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A global profile of glucose-sensitive endothelial-expressed long non-coding RNAs

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ABSTRACT

Hyperglycemia-related endothelial dysfunction is believed to be the crux of diabetes-associated micro- and macrovascular complications. We conducted a systematic transcriptional survey to screen for human endothelial long non-coding RNAs (lncRNAs) regulated by elevated glucose levels. LncRNAs and protein-coding transcripts from human umbilical vein endothelial cells (HUVECs) cultured under high (25 mmol/L) or normal (5 mmol/L) glucose conditions for 24 hours were profiled with the Arraystar Human LncRNA Expression Microarray V3.0. Of the 30,586 lncRNAs screened, 100 were significantly upregulated and 186 appreciably downregulated ($P<0.05$) in response to high glucose exposure. In the same HUVEC samples, 133 of the 26,109 mRNAs screened were upregulated and 166 downregulated. Of these 299 differentially expressed mRNAs, 26 were significantly associated with 28 differentially expressed long intergenic non-coding RNAs ($P<0.05$). Bioinformatics analyses indicated that the mRNAs most upregulated are primarily enriched in axon guidance signaling pathways; those most downregulated are notably involved in pathways targeting vascular smooth muscle cell contraction, dopaminergic signaling, ubiquitin-mediated proteolysis, and adrenergic signaling. This is the first lncRNA and mRNA transcriptome profile of high glucose-mediated changes in human endothelial cells. These observations may prove novel insights into novel regulatory molecules and pathways of hyperglycemia-related endothelial dysfunction and accordingly diabetes-associated vascular disease.

Abstract word count: 200

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INTRODUCTION

Long-term follow-up studies in large type 2 diabetes cohorts have demonstrated that diabetes-associated microvascular and macrovascular complications are directly related to the severity and duration of hyperglycemia (Hayward et al. 2015; Holman et al. 2008). A healthy endothelium is crucial to vascular health and anomalies in endothelial function, secondary to hyperglycemia (Avogaro et al. 2011), may underlie the clinical observations of elevated cardiovascular risk in individuals with diabetes relative to those without the disease (Booth et al. 2006). Recognized contributors of diabetes-associated endothelial dysfunction include protein kinase C (PKC), hexosamine and polyol pathways, and advanced glycation end-products (AGEs), all of which promote reactive oxygen species bioavailability and reduce nitric oxide production thereby altering endothelial cell structure and physiology (Giacco and Brownlee 2010; Shaw et al. 2014).

Long non-coding RNAs (lncRNAs), previously perceived as transcriptional noise due to their low expression and poor evolutionary conservation (Hangauer et al. 2013), have emerged as potential molecular determinants of a wide range of diseases. Similar to mRNAs, lncRNAs are transcribed by RNA-polymerase II, spliced, polyadenylated, and capped (Chew et al. 2013). They contain signature histone H3 on lysine 4 (H3K4) and Lys36 on histone (H3K36) trimethylation patterns that demarcate active promoters and transcribed regions (Fatica and Bozzoni 2014); and they can regulate nearby protein-coding genes in *cis* or those located further in *trans* (Rinn et al. 2007).

Transient hyperglycemia triggers gene-activating epigenetic changes and molecular cross-talks that are fundamental to the initiation and advancement of vascular anomalies (Cooper and El-Osta 2010). Morán and colleagues noted aberrant expression of the

lncRNAs *KCNQ10T1* and *HI-LNC45* in pancreatic islets from individuals with type 2 diabetes and upregulation of the long intergenic non-coding RNAs (lncRNAs) *HI-LNC78* and *HI-LNC80* in cultures of purified human pancreatic islets following exposure to a high glucose milieu (Moran et al. 2012). Another group also working with human pancreatic islets found significant associations between 17 lncRNAs and glycated hemoglobin levels (Fadista et al. 2014). Fibrovascular membranes of individuals with diabetes had higher levels of the lncRNA myocardial infarction-associated transcript (MIAT) than idiopathic epiretinal membranes from persons without diabetes (Yan et al. 2015). The notion that lncRNAs may regulate the diabetes-atherosclerosis axis is supported by reports that ANRIL, a lncRNA associated with risk for coronary atherosclerosis, carotid arteriosclerosis and peripheral artery disease, maps to an established type 2 diabetes susceptibility locus (Chen et al. 2014). The goal of this study was accordingly to address the potential that high glucose-associated changes in lncRNA expression may be fundamental to diabetes-associated vascular disorders.

