Global Analysis of Shear Stress-Responsive Genes in Vascular Endothelial Cells

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DNA microarray gene expression analysis was conducted in human umbilical vein endothelial cells (HUVECs) and coronary artery endothelial cells (HCAECs) exposed to laminar or turbulent shear stress. Approximately 3% of the total 5600 gene in HUVECs and HCAECs increased their expression more than two-fold or decreased it to less than half the static control in response to an arterial level of laminar shear stress (15 dynes/cm² for 24 hours). The proportions of shear-stress-responsive genes decreased to around 2% under the venous level of laminar shear stress (1.5 dynes/cm²) in both cell lines. Turbulent shear stress of 1.5 dynes/cm² altered the expression of 1.1% of all genes in the HCAECs. Laminar shear stress, but not turbulent shear stress, decreased the expression of a number of genes involved in DNA synthesis and the cell cycle in both HUVECs and HCAECs. Clustering analysis showed a variety of temporal profiles of gene expression in HUVECs exposed to laminar shear stress of 15 dynes/cm² for 3, 6, 12, 24, and 48 hours. Turbulent shear stress affected expression of many genes that play a role in vascular remodeling, including genes encoding plasminogen activators and their inhibitor, endothelin-1, transforming growth factor- β , collagen type IV, and ephrin A1. *J Atheroscler Thromb*, 2003; 10: 304–313.

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Introduction

The endothelial cells (ECs) lining blood vessels are constantly exposed to the mechanical force generated by flowing blood called shear stress, and ECs have properties that allow them to recognize changes in shear stress and alter their morphology and functions accordingly (1). The EC responses to shear stress are thought to play an important role in blood-flow-dependent phenomena,

such as angiogenesis, vascular remodeling, and atherogenesis. For instance, increases in shear stress *in vivo* induce capillary proliferation (2) and enlargement of artery diameter (3), while decreases in shear stress lead to a reduction in artery diameter (4) and EC apoptosis (5). Human atherosclerotic lesions preferentially occur at curving or branching sites in arteries, where blood flow stagnates, separates, and re-circulates, thereby exerting relatively low and turbulent shear stress on the vessel wall (6). Because of this, shear stress has been thought to play a role in the initiation and development of atherosclerotic lesions.

A number of *in vitro* studies using flow-loading devices and cultured ECs have revealed that ECs are sensitive to shear stress and undergo a variety of changes in func-

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tion. Shear stress affects the endothelial production of bioactive molecules that are involved in the regulation of vascular tone, such as nitric oxide (7), prostacyclin (8), C-type natriuretic peptide (9), adrenomedullin (10), and endothelin-1 (11, 12), in cell growth control, such as platelet-derived growth factor (13), heparin-binding epidermal growth factor (14), transforming growth factor- β (15), and fibroblast growth factor (16), in blood coagulation and fibrinolysis, such as thrombomodulin (17), tissue factor (18), tissue plasminogen activator (19), and thrombin receptors (20), and in cell-to-cell adhesion, such as vascular cell adhesion molecule-1 (21), intercellular adhesion molecule-1 (22), platelet endothelial cell adhesion molecule-1 (23), integrin (24), and connexin 43 (25). When EC function is altered by shear stress, expression of related genes is also usually altered (26), and thus far approximately 30 genes have been reported to undergo changes in expression in response to shear stress. However, the EC response to shear stress seems to comprise interactive networks involving far more than 30 genes, and thus a global analysis of gene responses would be needed to understand EC responses to shear stress. DNA microarray techniques have recently become available for this purpose (27), and this technology allows researchers to investigate more than several thousand genes at a time.

We used Affymetrix GeneChip Expression Analysis (28, 29) to identify genes altered by shear stress in human umbilical vein ECs (HUVECs) and human coronary artery ECs (HCAECs) and a clustering algorithm to analyze the temporal profiles of endothelial gene responses to shear stress. We also compared gene responses to laminar shear stress and turbulent shear stress, which might reveal atherosclerosis-related genes.

