Transcriptional Profiles of Valvular and Vascular Endothelial Cells Reveal Phenotypic Differences

Influence of Shear Stress

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***Objective*—**The similarities between valvular and vascular lesions suggest pathological initiation mediated through endothelium, but the role of hemodynamics in valvular endothelial biology is poorly understood.

***Methods and Results*—**Monolayers of porcine aortic endothelial cells (PAECs) or porcine aortic valve endothelial cells (PAVECs) were exposed to 20 dyne/cm^2 steady laminar shear stress for 48 hours, with static cultures serving as controls. Multiple microarray comparisons were made using RNA from sheared and control batches of both cell types. More than 400 genes were significantly differentially expressed in each comparison group. The resulting profiles were validated at the transcription and protein level and expression patterns confirmed in vivo by immunohistochemistry. PAVECs were found to be less intrinsically inflammatory than PAECs, but both cell types expressed similar antioxidant and antiinflammatory genes in response to shear stress. PAVECs expressed more genes associated with chondrogenesis, whereas PAECs expressed osteogenic genes, and shear stress had a protective effect against calcification.

***Conclusions*—**Transcriptional differences between PAVECs and PAECs highlight the valvular endothelial cell as a distinct organ system and suggest more attention needs to be given to valvular cells to further our understanding of similarities and differences between valvular and vascular pathology. **(*Arterioscler Thromb Vasc Biol*. 2006;26:69-77.)**

Key Words: aortic valve ■ shear stress ■ inflammation ■ calcification ■ endothelial cell

Aortic valve disease is associated with significant mortality and morbidity and is a strong risk factor for additional cardiovascular events.[1,2] Valvular degeneration is characterized by the development of stenosis or insufficiency, and by the time it is clinically manifested, it is usually only treatable by prosthetic valve replacement.[3] Explants of diseased valves reveal a wide spectrum of pathology, including sclerotic and calcific lesions, thrombus formations, bacterial vegetations, and fractured matrix fibers.[4,5] Aortic valve disease was originally thought to be the result of the continuous barrage of hemodynamic and mechanical forces over time, but recent evidence suggests a much more active biological progression involving inflammation, oxidation, angiogenesis, calcification, and osteogenesis.[6–8]

The vascular endothelium is a critical mediator of hemodynamic and humoral stimuli, and that endothelial inflammation and atherosclerosis occur preferentially at sites of disturbed or oscillatory flow.[9] Valvular endothelial dysfunction is also a hallmark of leaflet degeneration, and similarly characterized by the expression of proinflammatory adhesion receptors.[10,11] Interestingly, much of the aforementioned valvular pathology seems to occur preferentially on the aortic surface of the leaflet, which experiences a complex circulating flow that is different from the unidirectional flow on the ventricular side of the leaflet. This suggests that disturbed flow may play a causal role in the initiation of valvular pathology through activation of valvular endothelium, and likewise that valvular endothelium may be protected from dysfunctional activation by unidirectional flow, but as yet, no studies have been done to investigate this.

We have previously shown that valvular endothelial cells respond to shear stress by aligning perpendicular to the direction of flow, this in contrast to vascular endothelial cells, which align parallel to flow.[12] This alignment was associated with differences in focal adhesion arrangement and differential involvement of signal kinases, suggesting that these different endothelial cell types may interpret mechanical signals heterogeneously.

The objective of this article, therefore, is to characterize the similarities and differences between these endothelial popu-

Original received June 15, 2005; final version accepted November 1, 2005.

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***Arterioscler Thromb Vasc Biol.* is available at http://www.atvbaha.org** **DOI: 10.1161/01.ATV.0000196624.70507.0d**

lations through transcriptional profiles in static and shear conditions and identify putative mechanosensitive proteins that may be involved in the regulation of these differences. The resulting expression profiles suggest that valvular endothelium are similarly protected from oxidative stress, inflammatory stress, and calcification by shear stress, but intrinsic differences in the susceptibilities of these cells to the aforementioned pathologies may exist.

Materials and Methods

Cell Culture and Shear Exposure

Porcine aortic valve endothelial cells (PAVECs) and porcine aortic endothelial cells (PAECs) were isolated from tissue obtained at a local slaughterhouse and cultured as described previously.[12] Passage 5 monolayers of either PAVECs or PAECs were grown to confluence (48 hours) on glass slides coated with Collagen I (Becton-Dickenson; rat tail; 50 μg/mL). Slides were then placed in a parallel plate flow system and subjected to 20 dyne/cm^2 steady shear stress for 48 hours, whereas statically cultured slides served as controls (justification in online supplement, available at http://atvb.ahajournals.org).

