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Effects of Controlled Cortical Impact on the Mouse Brain Vasculome

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Abstract

Perturbations in blood vessels play a critical role in the pathophysiology of brain injury and neurodegeneration. Here, we use a systematic genome-wide transcriptome screening approach to investigate the vasculome after brain trauma in mice. Mice were subjected to controlled cortical impact and brains were extracted for analysis at 24 h post-injury. The core of the traumatic lesion was removed and then cortical microvesels were isolated from nondirectly damaged ipsilateral cortex. Compared to contralateral cortex and normal cortex from sham-operated mice, we identified a wide spectrum of responses in the vasculome after trauma. Up-regulated pathways included those involved in regulation of inflammation and extracellular matrix processes. Decreased pathways included those involved in regulation of metabolism, mitochondrial function, and transport systems. These findings suggest that microvascular perturbations can be widespread and not necessarily localized to core areas of direct injury *per se* and may further provide a broader gene network context for existing knowledge regarding inflammation, metabolism, and blood–brain barrier alterations after brain trauma. Further efforts are warranted to map the vasculome with higher spatial and temporal resolution from acute to delayed phase post-trauma. Investigating the widespread network responses in the vasculome may reveal potential mechanisms, therapeutic targets, and biomarkers for traumatic brain injury.

Key words: extracellular matrix; inflammation; microvessels; neurovascular unit; traumatic brain injury

Introduction

Traumatic brain injury (TBI) is a major public health issue.[1] Intense research has been directed to identify neuroprotective therapies to improve outcome after trauma. Unfortunately, clinical trials have not yielded any broadly efficacious treatments, and current care for TBI patients remains mostly supportive.[2–4]

Over the past decade, the concept of the neurovascular unit has emerged to suggest that treatments for stroke and neurodegeneration cannot be found by focusing on neurons alone. Instead, cell-cell signaling between all elements in neuronal, glial, and vascular compartments must be addressed in order to rescue function and improve outcomes in damaged and diseased brain.[5–7] Is it possible that for TBI, pure neuroprotection alone is also insufficient? In this study, we hypothesize that widespread vascular responses may occur in the context of TBI pathophysiology.

It was recently proposed that responses in blood microvessels (i.e., perturbations in the vasculome) mediate brain function and dysfunction.[7–10] The entire network of microvessels in the brain is no longer viewed as inert pipes. Instead, the cerebral endothelium is now recognized to be an active participant for cell-cell signaling in the neurovascular unit. Under normal conditions, endothelium may provide signals that support neurogenesis,[11,12] protect neurons against stress,[13,14] and mediate homeostasis in oligodendrocyte precursor pools.[15] When activated by injury, the endothelium may produce proinflammatory cytokines that magnify neuroinflammation and secondary injury.[16–18] Injured endothelial cells may no longer achieve the appropriate balance between coagulation and fibrinolysis, resulting in hemorrhage, microthrombin formation, and perturbations of cerebral blood flow.[19–21] Furthermore, disruption of endothelial tight junctions and basal lamina may lead to breakdown of the blood–brain barrier (BBB) with subsequent brain edema.[22,23] Stressed endothelium may also release measurable signals into the bloodstream, thus providing a potential source of biomarkers.[24] Ultimately, the entire brain endothelium plays a central role in substrate delivery, BBB function, flow-metabolism coupling, and trophic support for neurons and glia. Hence, mapping responses in the vasculome may offer new insights into mechanisms,

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therapeutic targets, and potential biomarkers in TBI. In this study, we used systematic genome-wide transcriptome screening approach to map the vasculome after controlled cortical impact (CCI) brain injury in mice.

Methods

Mouse controlled cortical impact

All CCI protocols were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male C57Bl/6 mice (10–12 weeks of age) were used for this model, as reported previously.[25] Briefly, mice were anesthetized with 4% isoflurane (Anaquest, Memphis, TN) in 70% N_2 and 30% O_2 and positioned in a stereotaxic frame. A 5-mm craniotomy was performed over the right cerebral hemisphere between the bregma and lambda by the use of a trephine drill, and the bone flap was removed. After craniotomy, mice were subjected to injury by a pneumatic cylinder with a 3-mm flat-tip impounder, 4.8 m/s of velocity, 0.6 mm of depth, and 150 ms of impact duration. Post-CCI, the scalp was sutured closed and mice were returned to their cages to recover from anesthesia. To confirm reproducible injury, mouse brains were cut and standard hematoxylin-eosin (H&E) stains were used to assess the distribution of traumatic injury.

Preparation of brain microvessels

Mice were sacrificed 24 h post-injury. After perfusion with phosphate-buffered saline (PBS), brains were harvested. Two sides of hemispheres (contra- and ipsilateral sides) were separated, removing the core lesion areas (which fall apart quite easily) and underlying white matter, then rolling on the filter paper to get rid of meninges and the remaining loose debris. After this, cortical tissue from each hemisphere was pooled from 4 mice, homogenized in cold PBS on ice with Knote Dounce glass tissue grinder (Part 885300-0002; Kimble Chase Life Science, Vineland, NJ), then centrifuged at 4°C, 500*g* for 5 min. The tissue pellet was suspended with 18% Dextran solution (molecular weight 60–90 kDa; USB Corporation, Cleveland, OH) in PBS and then centrifuged again at 4°C, 2500*g* for 20 min. The resulting pellet from this Dextran gradient centrifugation provides the raw sample of brain microvessels, as previously described.[26] To increase yield, the supernatant was centrifuged again and the pellets from two centrifugations were pooled, washed once in PBS, and then resuspended in PBS. The final suspension was filtered through a 40-μm cell strainer to get rid of remaining single cells or small cell clumps. The resulting microvessels on the top of the cell strainer were used directly for RNA extraction. To confirm purity, isolated microvessels were subjected to immunostaining for endothelial, smooth muscle cell, and glial markers. Primary antibodies anti-CD31, alpha-smooth muscle actin (α-SMA), and glial fibrillary acidic protein (GFAP) were used. Texas Red–labeled lycopersicon Esculentum (tomato) Lectin (Vector Laboratories, Burlingame, CA) was also used to stain vessel fragments. Note that in this study, we collected brain microvessel fragments using dextran gradient centrifugation, unlike the original vasculome paper where enzyme digestion and CD-31 magnetic bead isolation were required for the structurally tougher tissue from heart and kidney. We acknowledge that the comparative purity of endothelial cells may be different between these two methods. But according to reverse-transcriptase polymerase chain reaction (RT-PCR) verification, sufficient purity of microvessels was still obtained here.

Quantitative real-time polymerase chain reaction

Relative expressions (ipsilateral vs. contralateral [Ipsi/Cont] and ipsilateral vs. normal condition [Ipsi/Norm]) of some selected genes were verified by RT-PCR, a commonly used technique to quantify relative gene expression. First-strand complementary DNA (cDNA) was synthesized with the SuperScript VILO synthesis system (Invitrogen, Grand Island, NY), consisting of SuperScript III reverse transcriptase and Oligo(dT) primers. Specific gene expression level was quantified by real-time PCR on an ABI-7500 (Applied Biosystems, Foster City, CA) thermal cycler using predesigned Taqman primers with FAM fluorescent labeling (Applied Biosystems). Data normalization was performed by quantification of the endogenous 18S ribosomal RNA, and fold change was measured with $2^{-\Delta\Delta Ct}$ method.

Hematoxylin-eosin staining

To check the histomorphological condition of mouse brain hemisphere, standard H&E staining was processed by Massachusetts General Hospital Histopathology Research Core (Boston, MA).

Microarray sample analysis

Total RNA was extracted from the fresh preparation of brain microvasculature with the RNeasy Plus Micro kit (Qiagen, Hilden, Germany). Concentration of RNA was measured by Nanodrop, and integrity of RNA was tested with the RNA integrity number (RIN) score on the Agilent Bioanalyzer 2100 (Agilent Technologies, Inc., Santa Clara, CAQ). All samples had RIN scores larger than 8.0. Microarray hybridization and scanning was done by Expression Analysis Corporation with Affymetrix murine whole genome microarray M430 2.0, with 3-IVT (*in vitro* transcription) and Encore Biotin Module for amplification, fragmentation, and labeling. Raw expression data from each chip for each sample were summarized and normalized using the Robust Multi-Array algorithm. The quality of each chip was determined by manually checking mean values, variances, and paired scatter plots as well as principal component analysis plots. All chips passed the quality check. All statistical analyses were performed with the statistics software R (version 2.6.2; available from: http://www.r-project.org) and R packages developed by the BioConductor project (available from: http://www.bioconductor.org).

Microarray data analysis

After normalization and quality checking, samples were clustered using an unsupervised clustering method based on Pearson's correlation coefficient-derived distance matrix using all probes on the chip. Distances between clusters were determined using the complete link method. Next, differentially expressed genes were identified, based on both statistical significances, which were determined using the significance analysis of microarrays algorithm (a variant of *t*-test and specifically designed for microarray data), and fold change threshold. The genes with $p < 0.01$ and fold change >2 or <0.5 were considered as differentially expressed. The combination of fold change threshold and *p* values serves to eliminate most false positives, producing a more stringent list. Third, significantly changed gene sets in whole transcriptome were identified using the gene set enrichment analysis (GSEA) method.[27] The GSEA method requires two inputs: 1) a master list of genes ranked according to expression differences between two states and 2) *a priori*–defined gene sets (e.g., pathways) that consist of specific function-related genes. In this study, input 1 is a list of all genes presented on the Affymetrix mouse M430 2.0 platform and ranked according to the fold changes between two groups; input 2 is the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.genome.jp/kegg/) or Gene Ontology (GO) database (http://geneontology.org/).[28,29] Enrichment scores were calculated with standard parameters.

Results

Purity and cluster analysis of the traumatic brain injury vasculome

Mice were subjected to CCI ($n = 12$), and brains were removed at 24 h post-TBI. To prevent contamination of the vasculome by brain debris and nonspecific necrotic tissue, the center core of the TBI lesion was dissected away and tissue samples were isolated from nondirectly damaged ipsi- and contralateral cortex (Fig. 1). H&E staining confirmed that these nondirectly damaged cortical areas had no obvious damage (Fig. 1). For comparison, normal cortex was also obtained from sham-operated mice ($n = 6$).

Microvessels were then purified from all samples. Immunostaining demonstrated that isolated microvessel fragments were positive for endothelial CD31 markers and lectin staining and lacked contaminating signals from astrocytic GFAP or smooth muscle/pericyte α-SMA markers (Fig. 2A). RT-PCR confirmed purity of the isolated vasculome, with enriched levels of endothelial genes (Cdh5, eNOS, and CD31) and low levels of astrocyte (AQP4 and GFAP) and neuronal (MAP2 and Nrgn) genes (Fig. 2B), when compared to whole brain cortex RNA samples. There was also no significant contamination from blood; low levels of monocyte (Emr1 and Mac1) and neutrophil (MPO) genes were detected in our vasculome samples, when compared to blood samples (Fig. 2C).

Twelve hemisphere tissue samples per group were obtained for normal shams and contra- and ipsilateral groups, and four samples were pooled into one microarray chip, finally resulting in three chips per study group. Expression profiles were clustered based on the signals of all probes on each chip. Profiles from contralateral and normal cortex were similar to each other, suggesting that this CCI model of TBI did not significantly affect contralateral endothelium by 24 h post-injury (Fig. 3). However, all three samples from ipsilateral cortex were clustered together and clearly separated from normal and contralateral cortex (Fig. 3). This unsupervised clustering result indicated that the vasculome from nondirectly damaged cortical areas was significantly perturbed by TBI.

