Mol Cell Biochem (2017) 431:139–149
DOI 10.1007/s11010-017-2984-2

Endothelial long non-coding RNAs regulated by oxidized LDL

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Received: 17 August 2016 / Accepted: 24 February 2017 / Published online: 18 March 2017

Abstract Oxidized low-density lipoprotein (oxLDL) plays a central role in the pathogenesis of atherosclerosis, in part via an effect to promote endothelial dysfunction. In the present study, we evaluated the expression profiles of long non-coding RNAs (lncRNAs) and protein-coding mRNAs in endothelial cells following oxLDL stimulation. LncRNAs and mRNAs from human umbilical vein endothelial cells (HUVECs) were profiled with the Arraystar Human lncRNA Expression Microarray V3.0 following 24 h of oxLDL treatment (100 μg/mL). Of the 30,584 lncRNAs screened, 923 were significantly up-regulated and 975 significantly down-regulated ($P < 0.05$) in response to oxLDL exposure. In the same HUVEC samples, 518 of the 26,106 mRNAs screened were up-regulated and 572 were down-regulated. Of these differentially expressed lncRNAs, CLDN10-AS1 and CTC-459I6.1 were the most up-regulated (~87-fold) and down-regulated (~28-fold), respectively. Bioinformatic assignment of the differentially regulated genes into functional groups indicated that many are involved in signaling pathways among which are the cytokine receptor, chemokine, TNF, MAPK and Ras signaling pathways, olfactory transduction, and vascular smooth muscle cell function. This is the first report profiling oxLDL-mediated changes in lncRNA and mRNA expression in human endothelial cells. The novel targets

Electronic supplementary material The online version of this article (doi:10.1007/s11010-017-2984-2) contains supplementary material, which is available to authorized users.

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revealed substantially extend the list of potential candidate genes involved in atherogenesis.

Keywords LncRNA · Endothelial cells · OxLDL

Abbreviations

ANRIL	Antisense non-coding RNA in the INK4
CVD	Cardiovascular disease
DE	Differentially expressed
ENCODE	Encyclopedia of DNA Elements
GO	Gene ontology
HDL	High-density lipoprotein
HLA-DPB1	Major Histocompatibility Complex, Class II, DP Beta-1
HUVECs	Human umbilical vein endothelial cells
KEGG	Kyoto Encyclopedia of Genes and Genomes
lncRNA	Long non-coding RNAs
MIAT	Myocardial infarction-associated transcript
miRNA	MicroRNA
ncRNA	Non-coding RNAs
NO	Nitric oxide
NQO1	NAD(P)H dehydrogenase quinone 1
O_2^-	Oxygen radical
oxLDL	Oxidized low-density lipoprotein
piRNAs	PIWI-interacting RNAs
ROS	Reactive oxygen species
SMC	Smooth muscle cell
snoRNAs	Small nucleolar RNAs
t-UCRs	Transcribed ultra-conserved regions

Introduction

Atherosclerosis is a progressive inflammatory vascular disease that leads to atheromatous plaque development within the intima of the arteries [1–4]. Endothelial dysfunction plays a central role in the development and natural course of atherosclerosis [5–8]. Oxidized low-density lipoprotein cholesterol (oxLDL) is a well-established risk factor for atherothrombosis and exerts a plethora of effects to promote endothelial dysfunction, plaque progression, and inflammatory interactions between monocytes and the underlying vessel wall [9–11]. Understanding the molecular pinnings of how oxLDL incites endothelial activation may uncover novel approaches to limit atherosclerosis.

Non-coding RNAs (ncRNAs) form a high percentage of the mammalian genome. Two major subgroups of ncRNAs that have been identified are the long ncRNAs (lncRNAs) and the microRNAs (miRNAs) [12, 13]. LncRNAs are generally described as sequences that are longer than 200 nucleotides. Due to the absence of open reading frames, lncRNAs do not possess any translational ability but they are however able to alter gene expressions and signaling pathways [14]. Although lncRNAs do not appear to be as evolutionarily conserved as protein-coding genes, the available evidence strongly indicate that lncRNAs are intimately involved in the regulation of tissue homeostasis as well as a wide variety of cellular functions that include proliferation, migration, invasion, angiogenesis, differentiation, and survival [14].