Transcript Profiling Studies and Data Analyses

Four experimental groups were created for gene expression comparison: group 1 included PAVEC shear versus PAVEC static; group 2 included PAEC shear versus static; group 3 included PAVEC static versus PAEC static; and group 4, PAVEC shear versus PAEC shear (Figure I, available online at http://atvb.ahajournals.org). After shear or static conditions, total RNA was extracted from cell pellets pooled from 2 identical and independent experiments using the RNeasy mini column (Qiagen). Ten micrograms total RNA pools from 2 different experimental conditions were independently and randomly labeled with either Cy3 or Cy5 fluorophores using the Agilent Fluorescent Direct Label kit (Agilent Technologies). Labeled RNA pools were then competitively hybridized to Agilent Human 1 cDNA microarray slides (Agilent #G4100A), which contain 2 identical array areas. The above procedures were repeated 3 times using a different cell isolation batch each time, giving 6 arrays in an n=3 (biological replicates), n=2 (technical replicates) arrangement for each comparison group, for a total of 24 arrays. Fluorescence intensities of each hybridized spot were determined by using the Agilent Array Scanner and the Agilent Feature Extraction Software.[13] Two different statistical methods were used to determine differentially expressed genes: mixed ANOVA (MxANOVA) and significance analysis of microarrays (SAM).[14,15] The list of differentially expressed genes in each comparison group was analyzed for statistically enriched or depleted biological classifications using the GoMiner database engine.[16] Additional details are provided in the methods supplement.

Quantitative Real-Time RT-PCR, Western Blots, and Immunostaining

The expression trends of a panel of genes were confirmed at the transcript (quantitative real-time RT-PCR [QRT-PCR]) and protein levels (Western blot) using additional samples not used in the microarrays. Additional normal adult porcine valve leaflets and aortic wall tissue were excised, fixed in 10% neutral buffered formalin, paraffin embedded, and sectioned at 5 μm. Immunohistochemistry was performed for selected genes as indicated in the results using fluorescence-based detection methods (see online supplement).

Results

Transcriptional Response Differences Between Endothelial Cells

Microarray hybridizations were performed using sheared and static control samples of PAVECs and PAECs and analyzed statistically using MxANOVA. Shear exposure upregulated 545 and 324 genes in PAVECs and PAECs, respectively, and of those, 260 and 62 genes were unique to the valvular and vascular cell types. On the other hand, shear exposure downregulated expression of 311 and 157 genes in PAVECs and PAECs, respectively, with 184 and 37 genes unique to each cell type. A total of 236 and 615 genes were expressed more in PAVECs compared with PAECs in static culture and in response to shear stress, respectively, with 180 and 537 genes expressed more in PAECs under those same conditions. A comparison of the MxANOVA method with the permutation based system (SAM) and single ANOVA shows that the MxANOVA calls genes at a dramatically higher level of sensitivity compared with single ANOVA and maintained ≈75% fidelity with SAM (Table III, available online at http://atvb.ahajournals.org). Because SAM returned 3× more genes than the MxANOVA that were not present in both, we decided that the MxANOVA results were a more conservative estimate of the true called genes. Complete lists of significantly changed genes from each comparison group as determined by MxANOVA are available in the GEO database.

We next validated some of the microarray data at the mRNA and protein levels by QRT-PCR and Western blots, respectively (Figure 1). Based on the microarray data, we initially chose 3 known shear-regulated genes. Bone morphogenic protein 4 (BMP-4) downregulation in response to shear as shown by the microarray analysis was confirmed in both cell types and in each method ($P<0.05$; Figure 1A). Cytochrome P450 peptide 1A1 (CYP1A1) upregulation found in the microarray analysis (≈10-fold) by shear was also confirmed by QRT-PCR in both cell types ($P<0.05$), although the protein level was upregulated to a much smaller degree (1.6-fold upregulation; Figure 1B). As shown in Figure 1C, caveolin-1 (CAV-1) gene transcript level determined by the microarray was not affected by shear in PAECs but was downregulated by shear in PAVECs. However, QRT-PCR and Western blot studies showed that shear exposure decreased the mRNA and protein expression levels by 2.6 and 1.7-fold, respectively ($P<0.05$; Figure 1C).

We further confirmed the array data by Western blots for 2 additional genes: periostin (POSTN) and cadherin 11 (CAD11), in both cell types exposed to static and shear conditions (Figure 2). In total, the microarray trends were confirmed in 9 of 10 cases (BMP-4, CYP1A1, POSTN, and CAD11 in both cell types and CAV1 in PAVECs) but not in 1 case (CAV1 in PAECs) by QRT-PCR and Western blotting (Figures 1 and 2).