Differentially expressed genes

Applying the standard criteria of $p \leq 0.01$ and fold change ≥2 or ≤0.5, there were only 13 genes that were different between samples from contralateral versus normal cortex, again suggesting the lack of large effects in the contralateral mouse brain vasculome post-CCI. However, significant changes were detected in the vasculome from nondirectly damaged ipsilateral cortex; 141 genes were significantly up-regulated and 331 genes were significantly down-regulated compared to contralateral levels, whereas 191 significantly up-regulated genes and 238 significantly down-regulated genes compared to normal samples (see Supplementary Table 1 for full listing) (see online supplementary material at http://www.liebertpub.com). To further ensure that our microarray measurements were reliable, quantitative PCR was used to confirm changes of representative genes from various functional groups, including growth factors, extracellular matrix (ECM) proteinase and components, endothelial response genes, and cytokines. Although there were slight numerical differences in exact levels of quantitation, overall patterns of up- and down-regulation appeared to match between PCR and microarray analyses (Table 1).

After general confirmation of these changes in gene expression, the entire TBI vasculome was then assessed to obtain lists of differentially expressed genes in nondirectly damaged ipsilateral cortex. Up-regulated genes (greater than 5.0-fold change vs. sham) and down-regulated genes (less than 0.30-fold change vs. sham) were compiled (Tables 2 and 3, respectively). In general, these differentially expressed genes appeared to belong to prominent functional groups (e.g., matrix proteins, proteinase and their regulators, inflammation response genes and cytokines, and metabolism-related enzymes). Some patterns may also emerge. Genes belonging to matrix and inflammation responses tended to be up-regulated,

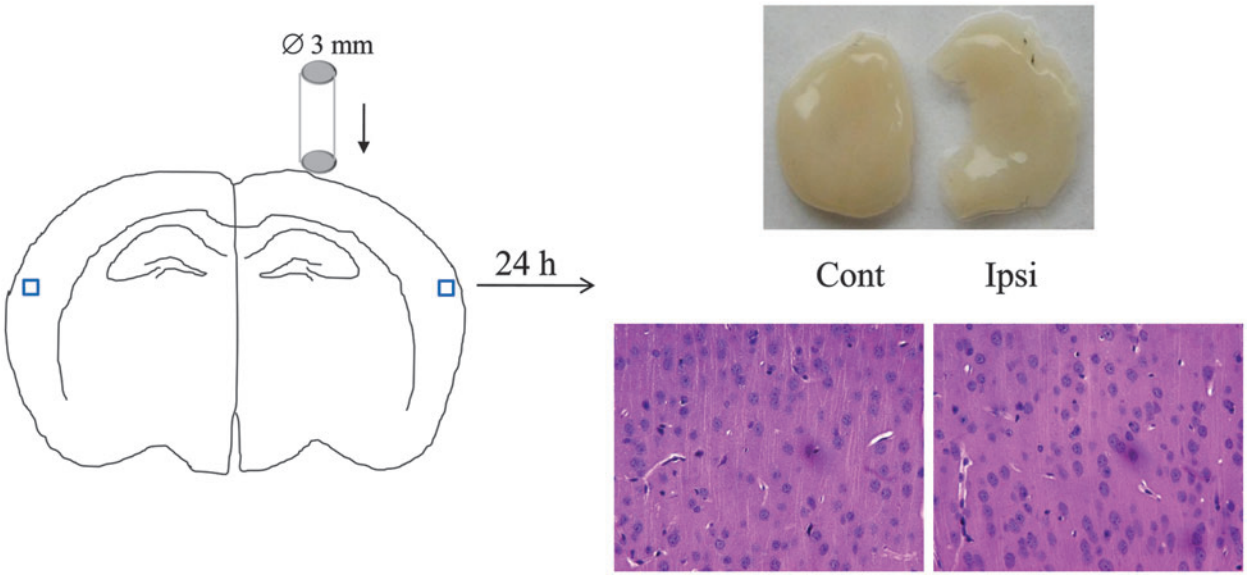

FIG. 1. Sketch of mouse CCI model and sample collection. Left panel shows the CCI model with controlled dropping impounder. Top right shows representative light images of cortexes for contralateral (Cont) and ipsilateral (Ipsi) samples, indicating the missing core area in Ipsi. Lower panel shows representative images of hematoxylin and eosin staining (lower, 400×), indicating no obvious damage in both Contr and Ipsi cortexes. CCI, controlled cortical impact. Color image is available online at www.liebertpub.com/neu

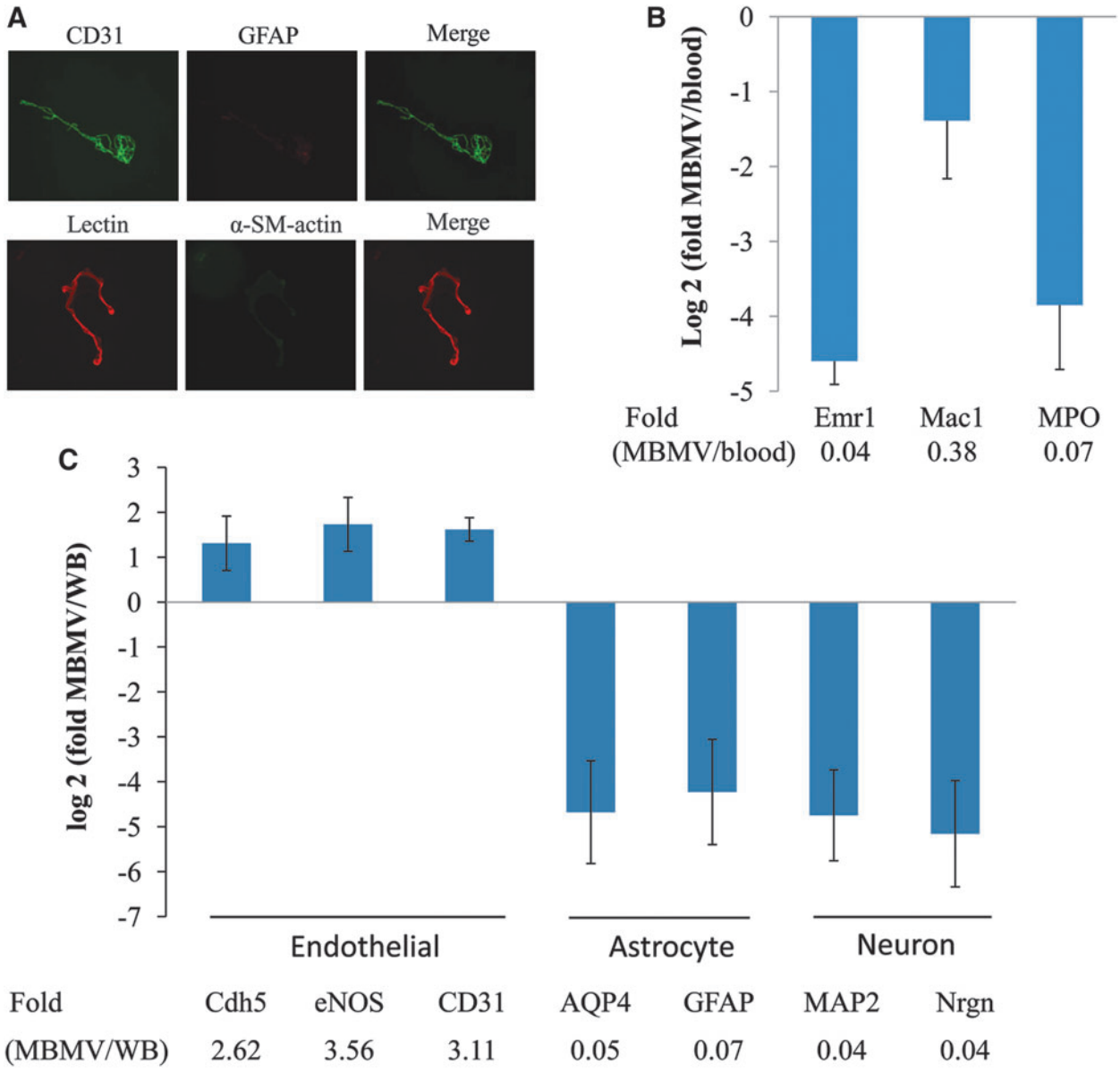

FIG. 2. Purity of brain microvessel fragments. Partial of fragments were used for fluorescent immunostaining (**A**), with cell markers for endothelial cells (CD31 and lectin), astrocyte (GFAP), and smooth muscle cells/pericyte (α-SM-actin). (**B**) Expression levels of brain cell markers are compared between microvessel fragments (MBMV) and whole brain (WB) by reverse-transcriptase polymerase chain reaction, and (**C**) expression levels of some leukocytes genes are compared between MBMV and mouse WB. Bar represents the mean ± standard deviation from three individual samples. AQP4, aquaporin 4; Emr1, endothelial growth factor–like module-containing mucin-like hormone receptor-like 1; eNOS, endothelial nitric oxide synthase; GFAP, glial fibrillary acidic protein; Mac1, macrophage antigen 1; MAP2, microtubule-associated protein 2; MPO, myeloperoxidase; Nrgn, neurogranin; α-SM-actin, alpha-smooth muscle actin. Color image is available online at www.liebertpub.com/neu

whereas most of the metabolic genes tended to show reduced expression post-TBI.

The fact that many vasculome genes were altered in the matrix category may be consistent with the overall concept of the neurovascular unit. Matrix proteins mediate communication between cells, interaction between cells and extracellular environment, and can also modulate the bioavailability of growth factors and other signaling molecules. For BBB regulation, for example, matrix metalloproteinase (MMP) 3 mediates BBB leakage and edema and hemorrhage.[30,31] Serpine1 (also known as PAI-1 [plasminogen activator inhibitor type 1]), a tissue plasminogen activator (tPA) inhibitor, is known to regulate the tightness of brain endothelial barriers.[32] The increase of angiopoietin (Ang) 2, or the disturbed ratio of Ang1/Ang2, is suspected of contributing to loss of BBB integrity after brain injury.[33,34] Tissue inhibitor of metalloproteinase (TIMP-1), as a general inhibitor to MMPs, may also ameliorate BBB leakage post-injury.[35] Perhaps these vasculome responses represent, in part, acute pathophysiology as well as the beginnings of endogenous repair post-TBI.

Another prominent aspect of the TBI vasculome may be related to the angiogenesis process[36,37]; many genes were altered in this category. Thrombospondin 1 (THBS-1) is one of the endogenous protein inhibitors of angiogenesis; Adamts (1 and 9) have potential antiangiogenic effects from their THBS-1 repeat

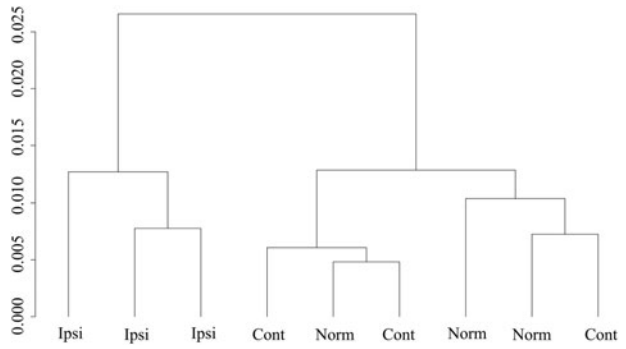

FIG. 3. Hierarchical cluster analysis of microarray data. The expression levels of all probes in each chip for individual sample were analyzed, as vasculome from normal (Norm), contralatral (Cont) and non-directly-damaged ipsilatral (Ipsi) samples, using unsupervised clustering algorithm. y-axis indicates sum of absolute distance between samples.

domains.[38] Transgenic mice with overexpressed tissue factor pathway inhibitor-2 show reduced neovascularization,[39] whereas PAI-1-deficient mice show markedly increased angiogenesis and enhanced wound healing,[40] and addition of PAI-1 reduced Sonic hedgehog–induced tube formation.[41] Cysteine-rich angiogenic inducer 61 has been identified as a novel, vascular endothelial growth factor (VEGF)-A-independent, clinically relevant proangiogenic factor response to hedgehog signaling, for tumor growth, tissue repair, and normal vascular development.[42–44] Syndecan 1 (Sdc1), a major component of the endothelial glycocalyx, is reported to participate in VEGF-induced angiogenesis[45] and might also negatively regulate fibroblast growth factor 2 (FGF2)-induced vascular growth,[46] suggesting the dual roles of Sdc1 in regulation of angiogenesis. Ang2 is considered as a proangiogenesis factor, facilitates VEGF-induced angiogenesis in the mature mouse brain compared to VEGF alone,[47,48] and is involved in brain development and tumor growth.[49,50] By human transcriptome and interactome analysis, CD44 was identified as one of six proteins at the center of an angiogenesis-associated network, suggesting its central role of crosstalk between protein families in regulation of angiogenesis.[51] Taken together, this broad spectrum of angiogenic genes suggests that the TBI vasculome may be surprisingly trying to remodel, even at this acute stage post-injury.

