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Original Research

Hemodynamic shear stress regulates the transcriptional expression of heparan sulfate proteoglycans in human umbilical vein endothelial cell

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Abstract: We have previously demonstrated the adaptive remodeling of endothelial glycocalyx under shear stress. However, the underlying mechanism in glycocalyx remodeling, especially the expression of the components heparan sulfate proteoglycans (HSPGs) under shear stress was not completely known. In the present study, we investigated the expression of those HSPGs (syndecan family and glypican-1) in human umbilical vein endothelial cells (HUVECs) responded to the distinct magnitude of shear stress, and performed a systematic and comprehensive analyze on the relationship between shear stress and HSPGs mRNA expression in a temporal manner. During the initial 0.5h of exposure, syndecan-1 mRNA was greatest upregulated by 4 dyn/cm^2 of shear stress, and syndecan-4 mRNA was significantly upregulated by 10 dyn/cm^2 and 15 dyn/cm^2. After 24h of exposure, the greatest increased HSPG mRNA was syndecan-4 under 4 dyn/cm^2, and was syndecan-3 under 15 dyn/cm^2. The adaptive remodeling of endothelial glycocalyx may due to the change in the expression of those molecules. Furthermore, the changes of those molecules that may associate with the vascular homeostasis and endothelial dysfunction revealed the potential candidate components of the glycocalyx in response to cardiovascular diseases.

Key words: Endothelial cell, glycocalyx, shear stress, syndecans, glypican-1, mRNA.

Introduction

The endothelial cells (ECs) constitute the single lining layer of the blood vessel (endothelium), which are continuously exposed to shear stress and transfer the force into cellular contributed to maintaining vascular homeostasis. The glycocalyx (GCX) forming a brushlike structure on the luminal surface of vascular endothelium plays vital roles in endothelial mechanotransduction for interacting directly with blood flow. The loss of one specific component of GCX could result in endothelial dysfunction (1-3). The GCX is rich in heparan sulfate proteoglycans (HSPGs) which carry heparan sulfate (HS) (4). It has been demonstrated that HSPGs attached HS participates in mechanotransduction of fluid shear stress in ECs (5-9). In recent, Zeng et al previously demonstrated that reorganization of the endothelial GCX in response to fluid shear stress (10, 11). During the initial 0.5h of shear exposure, the transmembrane protein syndecan-1 and the glypican-1 linked to caveolae is fixed with anchored HS, while the glypican-1 that bound to lipid rafts carrying HS moves to the cell boundary (10). After continued 24h exposure to shear stress, HS nearly uniformly distributed on ECs (11). Based on their findings, it can further hypothesize that HSPGs is distinctly expressed on ECs in a temporal and magnitudes dependent manner to shear stress.

HSPGs on ECs consist of the syndecan family and glypican-1 (3, 12). Syndecans comprise four members that are encoded by distinct genes that include divergent ectodomains, but a conserved transmembrane region and an intracellular domain (13, 14). Syndecans are divided into two structural and functional subfamilies: one includes syndecans-1 and -3 that share 30% sequence identity in the rat, another includes syndecan-2 and -4 that share 38% sequence identity in the rat (15, 16). In vascular ECs, syndecan-1 is a critical core protein in shear stress modulated signaling and phenotypic remodeling in endothelial cells (11, 17-20). It has demonstrated that knock-out of syndecan-1 abolished several key events of ECs in response to atheroprotective flow (21). Syndecan-3 is also expressed on ECs, which was well investigated in the nervous system and musculoskeletal tissues (22). Syndecan-3 could inhibit angiogenesis by reducing the migratory potential of ECs and may be a candidate for anti-angiogenic therapy (23).

Syndecan-2 interacts with specific proteins to actively participate in cytoskeleton organization, modulating cell adhesion (24). Syndecan-2 also retards angiogenesis by inhibiting endothelial cell migration (24), a key step in neovascularization (25). The presence of syndecan-4 on the cell surface is widely expressed during development, which is tightly linked to many cytoskeletal proteins, such as α-actinin and RhoA playing a central role in coordinating cytoskeletal changes that occur during focal adhesion and cell division (26-28). In addition, the binding of syndecan-4 attached HS chains with domain II of fibronectin is required for the formation and maturation of adhesion complexes in fibroblasts (29).

Glypican-1 is a GPI-anchored protein, which is the only one expressed on ECs in members of the glypican family (4). The glypican-caveolae-eNOS hypothesis is

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Table 1. Primer sequences for qRT-PCR detection of HSPGs.