Materials and Methods

Cell culture

Primary cultures of HUVECs were prepared from human umbilical cord veins by collagenase treatment, and HCAECs were obtained from Clonetics (San Diego, CA). The cultures were grown on a 1% gelatin-coated tissue culture flask in M199 supplemented with 15% FBS, 2 mmol/L L-glutamine (Gibco, San Diego, CA), 50 μ g/mL heparin, and 30 μ g/mL EC growth factor (BD Biosciences, San Jose, CA). The cells used in the present experiments were in the 7th and 10th passage.

Shear stress experiments

Confluent monolayers of HUVECs or HCAECs were exposed to well characterized hydrodynamically induced shear stresses in two types of apparatus. A parallel-plate-type apparatus was used to apply laminar shear stress to cells, as previously described (21). Briefly, one side of the flow chamber consisted of a glass plate on which the

cultured ECs rested, the other side was a polycarbonate plate, and these two flat surfaces were held 200 μ m apart by a Teflon™ gasket. The intensity of the shear stress (τ , dynes/cm²) acting on the EC layer was calculated by the formula $\tau = 6\mu Q/a^2b$, where μ is the viscosity of the perfusate (poise), Q is flow volume (ml/s), and a and b are cross-sectional dimensions of the flow path (cm). A closed circuit was arranged with a silicone tube, and medium was constantly circulated with a roller/tube pump (Atto Co., Tokyo, Japan) at 37°C in an atmosphere of 95% room air and 5% CO₂.

A cone-plate-type apparatus was used to apply turbulent shear stress to cells, as previously described by Sdougos *et al.* (30). Briefly, the apparatus consists of a stainless steel cone driven by an electric motor and a stage on which a 10-cm diameter culture dish with a glass plate inserted at the bottom is held. Rotation of the cone forces the fluid between the cone and glass plate to flow concentrically, exposing cells attached to the stationary glass plate to a fluid shear stress. The intensity of shear stress (τ , dynes/cm²) acting on the EC layer was calculated by the formula $\tau = \mu\omega/\alpha$, where μ is the viscosity of the perfusate (poise), ω the angular velocity of the cone, and α the cone angle in radians. The fluid shear stress is, therefore, constant over the entire plate surface. The modified Reynolds number, $\hat{R}$, was used to determine the appropriate experimental conditions to induce turbulent flow (31). The parameter $\hat{R}$ is calculated by the formula $\hat{R} = r^2\omega\alpha^2/12\nu$, where r is the radial distance from the apex of the cone, and ν the kinematic viscosity of the fluid. It is predicted from this parameter that flow is turbulent at $\hat{R} > 4$. In the present experiments, we used a 5° cone and a rotational velocity of 120 rpm. Since $\hat{R}$ is proportional to the radial dimension, turbulent flow was established at radii ≥ 2.4 cm, which corresponds to a $\hat{R} > 5$ and represented an average shear stress intensity of 1.5 dynes/cm². Thus, the cells for the turbulence experiments were harvested only from the outer portion of the glass plate (≥ 2.4 cm).

DNA microarray analysis

Microarray analysis of cDNA was performed according to the Technical Manual for Affymetrix GeneChip Expression Analysis (28, 29). Briefly, total RNA was extracted from ECs with ISOGEN (Nippon Gene, Tokyo, Japan) and converted into double-stranded cDNA with an oligo-dT primer containing a T7 RNA polymerase promoter. *In vitro* transcription was performed with biotinylated UTP and CTP (Enzo Diagnostics, Inc., Farmingdale, NY), resulting in a 40- to 8-fold linear amplification of RNA. Amplified cRNA was purified on an affinity resin column (RNeasy Mini Kit, Qiagen Inc., Valencia, CA) and randomly fragmented to sizes ranging from 50 to 150 bases before overnight hybridization to gene chips (HuGene FL array, Affymetrix Inc., Santa Clara, CA), which contains oligo-

nucleotide probe sets for approximately 5600 human genes. After 16-hour hybridization at 45°C, the gene chips were washed and stained with streptavidin/phycoerythrin (Molecular Probes, Eugene, OR), and read with a Hewlett-Packard GeneArray Scanner (Affymetrix Inc., Santa Clara, CA).