For metabolic enzymes, the majority of responses trended toward down-regulation, suggesting that the functional status of microvessels may be significantly altered even far away from directly damaged tissue. Apelin (Apln) regulates glucose metabolism and insulin sensitivity, partially through a nitric oxide (NO)-dependent pathway.[52,53] It is known to be beneficial to blood vessels by promoting angiogenesis, ensuring vascular integrity,[54–57] and regulating blood pressure.[58] Further, it is suggested that Apln has protective effects on neurons, by activating several prosurvival signaling and inhibiting excitotoxic signaling in neurons.[59,60] Gene xdh encodes for two enzymes, XDH and XO (xanthine oxidase), which could be converted by reversible sulfhydryl oxidation or irreversible proteolytic modification. Both are known for metabolism of purines and as a resource for oxygen radicals and have been implicated in the pathogenesis of endothelial injury and several diseases. There is an association between increased XO activity and negative clinical outcomes,[61–64] but the final balance may be complex given that recent reports also suggest that XO could a beneficial factor in those pathological conditions by inducing NO production.[65] Sphingosine phosphate phosphatase 2 (Sgpp2) is one of the metabolic enzymes essential for degradation of S1P (sphingosine 1-phosphate), which is a bioactive lipid molecule that acts as both an extracellular signaling mediator and an intracellular second messenger, suggesting that Sgpp2 participates in S1P-related functions, including angiogenesis and BBB regulation.[66]

For the general category of inflammatory response genes, the TBI vasculome showed a general increase in expression. Pentraxin 3 (PTX3), a hormonal mediator of innate immunity, regulates hyaluronic acid-enriched ECM assembly and inhibits FGF2-mediated angiogenesis.[67,68] PTX3 is induced under inflammatory conditions, and deficiency of PTX3 promotes vascular inflammation and atherosclerosis, suggesting the vascular protective effects

Table 1. Verification of Microarray Data by RT-PCR

	Microarray		*qPCR*		
Symbol	*Ipsi/norm*	*Ipsi/cont*	*Ipsi/norm*	*Ipsi/cont*	*Description*
Fmod	0.08	0.07	0.06	0.05	Fibromodulin
Cxcl12	0.25	0.24	0.51	0.39	Chemokine (C-X-C motif) ligand 12
VCAM-1	0.26	0.39	0.23	0.30	Vascular cell adhesion protein 1
PDGF-d	0.30	0.27	0.67	0.39	Platelet-derived growth factor, D polypeptide
IGF-1R	0.52	0.47	0.69	0.71	Insulin-like growth factor I receptor
ZO-1	0.71	0.63	0.62	0.44	Zona occludens protein 1
MMP-9	1.10	1.07	1.51	2.91	Matrix metallopeptidase 9
NOS-3	1.42	1.00	1.31	0.75	Nitric oxide synthase 3
BDNF	1.81	1.92	1.05	2.12	Brain-derived neurontrophic factor
IL-6	3.60	3.39	20.00	10.48	Interleukin-6
Tnc	6.41	4.89	26.01	18.41	Tenascin C
Thbs1	6.96	5.40	9.98	9.19	Thrombospondin 1
CD44	12.66	6.56	20.14	26.05	CD44 antigen
TIMP-1	15.03	5.49	8.73	7.45	Tissue inhibitor of metalloproteinase 1
Serpine1	20.87	12.02	31.5	22.3	Serine (or cysteine) peptidase inhibitor clade E, member 1
MMP-3	25.46	15.62	65.75	1882	Matrix metallopeptidase 3

The values fold change represent the mean of three samples in each group of nondirectly damaged ipsilateral (Ipsi), contralateral (Cont), and normal (Norm) microvessel fragments.

RT-PCR, reverse-transcriptase polymerase chain reaction; qPCR, quantitative polymerase chain reaction.

Table 2. List of the Most Increased Genes in Mouse TBI Vasculome Compared to Normal Brain Vasculome

Probe ID	Gene ID	Symbol	Description	Fold change		Function
				Ipsi/norm	Cont/norm	
418666_at	19288	Ptx3	Pentraxin-related gene	34.40	4.88	Inflammation
1418945_at	17392	Mmp3	Matrix metallopeptidase 3	25.46	1.63	ECM
1419149_at	18787	Serpine1	Serine (or cysteine) peptidase inhibitor, clade E, member 1	20.87	1.73	ECM
1416431_at	67951	Tubb6	Tubulin, beta 6	19.59	2.12	Membrane
1460227_at	21857	Timp1	Tissue inhibitor of metalloproteinase 1	15.03	2.74	ECM
1451038_at	30878	Apln	Apelin	14.93	1.36	Metabolism
1418547_at	21789	Tfpi2	Tissue factor pathway inhibitor 2	14.93	1.96	ECM
1434376_at	12505	Cd44	CD44 antigen	12.66	1.93	ECM
1437785_at	101401	Adamts9	A disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 9	10.28	1.47	ECM
1434046_at	433470	AA467197	Expressed sequence AA467197	8.93	1.05	
1437139_at	14654	Glra1	Glycine receptor, alpha 1 subunit	8.54	1.27	Transport
1449982_at	16156	Il11	Interleukin-11	7.92	1.24	Cytokine
1419100_at	20716	Serpina3n	Serine (or cysteine) peptidase inhibitor, clade A, member 3N	7.20	1.94	ECM
1421811_at	21825	Thbs1	Thrombospondin 1	6.96	1.29	ECM
1416342_at	21923	Tnc	Tenascin C	6.41	1.31	ECM
1422053_at	16323	Inhba	Inhibin beta-A	6.35	1.02	Inflammation
1448831_at	11601	Angpt2	Angiopoietin 2	6.15	1.69	Peptide
1437279_x_at	20969	Sdc1	syndecan 1	5.78	1.67	ECM
1450716_at	11504	Adamts1	A disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 1	5.71	1.13	ECM
1416529_at	13730	Emp1	Epithelial membrane protein 1	5.66	1.20	Membrane
1447839_x_at	11535	Adm	Adrenomedullin	5.23	1.33	Peptide
1416039_x_at	16007	Cyr61	Cysteine-rich protein 61	5.18	1.02	ECM
1421207_at	16878	Lif	Leukemia inhibitory factor	5.03	1.30	Cytokine
1448471_a_at	13024	Ctla2a	Cytotoxic T-lymphocyte-associated protein 2 alpha	5.01	1.59	Inflammation

TBI, traumatic brain injury; Ipsi, ipsilateral; Norm, normal; Cont, contralateral; ECM extracellular matrix.

of PTX3.[69] Similarly, PTX3 is reported to have a protective function in seizure-induced neurodegeneration.[70] Both interleukin (IL)-11 and leukemia inhibitory factor (LIF) are members of the Il-6 family of cytokines, and both are neuropoietic cytokines with trophic effects on subsets of neurons, with beneficial effects for tissue recovery post-injury.[71–74] IL-11 also shows thrombopoietic activity and cytoprotective effects.[75–77] It was reported that plasma IL-11 levels in spontaneous intracerebral hemorrhage (ICH) patients are highly associated with mortality and hydrocephalus occurring after ICH.[78] LIF is also a key regulator for stem cell proliferation and differentiation, such as for astrocytes, oligodendrocyte progenitor cells, and neural stem cells, in addition to endothelial cells.[79–81] Inhba is a subunit to several cytokines, including homodimer of Activin A, and heterodimer of Activin AB or Inhibin A, all belong to the transforming growth factor beta (TGF-β) superfamily, but Activins and Inhibins are functionally antagonists. Activin A is reported to be released early in acute systemic inflammation, increased in many disease conditions,[82] and strongly expressed during the repairing process of various tissues and organs, including the cardiovascular system and brain.[83] Activin A also exerts antiproliferation and anti-angiogenesis effects, but with neuroprotective effect.[84] It mediates the best documented neuroprotective growth factor, FGF2-induced neuroprotection.[85] Taken together, many of these up-regulated genes may represent enhancement of the inflammatory status in the TBI vasculome. However, there may be hints of pathway specificity, and not all responses were uniformly up-regulated. Some interesting signals were decreased. For example, chemokine (C-X-C motif) ligand 12 (Cxcl12; also known as SDF-1 [stromal cell-derived factor-1]), a chemoattractant for immune cells in response, regulates leukocyte recruitment and their transendothelial migration.[86,87] Vascular cell adhesion molecule (VCAM-1), an adhesion molecule, also functions as a scaffold for leukocyte (and other immune cells) migration and a trigger of endothelial signaling through nicotinamide adenine dinucleotide phosphate oxidase–generated reactive oxygen species.[88] Both Cxcl12 and VCAM-1 appeared to be reduced in nondirectly impacted microvessels, perhaps suggesting an endogenous response to help to reduce the leukocyte invasion around this ''histologically normal'' area. It is well accepted that neuroinflammation with TBI has both detrimental and beneficial effects,[89,90] and our vasculome responses may be consistent with this general principle as well.

A key function of cerebral endothelium is the regulation of permeability and hemodynamics. No significant changes were detected for the primary tight junction and adherens junction proteins, consistent with the fact that no explicit BBB damage occurred in the nondirectly damaged cortex. However, the TBI vasculome showed interesting perturbations in some permeability-associated genes. The membrane proteins, integral membrane protein 2A and epithelial membrane protein 1 (EMP1), have been reported to have specific expression in brain microvascular endothelial cells, compared to aortic endothelial cells, suggesting that they might be novel contributors to BBB function. EMP1 was further shown to be colocalized with occludin and this might facilitate its ability to coregulate BBB function under injury conditions.[91] For hemodynamic status, a larger list of genes was detected, including adrenomedullin (Adm) and endothelin 3 (Edn3), two autocrine peptides

Table 3. List of Most Decreased Genes in Mouse Brain TBI Vasculome Compared to Normal Brain Vasculome

Probe ID	Gene ID	Symbol	Description	Fold change		Function group
				Ipsi/norm	Cont/norm	
1456084_x_at	14264	Fmod	Fibromodulin	0.082	1.14	ECM
1450468_at	17926	Myoc	Myocilin	0.092	0.85	Membrane
1429286_at	68888	Gkn3	Gastrokine 3	0.16	0.96	ECM
1419663_at	18295	Ogn	Osteoglycin	0.16	1.00	ECM
1418979_at	105387	Akr1c14	Aldo-keto reductase family 1, member C14	0.16	1.10	Metabolism
1434734_at	623474	Rad54b	RAD54 homolog B (S.cerevisiae)	0.22	0.79	Cell response
1451006_at	22436	Xdh	Xanthine dehydrogenase	0.24	1.00	Metabolism
1423858_a_at	15360	Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	0.25	0.85	Metabolism
1416966_at	19879	Slc22a8	Solute carrier family 22 (organic anion transporter), member 8	0.25	1.28	Transport
1417574_at	20315	Cxcl12	Chemokine (C-X-C motif) ligand 12	0.25	1.07	Cytokine
1451322_at	69574	Cmbl	Carboxymethylenebutenolidase-like (*Pseudomonas*)	0.26	1.25	Metabolism
1415989_at	22329	Vcam1	Vascular cell adhesion molecule 1	0.26	0.68	Inflammation
1427345_a_at	20887	Sult1a1	Sulfotransferase family 1A, phenol-preferring, member 1	0.26	1.44	Metabolism
1426215_at	13195	Ddc	Dopa decarboxylase	0.26	1.52	Metabolism
1450699_at	20341	Selenbp1	Selenium binding protein 1	0.26	1.17	Transport
1438696_at	13616	Edn3	Endothelin 3	0.27	1.02	Active peptide
1457867_at	433323	Sgpp2	Sphingosine-1-phosphate phosphotase 2	0.27	1.02	Metabolism
1444139_at	73284	Ddit4l	DNA-damage-inducible transcript 4-like	0.28	0.82	Cell response
1422804_at	20708	Serpinb6b	Serine (or cysteine) peptidase inhibitor, clade B, member 6b	0.29	0.98	Cytosolic inhibitor
1451047_at	16431	Itm2a	Integral membrane protein 2A	0.29	1.18	Membrane
1439790_at	20723	Serpinb9	Serine (or cysteine) peptidase inhibitor, clade B, member 9	0.29	1.00	Cytosolic inhibitor
1440096_at	407800	Ecm2	Extracellular matrix protein 2	0.29	0.80	ECM
1416321_s_at	116847	Prelp	Proline arginine-rich end leucine-rich repeat	0.29	1.14	ECM

