Hyperglycemic conditions modulate connective tissue reorganization by human vascular smooth muscle cells through stimulation of hyaluronan synthesis

Introduction

Patients with diabetes are at a markedly increased risk for atherosclerosis and its sequelae compared with non-diabetic patients (Wilson 2001; Marso and Hiatt 2006; Air and Kissela 2007). Diabetic patients also represent a high-risk group for restenosis and unfavorable clinical outcome after percutaneous coronary interventions (PCI) (Kornowski et al. 1997). Although diabetic patients tend to have a higher frequency of classic cardiovascular risk factors such as dyslipidemia and hypertension, these factors can only partially explain why cardiovascular diseases and their event rates, including restenosis after PCI, are more common in diabetic patients than in non-diabetics (Fuller et al. 1983; Spijkerman et al. 2002; West et al. 2004). As it is natural, attention has been paid to the importance of hyperglycemia (Deedwania et al. 2008). Improved glycemic control, particularly early on in diabetes, has been shown to have an important and long-lasting beneficial influence in reducing the risk of cardiovascular complications both in type 1 and in type 2 diabetic patients (Nathan et al. 2005; Holman et al. 2008). Evidence is available that, in addition to high fasting blood glucose concentration, postprandial hyperglycemia is involved in the onset of coronary heart disease (DECODE Study Group 2001; Norhammer et al. 2002; Ceriello et al. 2008). Regarding restenosis, optimal glycemic control has demonstrated to improve the clinical outcome after PCI as well (Corpus et al. 2004). However, although abundant data are available to explain how hyperglycemia could contribute to the development of cardiovascular pathologies (Brownlee 2001), additional studies are required to fully understand the mechanisms of poor glycemic control leading to accelerated atherogenesis and worse clinical outcome after PCI among diabetic patients.

Changes in the composition and assembly of extracellular matrix (ECM) molecules are critically involved in the initial and later stages of atherosclerosis (Wight 1996; Raines 2000). For example, according to the response-to-retention hypothesis of atherosclerosis, accumulation of specific ECM molecules, namely proteoglycans and glycosaminoglycans (GAGs), has been postulated to favor the subendothelial retention of lipoproteins and to promote subsequent lesion formation (Williams and Tabas 1995; Tabas et al. 2007). Alterations in ECM molecules influence not only lipoprotein turnover, but they also modulate several other key events of atherogenesis including smooth muscle cell migration, proliferation and survival (Raines 2000). The above molecular and cellular events are also crucial in the process of restenosis fol-
lowing PCI (Farb et al. 2004; Toutouzas et al. 2004). Concerning the impact of diabetes on ECM of the vascular wall, both quantitative and qualitative ECM changes are evident as a result of hyperglycemia (Wasty et al. 1993; Heickendorff et al. 1994; Bobbink et al. 1997; Erikstrup et al. 2001; McDonald et al. 2007).

An ECM molecule that has been linked to cardiovascular diseases and restenosis is hyaluronan (HA), a large, nonsulfated GAG (Riessen et al. 1996; Chung et al. 2002; Kolodgie et al. 2002; Toole et al. 2002; Farb et al. 2004; McDonald et al. 2007; Nieuwdorp et al. 2007). It is synthesized by a family of enzymes called HA synthases (HASs), which in mammals comprises three members, named in the order of their discovery as HAS1–3 (Weigel and DeAngelis 2007). The HASs differ from each other, e.g., in their catalytic rates and the final molecular weight of HA synthesized by each of them (Itano et al. 1999). As regards factors influencing HA metabolism in diabetes, high glucose concentration has been shown to be a stimulator of HA production (Takeda et al. 2001; Wang and Hascall 2004), although opposing views have also been presented (Erikstrup et al. 2001). We have earlier demonstrated that the serum of patients with type 1 and type 2 diabetes stimulates HA synthesis by human vascular smooth muscle cells (VSMCs) cultured on plastic (Järveläinen et al. 1986, 1987). The proposed roles described for HA during atherogenesis and restenosis are numerous, including its inflammation potentiating action (de La Motte et al. 1999) as well as its stimulatory effect on platelet adhesion (Koshiishi et al. 1994) and smooth muscle cell proliferation and migration (Evanko et al. 1999; van den Boom et al. 2006).

In this study, we have used a three-dimensional CGC assay to examine whether high glucose concentration can directly modulate the capacity of VSMCs to reorganize ECM. Human genome microarrays were used to screen molecules involved in ECM modulation by high glucose concentration, and the expression of HASs and the production of HA were studied in more detail.

