57. (https://doi.org/10.1002/cpmb.57)
23 Paananen J & Fortino V. An omics perspective on drug target discovery platforms. *Briefings in Bioinformatics* 2020 **21** 1937–1953. (https://doi.org/10.1093/bib/bbz122)
24 Shi J, Xiao JC, Li JP, Wang XW, Her L, Sorensen MJ & Zhu HJ. FRACPRED-2D-PRM: a fraction prediction algorithm-assisted 2D liquid chromatography-based parallel reaction monitoring-mass spectrometry approach for measuring low-abundance proteins in human plasma. *Proteomics* 2020 **20** e2000175. (https://doi.org/10.1002/pmic.202000175)
25 Olivier M, Asmis R, Hawkins GA, Howard TD & Cox LA. The need for multi-omics biomarker signatures in precision medicine. *International Journal of Molecular Sciences* 2019 **20** 4781. (https://doi.org/10.3390/ijms20194781)
26 Itenov TS, Murray DD & Jensen JUS. Sepsis: personalized medicine utilizing 'omic' technologies – a paradigm shift? *Healthcare* 2018 **6** 111. (https://doi.org/10.3390/healthcare6030111)
27 Langley RJ & Wong HR. Early diagnosis of sepsis: is an integrated omics approach the way forward? *Molecular Diagnosis and Therapy* 2017 **21** 525–537. (https://doi.org/10.1007/s40291-017-0282-z)
28 Prucha M, Zazula R & Russwurm S. Sepsis diagnostics in the era of 'omics' technologies. *Prague Medical Report* 2018 **119** 9–29. (https://doi.org/10.14712/23362936.2018.2)
29 Pierrakos C, Velissaris D, Bisdorff M, Marshall JC & Vincent JL. Biomarkers of sepsis: time for a reappraisal. *Critical Care* 2020 **24** 287. (https://doi.org/10.1186/s13054-020-02993-5)
30 Teggert A, Datta H & Ali Z. Biomarkers for point-of-care diagnosis of sepsis. *Micromachines* 2020 **11** 286. (https://doi.org/10.3390/mi11030286)
31 Wong HR. Sepsis biomarkers. *Journal of Pediatric Intensive Care* 2019 **8** 11–16. (https://doi.org/10.1055/s-0038-1677537)
32 Vanska M, Koivula I, Hamalainen S, Pulkki K, Nousiainen T, Jantunen E & Juutilainen A. High PENTRAXIN 3 level predicts septic shock and bacteremia at the onset of febrile neutropenia after intensive chemotherapy of hematologic patients. *Haematologica* 2011 **96** 1385–1389. (https://doi.org/10.3324/haematol.2011.044925)
33 Wong HR, Cvijanovich N, Lin R, Allen GL, Thomas NJ, Willson DF, Freishtat RJ, Anas N, Meyer K, Checchia PA, *et al.* Identification of pediatric septic shock subclasses based on genome-wide expression profiling. *BMC Medicine* 2009 **7** 34. (https://doi.org/10.1186/1741-7015-7-34)
34 Scicluna BP, van Vught LA, Zwinderman AH, Wiewel MA, Davenport EE, Burnham KL, Nürnberg P, Schultz MJ, Horn J, Cremer OL, *et al.* Classification of patients with sepsis according to blood genomic endotype: a prospective cohort study. *Lancet: Respiratory Medicine* 2017 **5** 816–826. (https://doi.org/10.1016/S2213-2600(17)30294-1)
35 Sweeney TE, Azad TD, Donato M, Haynes WA, Perumal TM, Henao R, Bermejo-Martin JF, Almansa R, Tamayo E, Howrylak JA, *et al.* Unsupervised analysis of transcriptomics in bacterial sepsis across multiple datasets reveals three robust clusters. *Critical Care Medicine* 2018 **46** 915–925. (https://doi.org/10.1097/CCM.0000000000003084)
36 Zhang P & Itan Y. Biological network approaches and applications in rare disease studies. *Genes* 2019 **10**. (https://doi.org/10.3390/genes10100797)
37 van der Poll T, Shankar-Hari M & Wiersinga WJ. The immunology of sepsis. *Immunity* 2021 **54** 2450–2464. (https://doi.org/10.1016/j.immuni.2021.10.012)
38 Severino P, Silva E, Baggio-Zappia G, Brunialti M, Nucci LA, Rigato O, da Silva IDCG, Machado FR & Salomao R. Patterns of gene expression in peripheral blood mononuclear cells and outcomes from patients with sepsis secondary to community acquired pneumonia. *Shock* 2014 **41** 55–61. (https://doi.org/10.1371/journal.pone.0091886)