TBI, traumatic brain injury; Ipsi, ipsilateral; Norm, normal; Cont, contralateral; ECM extracellular matrix.

released from endothelial cells. Adm is a potent hypotensive hormone peptide, with high concentration in the cerebral circulation.[92] The vasculoprotective effect of Adm may involve vasodilation effects, reduction of oxidative stress, inhibition of endothelial cell apoptosis, promotion of angiogenesis, regulation of permeability, and control of fluid and electrolyte homeostasis.[92–96] Adm is also implicated as a promising plasma biomarker, so its presence in the TBI vasculome may warrant further investigation. Administration of exogenous Adm and its binding protein has shown neuroprotective effects against brain injury in experimental disease models.[97,98] Edn3, a vasoconstrictor from the endothelin family, is known to induce NO production, promote angiogenesis, regulate synthesis of inflammatory mediators and glutamate transporters, and control fluid and electrolyte homeostasis in the brain.[99–104] This broad spectrum of vascular responses again suggests a potentially wide distribution of microvessel reactions to injury in the entire ipsilateral cortex after TBI.

Pathway analysis

Although statistically probing the TBI vasculome for individually altered genes is a logical first step, another complementary way to assess changes over entire transcriptome is via pathway enrichment analysis by GSEA. Using GSEA analysis based on KEGG and GO terms, many statistically enriched pathways were found in nondirectly damaged cortex (Table 4). These clusters of gene responses included increased pathways of ECM, matrix peptidases and inhibitors, and cell communication, cytokine-receptor interactions, and inflammatory responses. Reduced pathways included those comprising mitochondrial function, oxidative phosphorylation, fatty acid metabolism, glutathione metabolism, gap junctions, and transport processes. Specific signaling pathways were also identified, including TGF-β and Wnt, both of which have important roles in the neurovascular unit, such as BBB permeability, angiogenesis, and neurogenesis.

Increased terms belonging to the cytokine signaling categories are consistent with known effects of TBI on neuroinflammation.[90,105,106] Reductions in mitochondria activity and oxidative phosphorylation are consistent with known detrimental effects of TBI on energy metabolism.[107,108] The present findings suggest that inflammatory and metabolic responses in endothelial cells may contribute to vascular dysfunction after TBI. Further, the reduced transport, tight junction, and gap junction categories all suggest a compromised BBB homeostasis in nondirectly damaged areas. Overall, GSEA analysis revealed alterations in pathways that correlate well with the single-gene up-/down-regulation analyses described earlier. Taken together, these mean that the deleterious vascular effects of TBI might be surprisingly diffuse, beyond core regions of directly impacted cortex.

Overlap with genome-wide association study genes

It has long been suggested that past TBI increases the subsequent incidence of chronic neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis.[109,110] Previously, we reported that the mouse brain

Table 4. Enriched Pathways in Mouse Brain TBI Vasculome Compared to Normal Brain Vasculome

Ipsi/norm			*Ipsi/norm*		
p *value*	*ES*	*Pathway (from KEGG)*	p *value*	*ES*	*Pathway (GO term)*
1.67E-12	–0.25	Oxidative phosphorylation	0.00E+00	–0.10	Integral to membrane
1.96E-08	–0.16	Wnt-signaling pathway	1.55E-15	–0.13	Mitochondrion
9.32E-08	–0.32	Valine, leucine, and isoleucine degradation	2.76E-07	–0.08	Transport
5.75E-07	–0.15	Axon guidance	4.03E-07	–0.07	Zinc ion binding
3.95E-06	–0.29	Fatty acid metabolism	1.64E-06	–0.28	Electron transport chain
4.62E-06	–0.33	Propanoate metabolism	3.76E-06	–0.12	Modification-dependent protein catabolic
1.04E-05	–0.13	Calcium-signaling pathway	6.44E-04	–0.04	Protein binding
1.42E-05	–0.17	Gap junction	1.20E-03	–0.48	Glutathione metabolic process
1.17E-04	–0.09	MAPK-signaling pathway	4.59E-03	–0.07	Metabolic process
9.34E-04	–0.10	Ubiquitin-mediated proteolysis			
2.28E-03	–0.13	TGF-β-signaling pathway	2.25E-10	0.10	Extracellular region
2.64E-03	–0.25	Glutathione metabolism	7.82E-08	0.19	Proteinaceous extracellular matrix
2.86E-03	–0.44	Thiamine metabolism	3.40E-06	0.21	Cytokine activity
3.68E-03	–0.29	beta-Alanine metabolism	7.51E-06	0.23	Inflammatory response
			1.30E-04	0.19	Serine-type endopeptidase activity
1.41E-05	0.17	Cell communication	1.60E-04	0.25	Chemotaxis
3.33E-03	0.09	Cytokine-cytokine receptor interaction	5.97E-04	0.21	Peptidase inhibitor activity

Analysis here is based on vasculomes from nondirectly damaged ipsilateral 24 h post-TBI and normal brains, based on KEGG pathway database or GO database. Negative ES (enrichment score) for decreased genes expression of such pathways; positive ES for increased genes expression of such pathways in TBI vasculome compared to normal brain.

TBI, traumatic brain injury; Ipsi, ipsilateral; Norm, normal; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; MAPK, mitogen-activated protein kinase; TGF, transforming growth factor.

vasculome contained many genes that are found in genome-wide association study (GWAS) datasets for human central nervous system (CNS) disorders, such as stroke, AD, and PD.[111] Is it possible that the TBI vasculome may also involve GWAS genes that would contribute to the vascular pathophysiology of brain injury? To assess this hypothesis, we compared our first draft of the TBI vacsulome with the three GWAS datasets. At 24 h post-trauma, differentially expressed genes in nondirectly damaged microvessels were significantly associated with stroke GWAS genes (p value of 8.25E-05 and odds ratio of 2.50). Similar linkages were detected for GWAS datasets in PD and AD as well. The list of significantly differentially expressed genes in the TBI vasculome overlapped with these disease GWAS databases are shown in Table 5.

Although the links between the TBI vasculome and GWAS data sets are intriguing, specific mechanisms remain to be defined. Nevertheless, a few genes may possess signaling roles that potentially underlie TBI pathophysiology. For example, stroke GWAS gene Cd44 is one with a significant increase after trauma. As an adhesion molecule as well as a signaling regulator, Cd44 may be involved in endothelial cell recognition, lymphocyte trafficking, actin cytoskeleton organization, angiogenesis, and vascular integrity.[51,112–114] Cd44 is also known to increase in the brain after ischemia,[115] and CD44 knockout mice showed reduced ischemic infarct area with improved neurological functions compared to that of wild-type mice.[116] Whether and how CD44 underlies vascular pathology after TBI should be rigorously explored.

Stroke GWAS gene suppressor of cytokine signaling 3 (SOCS3) is an inducible negative regulator of signaling by the Janus kinase/signal transduction and activator of transcription 3 pathway, and it is activated in many CNS injury conditions,[117–119] as part of an endogenous response to regulate endothelial cell death,[120,121] pathological angiogenesis, and inflammation response.[122–125] Together with CD93 and Apelin, SOCS3 is down-regulated in the brain after water deprivation.[126] At the same time, SOCS3 can also act as an intrinsic inhibitor for neural axon regeneration. Hence, crosstalk between the vasculome and neural plasticity remains a possibility.

Prostate transmembrane protein, androgen-induced 1 (PMEPA1) and CD93 are related with both stroke and PD. There are a few reports demonstrating that PMEPA1 can bind with E3 ubiquitin-protein ligase NEDD4 (neural precursor cell expressed developmentally down-regulated protein 4) to negatively regulate cell growth.[127] PMEPA1 is also a direct target gene of TGF-β signaling and participates in a negative feedback loop to control the duration and intensity of TGF-β/SMDH (mothers of decapentaplegic homologue) signaling.[128] Given that TGF-β signaling has been identified as a major pathway in the TBI vasculome, this linkage with GWAS genes further suggests that it may play a role in the pathophysiology of widespread vascular dysfunction in noninjured tissue.

CD93 is a membrane-associated glycoprotein, expressed in many types of cells, regulates phagocytosis of apoptotic cells during organ development, tissue repair, and maintenance of tissue homeostasis, and also regulates leukocyte migration and endothelial cell adhesion.[129] Recently, it is reported that as a novel regulator of inflammation, CD93 was highly induced in mouse brain after focal cerebral ischemia, especially in endothelial cells and selected macrophages and microglia.[130] CD93 knockout mice showed increased leukocyte infiltration into the brain, increased edema, and larger infarct volumes after ischemia and reperfusion, suggesting the potential neuroprotection from CD93.[130] Although human CD93, an epidermal growth factor (EGF)-like domain-containing transmembrane protein, is predominantly expressed in the vascular endothelium; soluble CD93 (with an EGF-like domain) has been detected in plasma.[131] Membrane-bound CD93 is proteolytically cleaved to soluble form in response to inflammatory signals, and soluble CD93 released from endothelium is a novel angiogenic factor.[131,132] The level of soluble CD93 in plasma has been proposed as a novel biomarker for coronary artery disease and myocardial infarction.[133] Whether soluble CD93

Table 5. List of Disease GWAS Genes Significantly Changed in TBI Vasculome

Gene ID	*Symbol*	*Related disease*	*Ipsi/norm*	*Description*
76459	Car12	Stroke	+	Carbonic anyhydrase 12
12505	Cd44	Stroke	+	CD44 antigen
17064	Cd93	Stroke, PD	+	CD93 antigen
14654	Glra1	Stroke	+	Glycine receptor, alpha 1 subunit
195646	Hs3st2	Stroke	+	Heparan sulfate (glucosamine) 3-*O*-sulfotransferase 2
18793	Plaur	Stroke	+	Plasminogen activator, urokinase receptor
65112	Pmepa1	Stroke, PD	+	Prostate transmembrane protein, androgen induced 1
277360	Prex1	Stroke	+	Phosphatidylinositol-3,4,5-trisphosphate–dependent Rac exchange factor 1
12702	Socs3	Stroke	+	Suppressor of cytokine signaling 3
72475	Ssbp3	Stroke	+	Single-stranded DNA-binding protein 3
217262	Abca9	Stroke	–	ATP-binding cassette, subfamily A (ABC1), member 9
330941	AI593442	Stroke	–	Expressed sequence AI593442
11982	Atp10a	Stroke	–	ATPase, class V, type 10A
320405	Cadps2	Stroke	–	Ca^{2+}-dependent activator protein for secretion 2
13176	Dcc	Stroke	–	Deleted in colorectal carcinoma
269109	Dpp10	Stroke	–	Dipeptidylpeptidase 10
74559	Elovl7	Stroke	–	ELOVL family member 7, elongation of long chain fatty acids (yeast)
217082	Hlf	Stroke	–	Hepatic leukemia factor
209378	Itih5	Stroke	–	Inter-alpha (globulin) inhibitor H5
243382	Ppm1k	Stroke	–	Protein phosphatase 1K (PP2C domain containing)
227545	Proser2	Stroke	–	Proline and serine rich 2
11514	Adcy8	AD	+	Adenylate cyclase 8
67801	Pllp	AD	+	Plasma membrane proteolipid
12826	Col4a1	PD	+	Collagen, type IV, alpha 1
15900	Irf8	PD	+	Interferon-regulatory factor 8
11994	Pcdh15	PD	+	Protocadherin 15
219257	Pcdh20	PD	–	Protocadherin 20
19279	Ptprr	PD	–	Protein tyrosine phosphatase, receptor type, R

The significantly changed gene list in mouse TBI vasculome is searched for disease GWAS gene, including stroke, AD, and PD. Plus sign (+) indicates significantly increased gene; minus sign (–) indicates significantly decreased gene in mouse TBI vasculome compared to normal brain.