Materials and methods

Cell culture

Human VSMCs derived from the human umbilical cord (Ihalainen et al. 2007) were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Paisley, Scotland) containing 10% (v/v) fetal bovine serum (FBS; Biochrom AG, Berlin, Germany), 100 IU/mL penicillin, 100 μg/mL streptomycin (Sigma, Saint Louis, MO, USA) and 2 mM L-glutamine (Sigma, Steinheim, Germany). In CGC assays, DMEM without antibiotics was used in order to avoid possible disturbances by drugs on the experiments. VSMCs were divided into two groups: normoglycemic (NG) group treated with normal concentration of d-glucose (5 mM) and hyperglycemic (HG) group treated with high concentration of d-glucose (25 mM). Cells were cultured in the above glucose concentrations at the minimum of three passages before their use to ensure that they had enough time to adapt to different glucose conditions. The glucose concentration of the NG group corresponds to a normal physiologic glucose condition, and the glucose concentration of the HG group mimics the condition of diabetics in highly poor glycemic control.

CGC assay

Collagen solutions for contraction assays were prepared using rat tail type I collagen (BD Biosciences, Bedford, USA) (Järveläinen et al. 2004). In addition to collagen, the solutions contained the following components: DMEM (Gibco) with normal (5 mM) or high glucose (25 mM) concentration, FBS (Biochrom AG) and L-glutamine. In control experiments for high glucose concentration, mannitol at the final concentration of 20 mM was added to 5 mM glucose containing DMEM medium to ensure that the results could not be attributed to the effect of different osmolality between the culture media with normal and high glucose concentrations. All the above solutions were always prepared for four simultaneous contraction assays, and the assays were conducted in the wells of 24-well culture plates (Nunc, Roskilde, Denmark).

The collagen lattices for CGC assays were prepared in three phases. In phases 1 and 2, the solutions containing 0.5 mg/mL type I collagen, 10% FBS and, alternatively, 5 mM glucose, 25 mM glucose or 5 mM glucose + 20 mM mannitol in the culture medium. The above solution was first layered on the well, and then VSMCs (12.5 × 103 VSMCs per gel) in the same solution were gently detached from the wall of the wells to initiate collagen contraction, and the cultures were maintained for 24 h at 37°C in 95% air/5% CO2. At the end of the experiments, the gels were fixed with 1% neutral-buffered formalin at room temperature. The degree of gel contraction was assessed by measuring the area of the gels with an imaging densitometer (MCID Image Analyzer). All experiments were performed at least three times, and similar results were always obtained.

In the experiments examining the role of HA in the efficacy of the cells to contract collagen gels, bovine testicular hyaluronidase (HAase) (Type IV-S, Sigma) at concentrations of 15–150 U/mL was added to the solutions and assayed for CGC. As a control, identical cultures without HAase were performed.

In addition, treatment of VSMCs with 4-methylumbelliferone (4-MU) (Sigma), an inhibitor of HA synthesis (Kultti et al. 2009; Vigetti et al. 2009), was used to evaluate the contribution of HA to CGC. HG cultures were treated with 0.5 mM of 4-MU, and the effect on CGC was examined.

Actin staining of VSMCs within collagen lattices

In selected experiments, actin cytoskeletons of NG- and HG-treated VSMCs in contracted collagen gels were labeled with phalloidin conjugated to Alexa Fluor 488 (Molecular Probes, Eugene, OR, USA) (Järveläinen et al. 2004). Briefly, the contracted and fixed gels were washed with phosphate buffered saline (PBS) twice for 15 min/time, permeabilized with acetone for 20 min at −20°C and then blocked with 1% bovine serum albumin (BSA) in PBS; whereafter, incubation with Phalloidin Alexa Fluor 488 (Molecular Probes) in PBS was conducted. After phalloidin labeling, the gels were washed three times for 30 min with PBS at room temperature and viewed by epifluorescence with a Leica DMRB microscope.
Particle-exclusion test

Pericellular matrix of VSMCs of NG and HG groups was visualized using a particle-exclusion test. VSMCs were seeded in 25 cm² culture flasks with DMEM containing 10% FBS. After 24 h, 500 μL of washed human red blood cells in PBS was added to both cultures, and the cultures were photographed. To demonstrate the presence of HA in the pericellular matrix, 75 U/mL of bovine testicular HAase (Sigma) was added to the culture medium for 1 h before performing the particle-exclusion test.