In addition, we validated the in vitro results in intact aortic valve (AV) tissues of normal pigs by immunohistochemical staining. We first used endothelial NO synthase (eNOS) as a positive control for endothelial cells and shear responses, although its transcript levels in our array studies could not be analyzed because of poor quality of some of the spots. The eNOS is expressed in the endothelium of the aorta and both sides of the aortic valve with higher expression at the ventricularis surface, where shear stress is believed to be more stable and unidirectional than that of fibrosa. BMP-4 was detected on the endothelium of normal aortas and valves

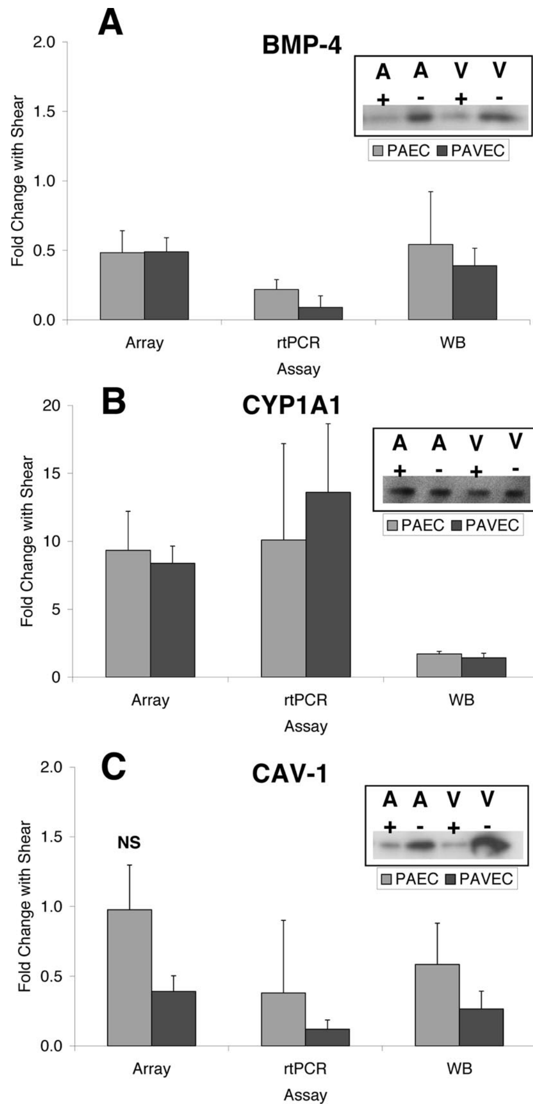

Figure 1. Confirmation of microarray data by real-time PCR and Western blot. NS denotes not significantly changed, whereas all other fold expressions are significantly different from matched controls ($P<0.05$). Boxes display Western blots. A indicates PAECs; V, PAVECs; +, shear; −, static.

(Figure 3). Expression was greater on the fibrosa side of the leaflet. Vascular cell adhesion molecule 1 (VCAM1) was not detected on normal aorta or aortic valves (data not shown). These results are similar to our previous report with aortic endothelial cells in response to laminar or oscillatory shear stresses.[17]

Analysis of Biological Classifications

The coordinated overexpression or underexpression of a group of genes in an ontological category may indicate certain functional responses to shear stress or differences between cell types. Biological classifications related to several important known endothelial functions using the GoMiner program were significantly changed (Table 1). For example, groups of genes related to oxioreductase activity, cell proliferation, apoptosis, cell migration, and cell signaling were significantly changed in PAVECs and PAECs in response to shear stress (groups 1 and 2) and differentially expressed between PAVECs and PAECs in static and shear flow conditions (groups 3 and 4). Surprisingly, there were also many biological classifications that were significantly changed in these groups related to developmental and differentiation events, including morphogenesis, angiogenesis, skeletal and muscle development. A complete list of the changed gene categories for each comparison group is available in the GEO database. Several additional classes of functional groups related to endothelial physiology and pathology were identified, including the genes involved in skeletal and mesenchymal development.

Expression of Antioxidant and Antiinflammatory Gene Transcripts in Aortic Valve Endothelium

As shown in Table 2, PAVECs expressed a similar number of antioxidant genes but less inflammatory ones compared with PAECs. PAVECs expressed 5 of 6 antiinflammatory genes to a greater degree than PAECs and 10 of 13 proinflammatory genes to a lesser degree than PAECs. PAECs expressed interleukins (IL-1A, IL-8), connexin 43 (GJA1), activated leukocyte adhesion molecule, BMP-4, and type III collagen (COL3A1) to a greater degree than PAVECs, all of which have been shown to contribute to vascular endothelial cell inflammatory atherosclerosis (see references in Table IV, available online at http://atvb.ahajournals.org). Expression of prooxidant and antioxidant genes was more evenly distributed between the 2 cell types, with 9 antioxidant genes expressed to a greater degree in PAECs and 8 in PAVECs. Antioxidant and antiinflammatory genes were uniformly upregulated in response to shear stress in both cell types, including peroxiredoxins (PRDX1 and PRDX2) superoxide dismutase (SOD2), and cytochromes (CYP1A1, CYP1B1). Shear stress regulated proinflammatory and prooxidant genes in a more complex and heterogeneous manner.