In recent years, lncRNAs have not only been implicated as novel regulators of multiple physiological and pathological conditions but also as potential therapeutic targets due to their ability to function as a molecular signal to regulate gene transcription and epigenetic modifications [15]. Indeed there is a growing appreciation of the role of lncRNAs in the regulation of cardiovascular diseases (CVDs) [16]. For example, the lncRNA Novlnc6 was recently found to be associated with acute myocardial infarction and another Mhrt has been linked with heart failure along with other lncRNAs that are involved in controlling hypertrophy, mitochondrial function, and cardiomyocyte death [16–19].

The endothelial-expressed lncRNAs MALAT1 and Tie-1-AS have been reported to control endothelial function in the vascular system [20, 21], and ANRIL has been demonstrated to regulate cell proliferation, cell adhesion, and apoptosis—cellular activities crucial for atherosclerosis [22]. Furthermore, a recent report noted the negative transcriptional regulatory role of lncRNA NAT APOA1-AS for APOA1, which is the main protein constituent of high-density lipoprotein (HDL), an important lipoprotein that is associated with reduced atherosclerosis [23].

Overall, these studies project lncRNAs as evolving regulators in CVDs and atherosclerosis. That said, our understanding of the underlying influence and function of lncRNAs in endothelial dysfunction and atherosclerosis remains still quite limited [24, 25]. In the present study, we performed the first transcriptome profiling of lncRNA expression upon oxLDL treatment in endothelial cells.

Materials and methods

Cell culture

Human umbilical vein endothelial cells (HUVECs, Lonza) and Human coronary artery endothelial cells (HCAECs, Lonza) were cultured in endothelial cell growth medium-2 (EGM™-2 Bulletkit™; Lonza) supplemented with growth factors, serum, and antibiotics at 37 °C in humidified 5% CO_2 incubator. Confluent HUVECs were maintained in 6-well plates for 24 h with or without the presence of oxLDL (100 µg/mL; Alfa Aesar). Cells were serum-starved overnight before they were treated with either oxLDL or the vehicle.

RNA preparation

Total RNA, isolated from HUVECs using the TRIzol™ (Invitrogen) reagent and according to the manufacturer's instructions, was quantified with the NanoDrop ND-1000 spectrophotometer. RNA integrity was confirmed by standard denaturing agarose gel electrophoresis.

Microarray profiling

The expression profile of 30,584 human lncRNAs and 26,106 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. In brief, 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 subsequently washed, fixed, and scanned using the Agilent DNA Microarray Scanner (Product#G2505C). Array images so collected were studied with the Agilent Feature Extraction software (version 11.0.1.1). We utilized the GeneSpring GX v11.5.1 software package (Agilent Technologies) to conduct quantile normalization and process the data. Statistical significance for differentially expressed (DE) genes was evaluated with the Student's t-test and adjusted for multiple testing by the Benjamini–Hochberg method to minimize the false discovery rate. 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) 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.

Validation qPCR

Confluent HUVECs and HCAECs were starved overnight and then maintained for 24 h with either the diluent or oxLDL (100 μg/mL). Total RNA was isolated and qPCR for lncRNAs was performed using standard protocols. The sequences for primers used to perform validation qPCR are described in Supplementary Table 1.

Results

Quality Assessment of lncRNAs and mRNAs data

RNA integrity and genomic DNA contamination of the six samples evaluated were measured by denaturing agarose gel electrophoresis. The intensity of the upper 28S ribosomal RNA band in all of the samples was about twice that of the lower 18S band, thereby confirming RNA integrity. The absence of smears above the 28S band attested to the purity of the RNA samples (Supplementary Fig. 1A). RNA quantity and purity were also confirmed with the NanoDrop ND-1000. All samples had an A260 /A280 ratio that was close to 2.0 and an A260/A230 ratio that exceeded 1.8 (Supplementary Fig. 1A). Box plots that included the 10th and 90th percentile values revealed comparable expression values after normalization (Supplementary Fig. 1B).

Scatter plots were generated to provide a profile of HUVEC lncRNAs (Fig. 1a) and mRNAs (Fig. 1b) that were up-regulated, down-regulated, or unaffected by oxLDL treatment. Overall, the average fold-changes for DE lncRNAs and mRNAs under the two study conditions were similar (Fig. 1c). Volcano plot filtering uncovered 923 significantly up-regulated and 975 significantly down-regulated lncRNAs in HUVECs treated with oxLDL in comparison to vehicle-treated control samples (Fig. 1d, $P < 0.05$). LncRNAs that demonstrated the greatest differences in expression ranged from 895 to 3307 bp. Specifically, CLDN10-AS1 (RNA length: 895 bp, chromosome 13) was the most up-regulated lncRNA (~87 fold) and CTC-459I6.1 (RNA length: 535 bp, chromosome 5) the most down-regulated (28 fold) in HUVECs subjected to oxLDL treatment. Table 1 lists the 10 most up-/down-regulated lncRNAs in response to oxLDL treatment. OxLDL also produced changes at the transcript level; specifically the levels of 1090 mRNAs were altered following oxLDL exposure with 518 up-regulated and 572 down-regulated (Fig. 1e, $P < 0.05$). Proinflammatory macrophage marker HLA-DPB1 (Major Histocompatibility Complex, Class II, DP Beta-1) was the most up-regulated (~241 fold) mRNA transcript after oxLDL stimulation in endothelial cells. Validation qPCR performed for 7 up-regulated and 5 down-regulated lncRNA showed similar trend for HUVECs and HCAECs after oxLDL treatment (Table 2).