Cell proliferation and viability determination

The proliferation of VSMCs treated with normal and high glucose concentration was assessed by plating identical cell counts (60,000 cells/well, approximately 40% confluence) on six-well plates. The number of cells attached on plastic was determined 24, 48 and 72 h later using a hemocytometer.

The viability of VSMCs in NG and HG groups was measured using a trypan blue exclusion test as described previously (Järvelläinen et al. 1985). Briefly, the cells were grown on plastic for 24 and 48 h in normo- and hyperglycemic conditions, and viable and dead cells were counted using a hemocytometer.

RNA extraction and microarray analysis

Total RNA was isolated from VSMCs using CsCl-centrifugation (Glisin et al. 1974), and the yield of RNA was determined by measuring the absorbance at 260 and 280 nm. To compare the effect of normal glucose concentration with that of high glucose concentration on the overall VSMC gene expression profile, Illumina Sentrix® Human-6 Expression BeadChips (Illumina, Inc., San Diego, CA, USA) were used. The microarray analyses were performed from two independently prepared specimens of separate experiments to ensure data reproducibility. Biotinylated complementary ribonucleic acid (cRNA) using the Illumina RNA Amplification kit (Ambion, Inc., Austin, TX, USA) was prepared using ~100 ng total RNA from both NG- and HG-treated VSMCs. The biotinylated cRNA was purified using the RNeasy kit (Qiagen, Valencia, CA, USA) and hybridized to the chips. The washing and scanning of the chips were performed according to standard Illumina protocols. The results were analyzed using Illumina’s BeadStudio software. Microarray results regarding the expression of HAS1–3 were confirmed by quantitative real-time polymerase chain reaction (RT-PCR) analysis as described below.

Quantitative RT-PCR

Quantitative PCR was performed in a MX3000P thermal cycler (Stratagene, La Jolla, CA). Messenger RNAs were analyzed with Absolute™ MAX 2-step QRT-PCR SYBR® Green kit (ABgene, Epsom, Surrey, UK), with specific primers shown in Table I. Acidic riboprotein PO (Arpo) was used as the control gene. Fold inductions were calculated using the formula 2−(ΔΔCt), where ΔΔCt is the ΔCt (treatment)−ΔCt (control). ΔCt is Ct Has1–3-Ct Arpo, and Ct is the cycle in which the detection threshold is crossed.

Enzyme-linked sorbent assay for hyaluronan

HA content in the culture media was measured with enzyme-linked sorbent assay (ELSA) as described (Hiltunen et al. 2002). Briefly, 96-well Maxisorp plates (Nunc, Roskilde, Denmark) were coated with 1 μg/mL of the HA binding complex of the cartilage aggrecan G1 domain and link protein (HABC), both of which were prepared in the Kuopio laboratory. HA standards (1–50 ng/mL) and samples diluted into 1% BSA in PBS were incubated in the wells for 1 h at 37°C. After washes, the wells were sequentially incubated with 1 μg/mL biotinylated HABC and horseradish peroxidase–streptavidin (1:20,000 in PBS, Vector Laboratories Inc., Burlingame, CA) for 1 h at 37°C, followed by 10 min incubation at room temperature with TMB substrate solution (0.01% 3,3′, 5,5′-tetramethylbenzidine, Sigma, and 0.005% H2O2 in 0.1 M sodium acetate, 1.5 mM citric acid buffer). The reaction was stopped with 50 μl of 2 M H2SO4, and the absorbances were measured at 450 nm.

Statistical analysis

Unpaired and paired Student’s t-tests were employed in statistical analyses. All P values <0.05 were considered statistically significant. The microarray data were analyzed with R language and environment for statistical computing program (R Development Core Team 2006). All genes that were downregulated or upregulated at least 0.7 times in a two-logarithmic scale in high glucose conditions compared with normal glucose conditions were considered to have biological relevance. Statistical analysis for the quantitative RT-PCR was performed as described above.

Results

Glucose inhibits contraction of type I collagen-rich gels by human vascular smooth muscle cells

Human VSMCs were used to examine the effect of glucose on the behavior of the cells. VSMCs were grown at the minimum of three passages in the culture medium containing either 5 mM d-glucose (nормoglycemic group, NG group) or 25 mM d-glucose (hyperglycemic group, HG group). The cells were seeded into type I collagen-rich matrix, and their elasticity was assessed by particle-exclusion test. VSMCs in HG group seem to reorganize actin into thinner fibers compared with their counterpart cells grown in HG conditions. Mannitol was used as an osmotic control and had no effect (Figure 1), eliminating the possibility that the difference in cell-mediated CGC between NG and HG conditions was due to osmotic reasons. The more powerful contraction of the gels by VSMCs of NG group was also seen at the cytoskeletal level as a more intense staining for filamentous actin (Figure 2). VSMCs in HG group seem to reorganize actin into thinner fibers compared with their counterpart cells grown in HG conditions.
and thus presumably weaker microfilaments resulting in cells that in HG group cannot contract collagen gel as potently as cells in the NG group.