MATERIALS AND METHODS

Cell Culture. Human umbilical vein endothelial cells (HUVECs from pooled donors, Lonza) were cultured in EC growth medium-2 (EGMTM-2 BulletkitTM; Lonza) supplemented with growth factors, serum and antibiotics at 37 °C in humidified 5% CO₂. Confluent HUVECs were starved overnight before exposure to study conditions. HUVECs designated to the control (normal glucose) group were maintained for 24 hours in 5 mmol/L glucose and 20 mmol/L mannitol to account for changes that may be triggered by osmolarity differences. The remaining HUVECs were maintained for the same duration in glucose-enriched (final glucose concentration 25 mmol/L) media.

Microarray Profiling. Total RNA was isolated from HUVECs using TRIzol reagent and quantified with the NanoDrop ND-1000 spectrophotometer. RNA integrity was confirmed by standard denaturing agarose gel electrophoresis. The expression profile of 30,586 human lncRNAs and 26,109 protein-coding transcripts was conducted with the Arraystar Human LncRNA Microarray V3.0. Sample labeling and array hybridization were performed on the Agilent Array platform. Briefly, total RNA from each sample was amplified and transcribed into fluorescent cRNA (Arraystar Flash RNA Labeling Kit, Arraystar) before 1 µg of each labeled cRNA was hybridized onto the microarray slide. The hybridized arrays were washed, fixed and scanned with the Agilent DNA Microarray Scanner (Product# G2505C). The acquired array images were analyzed with the Agilent Feature Extraction software (version 11.0.1.1). Quantile normalization and subsequent data processing were performed with the GeneSpring GX v11.5.1 software package (Agilent Technologies). Volcano plot filtering, set at a threshold of ≥ 2.0 folds, was used to screen for lncRNAs and

mRNAs that exhibited significantly different ($P < 0.05$; unpaired t-test adjusted for multiple testing with the Benjamini Hochberg method to minimize the false discovery rate) expression levels in the two study groups. Pathway analysis was based on the current Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Gene Ontology (GO) analysis was performed with the topGO package of bioconductor system.

qPCR Validation

The levels of select lncRNAs in HUVECs cultured as described above were evaluated using standard qPCR methods.

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RESULTS

The 6 samples evaluated had a 2:1 intensity ratio for their 28S:18S rRNA bands and OD260/OD280 ratios of >1.8 thereby verifying RNA integrity, purity and concentration (Supplementary Fig. 1). Box-and-Whisker plots constructed to visualize the distribution of the fluorescent intensities revealed very similar normalized log2 ratios for both lncRNA and mRNA, and accordingly comparable quality of the array data, across the board (Supplementary Fig. 1).

Scatter plots provided a profile of HUVEC lncRNAs (Fig. 1A) and mRNAs (Fig. 1B) that were upregulated, downregulated or unaffected by exposure to a high vs. normal glucose environment. Overall, the average fold changes of lncRNAs and mRNAs differentially expressed under the two study conditions were similar (Fig. 1C and 1D). Subsequent volcano plot filtering uncovered 100 significantly upregulated and 186 significantly downregulated lncRNAs in HUVECs cultured in high glucose media relative to expression in those maintained in media with normal glucose levels (Fig. 1E; $P < 0.05$). LncRNAs that demonstrated the greatest differences in expression ranged from 171 bp to 62.7 kb and are detailed in Table 1. Specifically, ENST00000421257 (RNA length: 825 bp, chromosome 20) was the most upregulated lncRNA and ENST00000559989 (RNA length: 7790 bp, chromosome 15) the most downregulated in HUVECs subjected to high vs. normal glucose culture conditions. Elevated glucose-associated changes were also noted amongst 299 mRNAs with 133 upregulated and 166 downregulated (Fig. 1F; $P < 0.05$). qPCR conducted on 5 select lncRNAs confirmed the trend observed with the lncRNA Array analysis (Table 2).

Supplementary Fig. 2 shows the dendrograms generated for hierarchical analysis of clustered lncRNAs and mRNAs that were differentially expressed in HUVECs cultured in media with high vs. normal glucose. Although glucose-sensitive lncRNAs were found in every human chromosome, most were located on chromosomes 1, 11 and 17 (Fig. 2A). Further probing revealed that while these differentially expressed lncRNAs are expressed along the entire length of the chromosomes (Fig. 2B), there is a notable abundance in lincRNAs (Fig. 2C).