39 Almansa R, Heredia-Rodriguez M, Gomez-Sanchez E, Andaluz-Ojeda D, Iglesias V, Rico L, Ortega A, Gomez-Pesquera E, Liu P, Aragón M, *et al.* Transcriptomic correlates of organ failure extent in sepsis. *Journal of Infection* 2015 **70** 445–456. (https://doi.org/10.1016/j.jinf.2014.12.010)
40 Almansa R, Tamayo E, Heredia M, Gutierrez S, Ruiz P, Alvarez E, Gomez-Sanchez E, Andaluz-Ojeda D, Ceña R, Rico L, *et al.* Transcriptomic evidence of impaired immunoglobulin G production in fatal septic shock. *Journal of Critical Care* 2014 **29** 307–309. (https://doi.org/10.1016/j.jcrc.2013.11.020)
41 Cvijanovich N, Shanley TP, Lin R, Allen GL, Thomas NJ, Checchia P, Anas N, Freishtat RJ, Monaco M, Odoms K, *et al.* Validating the genomic signature of pediatric septic shock. *Physiological Genomics* 2008 **34** 127–134. (https://doi.org/10.1152/physiolgenomics.00025.2008)
42 Burnham KL, Davenport EE, Radhakrishnan J, Humburg P, Gordon AC, Hutton P, Svoren-Jabalera E, Garrard C, Hill AVS, Hinds CJ, *et al.* Shared and distinct aspects of the sepsis transcriptomic response to fecal peritonitis and pneumonia. *American Journal of Respiratory and Critical Care Medicine* 2017 **196** 328–339. (https://doi.org/10.1164/rccm.201608-1685OC)
43 Gunawardana H, Romero T, Yao N, Heidt S, Mulder A, Elashoff DA & Valenzuela NM. Tissue-specific endothelial cell heterogeneity contributes to unequal inflammatory responses. *Scientific Reports* 2021 **11** 1949. (https://doi.org/10.1038/s41598-020-80102-w)
44 Wong HR, Sweeney TE, Hart KW, Khatri P & Lindsell CJ. Pediatric sepsis endotypes among adults with sepsis. *Critical Care Medicine* 2017 **45** e1289–e1291. (https://doi.org/10.1097/CCM.0000000000002733)
45 Sinha P, Furfaro D, Cummings MJ, Abrams D, Delucchi K, Maddali MV, He J, Thompson A, Murn M, Fountain J, *et al.* Latent class analysis reveals COVID-19-related acute respiratory distress syndrome subgroups with differential responses to corticosteroids. *American Journal of Respiratory and Critical Care Medicine* 2021 **204** 1274–1285. (https://doi.org/10.1164/rccm.202105-1302OC)
46 Pankla R, Buddhisa S, Berry M, Blankenship DM, Bancroft GJ, Banchereau J, Lertmemongkolchai G & Chaussabel D. Genomic transcriptional profiling identifies a candidate blood biomarker signature for the diagnosis of septicemic melioidosis. *Genome Biology* 2009 **10** R127. (https://doi.org/10.1186/gb-2009-10-11-r127)
47 Sutherland A, Thomas M, Brandon RA, Brandon RB, Lipman J, Tang B, McLean A, Pascoe R, Price G, Nguyen T, *et al.* Development and validation of a novel molecular biomarker diagnostic test for the early detection of sepsis. *Critical Care* 2011 **15** R149. (https://doi.org/10.1186/cc10274)
48 Howrylak JA, Dolinay T, Lucht L, Wang ZX, Christiani DC, Sethi JM, Xing EP, Donahoe MP & Choi AM. Discovery of the gene signature for acute lung injury in patients with sepsis. *Physiological Genomics* 2009 **37** 133–139. (https://doi.org/10.1152/physiolgenomics.90275.2008)
49 Osuchowski MF, Remick DG, Lederer JA, Lang CH, Aasen AO, Aibiki M, Azevedo LC, Bahrami S, Boros M, Cooney R, *et al.* Abandon the mouse

research ship? Not just yet! *Shock* 2014 **41** 463–475. (https://doi.org/10.1097/SHK.0000000000000153)
50 Jambusaria A, Hong ZG, Zhang LH, Srivastava S, Jana A, Toth PT, Dai Y, Malik AB & Rehman J. Endothelial heterogeneity across distinct vascular beds during homeostasis and inflammation. *eLife* 2020 **9** e51413. (https://doi.org/10.7554/eLife.51413)