The cells in HG conditions proliferated slightly faster, with a significantly higher number of cells at the 48-h time point (Figure 3). Different glucose concentrations had no influence on cell viability (data not shown). These results collectively excluded the possibility that the more powerful contraction of gels by VSMCs in NG conditions was due to increased number of cells.

**VSMCs cultured in normoglycemic and hyperglycemic conditions have differential gene expression profiles**

It is well established that cell-mediated CGC is a very complex process requiring the interplay of various cellular and extracellular molecules (Ngo et al. 2006). To obtain information about the candidate molecules involved in the inhibitory action of high glucose concentration on CGC, total RNA was isolated from VSMCs grown within collagen gels in NG or HG conditions and used for gene expression profiling applying Illumina Sentrix® Human-6 Expression BeadChips. The results demon-

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**Fig. 1.** Contraction of type I collagen-rich gels by VSMCs in normoglycemic (NG) and hyperglycemic (HG) conditions. (A) Representative images of collagen gels after 24-h contraction by cells of NG (5 mM glucose) or HG (25 mM glucose) groups. Mannitol (5 mM glucose + 20 mM mannitol) was used as an osmotic control and had no effect. Quantification of the collagen gels with an imaging densitometer (B). Statistically significant differences in gel contraction between NG and HG cultures are indicated by asterisk (**p < 0.001, unpaired Student’s t-test). Capped bars on top of the columns indicate standard deviations (n = 12).

**Fig. 2.** More powerful contraction of gels by VSMCs of NG group can be seen as a more abundant staining for filamentous actin by phalloidin labeled with Alexa Fluor 488. VSMCs cultured in NG conditions are shown in (A) and HG treated VSMCs in (B). Similar images were obtained from three independent experiments. Scale bars, 100 μm.
strated that out of 26,091 RefSeq genes represented on the microarray, 219 showed at least 0.7-fold change in expression on the two-logarithmic scale between the two groups. Of the regulated genes, 78 genes (36%) showed increased expression, and 141 genes (64%) showed decreased expression in HG conditions compared with NG conditions. Clustering analysis using Database for Annotation, Visualization, and Integrated Discovery (DAVID, http://david.abcc.ncifcrf.gov/home.jsp) (Dennis et al. 2003) led our interest to increased HAS2 expression. HAS2 is one of the three transmembrane glycosyltransferases that produce HA. Previously, we have shown that serum of type 1 and type 2 diabetic patients increases HA synthesis of VSMCs compared with the effect of serum of healthy controls (Järveläinen et al. 1986, 1987). Furthermore, in a recent study, perturbation of HA metabolism was shown to be associated with structural changes of the arterial wall of patients with diabetes (Nieuwdorp et al. 2007).

Expression of all three mammalian HA synthases is upregulated in VSMCs grown in hyperglycemic conditions

The result of the microarray analysis that HAS2 expression is increased in response to high glucose concentration was confirmed by quantitative RT-PCR (Figure 4B). Furthermore, RT-PCR analyses revealed that the expression of the two other HA synthases, HAS1 and 3, was also upregulated in VSMCs grown in HG conditions (Figure 4A and C). In VSMCs of HG group, relative HAS1, 2 and 3 expressions were increased by 1.5- to 2-fold compared with their expressions in the NG group.

Synthesis of HA is increased concomitantly with stimulated HAS1–3 expressions in VSMCs in response to high glucose concentration

The media from cultures of NG and HG groups were collected, and HA secreted in the culture medium was analyzed with an ELSA. The results showed that the amount of secreted HA in HG group was increased with statistical significance (Figure 5). This finding was in line with a higher content of HA also on cell surface, as the pericellular matrix, visualized with a particle-exclusion test, showed enlarged halo-like area around the VSMCs grown in HG conditions (compare Figure 6A and B). The matrix around VSMCs in HG group could be degraded by treatment with HAase (Figure 6C), an enzyme that randomly cleaves the β-N-acetylhexosamine-β 1,4 glycosidic bonds in HA.