Aortic and Aortic Valve Endothelial Cells Differentially Regulate Chondro/Osteogenic Genes

The analysis of significantly differentially expressed genes revealed an unexpected number of genes associated with chondrogenesis and osteogenesis (Table 2). Surprisingly, PAECs expressed 5 of 6 proosteogenic genes to a greater degree than PAVECs, whereas PAVECs expressed 6 of 8 chondrogenic genes to a greater degree than PAECs. PAVECs expressed CAD11 mRNA >100-fold greater than PAECs, whereas PAECs expressed POSTN mRNA >400-fold greater than PAVECs (Table 3). These POSTN and CAD11 array results were confirmed by Western blots using cell lysates. As shown in Figure 2, the 2 osteogenic proteins were expressed almost exclusively in 1 endothelial cell type. PAECs express 2 POSTN isoforms (75- and 77-kDa bands), whereas only the 75-kDa form was expressed in PAVECs (Figure 2A). The specificity of both bands has been confirmed by antigen competition blotting (Figure II, available online at http://atvb.ahajournals.org). Shear exposure downregulated the 77-kDa form but not the 75-kDa form, suggesting that the 77-kDa form corresponds to the shear-sensitive transcripts (Figure 2C). In contrast, CAD11 protein was highly expressed in PAVECs but not in PAECs (Figure 2B). The downregulation of CAD11 protein by shear in PAVEC was also confirmed (Figure 2D). These results are consistent

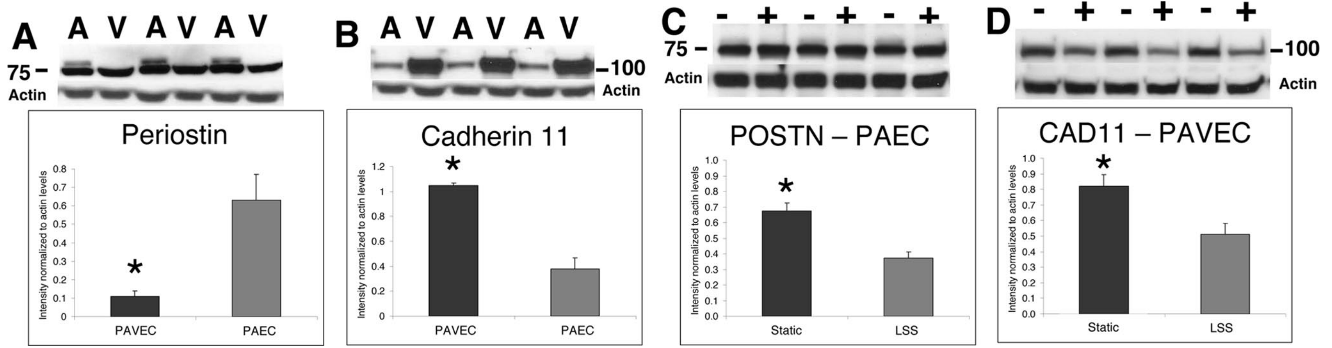

Figure 2. Divergent expression of mechanosensitive proteins involved in differentiation by aortic and aortic valve endothelial cells. A, Two POSTN isoforms (75 and 77kDa) are expressed in PAECs, whereas PAVECs express the 75-kDa form only. B, CAD11 expression is significantly higher in PAVECs than PAECs. C, POSTN is downregulated by shear in PAECs. D, CAD11 is downregulated by shear in PAVECs. Actin blots are used as loading controls. A indicates aortic endothelial cells; V, aortic valve endothelial cells; +, shear; −, static. *Denotes significance $P<0.05$.

with the notion that shear protects both endothelial cell types from chondro/osteogenic differentiation (Figure 2). The microarray and Western blot results of PAECs and PAVECs were further validated in vivo by immunostaining of normal porcine aorta and aortic valve (Figure 3). POSTN protein was easily detected in aortic endothelium (Figure 3P and 3Q) but not in valvular endothelium (Figure 3R through 3T). In contrast, CAD11 protein expression in aortic endothelium was not detectable (Figure 3K and 3L), whereas it was easily detected in valvular endothelium of fibrosa and ventricularis surfaces (Figure 3M through 3O).