GWAS, genome-wide association studies; TBI, traumatic brain injury; Ipsi, ipsilateral; Norm, normal; PD, Parkinson's disease; AD, Alzheimer's disease; ATP, adenosine triphosphate.

released from the TBI vasculome may also act as a biomarker remains to be investigated.

Discussion

The vasculome may provide a potentially useful conceptual framework for investigating how the vascular phenomenon may contribute to the pathophysiology of CNS injury and disease. Here, we mapped the initial draft of the TBI vasculome in a mouse model of CCI. Overall, we found that many genes and pathways were perturbed in ipsilateral cortex, even in remote areas far from the core lesion site.

In our study, the number of down-regulated genes was larger than up-regulated genes. This stands in contrast to previous TBI studies, where increased gene expression was more commonly reported. Von Gertten and colleagues used a cDNA microarray of 6200 gene probes to study expression changes induced by CCI in rat ipsilateral cortex, specifically within the impact area and surrounding cortex.[134] This study identified 150 genes that were significantly increased and 61 genes that were down-regulated compared to contralateral cortex at 24 h post-injury. In another relevant study, Natale and colleagues assessed gene expression in a rat model of fluid percussion and in mice with cortical impact injury, with measurements obtained within a 4-mm-diameter disk of parieto-occipital cortex containing core damaged tissue and surrounding contusion areas at 24 h post-injury.[135] In this study, 64 up-regulated genes and only 10 down-regulated genes were found in rat ipsilateral cortex after fluid percussion, and 207 up-regulated genes and 76 down-regulated genes in mouse ipsilateral cortex after cortical impact. Though the precise numbers may be without biological significance, the predominance of up- or down-regulation in gene expression may suggest different states of responses in the perturbed brain. There may be two reasons why our data differ. First, almost all previous studies examined total gene expression in macroscopically dissected brain. For a heterogenous multi-cell organ such as the brain, this aggregate response may be misleading. Because our TBI vasculome should be cell specific for cerebral endothelium in microvessels, the predominance of down-regulated genes may tell us something about the state of microvessels and vascular homeostasis in perturbed brain. A second potential issue may be related to the location of analysis. Previous studies tended to emphasize lesion cores and perilesional areas, whereas our study mapped the vasculome in the entire nondirectly damaged ipsilateral cortex. But at least one previous study attempted to examine TBI responses in nondamaged areas. Truettner and colleagues measured the expression of heat shock protein 70, brain-derived neurotrophic factor (BDNF), and nerve growth factor in rat brain after moderate fluid percussion injury. All three genes were rapidly up-regulated post-injury, even in cortex far from areas with histopathological damage. This study concluded that these gene expression patterns may be related with neuronal excitation and plasticity.[136] Our TBI vasculome suggests that hemispheric changes may be more common than previously thought, and the broad spectrum of down-regulated genes may suggest a widespread suppression of normal

function in all microvessels. These findings may be consistent with the emerging idea of diffuse responses in the brain post-TBI. Axonal perturbations in white matter are now thought to underline overall alternations in neuronal connectivity post-TBI.[137,138] The same may be true in terms of diffuse perturbations in vascular homeostasis. Altogether, the entire neurovascular unit may be affected throughout the hemisphere, and investigations into these network responses should be warranted.[139]

A major overall feature of the TBI vasculome may relate to the convergence of individual gene analysis and pathway analysis. Overall, genes and pathways related to matrix, cytokines, and inflammation were up-regulated, whereas those related to metabolism, transport, and barrier/hemodynamic function tended to be down-regulated. The decrease of metabolic processes in microvessels from nondirectly damaged cortex involved a wide array of effects, including reduced mitochondrion-related functions, oxidative phosphorylation for energy supply, and glutathione metabolic process. There also appeared to be a general reduction in tight junction and gap junction regulation, as well as transport systems at the neurovascular interface. At the same time, these ''remote'' microvessels showed an amplified response to trauma, including augmented inflammation response, cytokine activity, and receptor activities. Furthermore, many alterations were detected in ECM categories, all of which may influence cell-cell communication within the entire neurovascular unit. Taken together, these pathway findings suggest that microvessels from nondirectly damaged cortex are not quiescent post-injury, and widespread perturbations in vascular homeostasis may underlie an important mechanism throughout the TBI hemisphere.

The concept of the neurovascular unit emphasizes that cellular responses cannot occur in isolation and crosstalk between all neural, glial, and vascular compartments may be unavoidable. The same may apply to the TBI vasculome. Although, by definition, the vasculome is centered on vascular phenomena, many of our detected signals may connect with other cell types. For example, changes were observed in genes from the semaphorin family. Sema4A is an angiogenesis inhibitor, but it can also affect axon guidance. Fmod and Prelp (from a class II small leucine-rich repeat proteoglycans family) and TnC comprise important matricellular proteins in the neurovascular matrix, but all three are reported to regulate the environmental niche of stem cells, suggesting that they could promote angiogenesis as well as neurogenesis during tissue remodeling post-TBI.[140–143] Et-3 (Edn3) and its receptor, EDNRB, are found in endothelial systems, but may also regulate proliferation, differentiation, and migration of neural crest cells during development.[144] How the vasculome affects endogenous responses in other cell types post-TBI may be an interesting future direction.

Although our individual gene and pathway analyses revealed many cascades and potential targets, it is not easy to functionally interpret how the entire gene network is balanced between deleterious versus beneficial responses. For example, increases in THBS-1 and MMP-3, the decrease in the anti-inflammation gene Cxcl12, and the overall decrease of glutathione metabolism and oxidative phosphorylation may all point toward vascular dysfunction. In contrast, there was also up-regulation in protection signals, such as the anti-inflammatory IL-6 family (IL-6, IL-11, and LIF), and an elevation of TIMP-1 and growth factors, such as BDNF, VEGF, osteoglycin, and platelet-derived growth factor D. The same is true for some matrix-related signals, including tPA/PAI-1/MMP3. How the various ECM components and proteases coalesce to regulate neurovascular proteolysis versus homeostasis, angiogenesis versus edema, and other dynamic phenomena will have to be rigorously examined in future studies.

Taken together, our initial findings suggest that the TBI vasculome is broadly perturbed even in histological normal nondirectly damaged areas. However, there are a few caveats to keep in mind for this proof-of-concept study. First, for the sake of signal to noise, we mapped the vasculome in the entire cortex. It remains possible that even away from the core or perilesional rim, there may still exist gradients in vascular response. Further efforts to map the vasculome with more spatial precision, perhaps with laser-capture methods, may be interesting, especially for the purposes of distinguishing critical responses in the pericontusional rim from more distal profiles in the entire ipsilateral vasculome. Second, we only focused on acute 24-h post-TBI data in this proof-of-concept study. How the vasculome evolves over time may provide new insights into the progressive pathophysiology as tissue deteriorate and recovers. More detailed investigations are required to map how the TBI vasculome evolves from days to weeks post-trauma. Third, our technical approach concentrated on microvessels because these comprised the vast majority of vessels in the brain. However, changes in larger vessels may also be important, especially in terms of collateral supplies and vasculogenesis during recovery. How the vasculome differs at different levels of the vascular tree remains unknown. Fourth, beyond endothelial cells, multiple cell types also contribute to the neurovascular unit in terms of dysfunction and endogenous repair. For example, microglia cells are major participants in TBI response; mapping the microgliome and other cellular responses should also be considered. Finally, the TBI vasculome is expressed at many levels. We started by mapping the transcriptome, but whether gene patterns truly match protein patterns remains unknown. Our transcriptome findings may be quite relevant because recent studies now estimate that transcription can account for up to 80% of protein variance.[145–147] Nevertheless, future efforts to study the proteomic and metabolomic profiles of the TBI vasculome may also be warranted.

TBI is a highly complex and challenging problem. Increasingly, it is recognized that vascular responses may play an essential role in its pathophysiology.[148] In this proof-of-concept study, we mapped the 24-h post-TBI vasculome in a mouse model of CCI and showed that widespread microvessel perturbations are present even in nondirectly damaged areas. Further investigations into the vasculome may reveal new opportunities for pursuing targets and biomarkers for TBI.

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References

1. Centers for Disease Control and Prevention. (2013). CDC grand rounds: reducing severe traumatic brain injury in the United States. MMWR Morb. Mortal. Wkly. Rep. 62, 549–552.
2. Narayan, R.K., Michel, M.E., Ansell, B., Baethmann, A., Biegon, A., Bracken, M.B., Bullock, M.R., Choi, S.C., Clifton, G.L., Contant, C.F., Coplin, W.M., Dietrich, W.D., Ghajar, J., Grady, S.M., Grossman, R.G., Hall, E.D., Heetderks, W., Hovda, D.A., Jallo, J.,

Katz, R.L., Knoller, N., Kochanek, P.M., Maas, A.I., Majde, J., Marion, D.W., Marmarou, A., Marshall, L.F., McIntosh, T.K., Miller, E., Mohberg, N., Muizelaar, J.P., Pitts, L.H., Quinn, P., Riesenfeld, G., Robertson, C.S., Strauss, K.I., Teasdale, G., Temkin, N., Tuma, R., Wade, C., Walker, M.D., Weinrich, M., Whyte, J., Wilberger, J., Young, A.B., and Yurkewicz, L. (2002). Clinical trials in head injury. J. Neurotrauma 19, 503–557.