Data analysis and statistics

A single expression level for each gene was determined using 16-20 perfectly matched (PM) and mismatched (MM) control probes (32). The MM probes act as specificity controls that allow direct subtraction of both background and cross-hybridization signals. To determine the quantitative RNA levels, the average of the differences represented by PM minus MM for each gene-specific probe family was calculated after discarding the maximum, the minimum, and any outliers beyond 3 SDs. Genes whose average difference in the control was less than 100 were omitted from the study, because their expression was considered to be too low to be evaluated. The fold change in transcripts between the static control and the shear stressed sample was calculated with Affymetrix software.

Table 1. Percentages of shear-stress-responsive genes.

Cell line	Shear stress (dynes/cm ²)	Up (SD) > 2-fold	Down (SD) > 50%	%
HUVEC	Laminar 15	50 (21)	131 (33)	3.2
HUVEC	Laminar 1.5	32	86	2.1
HCAEC	Laminar 15	50 (1)	120 (4)	3.0
HCAEC	Laminar 1.5	25 (22)	88 (14)	2.0
HCAEC	Turbulent 1.5	23 (3)	40 (8)	1.1

n = 3 for HUVECs, *n* = 2 for HCAECs

Results

When exposed to laminar shear stress at the arterial level (15 dynes/cm²) for 24 hours, expression of approximately 3% of all genes in both the HUVECs and the HCAECs was up-regulated more than 2-fold or down-regulated to less than half (Table 1). The proportions of shear-stress-responsive genes decreased to around 2% under laminar shear stress of 1.5 dynes/cm² in both cell lines. Turbulent shear stress of 1.5 dynes/cm² altered the expression of 1.1% of all genes in the HCAECs.

The experiments in which HUVECs were subjected to laminar shear stress of 15 dynes/cm² for 24 hours were repeated three times, and the genes whose expression was reproducibly up-regulated more than 2-fold or down-regulated to less than 33% are summarized in Table 2 and 3. The up-regulated genes included the genes coding nicotinamide adenine dinucleotide phosphate (NADPH), heme oxygenase, and glucose-6-phosphate dehydrogenase (G6PDH), all of which are involved in antioxidant defense (Table 2). Thirteen (52%) of the twenty-five genes whose expression was down-regulated by shear stress were genes involved in DNA synthesis and the cell cycle, including the thymidylate synthase, cyclin B, CDK2, and thymidine kinase genes (Table 3).

The experiments in which HCAECs were subjected to laminar shear stress of 15 dynes/cm² for 24 hours were repeated twice, and the genes that were reproducibly up- or down-regulated are summarized in Table 4 and 5. A variety of genes involved in antioxidant defense (NADPH, heme oxygenase), anticoagulation (thrombomodulin), and vasodilation (endothelial nitric oxide synthase; eNOS) were up-regulated (Table 4). Many genes related to DNA synthesis and the cell cycle, including the cell cycle MCM2, cyclin B, and CDK2 genes,

Table 2. HUVEC genes up-regulated by laminar shear stress.