LncRNA chromosomal distribution and subtype analysis

Supplementary Fig. 2 shows the dendrograms generated for hierarchical analysis of clustered DE lncRNAs and mRNAs in the two study groups. Although lncRNAs modulated by oxLDL treatment were abundant and present on every chromosome, they were most commonly found on chromosomes 1, 2, and 5 (Fig. 2a). Further probing revealed that while these DE lncRNAs were generally expressed along the entire length of the chromosomes, there was notable clustering (Fig. 2b). LncRNA subgroup analysis, which helps identify the functional relationship between lncRNAs and their associated protein-coding genes, demonstrated that the majority (~50%) of the DE lncRNAs were intergenic in origin followed by intron and natural antisense lncRNAs (Fig. 2c).

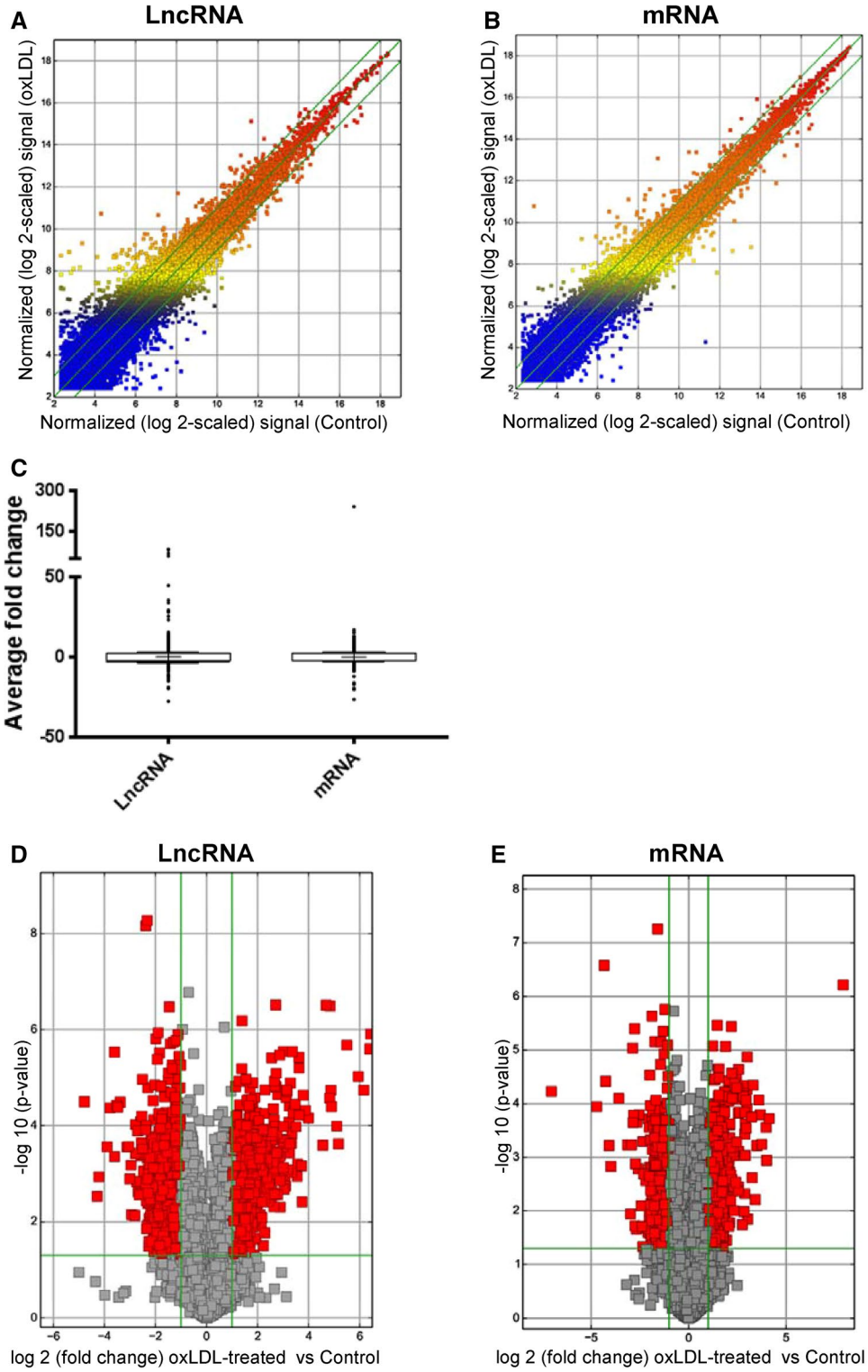

Fig. 1 LncRNA and mRNA expression profiles in HUVECs treated with oxLDL (100 µg/mL) vs. control. **a**, **b** Scatter plots comparing the variation in lncRNA and mRNA expression. The values plotted are the averaged normalized signal values (log2 scaled) for the control (*x* axis) and the oxLDL treatment (*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** Box-and-Whisker plots (10th, 90th percentile) showing average fold-change of lncRNAs and mRNAs. Median intensity is denoted with a "−" sign and mean intensity denoted with a "+" sign. **d**, **e** Volcano plots detailing magnitude of expression difference. The vertical green lines correspond to 2.0-fold up-regulation and 2.0-fold down-regulation of expression. The horizontal green line indicates a p value of ≤ 0.05. Red points represent lncRNAs and mRNAs with statistically significant differential expression (fold-change ≥ 2.0, $P \leq 0.05$)