3. Kabadi, S.V., and Faden, A.I. (2014). Neuroprotective strategies for traumatic brain injury: improving clinical translation. Int. J. Mol. Sci. 15, 1216–1236.
4. Algattas, H., and Huang, J.H. (2014). Traumatic brain injury pathophysiology and treatments: early, intermediate, and late phases post-injury. Int. J. Mol. Sci. 15, 309–341.
5. Arai, K., Lok, J., Guo, S., Hayakawa, K., Xing, C., and Lo, E.H. (2011). Cellular mechanisms of neurovascular damage and repair after stroke. J. Child Neurol. 26, 1193–1198.
6. Lo, E.H., Dalkara, T., and Moskowitz, M.A. (2003). Mechanisms, challenges and opportunities in stroke. Nat. Rev. Neurosci. 4, 399–415.
7. Lecrux, C., and Hamel, E. (2011). The neurovascular unit in brain function and disease. Acta Physiol. (Oxf.) 203, 47–59.
8. Iadecola, C. (2010). The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia. Acta Neuropathol. 120, 287–296.
9. Zlokovic, B.V. (2011). Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat. Rev. Neurosci. 12, 723–738.
10. Zacchigna, S., Lambrechts, D., and Carmeliet, P. (2008). Neurovascular signalling defects in neurodegeneration. Nat. Rev. Neurosci. 9, 169–181.
11. Zacchigna, S., Ruiz de Almodovar, C., and Carmeliet, P. (2008). Similarities between angiogenesis and neural development: what small animal models can tell us. Curr. Top. Dev. Biol. 80, 1–55.
12. Shen, Q., Goderie, S.K., Jin, L., Karanth, N., Sun, Y., Abramova, N., Vincent, P., Pumiglia, K., and Temple, S. (2004). Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. Science 304, 1338–1340.
13. Dugas, J.C., Mandemakers, W., Rogers, M., Ibrahim, A., Daneman, R., and Barres, B.A. (2008). A novel purification method for CNS projection neurons leads to the identification of brain vascular cells as a source of trophic support for corticospinal motor neurons. J. Neurosci. 28, 8294–8305.
14. Guo, S., Kim, W.J., Lok, J., Lee, S.R., Besancon, E., Luo, B.H., Stins, M.F., Wang, X., Dedhar, S., and Lo, E.H. (2008). Neuroprotection via matrix-trophic coupling between cerebral endothelial cells and neurons. Proc. Natl. Acad. Sci. U. S. A. 105, 7582–7587.
15. Arai, K., and Lo, E.H. (2009). An oligovascular niche: cerebral endothelial cells promote the survival and proliferation of oligodendrocyte precursor cells. J. Neurosci. 29, 4351–4355.
16. Pober, J.S., and Sessa, W.C. (2007). Evolving functions of endothelial cells in inflammation. Nat. Rev. Immunol. 7, 803–815.
17. Rossi, B., Angiari, S., Zenaro, E., Budui, S.L., and Constantin, G. (2011). Vascular inflammation in central nervous system diseases: adhesion receptors controlling leukocyte-endothelial interactions. J. Leukoc. Biol. 89, 539–556.
18. Pober, J.S., Min, W., and Bradley, J.R. (2009). Mechanisms of endothelial dysfunction, injury, and death. Annu. Rev. Pathol. 4, 71–95.
19. van Hinsbergh, V.W. (2012). Endothelium—role in regulation of coagulation and inflammation. Semin. Immunopathol. 34, 93–106.
20. Wallez, Y., and Huber, P. (2008). Endothelial adherens and tight junctions in vascular homeostasis, inflammation and angiogenesis. Biochim. Biophys. Acta 1778, 794–809.
21. Schouten, M., Wiersinga, W.J., Levi, M., and van der Poll, T. (2008). Inflammation, endothelium, and coagulation in sepsis. J. Leukoc. Biol. 83, 536–545.
22. Rosenberg, G.A. (2012). Neurological diseases in relation to the blood-brain barrier. J. Cereb. Blood Flow Metab. 32, 1139–1151.
23. Ayata, C., and Ropper, A.H. (2002). Ischaemic brain oedema. J. Clin. Neurosci. 9, 113–124.
24. Ning, M., Sarracino, D.A., Kho, A.T., Guo, S., Lee, S.R., Krastins, B., Buonanno, F.S., Vizcaino, J.A., Orchard, S., McMullin, D., Wang, X., and Lo, E.H. (2011). Proteomic temporal profile of human brain endothelium after oxidative stress. Stroke 42, 37–43.
25. Mori, T., Wang, X., Aoki, T., and Lo, E.H. (2002). Downregulation of matrix metalloproteinase-9 and attenuation of edema via inhibition of ERK mitogen activated protein kinase in traumatic brain injury. J. Neurotrauma 19, 1411–1419.
26. Song, L., and Pachter, J.S. (2003). Culture of murine brain microvascular endothelial cells that maintain expression and cytoskeletal association of tight junction-associated proteins. In Vitro Cell. Dev. Biol. Anim. 39, 313–320.
27. Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., and Mesirov, J.P. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. U. S. A. 102, 15545–15550.
28. Kanehisa, M., and Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 28, 27–30.
29. Harris, M.A., Clark, J., Ireland, A., Lomax, J., Ashburner, M., Foulger, R., Eilbeck, K., Lewis, S., Marshall, B., Mungall, C., Richter, J., Rubin, G.M., Blake, J.A., Bult, C., Dolan, M., Drabkin, H., Eppig, J.T., Hill, D.P., Ni, L., Ringwald, M., Balakrishnan, R., Cherry, J.M., Christie, K.R., Costanzo, M.C., Dwight, S.S., Engel, S., Fisk, D.G., Hirschman, J.E., Hong, E.L., Nash, R.S., Sethuraman, A., Theesfeld, C.L., Botstein, D., Dolinski, K., Feierbach, B., Berardini, T., Mundodi, S., Rhee, S.Y., Apweiler, R., Barrell, D., Camon, E., Dimmer, E., Lee, V., Chisholm, R., Gaudet, P., Kibbe, W., Kishore, R., Schwarz, E.M., Sternberg, P., Gwinn, M., Hannick, L., Wortman, J., Berriman, M., Wood, V., de la Cruz, N., Tonellato, P., Jaiswal, P., Seigfried, T., and White, R. (2004). The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res. 32, D258–D261.
30. Suzuki, Y., Nagai, N., Yamakawa, K., Kawakami, J., Lijnen, H.R., and Umemura, K. (2009). Tissue-type plasminogen activator (t-PA) induces stromelysin-1 (MMP-3) in endothelial cells through activation of lipoprotein receptor-related protein. Blood 114, 3352–3358.
31. Rosenberg, G.A., and Yang, Y. (2007). Vasogenic edema due to tight junction disruption by matrix metalloproteinases in cerebral ischemia. Neurosurg. Focus 22, E4.
32. Dohgu, S., Takata, F., Matsumoto, J., Oda, M., Harada, E., Watanabe, T., Nishioku, T., Shuto, H., Yamauchi, A., and Kataoka, Y. (2011). Autocrine and paracrine up-regulation of blood-brain barrier function by plasminogen activator inhibitor-1. Microvasc. Res. 81, 103–107.
33. Avraham, H.K., Jiang, S., Fu, Y., Nakshatri, H., Ovadia, H., and Avraham, S. (2014). Angiopoietin-2 mediates blood-brain barrier impairment and colonization of triple-negative breast cancer cells in brain. J. Pathol. 232, 369–381.
34. Chittiboina, P., Ganta, V., Monceaux, C.P., Scott, L.K., Nanda, A., and Alexander, J.S. (2013). Angiopoietins as promising biomarkers and potential therapeutic targets in brain injury. Pathophysiology 20, 15–21.
35. Fujimoto, M., Takagi, Y., Aoki, T., Hayase, M., Marumo, T., Gomi, M., Nishimura, M., Kataoka, H., Hashimoto, N., and Nozaki, K. (2008). Tissue inhibitor of metalloproteinases protect blood-brain barrier disruption in focal cerebral ischemia. J. Cereb. Blood Flow Metab. 28, 1674–1685.
36. Haass, C., Kaether, C., Thinakaran, G., and Sisodia, S. (2012). Trafficking and proteolytic processing of APP. Cold Spring Harb. Perspect. Med. 2, a006270.
37. Zhang, X., Kazerounian, S., Duquette, M., Perruzzi, C., Nagy, J.A., Dvorak, H.F., Parangi, S., and Lawler, J. (2009). Thrombospondin-1 modulates vascular endothelial growth factor activity at the receptor level. FASEB J. 23, 3368–3376.
38. Iruela-Arispe, M.L., Carpizo, D., and Luque, A. (2003). ADAMTS1: a matrix metalloprotease with angioinhibitory properties. Ann. N. Y. Acad. Sci. 995, 183–190.
39. Ivanciu, L., Gerard, R.D., Tang, H., Lupu, F., and Lupu, C. (2007). Adenovirus-mediated expression of tissue factor pathway inhibitor-2 inhibits endothelial cell migration and angiogenesis. Arterioscler. Thromb. Vasc. Biol. 27, 310–316.
40. Ebrahimian, T.G., Squiban, C., Roque, T., Lugo-Martinez, H., Hneino, M., Buard, V., Gourmelon, P., Benderitter, M., Milliat, F., and Tamarat, R. (2012). Plasminogen activator inhibitor-1 controls bone marrow-derived cells therapeutic effect through MMP9 signaling: role in physiological and pathological wound healing. Stem Cells 30, 1436–1446.
41. Teng, H., Chopp, M., Hozeska-Solgot, A., Shen, L., Lu, M., Tang, C., and Zhang, Z.G. (2012). Tissue plasminogen activator and plasminogen activator inhibitor 1 contribute to sonic hedgehog-induced in vitro cerebral angiogenesis. PLoS One 7, e33444.