Gene	Accession no.	Ratio	Function
NAD(P)H:menadione oxidoreductase	J03934	8.7	Antioxidant defense
Heme oxygenase	X06985	7.4	Antioxidant defense
Leukotriene B4 hydroxydehydrogenase	D49387	5.8	Inflammation
RTP: N-myc downstream regulated gene	D87953	4.4	Cell differentiation
PMP-22	D11428	4.4	Myelin protein
Tie 2:TEK tyrosine kinase receptor	L06139	3.9	Angiogenesis
PAI-2: urokinase inhibitor	M31551	3.2	Fibrinolysis
BENE protein	U17077	3	Myelin protein
Glucose-6-phosphate dehydrogenase	X55448	2.9	Antioxidant defense
γ-Glutamylcysteine synthetase	L35546	2.7	Cell protection
Lysosomal hyaluronidase	AJ000099	2.6	Extracellular matrix
DNase X	X90392	2.6	DNA degradation
Thioredoxin reductase	X91247	2.5	Electron carrier
TSC-22:TGFβ-stimulated protein	U35048	2.4	Transcription factor

Table 3. HUVEC genes down-regulated by laminar shear stress.

Gene	Accession no.	Ratio	Function
Chemokine HCC-1	Z49269	– 10.9	Inflammation
KIAA0101	D14657	– 9.2	Unknown
B-myb	X13293	– 7.0	Oncogene
PAF acetylhydrolase	D63391	– 6.8	Inflammation
Thymidylate synthase	D00596	– 6.7	DNA synthesis
KIAA0186	D80008	– 6.5	Unknown
Hydrogen carrier protein; glycine synthase	D00723	– 5.6	Protein synthesis
Topoisomerase	L47276	– 5.3	DNA synthesis
Mesoderm-specific transcript (MEST)	D78611	– 4.9	Maternal behavior
Metalloproteinase	Z50115	– 4.5	Extracellular matrix
Rad2	HG4074	– 4.2	Cell division
P1cdc47	D55716	– 4.2	DNA synthesis
Proliferating cell nuclear antigen (PCNA)	J05614	– 4.1	DNA synthesis
P1 protein	X62153	– 4.1	DNA synthesis
Cyclin B	M25753	– 3.9	Cell cycle
Cyclin-dependent kinase inhibitor 3 (CDK2)	L25876	– 3.8	Cell cycle
ADE2H1	X53793	– 3.7	Purine metabolism
Splicing factor SRp20	D28423	– 3.6	mRNA splicing
p55CDC	U05340	– 3.6	Cell cycle
KIAA0175	D79997	– 3.4	Unknown
Cyclin protein	M15796	– 3.4	Cell cycle
Thymidine kinase	M15205	– 3.3	DNA synthesis
hRlf beta subunit (p102 protein)	D38073	– 3.2	DNA synthesis
DNA (cytosin-5)-methyltransferase	X63692	– 3.1	DNA synthesis
Cyclin-selective ubiquitin carrier protein	U73379	– 3.0	Cell cycle

Table 4. HCAEC genes up-regulated by laminar shear stress.

Gene	Accession no.	Ratio	Function
SIP-1: sodium/hydrogen exchanger	U82108	5.8	Membrane ion transport
RTP: N-myc downstream regulated gene	D87953	5.1	Cell differentiation
VDUP1	S73591	4.6	Unknown
PMP-22	D11428	4.5	Myelin protein
Thrombomodulin	J02973	4.5	Anticoagulation
Heme oxygenase	X06985	4.3	Antioxiant defense
NAD(P)H:menadione oxidoreductase	J03934	4.1	Antioxiant defense
ST2 protein	D12763	3.5	Unknown
BENE protein	U17077	3.5	Myelin protein
LIM (PTB-BL)	X93510	3.2	Tyrosine phosphorylation
Leukotriene B4 hydroxydehydrogenase	D49387	3.0	Inflammation
eNOS	M93718	3.0	Nitric oxide production
KIAA0119	D17793	2.9	Unknown
Inositol 1,4,5-trisphosphate receptor	U01062	2.9	Calcium channel
Tie 2:TEK tyrosine kinase receptor	L06139	2.8	Angiogenesis
TSC-22: TGF- β stimulated protein	U35048	2.4	Transcription factor

were down-regulated by shear stress in HCAECs (Table 5), exhibiting the same tendency as seen in HUVECs.