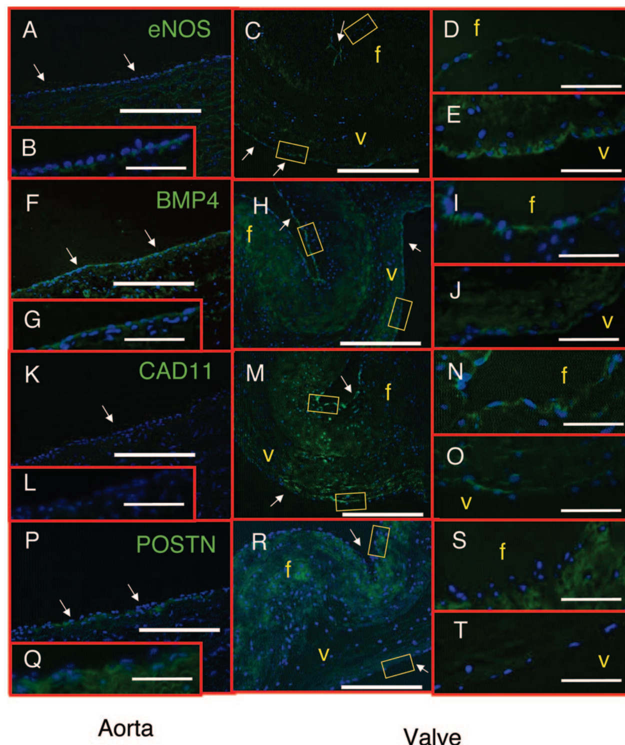

Figure 3. Confirmation of protein expression in vivo through immunohistochemistry. Panels A and B, F and G, K and L, and P and Q are from normal porcine ascending aortas, and the rest are of normal porcine aortic valve leaflets. Panels C, H, M, and R are the whole leaflet, whereas D, I, N, and S magnify the fibrosa side (f), and E, J, O, and T magnify the ventricularis (v) endothelium. Endothelial cells are marked with arrows. Panels A through E demonstrate eNOS expression; F through J, BMP-4 expression; K through O, Cad11 expression; and P through T, POSTN expression (green color). DAPI staining (blue) denotes cell nuclei. Bars=200 μm (B, C, G, and H) or 40 μm.

Discussion

Although it is well recognized that vascular endothelial cells are critical mediators of vascular function and dysfunction through response to changes in hemodynamics, a similar role for valvular endothelial cells has yet to be determined. The differences between embryonic valvular and vascular endothelial cells suggest that these cell types may represent unique phenotypes in adult tissues. Using unamplified RNA samples pooled from multiple experiments and 4 separate microarray comparisons, we generated a comprehensive picture of valvular and vascular endothelial transcriptional profiles in static and steady shear stress conditions. Many studies have used microarray analysis to investigate the response of vascular endothelial cells to steady shear stress.[18,19] A study by Farivar et al compared the transcriptional profile of valvular and vascular endothelial cells in static conditions and identified several genes with putative expression in 1 cell type or the other.[20] However, their study did not permit a statistical analysis of the generated expression profiles. A recent study by Simmons et al compared transcriptional profiles from endothelial cells isolated from both sides of the aortic valve leaflet and found side dependent differences in gene expression that may be implicated in valvular pathogenesis.[21] Our comparison system builds on these studies by enabling the profiling of both these cell types and their responses to shear stress using cells isolated from the same normal animals in the same statistically motivated experimental design. The transcriptional profiles coupled with the ontological profiles suggest that, like vascular endothelial cells, unidirectional shear stress inhibits oxidative and inflammatory responses of valvular endothelial cells. Our comparison group 2 (PAECs shear versus static) had many of the same genes significantly regulated as other published vascular microarray studies, including upregulation of antioxidants CYP1A1, PRDX1, and SOD2 and downregulation of a proinflammatory mediator BMP-4.[17–19] In addition, shear stress also protects both endothe-