Table 1 Ten most up- and down-regulated lncRNAs in HUVECs exposed to oxLDL (100 μg/mL) for 24 h vs. control conditions

Sequence name	RNA length	Chr.	Fold	*p* value
Up-regulated lncRNAs				
CLDN10-AS1	895	13	86.34	1.24984E–06
AL132709.5	737	14	84.24	2.5307E–06
RP4-669L17.4	498	1	71.62	1.81325E–05
AP001596.6	1078	21	61.44	9.40565E–06
RP11-152P17.2	801	8	45.10	2.11527E–06
ZNF295-AS1	1073	21	35.88	0.000239035
RP11-466I1.1	492	11	34.26	0.000102274
AC068282.3	2690	2	29.65	4.01824E–05
RP11-534G20.3	3307	10	29.22	1.82726E–05
HLA-DPB2	776	6	28.58	3.23277E–07
Down-regulated lncRNAs				
CTC-459I6.1	535	5	27.60	3.15432E–05
AX748283	2093	1	19.56	0.002942431
RP11-138B4.1	2114	4	18.71	0.001161294
AL832163	2799	8	14.90	0.000274343
DQ592442	2772	1	13.76	4.27425E–05
XLOC_007697	909	9	12.19	2.9438E-06
vPSORS1C3	593	6	12.16	0.000437692
RP11-676J12.6	585	17	11.54	3.74753E–05
BX284650.1	503	1	11.12	4.12326E–05
XLOC_014114	1975	21	10.43	3.2299E–05

Table 2 Validation qPCR for differentially expressed lncRNAs in HUVECs and HCAECs exposed to oxLDL (100 μg/mL) for 24 h vs. control conditions

Sequence name	Fold (HUVECs)	*p* value	Fold (HCAECs)	*p* value
Up-regulated lncRNAs				
CLDN10-AS1	7.74 ± 2.44	0.0069	4.44 ± 1.39	0.0343
AL132709.5	4.05 ± 0.06	0.0235	2.34 ± 0.22	0.0167
RP4-669L17.4	5.31 ± 0.20	0.0005	4.33 ± 0.17	0.0027
AP001596.6	2.88 ± 1.66	0.0137	2.20 ± 0.90	0.0055
ZNF295-AS1	3.60 ± 1.54	0.04817	4.12 ± 2.49	0.0153
RP11-466I1.1	4.82 ± 0.57	0.0103	2.70 ± 1.38	0.0332
RP11-534G20.3	2.09 ± 1.09	0.3370	4.12 ± 1.16	0.0422
Down-regulated lncRNAs				
CTC-459I6.1	3.20 ± 0.25	0.04043	2.35 ± 0.15	0.0452
AX748283	1.07 ± 0.18	0.4164	1.29 ± 0.21	0.0585
RP11-138B4.1	1.18 ± 0.11	0.0657	2.06 ± 0.18	0.04231
AL832163	2.29 ± 0.75	0.0304	2.12 ± 0.61	0.0254
RP11-676J12.6	1.24 ± 0.26	0.0425	1.21 ± 0.28	0.0436