42. Harris, L.G., Pannell, L.K., Singh, S., Samant, R.S., and Shevde, L.A. (2012). Increased vascularity and spontaneous metastasis of breast cancer by hedgehog signaling mediated upregulation of cyr61. Oncogene 31, 3370–3380.
43. Hilfiker-Kleiner, D., Kaminski, K., Kaminska, A., Fuchs, M., Klein, G., Podewski, E., Grote, K., Kiian, I., Wollert, K.C., Hilfiker, A., and Drexler, H. (2004). Regulation of proangiogenic factor CCN1 in cardiac muscle: impact of ischemia, pressure overload, and neurohumoral activation. Circulation 109, 2227–2233.
44. Mo, F.E., Muntean, A.G., Chen, C.C., Stolz, D.B., Watkins, S.C., and Lau, L.F. (2002). CYR61 (CCN1) is essential for placental development and vascular integrity. Mol Cell Biol 22, 8709–8720.
45. Rapraeger, A.C., Ell, B.J., Roy, M., Li, X., Morrison, O.R., Thomas, G.M., and Beauvais, D.M. (2013). Vascular endothelial-cadherin stimulates syndecan-1-coupled insulin-like growth factor-1 receptor and cross-talk between alphaVbeta3 integrin and vascular endothelial growth factor receptor 2 at the onset of endothelial cell dissemination during angiogenesis. FEBS J. 280, 2194–2206.
46. Yuan, K., Hong, T.M., Chen, J.J., Tsai, W.H., and Lin, M.T. (2004). Syndecan-1 up-regulated by ephrinB2/EphB4 plays dual roles in inflammatory angiogenesis. Blood 104, 1025–1033.
47. Zhu, Y., Lee, C., Shen, F., Du, R., Young, W.L., and Yang, G.Y. (2005). Angiopoietin-2 facilitates vascular endothelial growth factor-induced angiogenesis in the mature mouse brain. Stroke 36, 1533–1537.
48. Mochizuki, Y., Nakamura, T., Kanetake, H., and Kanda, S. (2002). Angiopoietin 2 stimulates migration and tube-like structure formation of murine brain capillary endothelial cells through c-Fes and c-Fyn. J. Cell Sci. 115, 175–183.
49. Vates, G.E., Hashimoto, T., Young, W.L., and Lawton, M.T. (2005). Angiogenesis in the brain during development: the effects of vascular endothelial growth factor and angiopoietin-2 in an animal model. J. Neurosurg. 103, 136–145.
50. Zagzag, D., Hooper, A., Friedlander, D.R., Chan, W., Holash, J., Wiegand, S.J., Yancopoulos, G. D., and Grumet, M. (1999). In situ expression of angiopoietins in astrocytomas identifies angiopoietin-2 as an early marker of tumor angiogenesis. Exp. Neurol. 159, 391–400.
51. Rivera, C.G., Bader, J.S., and Popel, A.S. (2011). Angiogenesis-associated crosstalk between collagens, CXC chemokines, and thrombospondin domain-containing proteins. Ann. Biomed. Eng. 39, 2213–2222.
52. Duparc, T., Colom, A., Cani, P.D., Massaly, N., Rastrelli, S., Drougard, A., Le Gonidec, S., Mouledous, L., Frances, B., Leclercq, I., Llorens-Cortes, C., Pospisilik, J.A., Delzenne, N.M., Valet, P., Castan-Laurell, I., and Knauf, C. (2011). Central apelin controls glucose homeostasis via a nitric oxide-dependent pathway in mice. Antioxid. Redox Signal. 15, 1477–1496.
53. Yue, P., Jin, H., Aillaud, M., Deng, A.C., Azuma, J., Asagami, T., Kundu, R.K., Reaven, G.M., Quertermous, T., and Tsao, P.S. (2010). Apelin is necessary for the maintenance of insulin sensitivity. Am. J. Physiol. Endocrinol. Metab. 298, E59–E67.
54. Sawane, M., Kidoya, H., Muramatsu, F., Takakura, N., and Kajiya, K. (2011). Apelin attenuates UVB-induced edema and inflammation by promoting vessel function. Am. J. Pathol. 179, 2691–2697.
55. Kidoya, H., Naito, H., and Takakura, N. (2010). Apelin induces enlarged and nonleaky blood vessels for functional recovery from ischemia. Blood 115, 3166–3174.
56. Eyries, M., Siegfried, G., Ciumas, M., Montagne, K., Agrapart, M., Lebrin, F., and Soubrier, F. (2008). Hypoxia-induced apelin expression regulates endothelial cell proliferation and regenerative angiogenesis. Circ. Res. 103, 432–440.
57. Cox, C.M., D'Agostino, S.L., Miller, M.K., Heimark, R.L., and Krieg, P.A. (2006). Apelin, the ligand for the endothelial G-protein-coupled receptor, APJ, is a potent angiogenic factor required for normal vascular development of the frog embryo. Dev. Biol. 296, 177–189.
58. Sonmez, A., Celebi, G., Erdem, G., Tapan, S., Genc, H., Tasci, I., Ercin, C.N., Dogru, T., Kilic, S., Uckaya, G., Yilmaz, M.I., Erbil, M.K., and Kutlu, M. (2010). Plasma apelin and ADMA Levels in patients with essential hypertension. Clin. Exp. Hypertens. 32, 179–183.
59. Cook, D.R., Gleichman, A.J., Cross, S.A., Doshi, S., Ho, W., Jordan-Sciutto, K.L., Lynch, D.R., and Kolson, D.L. (2011). NMDA receptor modulation by the neuropeptide apelin: implications for excitotoxic injury. J. Neurochem. 118, 1113–1123.
60. Zeng, X.J., Yu, S.P., Zhang, L., and Wei, L. (2010). Neuroprotective effect of the endogenous neural peptide apelin in cultured mouse cortical neurons. Exp. Cell Res. 316, 1773–1783.
61. Wang, G., Qian, P., Jackson, F.R., Qian, G., and Wu, G. (2008). Sequential activation of JAKs, STATs and xanthine dehydrogenase/oxidase by hypoxia in lung microvascular endothelial cells. Int. J. Biochem. Cell Biol. 40, 461–470.
62. Schroder, K., Vecchione, C., Jung, O., Schreiber, J. G., Shiri-Sverdlov, R., van Gorp, P.J., Busse, R., and Brandes, R.P. (2006). Xanthine oxidase inhibitor tungsten prevents the development of atherosclerosis in ApoE knockout mice fed a Western-type diet. Free Radic. Biol. Med. 41, 1353–1360.
63. Meneshian, A., and Bulkley, G.B. (2002). The physiology of endothelial xanthine oxidase: from urate catabolism to reperfusion injury to inflammatory signal transduction. Microcirculation 9, 161–175.
64. Yokoyama, Y., Beckman, J.S., Beckman, T.K., Wheat, J.K., Cash, T.G., Freeman, B.A., and Parks, D.A. (1990). Circulating xanthine oxidase: potential mediator of ischemic injury. Am. J. Physiol. 258, G564–G570.
65. Cantu-Medellin, N., and Kelley, E.E. (2013). Xanthine oxidoreductase-catalyzed reactive species generation: A process in critical need of reevaluation. Redox Biol. 1, 353–358.
66. Ogawa, C., Kihara, A., Gokoh, M., and Igarashi, Y. (2003). Identification and characterization of a novel human sphingosine-1-phosphate phosphohydrolase, hSPP2. J. Biol. Chem. 278, 1268–1272.
67. Leali, D., Inforzato, A., Ronca, R., Bianchi, R., Belleri, M., Coltrini, D., Di Salle, E., Sironi, M., Norata, G.D., Bottazzi, B., Garlanda, C., Day, A.J., and Presta, M. (2012). Long pentraxin 3/tumor necrosis factor-stimulated gene-6 interaction: a biological rheostat for fibroblast growth factor 2-mediated angiogenesis. Arterioscler. Thromb. Vasc. Biol. 32, 696–703.
68. Doni, A., Mantovani, G., Porta, C., Tuckermann, J., Reichardt, H.M., Kleiman, A., Sironi, M., Rubino, L., Pasqualini, F., Nebuloni, M., Signorini, S., Peri, G., Sica, A., Beck-Peccoz, P., Bottazzi, B., and Mantovani, A. (2008). Cell-specific regulation of PTX3 by glucocorticoid hormones in hematopoietic and nonhematopoietic cells. J. Biol. Chem. 283, 29983–29992.
69. Norata, G.D., Marchesi, P., Pulakazhi Venu, V.K., Pasqualini, F., Anselmo, A., Moalli, F., Pizzitola, I., Garlanda, C., Mantovani, A., and Catapano, A.L. (2009). Deficiency of the long pentraxin PTX3 promotes vascular inflammation and atherosclerosis. Circulation 120, 699–708.
70. Ravizza, T., Moneta, D., Bottazzi, B., Peri, G., Garlanda, C., Hirsch, E., Richards, G.J., Mantovani, A., and Vezzani, A. (2001). Dynamic induction of the long pentraxin PTX3 in the CNS after limbic seizures: evidence for a protective role in seizure-induced neurodegeneration. Neuroscience 105, 43–53.
71. Fujio, Y., Maeda, M., Mohri, T., Obana, M., Iwakura, T., Hayama, A., Yamashita, T., Nakayama, H., and Azuma, J. (2011). Glycoprotein 130 cytokine signal as a therapeutic target against cardiovascular diseases. J. Pharmacol. Sci. 117, 213–222.
72. Cakir, M., Altunbas, H., and Karayalcin, U. (2003). Hyperglycemia: an independent marker of in-hospital mortality in patients with undiagnosed diabetes. J. Clin. Endocrinol. Metab. 88, 1402; author reply, 1402.
73. Mahboubi, K., Biedermann, B.C., Carroll, J.M., and Pober, J.S. (2000). IL-11 activates human endothelial cells to resist immune-mediated injury. J. Immunol. 164, 3837–3846.
74. Thier, M., Hall, M., Heath, J.K., Pennica, D., and Weis, J. (1999). Trophic effects of cardiotrophin-1 and interleukin-11 on rat dorsal root ganglion neurons in vitro. Brain Res. Mol. Brain Res. 64, 80–84.
75. Kirkiles-Smith, N.C., Mahboubi, K., Plescia, J., McNiff, J.M., Karras, J., Schechner, J.S., Altieri, D.C., and Pober, J.S. (2004). IL-11 protects human microvascular endothelium from alloinjury in vivo by induction of survivin expression. J. Immunol. 172, 1391–1396.
76. Denis, C.V., Kwack, K., Saffaripour, S., Maganti, S., Andre, P., Schaub, R.G., and Wagner, D.D. (2001). Interleukin 11 significantly increases plasma von Willebrand factor and factor VIII in wild type and von Willebrand disease mouse models. Blood 97, 465–472.
77. Opal, S.M., and DePalo, V.A. (2000). Anti-inflammatory cytokines. Chest 117, 1162–1172

78. Fang, H.Y., Ko, W.J., and Lin, C.Y. (2005). Plasma interleukin 11 levels correlate with outcome of spontaneous intracerebral hemorrhage. Surg. Neurol. 64, 511–517; discussion, 517–518.
79. Deverman, B.E., and Patterson, P.H. (2012). Exogenous leukemia inhibitory factor stimulates oligodendrocyte progenitor cell proliferation and enhances hippocampal remyelination. J. Neurosci. 32, 2100–2109.
80. Bauer, S., and Patterson, P.H. (2006). Leukemia inhibitory factor promotes neural stem cell self-renewal in the adult brain. J. Neurosci. 26, 12089–12099.
81. Mi, H., Haeberle, H., and Barres, B.A. (2001). Induction of astrocyte differentiation by endothelial cells. J. Neurosci. 21, 1538–1547.
82. Jones, K.L., de Kretser, D.M., Patella, S., and Phillips, D.J. (2004). Activin A and follistatin in systemic inflammation. Mol. Cell. Endocrinol. 225, 119–125.
83. Hubner, G., Alzheimer, C., and Werner, S. (1999). Activin: a novel player in tissue repair processes. Histol. Histopathol. 14, 295–304.
84. Kaneda, H., Arao, T., Matsumoto, K., De Velasco, M.A., Tamura, D., Aomatsu, K., Kudo, K., Sakai, K., Nagai, T., Fujita, Y., Tanaka, K., Yanagihara, K., Yamada, Y., Okamoto, I., Nakagawa, K., and Nishio, K. (2011). Activin A inhibits vascular endothelial cell growth and suppresses tumour angiogenesis in gastric cancer. Br. J. Cancer 105, 1210–1217.
85. Tretter, Y.P., Hertel, M., Munz, B., ten Bruggencate, G., Werner, S., and Alzheimer, C. (2000). Induction of activin A is essential for the neuroprotective action of basic fibroblast growth factor in vivo. Nat. Med. 6, 812–815.
86. Man, S., Tucky, B., Cotleur, A., Drazba, J., Takeshita, Y., and Ransohoff, R.M. (2012). CXCL12-induced monocyte-endothelial interactions promote lymphocyte transmigration across an in vitro blood-brain barrier. Sci. Transl. Med. 4, 119ra114.
87. Liu, K.K., and Dorovini-Zis, K. (2009). Regulation of CXCL12 and CXCR4 expression by human brain endothelial cells and their role in CD4+ and CD8+ T cell adhesion and transendothelial migration. J. Neuroimmunol. 215, 49–64.
88. Cook-Mills, J.M., Marchese, M.E., and Abdala-Valencia, H. (2011). Vascular cell adhesion molecule-1 expression and signaling during disease: regulation by reactive oxygen species and antioxidants. Antioxid. Redox Signal. 15, 1607–1638.
89. Woodcock, T., and Morganti-Kossmann, M.C. (2013). The role of markers of inflammation in traumatic brain injury. Front. Neurol. 4, 18.
90. Kumar, A., and Loane, D. J. (2012). Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention. Brain Behav. Immun. 26, 1191–1201.
91. Bangsow, T., Baumann, E., Bangsow, C., Jaeger, M.H., Pelzer, B., Gruhn, P., Wolf, S., von Melchner, H., and Stanimirovic, D.B. (2008). The epithelial membrane protein 1 is a novel tight junction protein of the blood-brain barrier. J. Cereb. Blood Flow Metab. 28, 1249–1260.
92. Kis, B., Abraham, C.S., Deli, M.A., Kobayashi, H., Niwa, M., Yamashita, H., Busija, D.W., and Ueta, Y. (2003). Adrenomedullin, an autocrine mediator of blood-brain barrier function. Hypertens. Res. 26, Suppl., S61–S70.
93. Kato, J., Tsuruda, T., Kita, T., Kitamura, K., and Eto, T. (2005). Adrenomedullin: a protective factor for blood vessels. Arterioscler. Thromb. Vasc. Biol. 25, 2480–2487.
94. Ribatti, D., Nico, B., Spinazzi, R., Vacca, A., and Nussdorfer, G.G. (2005). The role of adrenomedullin in angiogenesis. Peptides 26, 1670–1675.
95. Temmesfeld-Wollbruck, B., Hocke, A.C., Suttorp, N., and Hippenstiel, S. (2007). Adrenomedullin and endothelial barrier function. Thromb. Haemost. 98, 944–951.
96. Samson, W.K. (1999). Adrenomedullin and the control of fluid and electrolyte homeostasis. Annu. Rev. Physiol. 61, 363–389.
97. Risso, F.M., Sannia, A., Gavilanes, D.A., Vles, H.J., Colivicchi, M., Ricotti, A., Li Volti, G., and Gazzolo, D. (2012). Biomarkers of brain damage in preterm infants. J. Matern. Fetal Neonatal Med. 25, Suppl. 4, 101–104.
98. Cheyuo, C., Yang, W.L., and Wang, P. (2012). The critical role of adrenomedullin and its binding protein, AMBP-1, in neuroprotection. Biol. Chem. 393, 429–439.
99. Minami, M., Yokokawa, K., Kohno, M., Yasunari, K., and Yoshikawa, J. (1998). Suppression of endothelin-3-induced nitric oxide synthesis by triglyceride in human endothelial cells. J. Cardiovasc. Pharmacol. 31, Suppl. 1, S467–S469.
100. Filipovich, T., and Fleisher-Berkovich, S. (2008). Regulation of glial inflammatory mediators synthesis: possible role of endothelins. Peptides 29, 2250–2256.
101. Rozyczka, J., Figiel, M., and Engele, J. (2004). Endothelins negatively regulate glial glutamate transporter expression. Brain Pathol. 14, 406–414.
102. Bek, E.L., and McMillen, M.A. (2000). Endothelins are angiogenic. J. Cardiovasc. Pharmacol. 36, S135–S139.
103. Hiyama, T.Y., Yoshida, M., Matsumoto, M., Suzuki, R., Matsuda, T., Watanabe, E., and Noda, M. (2013). Endothelin-3 expression in the subfornical organ enhances the sensitivity of Na(x), the brain sodium-level sensor, to suppress salt intake. Cell Metab. 17, 507–519.
104. Samson, W.K., Skala, K., Huang, F.L., Gluntz, S., Alexander, B., and Gomez-Sanchez, C.E. (1991). Central nervous system action of endothelin-3 to inhibit water drinking in the rat. Brain Res. 539, 347–351.
105. Lozano, D., Gonzales-Portillo, G.S., Acosta, S., de la Pena, I., Tajiri, N., Kaneko, Y., and Borlongan, C.V. (2015). Neuroinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. Neuropsychiatr. Dis. Treat. 11, 97–106.
106. Bigler, E.D. (2013). Neuroinflammation and the dynamic lesion in traumatic brain injury. Brain 136, 9–11.
107. Dai, W., Cheng, H.L., Huang, R.Q., Zhuang, Z., and Shi, J.X. (2009). Quantitative detection of the expression of mitochondrial cytochrome c oxidase subunits mRNA in the cerebral cortex after experimental traumatic brain injury. Brain Res. 1251, 287–295.
108. Davidson, S.M., and Duchen, M.R. (2007). Endothelial mitochondria: contributing to vascular function and disease. Circ. Res. 100, 1128–1141.
109. Faden, A.I., and Loane, D.J. (2015). Chronic neurodegeneration after traumatic brain injury: Alzheimer disease, chronic traumatic encephalopathy, or persistent neuroinflammation? Neurotherapeutics 12, 143–150.
110. Breunig, J.J., Guillot-Sestier, M.V., and Town, T. (2013). Brain injury, neuroinflammation and Alzheimer's disease. Front. Aging Neurosci. 5, 26.
111. Guo, S., Zhou, Y., Xing, C., Lok, J., Som, A.T., Ning, M., Ji, X., and Lo, E.H. (2012). The vasculome of the mouse brain. PLoS One 7, e52665.
112. Flynn, K.M., Michaud, M., Canosa, S., and Madri, J.A. (2013). CD44 regulates vascular endothelial barrier integrity via a PECAM-1 dependent mechanism. Angiogenesis 16, 689–705.
113. Lennon, F.E., and Singleton, P.A. (2011). Hyaluronan regulation of vascular integrity. Am. J. Cardiovasc. Dis. 1, 200–213.
114. Ponta, H., Sherman, L., and Herrlich, P.A. (2003). CD44: from adhesion molecules to signalling regulators. Nat. Rev. Mol. Cell Biol. 4, 33–45.
115. Wang, H., Zhan, Y., Xu, L., Feuerstein, G.Z., and Wang, X. (2001). Use of suppression subtractive hybridization for differential gene expression in stroke: discovery of CD44 gene expression and localization in permanent focal stroke in rats. Stroke 32, 1020–1027.
116. Wang, X., Xu, L., Wang, H., Zhan, Y., Pure, E., and Feuerstein, G.Z. (2002). CD44 deficiency in mice protects brain from cerebral ischemia injury. J. Neurochem. 83, 1172–1179.
117. Carow, B., and Rottenberg, M.E. (2014). SOCS3, a major regulator of infection and inflammation. Front. Immunol. 5, 58.
118. White, G.E., Cotterill, A., Addley, M.R., Soilleux, E.J., and Greaves, D.R. (2011). Suppressor of cytokine signalling protein SOCS3 expression is increased at sites of acute and chronic inflammation. J. Mol. Histol. 42, 137–151.
119. Baker, B.J., Akhtar, L.N., and Benveniste, E.N. (2009). SOCS1 and SOCS3 in the control of CNS immunity. Trends Immunol. 30, 392–400.
120. Yin, Y., Liu, W., Ji, G., and Dai, Y. (2013). The essential role of p38 MAPK in mediating the interplay of oxLDL and IL-10 in regulating endothelial cell apoptosis. Eur. J. Cell Biol. 92, 150–159.
121. Townsend, S.F., Dallman, M.F., and Miller, W.L. (1990). Rat insulin-like growth factor-I and -II mRNAs are unchanged during compensatory adrenal growth but decrease during ACTH-induced adrenal growth. J. Biol. Chem. 265, 22117–22122.