TABLE 1. Prominent Endothelial Gene Classifications Identified by GOMiner*

Classification	Rank	Total	Change	*P* Value
Group 1: PAVEC Shear vs Static				
Rho guanyl-nucleotide exchange factor activity	31	6	4	0.002
Phospholipase D activity	51	8	4	0.008
Cell proliferation	52	764	108	0.009
muscle development	62	76	16	0.011
Phospholipid metabolism	67	34	9	0.012
Heat shock protein activity	80	52	12	0.013
Phospholipase activity	107	29	8	0.014
Protein kinase inhibitor activity	123	10	4	0.021
Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	169	34	8	0.035
Membrane lipid metabolism	173	53	11	0.036
mesoderm development	215	17	5	0.038
Establishment and/or maintenance of cell polarity	221	12	4	0.04
Morphogenesis	231	624	85	0.046
Organogenesis	232	552	76	0.047
Group 2: PAEC Shear vs Static				
Extracellular matrix structural constituent	5	37	12	9E-04
Superoxide metabolism	8	3	3	0.002
Regulation of cell migration	12	9	5	0.002
Enzyme inhibitor activity	21	87	19	0.006
Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	29	26	8	0.009
Anti-apoptosis	47	28	8	0.015
Glucan biosynthesis	72	9	4	0.016
Skeletal development	80	53	12	0.021
Muscle development	85	54	12	0.024
Regulation of angiogenesis	91	6	3	0.026
Development	98	596	86	0.03
Wnt receptor activity	110	3	2	0.04
Regulation of lymphocyte differentiation	115	3	2	0.04
Regulation of S phase of mitotic cell cycle	116	3	2	0.04
Group 3: PAVEC vs PAEC Static				
Response to reactive oxygen species	3	2	2	0.003
Blood vessel development	6	49	8	0.003
Cellular morphogenesis	14	59	8	0.009
Regulation of angiogenesis	17	10	3	0.012
Connexin channel activity	20	10	3	0.012
Cell migration	21	76	9	0.014
Angiogenesis	41	45	6	0.024
Cell adhesion	44	381	28	0.028
Regulation of cell shape	50	14	3	0.031
Cell fate specification	53	6	2	0.033
Negative regulation of angiogenesis	56	6	2	0.033
Programmed cell death	62	259	20	0.037
Mesoderm cell fate commitment	68	7	2	0.045
Post-embryonic morphogenesis	69	7	2	0.045

TABLE 1. Continued

Classification	Rank	Total	Change	*P* Value
Group 4: PAVEC vs PAEC Shear				
NADPH regeneration	13	5	4	0.015
Collagen type IV	16	5	4	0.015
Extracellular matrix structural constituent	26	40	16	0.024
Oxygen and reactive oxygen species metabolism	28	37	15	0.025
Regulation of cell migration	31	8	5	0.026
Cell differentiation	38	92	31	0.033
NADPH metabolism	41	6	4	0.037
DNA replication factor C complex	44	6	4	0.037
Morphogenesis	46	433	123	0.038
Embryonic morphogenesis	48	20	9	0.039
Cell-matrix adhesion	55	30	12	0.047
Superoxide metabolism	57	4	3	0.05
Wnt receptor activity	61	4	3	0.05
Regulation of angiogenesis	65	4	3	0.05

*Shown are the top unique biological themes overexpressed in each comparison group and those related to endothelial physiology and pathology. Rank indicates category rank in ascending *P* value order; total, total number of genes expressed on the array; change, number of differentially expressed genes in that comparison group. *P* value from Fisher Exact probability. See supplemental methods for additional information.

lial cell types from chondro/osteogenic differentiation, which is in accordance with the ventricular endothelial protection from this differentiation suggested by the study by Simmons et al.[21] We also found novel differences between the endothelial cell types including the increased expression of chondrogenic factors in PAVECs but osteogenic factors in PAECs, The expression trends of genes implicated in each of these functional areas were confirmed at the transcript and protein level, and conforming expression patterns were found in vivo in adult tissue. These data point to important valvular endothelial functions that may not be mimicked by vascular endothelium and suggest that valvular endothelium acts as a distinct organ system.

The similar regulation of oxidative and inflammatory genes by PAVECs and PAECs by shear stress suggests that hemodynamics may play a similarly important role in the pathogenesis of valvular diseases as it does in the vasculature. The hemodynamics experienced by the ventricular and aortic surfaces of aortic leaflets are distinctly different, with changes in flow direction on the aortic surface similar to those seen in vascular bifurcations.[22] Mapping of the TIE1 promoter in mice localized to regions of vasculature that were atheroprone, including at bifurcations and on the aortic surface of the aortic valve,[23] suggesting that both of these regions may be similarly susceptible to inflammatory disease. Early valvular lesions are primarily localized to the aortic (fibrosa) surface and characterized by endothelial expression of adhesion receptors such as VCAM1, intercellular adhesion molecule, and E-selectin.[10,11] The more abundant expression of proinflammatory genes by PAECs compared with PAVECs (and less abundant expression of antiinflammatory genes by PAECs) suggests that aortic valve endothelial cells may be more intrinsically antiinflammatory than aortic endothelial cells.

More advanced lesions on both aortic valve leaflets and aortas are characterized by calcification of underlying tissue. Although calcification of valvular interstitial cells and vascular smooth muscle cells is mediated by apoptosis and enhanced with transforming growth factor-β stimulation,[24,25] the role of endothelial cells in this process is not completely understood. Increasing evidence suggests that endothelial cells may play a critical role in regulating this process. Endothelial cells in calcification-susceptible regions express calcification stimulating factors such as BMP-4 and reduced expression of inhibitory factors such as osteoprotegrin and osteopontin.[21,26] We show shear stress downregulates initiators of calcification such as BMP-4 in vascular and valvular endothelial cells, as well as other genes, suggesting that hemodynamics may also mediate these events. We also discovered the almost exclusive expression of CAD11 in PAVECs and POSTN in PAECs, and both of these genes were significantly downregulated by steady shear stress. CAD11 is a member of the cadherin adhesion receptor family and is expressed in a variety of mesenchymal cells.[27] Transfection of embryonic stem cells with CAD11 directly induces differentiation to chondrogenic and osteogenic phenotypes mediated by cell–cell contacts.[28] Laminar shear inhibition of CAD11 may be critical for inhibition of calcification in valvular tissue. POSTN is a fibrous extracellular matrix protein with repeating fasciclin domains and 4 known isoforms, each of which appears to be a positive regulator of osteogenesis in preosteoblastic cells.[29] Interestingly, POSTN is initially expressed in the mesenchyme of developing valve cushions, yet its expression is reduced in adult leaflets in comparison to the aorta by birth.[30] We found 2 POSTN isoforms expressed in both endothelial cells, the 77-kDa