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
Syndecan-1	TCTTTGCTGTGTGCCTGGTG	CCTCCTGTTTGGTGGGCTTC
Syndecan-2	TGTCATTGCTGGTGGAGTTATTG	GCACTGGATGGTTTGCGTTC
Syndecan-3	GCAGGAGTCGGGCATTGAGA	AGGCTGGATGTTCGTGGTGG
Syndecan-4	CCTCTTTGCCGTCTTCCTGA	GGGCTTTCTTGTAGATGGGTTTC
Glypican-1	GATGGCTGTCTGGATGACCTCT	GTCTTCTGTCCTTCCTGCTCTGA
GAPDH	CTTTGGTATCGTGGAAGGACTC	GTAGAGGCAGGGATGATGTTCT

that upon exposure to shear stress, the glypican core protein of the EC glycocalyx transmits mechanical force to the caveolae, where eNOS is phosphorylated, ultimately leading to NO production. This likely involves intermediate signaling through Src and downstream cascades (30). In a recent study, Ebong et al (18) also reported that knockdown of glypican-1, not of syndecan-1, blocked 15 dyn/cm^2 of shear stress-induced eNOS activation in cultured bovine aortic endothelial cells.

Cardiovascular disease seriously threatens the health of people, which often occurs in the endothelial dysfunction region (at curvatures and bifurcations) developed with low magnitude of wall shear stress (<4 dyn/cm^2), but not in the straight parts of the aorta with high magnitude of wall shear stress (10~40 dyn/cm^2) (11, 20). For further analyzing the role of HSPGs in abnormal hemodynamic response to cardiovascular disease, in the present study, we tested the gene expression of all the HSPGs in human umbilical vein endothelial cells (HUVECs) responded to the distinct magnitude of the shear stress with short-term or long-term exposure. Our findings provide preliminary data for the complete understanding of the response of HSPGs to shear stress, which is important for clarifying the role of HSPGs in the potential adaptive remodeling of the GCX in cardiovascular disease.

Materials and Methods

Cell Culture

Human umbilical vein endothelial cells (HUVECs) and all cell culture reagents were purchased from Allcells, China. In brief, HUVECs were cultured in HUVEC medium with 10% FBS and 10% growth factor supplement containing EGF, FGF-2, cAMP, heparin, hydrocortisone, penicillin, streptomycin, and amphotericin-B (HUVEC-004, Allcells). Cells at passages 3~6 were plated on 25 μg human fibronectin (Life Technologies, USA) pre-coated glass slides at a density of 1×10^5 cells/cm^2 and cultured for 4–6 days until they attained confluence.

Shear Application

Shear stress was applied to the HUVEC monolayers using a parallel-plate flow chamber that has been described previously (10, 11). Briefly, the flow chamber was connected to a recirculation flow loop consisting of a glass reservoir, a peristaltic pump (WT600F, Leadfluid, China) and tubes. The reservoir was placed in 37 °C water bath and was connected to a humidified 5%/95% CO_2/air incubator.

In the present study, cells were subjected to different magnitudes of shear stress: 4 dyn/cm^2, 10 dyn/cm^2 and 15 dyn/cm^2 with 0.5h, 2h, 8h and 24h, respectively. For shear stress application, HUVEC basal medium (HUVEC-004B, Allcells) with an addition of 5% FBS and 0.5% BSA was used as circulating medium. The flow system was kept at 37°C in a humidified 5%/95% CO_2 air incubator. After shear stress exposure, the expression of HSPGs was determined by qRT-PCR.

Quantitative RT-PCR Analysis

After shear stress application immediately, total RNAs in HUVECs were isolated using TRIZOL (Invitrogen Inc., USA). The RNA concentration was measured using Nanodrop 2000 Spectrophotometer (Thermo Scientific, USA). Reverse transcription reaction and cDNA synthesis were performed according to the manufacturer's instructions (Takara, China). The qPCR reactions were performed on a CFX96 touch q-PCR system (BIO-RAD, USA) using SYBR Premix Ex TaqIIkit (Takara, China). Gene expression of HSPGs was normalized to the level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) within each sample using the $2^{-\Delta\Delta CT}$ methods as previously described (31). Gene expression is shown as a relative expression of indicated controls. All the primer sequences used in the present study are given in Table 1.

Statistical Analysis

All data were collected from at least three independent experiments and all results are expressed as the mean ± SD. Using SPSS 20.0 software package (IBM SPSS Software, USA), statistical analysis was performed by one-way analysis of variance (ANOVA) with either the least significant difference (LSD) test or Tamhane's T2 test (depending on Levene's statistic for homogeneity of variance). Differences in means were considered significant if $P<0.05$. The figures were performed using the GraphPad Prism version 6.0 (GraphPad Software, USA).