LncRNAs and associated protein-coding transcripts

We conducted additional investigations to gather further insights into the DE lncRNAs and their associated protein-coding transcripts. The 10 most up- and down-regulated lncRNAs with their known associated protein-coding genes are summarized in Fig. 3. Interestingly, all 20 lncRNAs demonstrated a direct correlation in fold-change with its associated mRNA (Fig. 3).

Bioinformatics analyses

Pathway analysis with the current KEGG database yielded several pertinent findings (Tables 3, 4). Briefly, lncRNAs up-regulated in response to oxLDL treatment are most commonly associated with the cytokine–cytokine receptor interface, chemokine signaling pathway, TNF signaling pathway, and estrogen signaling pathway (Table 3). The most down-regulated lncRNAs are notably involved in olfactory transduction, MAPK signaling pathway, Ras signaling pathway, cytoskeletal actin regulation, and vascular smooth muscle contraction (Table 4).

Table 5 details the results of the GO analysis that grouped the DE mRNAs under the following three categories: Biological Processes, Cellular Component, and Molecular Function. GO terms most broadly associated with up-regulated mRNAs were regulation of biological processes, extracellular space, and binding (Table 5). GO terms associated with down-regulated mRNA were mainly enriched in single-organism process, membrane components, and carbohydrate derivative binding (Table 5).

Discussion

We have come a long way since the initial description of how modified LDL is involved in the transformation of macrophages to foam cells in the atherosclerotic process [26, 27]. It is now well established that foam cells release proinflammatory cytokines, reactive oxygen species (ROS), and matrix degrading proteolytic enzymes, which together promote plaque formation and destabilization [4]. These observations provided the impetus behind the notion that oxidative modification of LDL alters its biological signature such that it acquires the ability to nurture the atherosclerotic process via multiple avenues [9, 28]. Specifically, oxLDL is capable of inciting endothelial cell dysfunction, proliferation, apoptosis, and necrosis, all of which are critical components of the atherosclerotic state [29, 30]. Under physiological conditions, endothelial cells release nitric oxide (NO), which serves to maintain vascular tone [31]. In the presence of oxLDL, however, NO release is inhibited and the NO that is generated is quickly inactivated by the