51 Cleuren ACA, van der Ent MA, Jiang H, Hunker KL, Yee A, Siemieniak DR, Molema G, Aird WC, Ganesh SK & Ginsburg D. The in vivo endothelial cell translatome is highly heterogeneous across vascular beds. *PNAS* 2019 **116** 23618–23624. (https://doi.org/10.1073/pnas.1912409116)
52 Chen LS, Singh SP, Mueller G, Bornstein SR & Kanczkowski W. Transcriptional analysis of sepsis-induced activation and damage of the adrenal endothelial microvascular cells. *Frontiers in Endocrinology* 2019 **10** 944. (https://doi.org/10.3389/fendo.2019.00944)
53 Niethamer TK, Stabler CT, Leach JP, Zepp JA, Morley MP, Babu A, Zhou S & Morrisey EE. Defining the role of pulmonary endothelial cell heterogeneity in the response to acute lung injury. *eLife* 2020 **9** e53072. (https://doi.org/10.7554/eLife.53072)
54 Paik DT, Tian L, Williams IM, Rhee S, Zhang H, Liu C, Mishra R, Wu SM, Red-Horse K & Wu JC. Single-cell RNA sequencing unveils unique transcriptomic signatures of organ-specific endothelial cells. *Circulation* 2020 **142** 1848–1862. (https://doi.org/10.1161/CIRCULATIONAHA.119.041433)
55 Wang PL, Meng X, Li JJ, Chen YF, Zhang D, Zhong HR, Xia P, Cui L, Zhu G & Wang H. Transcriptome profiling of avian pathogenic Escherichia coli and the mouse microvascular endothelial cell line bEnd.3 during interaction. *PeerJ* 2020 **8** e9172. (https://doi.org/10.7717/peerj.9172)
56 Toledo AG, Golden G, Campos AR, Cuello H, Sorrentino J, Lewis N, Varki N, Nizet V, Smith JW & Esko JD. Proteomic atlas of organ vasculopathies triggered by Staphylococcus aureus sepsis. *Nature Communications* 2019 **10** 4656. (https://doi.org/10.1038/s41467-019-12672-x)
57 Xu X, Zhu Q, Zhang R, Wang Y, Niu F, Wang W, Sun D & Wang A. ITRAQ-based proteomics analysis of acute lung injury induced by oleic acid in mice. *Cellular Physiology and Biochemistry* 2017 **44** 1949–1964. (https://doi.org/10.1159/000485885)
58 Gallart-Palau X, Serra A & Sze SK. System-wide molecular dynamics of endothelial dysfunction in Gram-negative sepsis. *BMC Biology* 2020 **18** 175. (https://doi.org/10.1186/s12915-020-00914-0)
59 Azimzadeh O, Sievert W, Sarioglu H, Merl-Pham J, Yentrapalli R, Bakshi MV, Janik D, Ueffing M, Atkinson MJ, Multhoff G, *et al.* Integrative proteomics and targeted transcriptomics analyses in cardiac endothelial cells unravel mechanisms of long-term radiation-induced vascular dysfunction. *Journal of Proteome Research* 2015 **14** 1203–1219. (https://doi.org/10.1021/pr501141b)
60 Philipp J, Sievert W, Azimzadeh O, von Toerne C, Metzger F, Posch A, Hladik D, Subedi P, Multhoff G, Atkinson MJ, *et al.* Data independent acquisition mass spectrometry of irradiated mouse lung endothelial cells reveals a STAT-associated inflammatory response. *International Journal of Radiation Biology* 2020 **96** 642–650. (https://doi.org/10.1080/09553002.2020.1712492)
61 Sakaue T, Shikata F, Utsunomiya K, Fukae S, Kurata M, Nakaoka H, Okazaki M, Kawanishi Y, Kojima A, Higashiyama S, *et al.* Proteomics-based analysis of lung injury induced proteins in a mouse model of common bile duct ligation. *Surgery* 2017 **161** 1525–1535. (https://doi.org/10.1016/j.surg.2016.12.017)
62 Liu H, Huang CX, He Q, Li D, Luo MH, Zhao F & Lu W. Proteomics analysis of HSV-1-induced alterations in mouse brain microvascular endothelial cells. *Journal of NeuroVirology* 2019 **25** 525–539. (https://doi.org/10.1007/s13365-019-00752-z)
63 Li H, Liu L, Zhang D, Xu J, Dai H, Tang N, Su X & Cao B. SARS-CoV-2 and viral sepsis: observations and hypotheses. *Lancet* 2020 **395** 1517–1520. (https://doi.org/10.1016/S0140-6736(20)30920-X)
64 Su YP, Chen D, Yuan D, Lausted C, Choi J, Dai CL, *et al.* Multi-omics resolves a sharp disease-state shift between mild and moderate COVID-19. *Cell* 2020 **183** 1479.e20–1495.e20. (https://doi.org/10.1016/j.cell.2020.10.037)