Results

The mRNA expression level of syndecan-1 in HUVECs is regulated by hemodynamic shear stress with duration and magnitudes

As shown in Figure 1, there are obvious differences in the mRNA expression levels of syndecan-1 among HUVECs that exposed to shear stress with different magnitudes. Under 4 dyn/cm^2 of shear stress (Figure 1A), there was a 2-fold increase of syndecan-1 mRNA expression at 0.5h, showing an early and strong response. Then, the mRNA expression level of syndecan-1 was weakened at 2h, but was significantly increased to 3 folds at 8h. After 24h exposure, the syndecan-1 mRNA expression level dropped down to the level at static conditions.

Under both 10 dyn/cm^2 (Figure 1B) and 15 dyn/cm^2 (Figure 1C) of shear stress, the mRNA expression levels of syndecan-1 did not significantly changed in 0.5h, it is consistent with the previous study (10) that suggested syndecan-1 did not significantly change during the first 0.5h exposure of 15 dyn/cm^2 of shear stress. After continued exposure to shear stress, the syndecan-1 mRNA expression levels were obviously elevated at 2h under the both shear stress magnitudes, and then it was further increased at 8h and was decreased at 24h under 10 dyn/cm^2 of shear stress while it was decreased after 8h of 15 dyn/cm^2 exposure.

Overall, the expression of syndecan-1 in transcriptional level is regulated by distinct magnitudes of shear stress (Figure 1D).

Syndecan-2 mRNA expression is remarkably regulated by 10 dyn/cm^2 of shear stress

Then, we examined the mRNA expression of syndecan-2, which was obviously regulated by 10 dyn/cm^2 and 15 dyn/cm^2 of shear stress, rather than 4 dyn/cm^2 (Figure 2). Compared to a static condition, we didn't find any significant difference in the expression of syndecan-2 at all durations of 4 dyn/cm^2 of shear stress (Figure 2A). However, under 10 dyn/cm^2 of shear stress (Figure 2B), syndecan-2 mRNA was firstly downregulated after 0.5h exposure and then gradually upregulated in a time-dependent manner. After 24h stimulation, the result showed a most striking 3.2-fold increase in the expression of syndecan-2, compared with static controls.

Under 15 dyn/cm^2 of shear stress (Figure 2C), the mRNA expression level of syndecan-2 was slightly (relative to 10 dyn/cm^2) and significantly decreased after 2h and 8h exposure, but was significantly increased to 1.7-fold at 24h, relative to static controls.

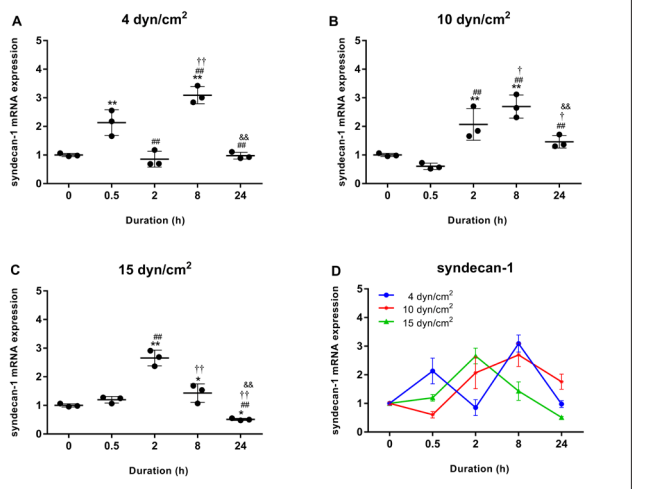

Figure 1. Syndecan-1 mRNA expression in HUVECs under shear stress. After exposure to hemodynamic shear stress (A) 4 dyn/cm^2, (B) 10 dyn/cm^2 and (C) 15 dyn/cm^2 for 0h, 0.5h, 2h, 8h and 24h, respectively, the mRNA expressions of syndecan-1 in HUVECs were determined by qRT-PCR and normalized to GAPDH mRNA (n=3). The graphs show fold changes in expression of syndecan-1 in HUVECs under shear stress with respect to that in static conditions. (D) For comparison, a summary of the gene expression levels of syndecan-1 under hemodynamic shear stress was present. **P*<0.05, ***P*<0.01 vs. 0h; #*P*<0.05, ##*P*<0.01 vs. 0.5h; †*P*<0.05, ††*P*<0.01 vs. 2h; &*P*<0.05, &&*P*<0.01 vs. 8h.