TABLE 2. Differential Genes Related to Oxidation and Inflammation*

Gene	Accession No.	Group 1	Group 2	Group 3	Group 4	Effect	VEC	AEC	Exp.
Genes related to oxidation									
CAMK2D	AK025368	**0.565**	**0.404**	**0.526**	—	Pro	–	–	**A**
HMOX1	BI596354	**2.856**	**5.447**	1.563	**1.967**	Pro	+	+	**V**
NP	X00737	1.729	**2.487**	**2.535**	**2.241**	Pro		+	**V**
ALDH1A1	AV649527	0.845	1.016	**0.256**	**0.343**	Anti			**A**
CYP1B1	U03688	1.050	**2.170**	0.816	0.330	Anti		–	
CYP1A1	K03191	**8.381**	**9.328**	1.634	**2.891**	Anti	–	–	**V**
FTL	BE394443	—	**2.425**	**0.776**	—	Anti		–	**A**
GCLC	M90656	6.297	—	—	**2.011**	Anti			**V**
GPX1	BI908630	1.125	1.372	**2.316**	**2.956**	Anti			**V**
GSR	AF228704	**2.285**	—	—	**1.738**	Anti	–		**V**
GUCY1A3	Y15723	1.074	1.080	**0.326**	—	Anti			**A**
LTB4DH	D49387	1.367	—	**0.510**	**0.473**	Anti			**A**
MT1A	K01383	—	1.107	**0.138**	**0.168**	Anti			**A**
MT1E	H72532	—	1.187	**0.177**	**0.205**	Anti			**A**
MT1F	M13003	1.016	1.063	**0.255**	**0.239**	Anti			**A**
MT1X	BF130769	1.015	—	—	**0.138**	Anti			**A**
NDUFV1	AW250734	**2.023**	2.357	2.126	**2.259**	Anti	–		**V**
POR	AF258341	**1.671**	**2.204**	—	**1.376**	Anti	–	–	**V**
PRDX1	BC007063	**4.232**	**4.506**	0.715	**1.297**	Anti	–	–	**V**
SOD2	BG773219	N/A	**3.2328**	2.0057	**1.9914**			–	**V**
Genes related to inflammation									
MMP2	J03210	2.332	**3.001**	0.349	0.580	Pro		+	
VCAM1	AL037831	**0.800**	**—**	0.909	0.725	Pro	–		
ALCAM	AI050952	**0.677**	**0.401**	0.667	**0.789**	Pro	–	–	**A**
BMP4	NM_001202	**0.489**	**0.483**	**0.579**	**0.435**	Pro	–	–	**A**
COL3A1	X14420	1.036	0.603	**0.149**	—	Pro			**A**
CTSS	BC002642	0.945	0.978	—	**0.235**	Pro			**A**
DCBLD2	AK001362	0.861	0.889	**0.391**	**0.442**	Pro			**A**
G1P2	AI739106	0.885	—	**0.178**	—	Pro			**A**
GJA1	X52947	—	0.762	**0.239**	**0.260**	Pro			**A**
HDC	M60445	—	0.717	—	**0.468**	Pro			**A**
IL1A	X02851	—	**6.452**	—	**0.481**	Pro		+	**A**
IL8	BG777366	**5.847**	**5.287**	**0.024**	0.053	Pro	+	+	**A**
GJA4	M96789	0.800	**0.612**	**3.952**	**4.610**	Pro		–	**V**
SDC2	J04621	—	0.275	2.004	**2.403**	Pro			**V**
YARS	BC004151	**1.768**	**2.255**	**1.964**	**2.018**	Pro	+	+	**V**
ANXA1	AU139496	—	**2.116**	1.333	0.960	Anti		–	
CTSC	AV717480	—	**2.362**	1.416	**1.982**	Anti		–	**V**
FST	M19480	**2.024**	—	**0.565**	**1.783**	Anti	–		**V**
GJA5	AF074995	**3.152**	—	**1.818**	0.947	Anti	–		**V**
HNRPR	AL538660	**2.076**	1.874	1.480	**1.793**	Anti	–		**V**
MYH11	NM_002474	—	1.980	**2.116**	**3.041**	Anti			**V**
PTGIS	D38145	**1.263**	1.352	2.264	**2.294**	Anti	–		**V**