HUVECs were exposed to laminar shear stress of 15 dynes/cm² for 3, 6, 12, 24, and 48 hours, and the temporal profiles of gene expression were analyzed by a clustering method. Three-hundred seventy-nine genes whose expression increased more than two-fold or decreased to less than 50% at least at some point were selected, and their cluster images were obtained (Fig.1). The temporal profiles of the gene responses to shear stress were classified into eleven clusters. Among the up-regulation responses, Cluster C was the highest in frequency (50.9%), and Cluster D was second (20.6%) (Fig.1A). Among the down-regulation responses, Cluster K was highest in frequency (35.4%), and Cluster J was second (31.8%) (Fig.1B). These findings indicate the existence of a variety of temporal patterns of gene responses to shear stress.

HCAECs were exposed to laminar or turbulent shear stress (1.5 dynes/cm² for 24 hours), and the genes whose turbulent/laminar expression ratio was more than two or

less than half were identified (Table 6 and 7). Turbulent shear stress affected the expression of genes that are involved in vascular remodeling, including genes encoding tissue and urokinase plasminogen activator (tPA and uPA), and their inhibitor (PAI-1), transforming growth factor- β , endothelin-1, collagen type IV, and ephrin A1.

Discussion

The results of this study demonstrated that approximately 3% of the all HUVEC and HCAEC genes increase their expression more than two-fold or decrease it to less than half in response to arterial levels of laminar shear stress (15 dynes/cm², 24 hours). Our previous study using mRNA differential display showed that around 4% of the all mRNAs detected in HUVECs were responsive to shear stress (33). In reviewing our data and those reported by others (31, 34–36), the ratio of shear stress-responsive EC genes appeared to range from 1.3% to 6%. The ratio decreased at a venous level of laminar shear stress (1.5 dynes/cm²), and decreased further at a turbu-

Table 5. HCAEC genes down-regulated by laminar shear stress.

Gene	Accession no.	Ratio	Function
Cell cycle MCM2	D21063	– 21.4	Cell cycle
Chemokine HCC-1	Z49269	– 16.3	Inflammation
T-cell receptor active beta-chain	M12886	– 14.7	Antigen recognition
DNA polymerase δ small subunit	U21090	– 13.1	DNA synthesis
KIAA0101	D14657	– 12.9	Unknown
PAF acetylhydrolase IB γ -subunit	D63391	– 12.5	Brain development
Topoisomerase	L47276	– 8.3	DNA synthesis
Mesoderm-specific transcript (MEST)	D78611	– 7.6	Maternal behavior
Cyclin B	M25753	– 7.5	Cell cycle
Thymidylate synthase	D00596	– 6.9	DNA synthesis
Cyclin-dependent kinase inhibitor 3(CDK2)	L25876	– 6.9	Cell cycle
KIAA0168	D79990	– 5.4	Unknown
p55CDC	U05340	– 5.1	Cell cycle
P311 HUM (3.1)	U30521	– 4.7	Neuronal protein
Mesothelial keratin K7 (type II)	M13955	– 4.3	Cytoskeleton
Transformer-2 beta (htra-2 beta)	U68063	– 3.9	mRNA splicing
Cyclin-selective ubiquitin carrier protein	U73379	– 3.9	Cell cycle
Glia maturation factor	AB001106	– 3.6	Glia maturation
Nonmuscle myosin heavy chainB (MYH10)	M69181	– 3.4	Cell locomotion
H2A histone family, member X	X14850	– 3.2	DNA synthesis
hRlf beta subunit (p102 protein)	D38073	– 3.2	DNA synthesis
Ephrin A1	M57730	– 3.2	Angiogenesis
Fatty acid binding protein homologue	M94856	– 3.2	Lipid metabolism
Cytosolic aldehyde dehydrogenase	M31994	– 3.1	Alcohol metabolism
Antiquitin turgor protein	S74728	– 3.0	Alcohol metabolism
Osteoblast specific factor 2 (OSF-2)	D13666	– 3.0	Unknown

lent shear stress of 1.5 dynes/cm², indicating that the number of genes that respond to flow depends on the magnitude or nature of the shear stress.