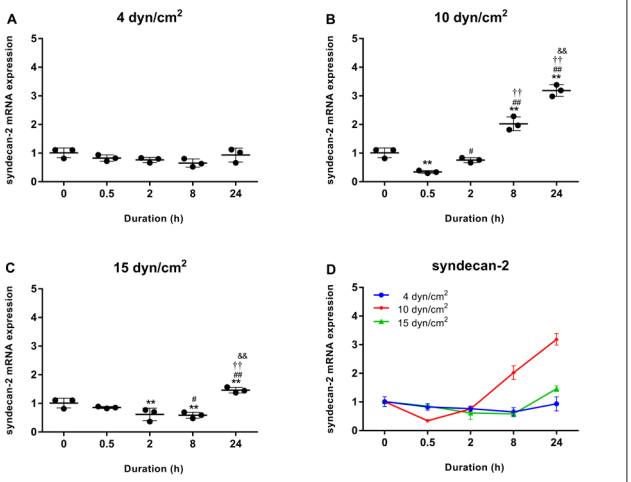

Figure 2. Syndecan-2 mRNA expression in HUVECs under shear stress. After exposure to hemodynamic shear stress (A) 4 dyn/cm^2, (B) 10 dyn/cm^2 and (C) 15 dyn/cm^2 for 0h, 0.5h, 2h, 8h and 24h, respectively, the mRNA expressions of syndecan-2 in HUVECs were determined by qRT-PCR and normalized to GAPDH mRNA (n=3). The graphs show fold changes in expression of syndecan-2 in HUVECs under shear stress with respect to that in static conditions. (D) For comparison, a summary of the gene expression levels of syndecan-2 under hemodynamic shear stress was present. **P*<0.05, ***P*<0.01 vs. 0h; #*P*<0.05, ##*P*<0.01 vs. 0.5h; †*P*<0.05, ††*P*<0.01 vs. 2h; &*P*<0.05, &&*P*<0.01 vs. 8h.

In general, the change in mRNA expression level of syndecan-2 under 10 dyn/cm^2 of shear stress was greater than that under 15 dyn/cm^2 of shear stress: greater decrease after short-term exposure (0.5h) and greater increase after long-term (24h).

Syndecan-3 mRNA expression is different from short-term and long-term shear exposure

As shown in Figure 3, there were obvious differences in the mRNA expression levels of syndecan-3 among HUVECs that exposed to short-term and long-term shear stress. During the first 0.5h, the syndecan-3 mRNA expression level relative to control was significantly decreased under 4 dyn/cm^2 and 10 dyn/cm^2 of shear application, but was not significantly decreased under 15 dyn/cm^2. From 2h up to 24h, under 4 dyn/cm^2 of shear stress (Figure3A), we observed that the syndecan-3 mRNA expression level was gradually increased and appeared in 3.5-fold after 24h exposure. Under 10 dyn/cm^2 of shear stress (Figure 3B), the mRNA expression level of syndecan-3 was descended slightly, but significantly at 2h and 8h time points, and was substantially ascended at 24h to the baseline level. Under 15 dyn/cm^2 of shear stress (Figure 3C), the transcription level of syndecan-3 was obviously increased to 4.3-, 2.5- and 7-fold at 2h, 8h, and 24h, respectively.

Overall, the syndean-3 mRNA expression level was dependent on the magnitudes of shear stress, especially the long-term pattern.

The mRNA expression level of syndecan-4 in HUVECs is regulated by hemodynamic shear stress

Figure 4 provides the mRNA expression levels of syndecan-4 in HUVECs exposed to shear stress. Under 4 dyn/cm^2 of shear stress (Figure 4A), the mRNA expression of syndecan-4 was significantly upregulated at