Hyaluronidase and 4-methylumbelliferone counteract the diminished collagen gel contraction efficacy of VSMCs grown in hyperglycemic conditions

To investigate whether HA plays a causal role in the diminished CGC efficacy of VSMCs grown in HG conditions, the effects of HAase and 4-MU treatments on cell-mediated gel contraction were examined. Treatment of the cells with 15–150 U/mL of HAase dose-dependently enhanced the contraction of type I collagen-rich gels by VSMCs of HG group. Actually, HAase treatment restored the diminished CGC efficacy of cells grown in HG conditions to the NG control level (Figure 7). HAase treatment also potentiated markedly CGC by VSMCs of NG group (Figure 7), further supporting the crucial importance of HA as a regulator of ECM reorganization. In conformity with this, treatment of VSMCs with 4-MU, a relatively specific inhibitor of HA synthesis, resulted in a modest but statistically significant increase in the capacity of the cells of HG group to contract collagen gels (Figure 8A) and decrease the synthesis of HA (Figure 8B). This also supports the causal role of HA in the remodeling process of ECM.

Discussion

In this study, we used a CGC assay (Järveläinen et al. 2004) to elucidate whether glucose can directly influence ECM reorganization by VSMCs. We demonstrated that, in comparison with normal glucose concentration, high glucose concentration has an inhibitory effect on the efficacy of VSMCs to contract collagen gels and that this effect is associated with altered form of actin cytoskeleton. The possibility that the diminished CGC in HG conditions was due to osmotic reasons was excluded by demonstrating that a corresponding concentration of mannitol did not influence contraction. Analogous to our results, the contraction phase during wound healing has been shown to be impaired in diabetes (Velander et al. 2008). Furthermore, in diabetes the intimal area of vascular wall has been demonstrated to be increased (McDonald et al. 2007), and the structure of the tunica media altered due to changes of ECM assembly (Heickendorff et al. 1994).
Fig. 4. Expression of HAS1–3 is upregulated in VSMCs cultured in HG conditions. Results of the quantitative RT-PCR are shown as relative HAS1–3 expression (A–C) by VSMCs in HG conditions compared with NG conditions. Expression of HASs in NG conditions was marked as 100% in all experiments. The levels of HASs in HG conditions were calculated by using the same coefficients as for their counterparts in NG conditions. Capped bars on top of the columns indicate standard deviations (n = 3).

Fig. 5. Production of HA is increased by VSMCs in the HG group. HA content in the culture medium was measured with ELSA. Results are shown as nanograms HA per cell per 48 h. Statistically significant differences in the content of HA between NG and HG cultures are indicated by asterisk (***P < 0.01, unpaired Student’s t-test). Capped bars on top of the columns indicate standard deviations (n = 3).
To get an overall view on the candidate molecules involved in the altered contraction phenomenon by VSMCs in response to HG conditions, we used whole human genome microarrays to screen the gene expression profiling of cells in different glycemic conditions. An altered expression pattern was found in over 200 genes. From the upregulated genes, we chose HAS2 for more detailed examination since its upregulation was in agreement with our previous studies showing that serum from patients with diabetes stimulates HA synthesis of human aortic smooth muscle cells cultured on plastic (Jarvelainen et al. 1986, 1987). HAS2 is one of the three known HASs, and its expression has previously been shown to be increased, e.g., in human mesangial cells in HG conditions (Yevdokimova 2006). Using quantitative RT-PCR, we confirmed the upregulation of HAS2 and found that actually the expression of all three HASs (HAS1–3) was increased. The increased expression of HASs led to a higher level of HA production, an observation consistent with a number of previous studies indicating transcriptional regulation of HA synthesis (Jacobson et al. 2000; Evanko et al. 2001; Karvinen et al. 2003; Sussmann et al. 2004; Saavalainen et al. 2007). While our experimental setting did not reveal the relative importance of the three HASs, earlier studies have suggested that HAS2 is the dominant HAS expressed in vascular smooth muscle cells (Evanko et al. 2001; Sussmann et al. 2004). The same has been shown for other cell types of mesodermal origin such as lung fibroblasts (Jacobson et al. 2000). Furthermore, treatment of arterial smooth muscle cells by different external stimuli, e.g., platelet-derived growth factor (Evanko et al. 2001), vasodilatory prostaglandins (Sussmann et al. 2004) or glucose (Yevdokimova 2006), has been shown to increase HAS2 expression. Thus, it is probable that HAS2 is also playing a major role in our results.