65 Tang Y, Soroush F, Sheffield JB, Wang B, Prabhakarpandian B & Kiani MF. A biomimetic microfluidic tumor microenvironment platform mimicking the EPR effect for rapid screening of drug delivery systems. *Scientific Reports* 2017 **7** 9359. (https://doi.org/10.1038/s41598-017-09815-9)
66 Burghoff S & Schrader J. Secretome of human endothelial cells under shear stress. *Journal of Proteome Research* 2011 **10** 1160–1169. (https://doi.org/10.1021/pr100937a)
67 Mohr T, Haudek-Prinz V, Slany A, Grillari J, Micksche M & Gerner C. Proteome profiling in IL-1 beta and VEGF-activated human umbilical vein endothelial cells delineates the interlink between inflammation and angiogenesis. *PLoS ONE* 2017 **12** e0179065. (https://doi.org/10.1371/journal.pone.0179065)
68 Gautier V, Cayrol C, Farache D, Roga S, Monsarrat B, Burlet-Schiltz O, Gonzalez de Peredo A & Girard J. Extracellular IL-33 cytokine, but not endogenous nuclear IL-33, regulates protein expression in endothelial cells. *Scientific Reports* 2016 **6** 34255. (https://doi.org/10.1038/srep34255)
69 Kwon OK, Lee W, Kim SJ, Lee YM, Lee JY, Kim JY, Bae JS & Lee S. In-depth proteomics approach of secretome to identify novel biomarker for sepsis in LPS-stimulated endothelial cells. *Electrophoresis* 2015 **36** 2851–2858. (https://doi.org/10.1002/elps.201500198)
70 Yang Q, Wijerathne H, Langston JC, Kiani MF & Kilpatrick LE. Emerging approaches to understanding microvascular endothelial heterogeneity: a roadmap for developing anti-inflammatory therapeutics. *International Journal of Molecular Sciences* 2021 **22** 7770. (https://doi.org/10.3390/ijms22157770)
71 Tkacova Z, Pulzova LB, Mochnacova E, Jimenez-Munguia I, Bhide K, Mertinkova P, Majerová P, Kulkarni A, Kováč A & Bhide M. Identification of the proteins of Borrelia garinii interacting with human brain microvascular endothelial cells. *Ticks and Tick-Borne Diseases* 2020 **11** 101451. (https://doi.org/10.1016/j.ttbdis.2020.101451)
72 Ren C, Yao RQ, Zhang H, Feng YW & Yao YM. Sepsis-associated encephalopathy: a vicious cycle of immunosuppression. *Journal of Neuroinflammation* 2020 **17** 14. (https://doi.org/10.1186/s12974-020-1701-3)
73 McCormick SM, Eskin SG, McIntire LV, Teng CL, Lu CM, Russell CG & Chittur KK. DNA microarray reveals changes in gene expression of shear stressed human umbilical vein endothelial cells. *PNAS* 2001 **98** 8955–8960. (https://doi.org/10.1073/pnas.171259298)
74 Chen BPC, Li YS, Zhao YH, Chen KD, Li S, Lao JM, Yuan S, Shyy JY & Chien S. DNA microarray analysis of gene expression in endothelial cells in response to 24-h shear stress. *Physiological Genomics* 2001 **7** 55–63. (https://doi.org/10.1152/physiolgenomics.2001.7.1.55)
75 Dolan JM, Meng H, Sim FJ & Kolega J. Differential gene expression by endothelial cells under positive and negative streamwise gradients of high wall shear stress. *American Journal of Physiology: Cell Physiology* 2013 **305** C854–C866. (https://doi.org/10.1152/ajpcell.00315.2012)
76 Conway DE, Williams MR, Eskin SG & McIntire LV. Endothelial cell responses to atheroprone flow are driven by two separate flow components: low time-average shear stress and fluid flow reversal. *American Journal of Physiology: Heart and Circulatory Physiology* 2010 **298** H367–H374. (https://doi.org/10.1152/ajpheart.00565.2009)
77 Chu TJ & Peters DG. Serial analysis of the vascular endothelial transcriptome under static and shear stress conditions. *Physiological Genomics* 2008 **34** 185–192. (https://doi.org/10.1152/physiolgenomics.90201.2008)