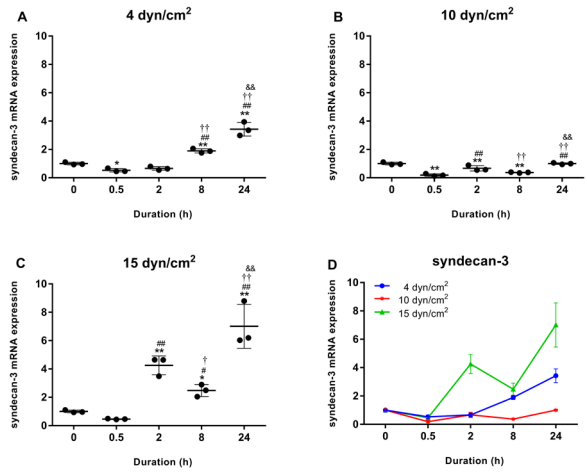

Figure 3. Syndecan-3 mRNA expression in HUVECs under shear stress. After exposure to hemodynamic shear stress (A) 4 dyn/cm^2, (B) 10 dyn/cm^2 and (C) 15 dyn/cm^2 for 0h, 0.5h, 2h, 8h and 24h, respectively, the mRNA expressions of syndecan-3 in HUVECs were determined by qRT-PCR and normalized to GAPDH mRNA (n=3). The graphs show fold changes in expression of syndecan-3 in HUVECs under shear stress with respect to that in static conditions. (D) For comparison, a summary of the gene expression levels of syndecan-3 under hemodynamic shear stress was present. **P*<0.05, ***P*<0.01 vs. 0h; #*P*<0.05, ##*P*<0.01 vs. 0.5h; †*P*<0.05, ††*P*<0.01 vs. 2h; &*P*<0.05, &&*P*<0.01 vs. 8h.

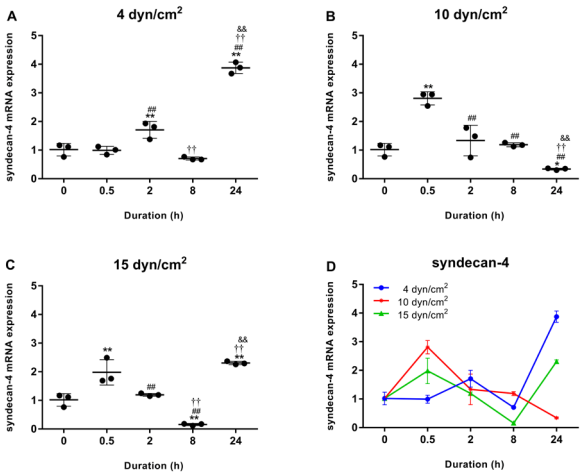

Figure 4. Syndecan-4 mRNA expression in HUVECs under shear stress. After exposure to hemodynamic shear stress (A) 4 dyn/cm^2, (B) 10 dyn/cm^2 and (C) 15 dyn/cm^2 for 0h, 0.5h, 2h, 8h and 24h, respectively, the mRNA expressions of syndecan-4 in HUVECs were determined by qRT-PCR and normalized to GAPDH mRNA (n=3). The graphs show fold changes in expression of syndecan-4 in HUVECs under shear stress with respect to that in static conditions. (D) For comparison, a summary of the gene expression levels of syndecan-4 under hemodynamic shear stress was present. **P*<0.05, ***P*<0.01 vs. 0h; #*P*<0.05, ##*P*<0.01 vs. 0.5h; †*P*<0.05, ††*P*<0.01 vs. 2h; &*P*<0.05, &&*P*<0.01 vs. 8h.

2h, and substantially downregulated at 8h to the baseline level, but the expression was increased steeply to 4-fold at 24h, relative to controls.

Under both 10 dyn/cm^2 (Figure 4B) and 15 dyn/cm^2 (Figure 4C) of shear stress, the mRNA expression levels of syndecan-4 presented a transient significant increase in 0.5h, whereas they declined toward the baseline level from 2h to 8h time points. After 8h exposures, we observed that the syndecan-4 mRNA expression was closed to the baseline level under 10 dyn/cm^2 of shear stress (Figure 4B), but was severely decreased under 15 dyn/cm^2 of shear stress to only 10% of static controls (Figure 4C).

After 24h long-term exposure, the syndecan-4 mRNA expression was increased sharply under 4 and 15 dyn/cm^2 of shear stress, respectively (Figure 4A and C), interestingly, the syndecan-4 expression level was further decreased under 10 dyn/ cm^2 of shear stress.

As shown in Figure 4D, the 8h time point of shear stress exposure is the key point for syndecan-4 mRNA in HUVECs, indicating an important role of syndecan-4 in responding to the magnitudes of shear stress.

The glypican-1 mRNA expression is more sensitive to long-term shear application

In the previous study (10), Zeng et al reported that they did not observe the expression of glypican-1 at short-term (0.5h) by Western blot or immunostaining, but detected an increased fluorescence intensity at long-term (24h) by immunofluorescence staining and confocal microscopy in 15 dyn/cm^2-exposed endothelial cells, and indicated the expression of glypican-1 contributed to the adaptive changes of glycocalyx at long-term.