Hyaluronan is known to be retained on the surface of cells by remaining attached to HASs (Kulitti et al. 2006) or bound to specific receptors like CD44 and to form a coat around the cells (Evanko et al. 2007). In our study, HG conditions greatly expanded a HA-dependent pericellular matrix around the VSMCs, as detected with the particle-exclusion test. Interestingly, such a HA-rich pericellular matrix has been suggested to be required for proliferation and migration of VSMCs (Evanko et al. 1999). Accordingly, a slightly enhanced proliferation rate of VSMC by HG was also found in the present study.

The degradation of HA by HAase in the CGC assay in both HG and NG conditions increased the ability of the cells

![Fig. 6. Pericellular matrices of VSMCs visualized by particle-exclusion test. Cells were seeded on 25 cm² culture flasks and cultured for 24 h. In NG group (A), only a small pericellular matrix was seen compared with a prominent halo around the VSMCs in HG group (B). Treatment with HAase degraded the pericellular matrix of HG cells, confirming its dependence on HA (C). Arrows indicate the borders of the pericellular matrix, bars = 100 μm.](https://academic.oup.com/glycob/article-abstract/20/9/1117/1988892)

![Fig. 7. Exogenous HAase potentiates CGC of VSMCs in HG and NG groups and restores the contraction of cells in HG conditions to the NG control level. Columns represent quantification of the collagen gels after HAase treatment (15–150 U/mL). Statistically significant differences in gel contraction between NG control and HG cultures are indicated by asterisk (***P < 0.001, unpaired Student’s t-test). Note that the treatment of HG culture with 150 U/mL HAase results in similar contraction as NG control (dashed line). HAase treatment also potentiates the contraction of NG cultures with statistical significance (for 75 and 150 U/mL, P < 0.01, asterisks are not shown). Capped bars on top of the columns indicate standard deviations (n = 12).](https://academic.oup.com/glycob/article-abstract/20/9/1117/1988892)
to contract collagen gels, thus emphasizing the causal role of HA in the regulation of VSMC-mediated connective tissue reorganization. Because the type of hyaluronidase used in this study can also use chondroitin sulfate as a substrate, it is possible that proteoglycans containing chondroitin sulfate such as versican (Wight and Merrilees 2004) can contribute to the change in gel contraction. However, since the contraction was also enhanced by 4-MU, a relatively specific inhibitor of HA synthesis in VSMC (Vigetti et al. 2009), we believe that HA was mainly responsible for the effect. Since 4-MU does not inhibit the synthesis of HA completely and does not remove preexisting HA, its effect is not expected to be as strong as that of HAase.

The pathways whereby hyperglycemia leads to increased HA synthesis by VSMCs, which in turn causes inhibition in CGC, are not known. However, it has been documented earlier that diabetic vascular complications in response to hyperglycemia can result via four main pathways: increased polyl pathway flux, increased advanced end-product (AGE) formation, activation of protein kinase C isoforms and increased hexosamine pathway flux (Brownlee 2001). Because HA is composed of repeating units of D-glucuronic acid and N-acetyl-glucosamine linked together through alternating β1,4 and β1,3 glycosidic bonds, it is tempting to speculate that hexosamine pathway forms the mechanistical basis for our results. On the other hand, HA has been demonstrated to modulate cell adhesion and migration involving CD44-mediated signaling through ERK1/2 (Vigetti et al. 2008).

In conclusion, we demonstrated that in HG conditions the gene expression profile of VSMCs is remarkably altered and that the HASs are among the upregulated genes resulting in an increase in HA production. We also showed that increased HA synthesis modulates the ability of the cells in HG conditions to reorganize collagen-rich matrix. This provides a new mechanistic explanation behind macrovascular complications in diabetes.

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**Conflict of interest statement**

None declared.
Abbreviations

BSA, bovine serum albumin; CGC, collagen gel contraction; cRNA, complementary ribonucleic acid; DME/M, Dulbecco’s modified Eagle’s essential medium; ECM, extracellular matrix; ELSA, enzyme-linked sorbent assay; FBS, fetal bovine serum; GAG, glycosaminoglycan; HA, hyaluronan; HAase, hyaluronidase; HABC, HA binding complex of the cartilage aggrecan G1 domain and link protein; HAS, hyaluronan synthesase; HG, hyperglycemic; 4-MU, 4-methylumbelliferone; NG, normoglycemc; PBS, phosphate buffered saline; PC1, percutaneous coronary intervention; RT-PCR, real-time polymerase chain reaction; VSMCs, vascular smooth muscle cells.

References