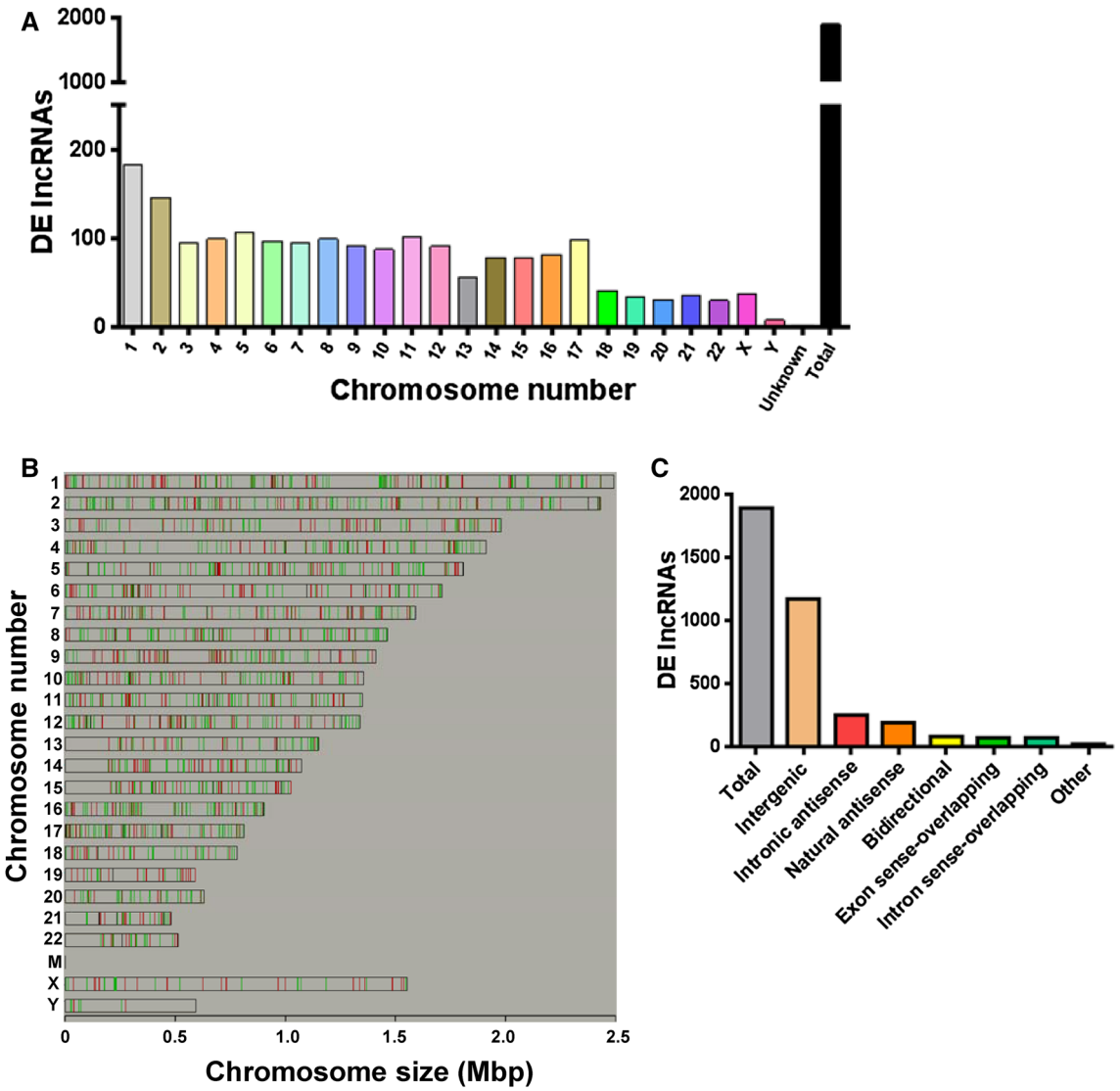

Fig. 2 Distribution, location, and classification of differentially expressed lncRNAs in HUVECs treated with oxLDL (100 µg/mL) versus control. Demonstration of **a** numbers and **b** chromosomal location of DE lncRNAs on different chromosomes. **c** *Bar graph* representing types of differently expressed lncRNAs, depending upon their genomic location

enhanced production of ROS [31–33]. OxLDL-associated endothelial cell loss—either via necrosis or apoptosis—not only augments vascular permeability and promotes smooth muscle cell (SMC) proliferation but also amplifies coagulation which together aid in the process of atherogenesis [34, 35].

In addition to its role in atherogenesis, oxLDL has been highlighted as a biomarker for CVD in recent years [36, 37]. Mechanisms of oxLDL-mediated endothelial dysfunction have been well studied [38, 39]. Although the molecular mechanisms have been studied for many years, the detailed epigenetic alterations with a special emphasis on the crosstalk between oxLDL and lncRNAs have remained unknown.

Although several thousands of lncRNAs have been recognized in mammals, our understanding of regulation and function of lncRNAs is still limited. However, due to recent rapid advancements in the molecular biology field, immense attention has been reaped by lncRNAs and their roles. The lncRNAs have already been reported in a broad range of physiological and pathological conditions but their function in the development of CVDs and especially in atherosclerosis is inadequately understood. MIAT and ANRIL were the earliest lncRNAs identified as a risk factor for CVDs [40–42]. ANRIL regulates genes involved in cell proliferation, cell adhesion, and apoptosis, and also correlates with the gravity of atherosclerosis in humans [22, 41]. Although these observations imply that lncRNAs can