We conducted additional profiling and found that 28 lincRNAs that were differentially expressed in response to elevated glucose are in close proximity (<300 kb) to 26 similarly regulated mRNAs. The fold change calculated for each lincRNA and its associated mRNA is summarized in Fig. 3A. Of note, 15 of the 28 lincRNAs demonstrated a direct correlation in fold change with its associated mRNA, whereas the remaining 13 displayed an inverse correlation.

Pathway analysis with the current KEGG database yielded several pertinent findings (Fig. 3B). In brief, lncRNAs upregulated in response to elevated glucose are involved in axon guidance while those downregulated have known associations with vascular smooth muscle cell contraction, dopaminergic synaptic signaling, ubiquitin mediated proteolysis, adrenergic signaling in cardiomyocytes, insulin signaling, and apoptosis. Table 3 lists the biological pathways that have been linked with the mRNAs found to be differentially expressed in HUVECs cultured in media with elevated vs. normal glucose.

GO analysis grouped the differentially expressed mRNAs under the following three categories: Biological Processes, Cellular Component, and Molecular Function (Table 3). GO terms most appreciably associated with differentially enriched mRNAs were cellular and

metabolic processes (Biological Processes); cell part, cell, intracellular part; and intracellular (Cellular Component); and binding and protein binding (Molecular Function).

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DISCUSSION

This is the first report providing a complete global profile of glucose-regulated human endothelial cell-expressed lncRNAs and mRNAs. Specifically, our transcriptome survey revealed that the expression of 286 lncRNAs and 299 mRNAs were significantly different in HUVECs exposed to a high vs. normal glucose environment for 24 hours. Amongst the differentially expressed mRNAs, 26 were associated with 28 differentially expressed lncRNAs. The most upregulated mRNAs were commonly associated with axon guidance signaling pathways while those most downregulated were associated with vascular smooth muscle cell contraction, dopaminergic signaling, ubiquitin-mediated proteolysis, and adrenergic signaling networks.

Contrary to the tenet that protein-coding genes form the sole basis of gene regulation, growing evidence positions mutated lncRNAs and dysregulated lncRNA function within the scaffold of a wide range of diseases (Wahlestedt 2013; Wapinski and Chang 2011). Previous reports contend that the expression of some lncRNAs, arbitrarily defined as non-coding RNAs greater than 200 nucleotides in length (Ma et al. 2013), fluctuates with transitions from a euglycemic to hyperglycemic environment (Fadista et al. 2014; Moran et al. 2012; Yan et al. 2015). Others have shown that insulin and insulin-like growth factors transiently downregulated nuclear transcripts of the lncRNA colorectal neoplasia differentially expressed (CRNDE) gene in human colorectal and MCF-7 breast cancer cell lines (Ellis et al. 2014). Notwithstanding the morphological and functional differences between human and rodent islets and the weak conservation of human lncRNAs in rodent and *in vitro* models, it is worth noting that the compromised pancreatic islet structure and function observed in murine gestational diabetes were attributed to

dysregulated lncRNA H19 (Ding et al. 2012); 303 lncRNAs were aberrantly expressed in the retinas of mice with early diabetic retinopathy (Yan et al. 2014); and the evolutionary well conserved lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) was appreciably upregulated in RF/6A choroidal endothelial cells previously exposed to high glucose (Yan et al. 2014). The results described herein therefore extend these earlier findings that support a lncRNA-diabetes relationship and also provide a potential molecular account for why endothelial dysfunction often presents in the metabolic milieu of diabetes (Booth et al. 2006).

Sequence analysis indicated that lincRNAs had a dominant presence amongst the differentially expressed lncRNAs. Although intergenic regions are robustly enriched in disease-associated polymorphisms (Li et al. 2014), whether any, some or all of this subgroup of lincRNAs have functional relevance under altered glucose conditions and diabetes remains to be confirmed. Interestingly, expression associations were only detected for 28 of the 170 differentially expressed lincRNAs and with 26 differentially expressed mRNAs. This is not a surprising finding given that our knowledge of the biological significance, mechanism(s) of action, and target(s) of lncRNAs have not kept pace with our identification of novel lncRNAs. That said, our observations that the expression of 13 of the aforementioned lincRNAs directly correlated with that of their differentially expressed "partner" mRNA while an inverse correlation was noted for the remaining 15 lincRNAs and their associated protein-coding transcript is consistent with the understanding that lncRNAs both positively and negatively regulate gene expression (Wahlestedt 2013).