Here, we further detected the mRNA expression of glypican-1 during a different exposure time under distinct magnitudes of shear stress (Figure 5). Notably, all the three magnitudes of shear stress resulted in a sharply significant increase in mRNA expression of glypican-1 after 24h long-term exposure. Compared with static control, there was a 1.7-, 3.4- and 4.1-fold increase at long-term (24h) under 4, 10, 15 dyn/cm^2, respectively, but was not significantly changed at short-term (0.5h). This is consistent with the previous results (10).

At both 2h and 8h, the mRNA expression levels of glypican-1 did not significantly changed under 4 dyn/cm^2 (Figure 5A) and 10 dyn/cm^2 (Figure 5B) of shear stress, respectively, while those were obviously elevated under 15 dyn/cm^2 (Figure 5C), suggesting that the glypican-1 mRNA expression is dependent on the shear stress magnitude.

HSPGs mRNA expression after short-term and long-term shear stress exposures

To address the importance of HSPGs components, we further investigated the difference in mRNA expression level between syndecans and glypican-1 (Figure 6). At static condition, syndecans mRNA and glypican-1 mRNA expressed in the order: syndecan-1 > syndecan-4 > syndecan-3 > syndecan-2 > glypican-1.

At 0.5h (Figure 6A and B), syndecan-1 mRNA was the most expressed one among those HSPGs under 4 dyn/cm^2 of shear stress, and syndecan-4 mRNA level was the most elevated one under 10 dyn/cm^2 of shear stress. Both syndecan-1 and syndecan-4 mRNAs under 15 dyn/cm^2 of shear stress were at high level. Although the syndecan-2 and -3 mRNAs were significantly changed under shear stress, the changes in their mRNA levels were relatively smaller than the others in those conditions.

At 24h (Figure 6C and D), the gypican-1 mRNA was increased with shear stress magnitudes (under 4 dyn/cm^2 vs. 10 or 15 dyn/cm^2, P<0.05), but it, not the greatest

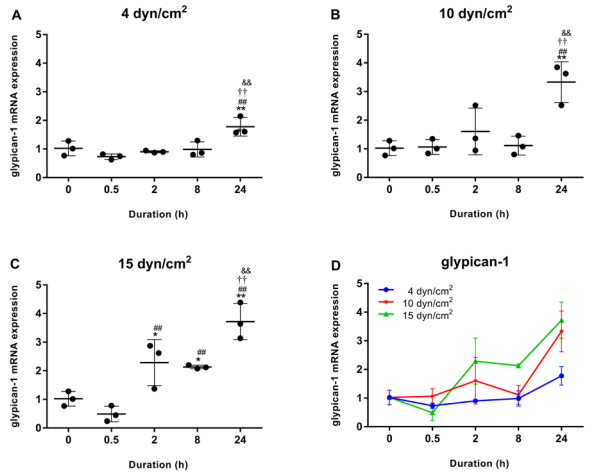

Figure 5. Glypican-1 mRNA expression in HUVECs under shear stress. After exposure to hemodynamic shear stress (A) 4 dyn/cm^2, (B) 10 dyn/cm^2 and (C) 15 dyn/cm^2 for 0h, 0.5h, 2h, 8h and 24h, respectively, the mRNA expressions of glypican-1 in HUVECs were determined by qRT-PCR and normalized to GAPDH mRNA (n=3). The graphs show fold changes in expression of glypican-1 in HUVECs under shear stress with respect to that in static conditions. (D) For comparison, a summary of the gene expression levels of glypican-1 under hemodynamic shear stress was present. **P*<0.05, ***P*<0.01 vs. 0h; #*P*<0.05, ##*P*<0.01 vs. 0.5h; †*P*<0.05, ††*P*<0.01 vs. 2h; &*P*<0.05, &&*P*<0.01 vs. 8h.