78 Ziegler T, Bouzourene K, Harrison VJ, Brunner HR & Hayoz D. Influence of oscillatory and unidirectional flow environments on the expression of endothelin and nitric oxide synthase in cultured endothelial cells. *Arteriosclerosis, Thrombosis, and Vascular Biology* 1998 **18** 686–692. (https://doi.org/10.1161/01.atv.18.5.686)

79 Li MM, Liu XX, Zhang Y, Di MX, Wang H, Wang L, Chen Y, Liu X, Cao X, Zeng R, *et al.* Upregulation of Dickkopf1 by oscillatory shear stress accelerates atherogenesis. *Journal of Molecular Medicine* 2016 **94** 431–441. (https://doi.org/10.1007/s00109-015-1369-9)
80 Lupu F, Kinasewitz G & Dormer K. The role of endothelial shear stress on haemodynamics, inflammation, coagulation and glycocalyx during sepsis. *Journal of Cellular and Molecular Medicine* 2020 **24** 12258–12271. (https://doi.org/10.1111/jcmm.15895)
81 Kanter JE. FOXP1: a gatekeeper of endothelial cell inflammation. *Circulation Research* 2019 **125** 606–608. (https://doi.org/10.1161/CIRCRESAHA.119.315687)
82 Zhuang T, Liu J, Chen XL, Zhang L, Pi JJ, Sun HM, Li L, Bauer R, Wang H, Yu Z, *et al.* Endothelial Foxp1 suppresses atherosclerosis via modulation of Nlrp3 inflammasome activation. *Circulation Research* 2019 **125** 590–605. (https://doi.org/10.1161/CIRCRESAHA.118.314402)
83 Bryan MT, Duckles H, Feng S, Hsiao ST, Kim HR, Serbanovic-Canic J & Evans PC. Mechanoresponsive networks controlling vascular inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2014 **34** 2199–2205. (https://doi.org/10.1161/ATVBAHA.114.303424)
84 Soleimani S, Shamsi M, Ghazani MA, Modarres HP, Valente KP, Saghafian M, Ashani MM, Akbari M & Sanati-Nezhad A. Translational models of tumor angiogenesis: a nexus of in silico and in vitro models. *Biotechnology Advances* 2018 **36** 880–893. (https://doi.org/10.1016/j.biotechadv.2018.01.013)
85 Andreoni C, Orsi G, De Maria C, Montemurro F & Vozzi G. In silico models for dynamic connected cell cultures mimicking hepatocyte-endothelial cell-adipocyte interaction circle. *PLoS ONE* 2014 **9** e111946. (https://doi.org/10.1371/journal.pone.0111946)
86 Koo A, Nordsletten D, Umeton R, Yankama B, Ayyadurai S, Garcia-Cardena G & Dewey CF. In silico modeling of shear-stress-induced nitric oxide production in endothelial cells through systems biology. *Biophysical Journal* 2013 **104** 2295–2306. (https://doi.org/10.1016/j.bpj.2013.03.052)
87 Gorick CM, Saucerman JJ & Price RJ. Computational model of brain endothelial cell signaling pathways predicts therapeutic targets for cerebral pathologies. *Journal of Molecular and Cellular Cardiology* 2022 **164** 17–28. (https://doi.org/10.1016/j.yjmcc.2021.11.005)
88 Bloomingdale P, Nguyen VA, Niu J & Mager DE. Boolean network modeling in systems pharmacology. *Journal of Pharmacokinetics and Pharmacodynamics* 2018 **45** 159–180. (https://doi.org/10.1007/s10928-017-9567-4)
89 Ramazi S & Zahiri J. Post-translational modifications in proteins: resources, tools and prediction methods. *Database* 2021 **2021** baab012. (https://doi.org/10.1093/database/baab012)
90 Soni AS, Jenkins JW & Sundaram SS. Determination of critical network interactions: an augmented Boolean pseudo-dynamics approach. *IET Systems Biology* 2008 **2** 55–63. (https://doi.org/10.1049/iet-syb:20070025)
91 Hannig J, Giese H, Schweizer B, Amstein L, Ackermann J & Koch I. isiKnock: in silico knockouts in signaling pathways. *Bioinformatics* 2019 **35** 892–894. (https://doi.org/10.1093/bioinformatics/bty700)
92 Deshpande RR, Tiwari AP, Nyayanit N & Modak M. In silico molecular docking analysis for repurposing therapeutics against multiple proteins from SARS-CoV-2. *European Journal of Pharmacology* 2020 **886** 173430. (https://doi.org/10.1016/j.ejphar.2020.173430)

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