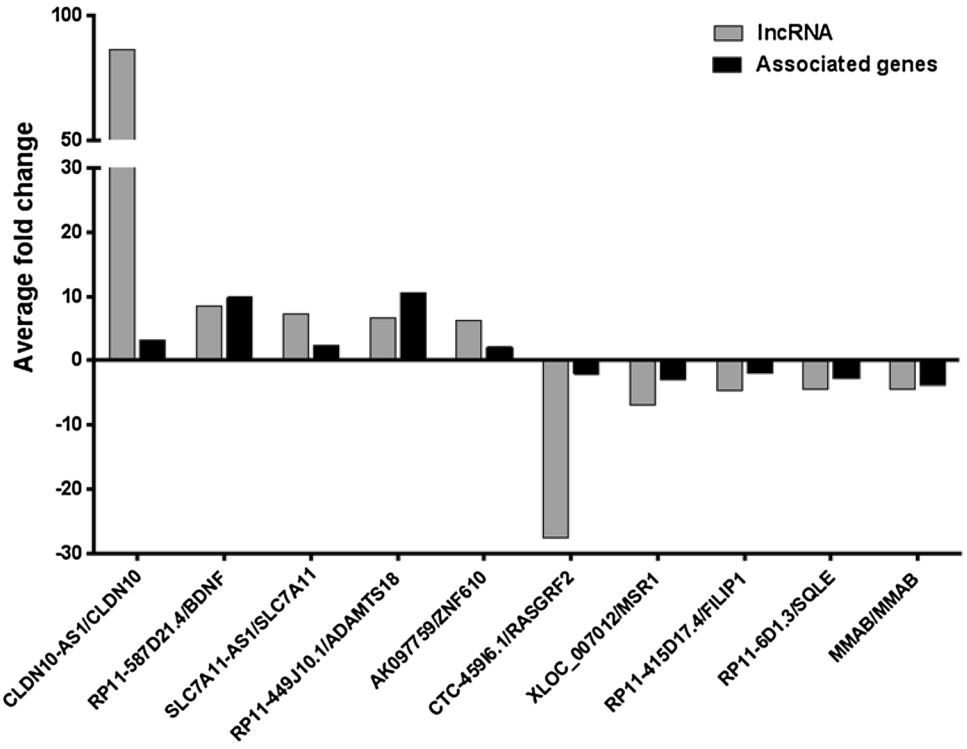

Fig. 3 Network co-expression and bioinformatics analyses of samples from HUVECs treated with oxLDL (100 μg/mL) vs. control. Representation of DE lncRNAs and associated genes with respect to fold-change. Ten significantly up-regulated and 10 down-regulated lncRNAs with known target genes were selected for presentation in the figure

Table 3 Bioinformatics analyses of up-regulated pathways in HUVECs following oxLDL (100 μg/mL) exposure

Pathway analysis	Up-regulated gene count	*p* value
Cytokine–cytokine receptor interaction	271	0.0001
Chemokine signaling pathway	189	0.0114
TNF signaling pathway	110	0.0129
Estrogen signaling pathway	100	0.0270
Rheumatoid arthritis	91	0.0011
Salmonella infection	86	0.0138
p53 signaling pathway	68	0.0195
Inflammatory bowel disease (IBD)	67	0.0042
Steroid hormone biosynthesis	57	0.0412
Legionellosis	55	0.0082
Butanoate metabolism	26	0.0003

modulate numerous processes linked to CVDs including cell proliferation, endothelial function, lipid metabolism, and inflammation, comprehensive information on endothelial lncRNAs regulated by oxLDL was missing.

Therefore, for investigating the outcome of oxLDL treatment on endothelial cell transcriptome, we performed lncRNA and mRNA microarray analysis on total RNA isolated from oxLDL-stimulated HUVECs. We identified novel lncRNAs and target genes providing insights into the differential regulation of lncRNAs and mRNAs by oxLDL in endothelial cells. A total of 30,584 lncRNAs were screened, where 923 were notably up-regulated and 975 were appreciably down-regulated ($P < 0.05$) in response to oxLDL in HUVECs. In a total of 26,106 mRNAs screened, 518 were significantly up-regulated and 572 significantly down-regulated. The validation qPCR performed for 10 most up- and down-regulated lncRNAs showed similar trend for 7/10 up-regulated and 5/10 down-regulated lncRNAs (Table 2). The DE lncRNAs were dispersed over all the chromosomes, with maximum number identified for chromosome 1 (Fig. 2a, b). Majority of DE lncRNAs were intergenic in nature (Fig. 2c). Our data show that the first 20 lncRNAs with known target mRNA demonstrated a direct correlation in fold-change with its associated mRNA (Fig. 3). For most functional groups, it is challenging to predict the overall effects of oxLDL treatment on HUVECs, since a variety of genes with diverse functional roles were differentially regulated simultaneously. However, pathway analysis revealed that DE mRNAs up-regulated in response to oxLDL treatment are primarily involved in cytokine–cytokine receptor interface and pathways such as chemokine signaling, TNF signaling, and estrogen signaling (Table 3). The most down-regulated DE mRNAs are notably involved in olfactory transduction, MAPK signaling, Ras signaling, cytoskeletal actin regulation, and vascular smooth muscle contraction (Table 4). Interestingly, profile of the DE genes assessed in this study showed some similarities to other reports by Deng et al. and Minta et al. on DE genes in oxLDL-treated SMCs [43, 44]. Among the top 15 up-regulated genes, HMOX1 was