122. Stahl, A., Joyal, J.S., Chen, J., Sapieha, P., Juan, A.M., Hatton, C.J., Pei, D.T., Hurst, C.G., Seaward, M.R., Krah, N.M., Dennison, R.J., Greene, E.R., Boscolo, E., Panigrahy, D., and Smith, L.E. (2012). SOCS3 is an endogenous inhibitor of pathologic angiogenesis. Blood 120, 2925–2929.
123. Stratton, C.W., Weeks, L.S., and Tausk, F. (1987). Beta-lactamase induction and aminoglycoside susceptibility in *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. 19, 21–25.
124. Sands, W.A., Woolson, H.D., Milne, G.R., Rutherford, C., and Palmer, T.M. (2006). Exchange protein activated by cyclic AMP (Epac)-mediated induction of suppressor of cytokine signaling 3 (SOCS-3) in vascular endothelial cells. Mol. Cell. Biol. 26, 6333–6346.
125. Wong, P.K., Egan, P.J., Croker, B.A., O'Donnell, K., Sims, N.A., Drake, S., Kiu, H., McManus, E.J., Alexander, W.S., Roberts, A.W., and Wicks, I.P. (2006). SOCS-3 negatively regulates innate and adaptive immune mechanisms in acute IL-1-dependent inflammatory arthritis. J. Clin. Invest. 116, 1571–1581.
126. Tang, C., Zelenak, C., Volkl, J., Eichenmuller, M., Regel, I., Frohlich, H., Kempe, D., Jimenez, L., Le Bellego, L., Vergne, S., and Lang, F. (2011). Hydration-sensitive gene expression in brain. Cell. Physiol. Biochem. 27, 757–768.
127. Xu, L.L., Shi, Y., Petrovics, G., Sun, C., Makarem, M., Zhang, W., Sesterhenn, I.A., McLeod, D.G., Sun, L., Moul, J.W., and Srivastava, S. (2003). PMEPA1, an androgen-regulated NEDD4-binding protein, exhibits cell growth inhibitory function and decreased expression during prostate cancer progression. Cancer Res. 63, 4299–4304.
128. Watanabe, Y., Itoh, S., Goto, T., Ohnishi, E., Inamitsu, M., Itoh, F., Satoh, K., Wiercinska, E., Yang, W., Shi, L., Tanaka, A., Nakano, N., Mommaas, A.M., Shibuya, H., Ten Dijke, P., and Kato, M. (2010). TMEPAI, a transmembrane TGF-beta-inducible protein, sequesters Smad proteins from active participation in TGF-beta signaling. Mol. Cell. 37, 123–134.
129. Greenlee, M.C., Sullivan, S.A., and Bohlson, S.S. (2008). CD93 and related family members: their role in innate immunity. Curr. Drug Targets 9, 130–138.
130. Harhausen, D., Prinz, V., Ziegler, G., Gertz, K., Endres, M., Lehrach, H., Gasque, P., Botto, M., Stahel, P.F., Dirnagl, U., Nietfeld, W., and Trendelenburg, G. (2010). CD93/AA4.1: a novel regulator of inflammation in murine focal cerebral ischemia. J. Immunol. 184, 6407–6417.
131. Kao, Y.C., Jiang, S.J., Pan, W.A., Wang, K.C., Chen, P.K., Wei, H.J., Chen, W.S., Chang, B.I., Shi, G.Y., and Wu, H.L. (2012). The epidermal growth factor-like domain of CD93 is a potent angiogenic factor. PLoS One 7, e51647.
132. Greenlee, M.C., Sullivan, S.A., and Bohlson, S.S. (2009). Detection and characterization of soluble CD93 released during inflammation. Inflamm. Res. 58, 909–919.
133. Malarstig, A., Silveira, A., Wagsater, D., Ohrvik, J., Backlund, A., Samnegard, A., Khademi, M., Hellenius, M.L., Leander, K., Olsson, T., Uhlen, M., de Faire, U., Eriksson, P., and Hamsten, A. (2011). Plasma CD93 concentration is a potential novel biomarker for coronary artery disease. J. Intern. Med. 270, 229–236.
134. von Gertten, C., Flores Morales, A., Holmin, S., Mathiesen, T., and Nordqvist, A.C. (2005). Genomic responses in rat cerebral cortex after traumatic brain injury. BMC Neurosci. 6, 69.
135. Natale, J.E., Ahmed, F., Cernak, I., Stoica, B., and Faden, A.I. (2003). Gene expression profile changes are commonly modulated across models and species after traumatic brain injury. J. Neurotrauma 20, 907–927.
136. Truettner, J., Schmidt-Kastner, R., Busto, R., Alonso, O.F., Loor, J.Y., Dietrich, W.D., and Ginsberg, M.D. (1999). Expression of brain-derived neurotrophic factor, nerve growth factor, and heat shock protein HSP70 following fluid percussion brain injury in rats. J. Neurotrauma 16, 471–486.
137. Sharp, D.J., Scott, G., and Leech, R. (2014). Network dysfunction after traumatic brain injury. Nat. Rev. Neurol. 10, 156–166.
138. Smith, D.H., Meaney, D. F., and Shull, W. H. (2003). Diffuse axonal injury in head trauma. J. Head Trauma Rehabil. 18, 307–316.
139. Werner, C., and Engelhard, K. (2007). Pathophysiology of traumatic brain injury. Br. J. Anaesth. 99, 4–9.
140. Tsuru, M., Soejima, T., Shiba, N., Kimura, K., Sato, K., Toyama, Y., and Nagata, K. (2013). Proline/arginine-rich end leucine-rich repeat protein converts stem cells to ligament tissue and Zn(II) influences its nuclear expression. Stem Cells Dev. 22, 2057–2070.
141. Bi, Y., Ehirchiou, D., Kilts, T.M., Inkson, C.A., Embree, M.C., Sonoyama, W., Li, L., Leet, A.I., Seo, B.M., Zhang, L., Shi, S., and Young, M.F. (2007). Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat. Med. 13, 1219–1227.
142. Garcion, E., Halilagic, A., Faissner, A., and ffrench-Constant, C. (2004). Generation of an environmental niche for neural stem cell development by the extracellular matrix molecule tenascin C. Development 131, 3423–3432.
143. Zagzag, D., Friedlander, D.R., Dosik, J., Chikramane, S., Chan, W., Greco, M.A., Allen, J.C., Dorovini-Zis, K., and Grumet, M. (1996). Tenascin-C expression by angiogenic vessels in human astrocytomas and by human brain endothelial cells in vitro. Cancer Res. 56, 182–189.
144. Heuckeroth, R.O. (2003). Finding your way to the end: a tale of GDNF and endothelin-3. Neuron 40, 871–873.
145. Li, J.J., Bickel, P.J., and Biggin, M.D. (2014). System wide analyses have underestimated protein abundances and the importance of transcription in mammals. PeerJ 2, e270.
146. Li, J.J., and Biggin, M.D. (2015). Gene expression. Statistics requantitates the central dogma. Science 347, 1066–1067.
147. Schwanhausser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., Chen, W., and Selbach, M. (2011). Global quantification of mammalian gene expression control. Nature 473, 337–342.
148. Lo, E.H., Lok, J., Ning, M., and Whalen, M.J. (eds). (2014). *Vascular Mechanisms in CNS Trauma*. Springer series in translational stroke research 5. Springer Science+Business Media: New York.

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