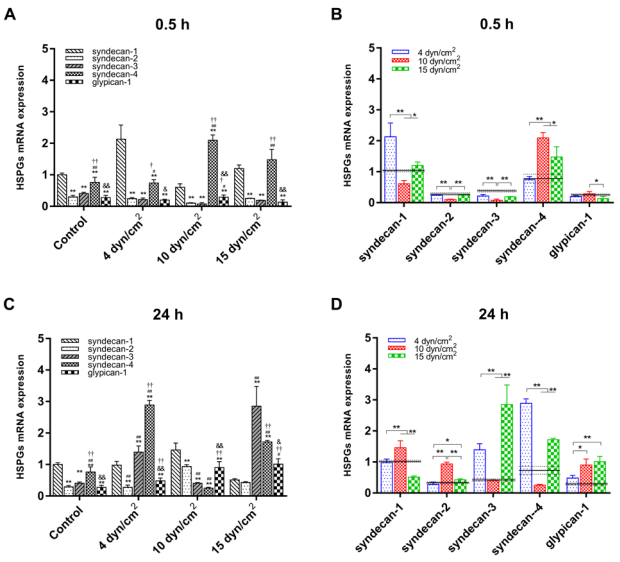

Figure 6. The mRNA expressions of HSPGs in HUVECs during short-term and long-term shear exposure. After exposure to three different magnitudes hemodynamic shear stress for 0.5h (A and B) or 24h (C and D), respectively, the mRNA expressions of syndecans and glypican-1 in HUVECs were determined by qRT-PCR and normalized to GAPDH mRNA (n=3). The final results present the fold changes in mRNA expression of HSPGs in HUVECs under shear stress to the mRNA level of syndecan-1 at static conditions. The static mRNA level of each gene was shown in B and D panels using the solid and dotted lines (Mean ± SD). (A) and (C) **P*<0.05, ***P*<0.01 vs. syndecan-1; #*P*<0.05, ##*P*<0.01 vs. syndecan-2; †*P*<0.05, ††*P*<0.01 vs. syndecan-3; &*P*<0.05, &&*P*<0.01 vs. syndecan-4. (B) and (D) **P*<0.05, ***P*<0.01.

changed HSPG mRNA. The greatest increased HSPG mRNA was syndecan-4 under 4 dyn/cm^2, and was syndecan-3 under 15 dyn/cm^2. The syndecan-1 mRNA was downregulated under 15 dyn/cm^2, while that was closed to the static level under 4 dyn/cm^2 and upregulated under 10 dyn/cm^2. Also, the increased extent of syndecan-2 mRNA was relatively smaller than others in those conditions.

Overall, the gene expression of HSPGs was different with duration and magnitudes of hemodynamic shear stress, which indicated distinct roles of the changed HSPGs in the endothelium dysfunction and homeostasis.

Discussion

Although numerous studies have demonstrated that HSPGs play a vital role in EC mechanotransduction of hemodynamic shear stress, how HSPGs expression regulated by shear stress was still unknown. In the present study, we demonstrated the temporal and magnitude effects of shear stress on HSPGs mRNA expression in HUVECs.

During the initial 0.5h of exposure, the mRNA expression levels of syndecan-1 were upregulated by 4 dyn/cm^2 shear stress (Figure 1A), but was not significantly changed by 10 dyn/cm^2 and 15 dyn/cm^2 (Figure 1B and C). This is consistent with the previous study that not observed a significant change in syndecan-1 intensity by immunostaining on ECs in response to 15 dyn/cm^2 shear stress for 0.5h (10). Among all those HSPGs, syndecan-1 was greatest upregulated by 4 dyn/cm^2 of shear stress in the short-term (0.5h, Figure 6A and B). Usually, 4 dyn/cm^2 of shear stress is presented in the atherosclerotic lesions at an early stage. The changes of syndecan-1 may associate with endothelial dysfunction and the development of atherosclerotic lesions. Another study has identified that syndecan-1 play an important role in maintaining physiological vascular function. They found that knock-out of syndecan-1 in endothelial cells abolished several signaling events in response to acute exposure (5-15 min) of atheroprotective flow (12 dyn/cm^2 of steady shear stress) (21).

Under 15 dyn/cm^2, although the expression syndecan-1 mRNA was at a high level, but not significantly increased compared to controls. Besides syndecan-1, the mRNA expression levels of syndecan-4 were significantly upregulated by 10 dyn/cm^2 and 15 dyn/cm^2 at 0.5h (Figure4 B and C). The differential expression of syndecan-4 between the low (4 dyn/cm^2) and the high (10 dyn/cm^2 and 15 dyn/cm^2) magnitudes of shear stress may imply its importance to homeostasis of blood vessels. It was reported that syndecan-4 acts as a central role in coordinating cytoskeletal changes that occur during focal adhesion and cell proliferation (26, 27). A previous research of Zeng et al (11) has been proved that the increase and redistribution of F-actin emerged after shear exposure of 30 min. Thus, the alterations of syndecan-4 induced by short-term shear stress may link with the changes of the cytoskeleton.