It was noteworthy that the laminar shear stress (15 dynes/cm², 24 hours) decreased the expression of many genes involved in DNA synthesis and the cell cycle with the percentage of such genes reaching 52% and 39% of all genes reproducibly down-regulated to less than 33% in HUVECs and HCAECs, respectively. These results suggest that laminar shear stress exerts an inhibitory ef-

fect on EC growth. Several in vitro studies have shown that laminar shear stress actually suppresses EC growth. Levesque *et al.* demonstrated that the proliferation of bovine aortic ECs decreased in response to increases in laminar shear stress (37), and Akimoto *et al.* reported that ³H-thymidine uptake decreased markedly when a confluent monolayer of HUVECs was exposed to laminar shear stress (38). The genes up-regulated by laminar shear stress included those that play a role in a variety of EC functions, including anti-oxidative defense, anti-

Fig.1. Cluster image showing the different classes of gene expression profiles. HUVECs were exposed to laminar shear stress of 15 dynes/cm² for 3, 6, 12, 24, and 48 hours, and three hundred seventy-nine genes whose expression increased more than 2-fold or decreased to less than half at least at one time point were selected. These genes were clustered hierarchically into groups on the basis of the similarity of their expression profiles by using Genespring (Silicon Genetics, Redwood City, CA). The similarity tree (dendrogram) has been divided into 8 levels of branching depth. Division of the tree at branching level 3 divides the genes into eleven clusters of gene expression. A: up-regulated genes, B: down-regulated genes. The expression pattern of each gene is displayed as a horizontal strip. The ratio of the expression of a gene in HUVECs at the indicated time after shear stress stimulation to its level in the static control is represented in color. The graphs show the average normalized expression pattern over the time points for all of the genes in each cluster indicated by the letters A to K and the frequency of each cluster.

thrombosis, and vasodilatation. These findings seem to be consistent with the general concept that laminar shear stress has a vasoprotective effect. About half of the genes that responded to shear stress were the same in HUVECs and HCAECs, and the other half were different, meaning that shear stress-responsive genes differ among ECs derived from different vessels.

The cluster analysis showed that the temporal profiles of the gene response to shear stress are variable instead of uniform, and they were classified into eleven clusters. These results suggest the presence of a variety of signal transduction pathways between the recognition of shear stress and gene expression in ECs. Actually, shear stress has been found to activate multiple pathways that lead to the activation of transcription factors, such as AP-1, NF κ B, Egr-1, and Sp1, in which various molecules, such as ion channels, G-protein coupled receptors, integrins and many protein kinases, are involved. It remains unclear, however, which pathways are primary and which are secondary. Integration of the data concerning the

temporal profile of gene responses may allow resolution of this question.

The genes encoding tPA and uPA increased their expression in response to turbulent shear stress, whereas their inhibitor, the PAI-1 gene, decreased its expression. Plasminogen activators catalyze the production of plasmin, thereby stimulating fibrinolysis and extracellular proteolysis. Recent studies have indicated that tPA and uPA are involved in the migration and proliferation of vascular smooth muscle cells (39, 40) and that uPA expression is elevated in atherosclerotic human or rabbit aorta (41). Thus, the up-regulation of the tPA and uPA genes and the down-regulation of the PAI-1 gene by turbulent shear stress may be associated with atherosclerotic vascular remodeling, because atherosclerotic lesions preferentially occur in vessel regions exposed to such stress. By contrast, uPA and uPA expression did not increase in response to laminar shear stress. These findings indicate that ECs may differentially recognize laminar and turbulent shear stress and may use distinct transcription fac-

Table 6. HCAEC genes up-regulated by turbulent shear stress.

