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.

Key words: Hemodynamic force, Gene chip, DNA microarray, Turbulent flow

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 atherosclerosis. 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-
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 μg/mL heparin, and 30 μg/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 μm 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 \( \mu \) 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 (\( \tau \), dynes/cm²) acting on the EC layer was calculated by the formula \( \tau = \mu \omega r / \alpha \), where \( \mu \) is the viscosity of the perfusate (poise), \( \omega \) the angular velocity of the cone, and \( \alpha \) the cone angle in radians. The fluid shear stress is, therefore, constant over the entire plate surface. The modified Reynolds number, \( \tilde{R} \), was used to determine the appropriate experimental conditions to induce turbulent flow (31). The parameter \( \tilde{R} \) is calculated by the formula \( \tilde{R} = r^2 \omega c / 12 \nu \), where \( r \) is the radial distance from the apex of the cone, and \( \nu \) the kinematic viscosity of the fluid. It is predicted from this parameter that flow is turbulent at \( \tilde{R} > 4 \). In the present experiments, we used a 5° cone and a rotational velocity of 120 rpm. Since \( \tilde{R} \) is proportional to the radial dimension, turbulent flow was established at radii \( \geq 2.4 \) cm, which corresponds to a \( \tilde{R} > 5 \) 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 (\( \geq 2.4 \) 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.

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, CDK2, and thymidine kinase genes (Table 3).

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

| Cell line | Shear stress (dynes/cm²) | Up (SD) | Down (SD) |
|-----------|-------------------------|---------|-----------|
| 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

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 (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    | Antioxidant defense           |
| NAD(P)H:menadione oxidoreductase          | J03934        | 4.1    | Antioxidant 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 effect 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-

![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.](image-url)
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, NFκB, 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-

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**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 atherosclerosis.

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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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