Similar to Yan et al. who worked with RF/6A choroidal endothelial cells (Yan et al. 2014), Puthanveetil et al. reported significantly higher MALAT1 transcripts in HUVECs incubated with 25 mmol/L D-glucose vs. 25 mmol/L L-glucose (osmotic control) and 5 mmol/L glucose (normal glucose) (Puthanveetil et al. 2015). However, while MALAT1 levels increased with time in the former study, the rise was transient in the latter peaking at the 12-hour incubation mark. Whether the contrasting outcomes were due to the different cell types studied or the different concentration of glucose (50 vs. 25 mmol/L) applied remains to be determined. Unlike these investigators, we did not detect a difference in MALAT1 expression between our normal and high glucose-treated HUVECs. Our experimental set up was the same as that of Puthanveetil's group but as we only harvested samples at the 24-hour point, we are unable to speak to the potential temporal changes in MALAT1 levels that may have also occurred in our HUVEC samples. Regardless, the collective evidence would appear to suggest that high glucose-regulated MALAT1 expression is time-dependent.

KEGG analysis revealed significant downregulation of coding transcripts that are involved in the ubiquitin-proteasomal system (UPS), a tightly controlled machinery involved in intracellular protein degradation and turnover (Jung et al. 2009). These findings are in line with an earlier report demonstrating predominant downregulation of genes of the UPS in pancreatic islets from individuals with type 2 diabetes vs. without diabetes (Bugliani et al. 2013). That deubiquitinating enzyme ubiquitin specific protease 7 is critically involved in stabilizing insulin receptor substrate (IRS)-1 and IRS-2, key mediators of insulin/insulin-like growth factor signaling, (Yoshihara et al. 2012) lends further credence to the concept of a modulatory relationship existing between the UPS and

proteins involved with glycemia control. Indeed, pathway analysis also detected decreased expression of the calcium channel voltage-dependent, L type $\alpha 1C$ subunit (*CACNA1C*) which in mice is critical for insulin secretion (Barg et al. 2002). In human islets and β -cells, however, *CACNA1C* mRNA molecules were present at much lower levels than *CACNA1D* transcripts although there was a trend toward lower *CACNA1C* expression in the islets from patients with type 2 diabetes vs. without the disease (Reinbothe et al. 2013). Accordingly, whether or not *CACNA1C* plays a role in influencing insulin secretion or the insulin network in humans remains unknown.

CACNA1C, however, also encodes voltage-dependent L-type Ca^{2+} channels (CaV1.2) which serve as the primary Ca^{2+} entry pathway in vascular smooth muscle cells (Bannister et al. 2013). It may therefore regulate vasomotor tone independent of its potential metabolic effects.

The present transcriptome analysis found that several genes involved in vascular smooth muscle contraction AGEs can act via a variety of routes to propagate diabetes-associated vascular complications. On the one hand, they may amass in the extracellular space and within the vessel wall to induce oxidative stress and ramp up the atherosclerosis process (Basta et al. 2004). On the other, they may promote endothelial (progenitor) cell apoptosis (Lan et al. 2015; Shen et al. 2010). Consistent with this, we found that the apoptosis inhibitors BIRC7 and BIRC8, were both downregulated in HUVECs that had been subjected to a high glucose environment. Taken together, these data suggest that lncRNAs may exert vascular protection by downregulating anti-apoptotic genes that participate in AGE-dependent cell demise.

The nervous and circulatory systems are closely intertwined both anatomically and functionally, and it has been proposed that axon and capillary sprouting may be more alike than dissimilar (Klagsbrun and Eichmann 2005). Indeed, several proteins that are known to mediate axon guidance have since been determined to also be intimately involved with angiogenic processes (Klagsbrun and Eichmann 2005). Our bioinformatic analysis indicate an upregulation of genes involved with axon guidance. Given the potential premise of axons and capillaries sharing the same branching prompts, the enhanced levels of axon guidance-related genes in HUVECs exposed to a high glucose milieu may account for the pathogenic angiogenesis typically observed in proliferative diabetic retinopathy.