After 24h long-term exposure, the syndecan-1 mRNA expression level was downregulated under 15 dyn/cm^2 of shear stress, while upregulated under 10 dyn/cm^2 (Figure 1C). It seems to be contradictory to the previous immunofluorescence results that syndecan-1 increased significantly after exposure to 15 dyn/cm^2 shear stress at 24h (11). In fact, the syndecan-1 mRNA expression level under 15 dyn/cm^2 was dramatically

elevated at 2h, was decreased to 1.43 fold of control at 8h, and was further decreased to 0.51 fold of control at 24h. This also seems contradictory to some literature. It has suggested that syndecan-1 is an important atheroprotective molecule. Voyvodic et al (21) found that syndecan-1 knock-out endothelial cells after exposure to 12 dyn/cm^2 of shear stress for 24h shifted to an inflammatory/pro-atherosclerotic phenotype, in contrast to the atheroprotective phenotype of wild-type cells. Koo et al (17) also demonstrated that syndecan-1 expression was specifically reduced in endothelial cells under atheroprone flow condition for 7 days, but increased under atheroprotective flow condition for 7 days. The differences between mRNA levels that presented in the present study and protein levels that reported by those literatures are possible due to the different flow pattern and duration, delay of the translational expression, or distinct post-transcriptional regulation patterns. To clear the point, the protein levels of HSPGs under distinct magnitudes of shear stress will carry out in our future study.

Syndecan-3 is expressed at a relatively low level on ECs. Although syndecan-3 has microdomain similarity with syndeccan-1 (13, 14), the change of syndecan-3 was very different from that of syndecan-1. Under both 4 dyn/cm^2 (Figure 3B) and 15 dyn/cm^2 (Figure 3C) of shear stress, the mRNA expression levels of syndecan-3 presented a significant increase at 24h. Interestingly, syndecan-3 mRNA was expressed highest among all those HSPGs under 15 dyn/cm^2 of shear stress (24h, Figure 6C and D). It is suggested that syndecan-3 play an important role in response to the long-term high magnitude of shear stress in ECs.

Syndecan-4 was the expressed greatest HSPG under 4 dyn/cm^2 of shear stress at 24h. As mentioned above, in the short-term, syndecan-1 was greatest upregulated by 4 dyn/cm^2 of shear stress. It is indicated that syndecan-4 shows profound importance in the long-term exposure of low magnitude of shear stress in ECs.

Although the syndecan-2 and -3 mRNAs were significantly changed under different magnitudes of shear stress with 0.5h short-term exposure, respectively, the changes in their mRNA levels were relatively smaller than that in syndecan-1 and -4 in those conditions (Fig 6B), indicating a significant but rather mild contribution of them in the initial mechanism of endothelial mechanotransduction of shear stress.

For glypican-1, most researchers focused on upstream and downstream of glypican-1 molecular events, but they ignore the expression characteristics itself. The previous experimental evidence, analyzing by Western blot and immunofluorescence suggested that glypican-1 significantly increased at 24h but not 30 min in ECs that subjected to 15 dyn/cm^2 of shear stress (10, 11). These results are consistent with our data. In long-term exposure, glypican-1 is associated with NO signaling. Glypican-1 mediated flow (15 dyn/cm^2) -induced NO signaling via eNOS activation at 24h (18, 32).

It was demonstrated the triphasic adaptive response (induction period, early adaptation, and late remodeling) of the endothelial transcriptome under shear stress (33). Interestingly, syndecan-1 mRNA expression levels were increased under 4 dyn/cm^2 for 0.5 and 8h, showing a "bi-Peak" form (Fig. 1A). Similar "bi-Peak" form could also observe in the syndecan-3 mRNA expression level under 15 dyn/cm^2 (Fig. 3C), the syndecan-4 mRNA expression level under 4 or 15 dyn/cm^2 (Fig. 4A and C), and the glypican-1 mRNA expression level under 10 and 15 dyn/cm^2 (Fig. 5B and C). On the other hand, "one-Peak" form presented in the syndecan-1 mRNA expression levels under 10 and 15 dyn/cm^2 (Fig. 1B and C), the syndecan-2 mRNA expression levels under 10 and 15 dyn/cm^2 (Fig. 2B and C), the syndecan-3 mRNA expression levels under 4 dyn/cm^2 (Fig. 3A), the syndecan-4 mRNA expression levels under 10 dyn/cm^2 (Fig. 4B), and the glypican-1 mRNA expression levels under 4 dyn/cm^2 (Fig. 5A). These findings suggested that the adaptation process for each HSPG is quite distinct. It is possible that HSPGs have distinctly different role in mechanotransduction mechanism for the distinct magnitude of the shear stress, and final results in various responses of endothelial cells.

In conclusion, this study performed a systematic and comprehensive analyze of the relationship between shear stress and HSPGs mRNA expression in a temporal manner. Although only the transcriptional expression patterns of HSPGs were investigated here and translational or post-translational expression patterns, as well as their associated function, need to further study, we revealed the potential candidate components of the GCX in response to cardiovascular disease.

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