Gene	Accession no.	Ratio*	Function
Transglutaminase (TGase)	M55153	5.1	Metabolic regulation
tPA	K03021	3.7	Fibrinolysis and proteolysis
Microfibril-associated glycoprotein (MFAP2)	U19718	3.4	Extracellular matrix
RNPL	U28686	3.2	RNA binding protein
Thimet oligopeptidase (metalloproteinase)	Z50115	2.8	Amyloidogenic processing
KIAA0124	D50914	2.7	Unknown
Chorionic gonadotropin beta subunit	K03189	2.7	Reproduction
Initiation factor eIF-5A	U17969	2.7	Protein synthesis
Dynamin (DNM)	L36983	2.6	Endocytosis
T-cell receptor active beta-chain	M12886	2.6	Antigen recognition
Topoisomerase	L47276	2.6	DNA replication
Cytochrome c-1	J04444	2.5	Electron transfer
Protein phosphatase 2A B56-delta	L76702	2.5	Protein phosphorylation
HPXEL	U16660	2.5	Fatty acid metabolism
1-8D gene from interferon-inducible gene family	X57351	2.5	Unknown
uPA	X02419	2.4	Fibrinolysis and proteolysis
Protein tyrosine phosphatase (CIP2)	L25876	2.3	Protein phosphorylation
APM2 : adipose specific 2	D45370	2.3	Unknown
26S proteasome subunit p55	AB003103	2.2	Unknown
Peroxisome Proliferator Activated Receptor	HG3355	2.2	Lipid metabolism
Dihydropteridine reductase (hDHPR)	M16447	2.2	Metabolic regulation
Bmx mRNA for cytoplasmic tyrosine kinase	X83107	2.2	Signal transduction
Isolate JuSo MUC18 glycoprotein	M29277	2.2	Cell adhesion
Disintegrin	U41767	2.2	Cell adhesion
DNA (cytosine-5)-methyltransferase	X63692	2.2	DNA methylation
hRlf beta subunit (p102 protein)	D38073	2.2	DNA repair

Genes whose ratio exceeded 2.2 are shown. * Turbulent versus laminar

tors and their binding sites on gene promoters, i.e., shear stress-responsive elements, that are involved in shear stress-mediated regulation of gene transcription. The clarification of the mechanisms underlying the differential effects of laminar or turbulent shear stress on EC gene expression will provide new insight and allow better understanding of atherogenesis.

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Table 7. HCAEC genes down-regulated by turbulent shear stress.

Gene	Accession no.	Ratio*	Function
ST2 protein	D12763	– 5.2	Unknown
PolyA	Z24724	– 4.5	mRNA stabilization
MT-1	X76717	– 3.7	Unknown
TGF- β superfamily protein	AB000584	– 3.4	Cell growth
Alcohol dehydrogenase	U73514	– 3.1	Alcohol metabolism
Metallothionein	X64177	– 3.0	Metal (Zn) homeostasis
Metallothionein	V00594	– 2.9	Metal (Zn) homeostasis
Spermidine/spermine N1-acetyltransferase	U40369	– 2.8	Polyamine catabolism
JAK1	M64174	– 2.8	Signal transducers
HLH 1R21	X69111	– 2.7	Vascular formation
AF1q	U16954	– 2.6	Leukemogenesis
Tyrosine phosphatase	L77886	– 2.5	Signal transduction
α -2 collagen type IV (COL4A2)	M24766	– 2.4	Extracellular matrix
PAI-1	X04729	– 2.4	Fibrinolysis and proteolysis
Ubiquitin-binding protein P62	U46751	– 2.3	Protein degradation
B61(Ephrin A1)	M57730	– 2.3	Vascular formation
Endothelin-1	J05008	– 2.1	Vascular tone regulation
α -type IV collagen	M26576	– 2.1	Extracellular matrix
Dihydropyrimidinase	D78014	– 2.1	Pyrimidine biosynthesis
4F2HC antigen	M21904	– 2.1	Hormone secretion

* Turbulent versus laminar

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