Although our study is limited by the relatively low number of replicates ($n=3$) and the reliance on a single endothelial cell type (HUVECs), we provide the first transcriptome analysis identifying endothelial lncRNAs that are differentially expressed following high glucose exposure. Inasmuch as clinical practice guidelines are unified in emphasizing the importance of early and aggressive glucose-lowering strategies to slow the development and progression of vascular complications (American Diabetes Association 2015; Canadian Diabetes Association Clinical Practice Guidelines Expert Committee 2013), these data may provide critical insights into putative biomarkers and therapeutic targets that could pave the way for novel strategies to complement the currently available vascular protective armamentarium for diabetes.

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DISCLOSURES

Nothing to disclose. No relevant relationship with industry exists.

CONFLICT OF INTEREST

None declared.

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Table 1

Most differentially expressed lncRNAs in HUVECs cultured in elevated vs. normal glucose conditions

	Sequence Name	RNA Length	Chromosome	Fold	P
<i>Upregulated lncRNAs</i>	ENST00000421257	825	20	3.8073319	0.0238969
	TCONS_00007280	777	3	3.4405895	0.0373811
	TCONS_00016146	460	9	3.4268607	0.0383556
	ENST00000547721	779	12	3.2655793	0.0185213
	NR_047512	2267	11	3.2218557	0.0419678
	TCONS_00008208	291	4	3.0359229	0.0168951
	TCONS_00019769	254	11	3.0282746	0.0050136
	ENST00000565041	545	16	2.9479401	0.0134413
	ENST00000421891	831	6	2.9340224	0.0356850
	NR_024091	3544	11	2.8919421	0.04260510
<i>Downregulated lncRNAs</i>	ENST00000559989	7790	15	11.1682717	0.0041190
	ENST00000458351	1269	2	8.0587365	0.0467283
	uc010zan.1	2070	2	7.2847266	0.0477430
	ENST00000465390	517	21	6.9831873	0.0492896
	ENST00000538550	553	11	6.6305	0.0483391
	TCONS_00015989	218	9	5.9731484	0.0420295
	TCONS_00000891	236	1	5.8343598	0.0164555
	uc001vvu.3	1388	14	5.6585137	0.0378370
	ENST00000432411	447	21	5.3563798	0.0460850
	uc001yvq.2	3358	15	5.139614	0.0160024

Table 2

Comparison of lncRNA array and qPCR results for select lncRNAs in HUVECs cultured in elevated vs. normal glucose conditions

Sequence Name	Primer Sequences (5'-3')	Fold (lncRNA array)	Fold (validation qPCR)	p-value
TCONS_00007280	F-gcagttgcatccattagc R-ccctccaggtggttttaca	3.44	2.83	0.041
NR_047512	F-cttctgctgccacatgttct R-ggcctggaccagttcctc	3.22	2.31	0.043
TCONS_00008208	F-gctgagctccttgctaattg R-ggtagcttgagtcattgtgaggt	3.04	3.73	0.006
ENST00000421891	F-gtctcctgcgtgctcacc R-cttggttttcaggcagttac	2.93	1.61	0.013
NR_024091	F-tgactgctcttacggaatcctat R-cggtcagctcactccactg	2.89	3.37	0.039

F and R denote the forward and reverse primers, respectively.

Table 3

Results of bioinformatic analyses on lncRNAs and mRNAs from HUVECs cultured in elevated vs. normal glucose conditions

KEGG pathway analysis item	Count	Genes
Prion diseases	3	C6, NCAM1, PRKACB
Vibrio cholerae infection	3	ATP6V1G2, ATP6V1H, PRKACB
Autoimmune thyroid disease	3	HLA-F, IL5, TSHB
Vascular smooth muscle contraction	4	CACNA1C, NPPC, PLA2G1B, PRKACB
Dopaminergic synapse	4	CACNA1C, DRD3, PPP2R5D, PRKACB
Ubiquitin mediated proteolysis	4	BIRC7, BIRC8, FBXO4, KLHL13
Apoptosis	3	BIRC7, BIRC8, PRKACB
Insulin secretion	3	CACNA1C, GCG, PRKACB
Hematopoietic cell lineage	3	CD33, CR1, IL5

GO classification term	Count	Proportion of differentially expressed genes	<i>P</i>
Cellular process	108	93.10345	0.0498715
Metabolic process	107	76.97842	0.032899
Organic substance metabolic process	102	73.38129	0.0132884
Primary metabolic process	99	71.22302	0.0175482
Cellular metabolic process	80	68.96552	0.0455353