*Bold indicates differentially expressed ($P<0.005$) in that comparison group. VEC indicates putative valvular endothelial cell shear stress effect; AEC, putative aortic endothelial shear stress effect. + denotes proinflammatory effect; Exp, which cell type expresses the protein more (A denotes aortic endothelial cells; V, valve endothelial cells) as determined from group 3 and group 4. — Expression of that gene not determinable in that comparison group. Refer to supplemental Table IV.

TABLE 3. Differentially Expressed Genes Related to Calcification*

Gene	Accession No.	Group 1	Group 2	Group 3	Group 4	VEC	AEC	Exp	Ref.
Differentially Expressed Genes Related to Chondrogenesis									
CDH11	BI766088	**0.599**	—	**113.161**	—	−		**V**	1
CHAD	AF371328	—	0.871	**3.469**	**2.224**			**V**	2, 3
CHI3L1	AL035737	**2.386**	—	3.691	**2.779**	+		**V**	4
HSPG2	NM_005529	1.179	**0.501**	**0.388**	0.839		−	**A**	5
ITM2A	AA010378	0.656	0.486	**3.208**	**2.858**			**V**	6
MAF	AF055376	0.871	—	**10.766**	—			**V**	7
SOX9	BC007951	0.886	1.112	**2.213**	**1.344**			**V**	8
ASPN	AK000136	—	—	**0.164**	**0.220**			**A**	9
Differentially Expressed Genes Related to Osteogenesis/Calcification									
BMP4	NM_001202	**0.489**	**0.483**	**0.579**	**0.435**	−	−	**A**	10, 11
FMOD	AI249821	1.008	**0.598**	**0.297**	**0.491**		−	**A**	12
MEIS2	AF179896	**0.604**	0.465	0.438	**0.413**	−		**A**	13
POSTN	AW608422	—	**0.319**	**0.002**	—		−	**A**	14
EBP	Z37986	**1.500**	**2.100**	0.972	0.960	−	−		15
MGP	AW999947	—	**0.174**	**0.182**	**0.355**		−	**A**	16

*Bold indicates differentially expressed ($P<0.005$) in that comparison group. VEC indicates putative valvular endothelial cell shear stress effect; AEC, putative aortic endothelial shear stress effect. + denotes procalcification effect; Exp, which cell type expresses the protein more (A denotes aortic endothelial cells; V, valve endothelial cells) as determined from group 3 and group 4. — denotes expression of that gene not determinable in that comparison group. Refer to supplemental Table III.

isoform conforming to the microarray data (Figure 2; Figure II). We detected POSTN only in the aortic endothelium in vivo but not in the aortic valve endothelium. POSTN is dramatically increased during vascular injury and dilated cardiomyopathy and enhanced by BMP stimulation,[31,32] suggesting its role in pathogenic cardiovascular remodeling. The downregulation of POSTN by shear stress in PAECs suggests that hemodynamics may also play a role in the regulation of POSTN in a calcific resistive manner in vascular tissue. It was also interesting to note that the preponderance of calcification genes expressed by PAVECs were chondrogenic, whereas osteogenic in PAECs. There have been rare occurrences of complete transformation of aortic valves into cartilage in humans,[33,34] and cartilaginous tissue has been found in explants of bioprosthetic valves.[35] The authors of these reports suggested that the formation of this cartilage was associated with tissue stress levels and represented a repair of ossified tissue. The interaction between valvular endothelial cells and interstitial cells leading to this transformation are unclear but suggest a unique result of pathogenic hemodynamic stimuli in valvular tissue.

In summary, transcriptional profile comparisons of valvular and vascular endothelial cells in different hemodynamic environments suggest that these cell types are distinctly different with respect to important biological functions but respond similarly to unidirectional shear stress to maintain a mature quiescent phenotype. The results of these studies raise important questions about endothelial phenotypes and the role hemodynamics play in regulating them, and the presented data provide a rich foundation from which more detailed investigation can progress.

Acknowledgments

This research was funded by the American Heart Association Southeast Affiliate (J.B., predoctoral fellowship award 0315103B; G.S., postdoctoral fellowship), the Georgia Tech/Emory Center for the Engineering of Living Tissues, NSF Grant EEC-9731643 (R.N.), and the grant HL71014 from the National Institutes of Health (H.J.). We gratefully acknowledge the assistance of Guoshen Wang and Wenli Wang and the rest of the staff at the Cardiovascular Research Institute of the Morehouse School of Medicine in processing the microarrays.

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