Biological Processes

	Macromolecule metabolic process	72	62.06897	0.0103074
	Cellular macromolecule metabolic process	68	58.62069	0.0027253
	Signaling	62	44.60432	0.0257421
	Single organism signaling	62	44.60432	0.0257421
	Cell communication	62	44.60432	0.0334142
Cellular Component	Cell part	113	94.16667	0.0090424
	Cell	113	94.16667	0.0090848
	Intracellular part	101	84.16667	0.0040257
	Intracellular	101	84.16667	0.0065194
	Organelle	95	79.16667	0.0166605
	Membrane-bounded organelle	91	75.83333	0.0062574
	Intracellular organelle	90	75	0.0066086
	Intracellular membrane-bounded organelle	86	71.66667	0.0011978
	Cytoplasm	77	64.16667	0.0441913
Molecular Function	Binding	97	88.99083	0.0096456
	Protein binding	70	64.22018	0.0139664

FIGURE LEGENDS

Fig 1. LncRNA and mRNA expression profiles in HUVECs exposed to normal (5 mmol/L) vs. high (25 mmol/L) glucose.

A, B Scatter plots comparing the variation in lncRNA and mRNA expression. The values plotted are the averaged normalized signal values (log2 scaled) for the normal glucose (x-axis) and the high glucose (y-axis) groups. The green lines indicate fold change. LncRNAs and mRNAs above the top green line and below the bottom green line exhibit at least a 2.0-fold difference between the two study groups. **C, D** Box-and-Whisker plots (10th, 90th percentile) showing average fold change of lncRNAs and mRNAs. Median intensity is denoted with a –; mean intensity with a +. **E, F** Volcano plots detailing magnitude of expression difference. The vertical green lines correspond to 2.0-fold upregulation and 2.0-fold downregulation of expression. The horizontal green line indicates a *P* value of 0.05. Red points represent lncRNAs with statistically significant differential expression (fold change $\geq$ 2.0, *P* $\leq$ 0.05).

Fig 2. Distribution, location and classification of lncRNAs differentially expressed in HUVECs exposed to normal (5 mmol/L) vs. high (25 mmol/L) glucose.

A Chromosomal distribution. **B** Chromosomal location. **C** LncRNA classification.

Fig 3. Network co-expression and bioinformatics analyses of samples from HUVECs exposed to normal (5 mmol/L) vs. high (25 mmol/L) glucose.

A LincRNAs and associated protein-coding transcripts. **B** Pathway analysis of differentially expressed protein-coding genes. Red bars denote those associated with downregulated mRNAs; green bars denote those associated with upregulated mRNAs.

Draft

Figure 1

Fig 1. LncRNA and mRNA expression profiles in HUVECs exposed to normal (5 mmol/L) vs. high (25 mmol/L) glucose.

A, B Scatter plots comparing the variation in LncRNA and mRNA expression. The values plotted are the averaged normalized signal values (log₂ scaled) for the normal glucose (x-axis) and the high glucose (y-axis) groups. The green lines indicate fold change. LncRNAs and mRNAs above the top green line and below the bottom green line exhibit at least a 2.0-fold difference between the two study groups. C, D Box-and-Whisker plots (10th, 90th percentile) showing average fold change of LncRNAs and mRNAs. Median intensity is denoted with a $-$; mean intensity with a $+$. E, F. Volcano plots detailing magnitude of expression difference. The vertical green lines correspond to 2.0-fold upregulation and 2.0-fold downregulation of expression. The horizontal green line indicates a P value of 0.05. Red points represent LncRNAs with statistically significant differential expression (fold change ≥ 2.0 , $P \leq 0.05$).

190x254mm (300 x 300 DPI)

Draft

Figure 2

Fig 2. Distribution, location and classification of lncRNAs differentially expressed in HUVECs exposed to normal (5 mmol/L) vs. high (25 mmol/L) glucose.
A Chromosomal distribution. B Chromosomal location. C LncRNA classification.

225x168mm (300 x 300 DPI)

Figure 3

Fig 3. Network co-expression and bioinformatics analyses of samples from HUVECs exposed to normal (5 mmol/L) vs. high (25 mmol/L) glucose.

A LincRNAs and associated protein-coding transcripts. B Pathway analysis of differentially expressed protein-coding genes. Red bars denote those associated with downregulated mRNAs; green bars denote those associated with upregulated mRNAs.

190x254mm (300 x 300 DPI)