Table 4 Bioinformatics analyses of down-regulated pathways in HUVECs following oxLDL (100 μg/mL) exposure

Pathway analysis	Down-regulated gene count	*p* value
Olfactory transduction	405	0.0318
MAPK signaling pathway	257	0.0002
Ras signaling pathway	227	0.0175
Regulation of actin cytoskeleton	215	0.0018
Vascular smooth muscle contraction	131	0.0158
Axon guidance	127	0.0351
Prostate cancer	89	0.0048
PPAR signaling pathway	69	0.0042
Steroid biosynthesis	20	0.0025

up-regulated in both studies conducted in SMCs along with our study in HUVECs [43, 44]. In another study, oxLDL treatment in human coronary artery SMCs induced a gene regulation profile similar to the gene appearance pattern observed in the aortas of apoE$^{-/-}$ mice [45]. In accordance with Reeve et al. and Minta et al., our data also documented that oxLDL induces expression of NQO1 [NAD(P)H dehydrogenase quinone 1] not only in SMCs but also in endothelial cells [43, 45]. This understanding further backs the proposition that the effect of oxLDL on endothelial cell assumes great significance for the development of atherosclerosis. Results of bioinformatics GO analysis, as described in Table 5, grouped the DE mRNAs under the following three categories: Biological Processes, Cellular Component, and Molecular Function. GO terms most broadly associated with up-regulated DE mRNAs were in regulation of biological, extracellular space, and binding (Table 5). GO terms associated with down-regulated DE mRNA were mainly enriched in single-organism process, membrane, and carbohydrate derivative process (Table 5). This is the first lncRNA and mRNA transcriptome profile of oxLDL-mediated changes in human endothelial cells. To confirm that our data are not HUVEC-specific, we also treated HCAECs with oxLDL and performed qPCR for 10

Table 5 Results of bioinformatics GO (gene ontology) enrichment analyses to determine the roles of differentially expressed mRNAs in GO terms

	Up-regulated				Down-regulated			
	GO term	Count	% of total DE genes	*p* value	GO term	Count	% of total DE genes	*p* value
Biological process	Regulation of biological process	260	66.0	0.0293	Single-organism process	376	83.4	0.016
	Regulation of metabolic process	167	42.3	0.0219	Biological regulation	307	68.3	0.036
	Regulation of primary metabolic process	154	39.0	0.0135	Regulation of biological process	295	65.7	0.028
	Regulation of macromolecule metabolic process	147	37.3	0.0067	Response to stimulus	270	60.1	2.18E–07
	Regulation of nitrogen compound metabolic process	120	30.4	0.0426	Cellular response to stimulus	216	48.1	8.12E–05
Cellular component	Extracellular space	39	9.5	0.0293	Membrane	258	53.8	0.027
	Extracellular matrix	17	4.2	0.0170	Membrane part	202	42.1	0.007
	Endocytic vesicle	11	2.7	0.0128	Intrinsic component of membrane	182	37.9	0.0015
	Integral component of organelle membrane	10	2.4	0.0389	Integral component of membrane	179	37.3	0.001
	Integral component of endoplasmic reticulum membrane	7	1.7	0.0126	Cell periphery	158	32.9	0.001
Molecular function	Binding	318	85.0	0.0075	Carbohydrate derivative binding	78	17.6	0.011
	Ion binding	163	43.5	0.0112	Ribonucleotide binding	66	15.0	0.021
	Cation binding	125	33.4	0.0007	Purine nucleotide binding	65	14.7	0.030
	Metal ion binding	123	32.8	0.0008	Purine ribonucleotide binding	64	14.5	0.034
	DNA binding	89	23.7	4.38E–06	Purine ribonucleoside triphosphate binding	63	14.2	0.031

most up- and down-regulated lncRNAs, which showed similar trend (Table 2).

Although interest in the contribution of lncRNAs to human health and disease is booming, the mechanism of action has only been pinpointed for a limited number of lncRNAs. Collaborative initiatives, such as the Encyclopedia of DNA Elements (ENCODE) project, aiming to recognize every functional element in the human genome are required [46]. However, the lack of defined functional motifs and regulatory regions and low expression levels of some lncRNAs are the major challenges. Majority of the lncRNAs are expressed as countless transcript alternates and the fact that they are poorly conserved challenges defining their specific biological roles and mechanisms of activity. Budding genomic, epigenomic, and bioinformatics approaches will be central in characterizing the lncRNAs. In order to avoid confusion and to facilitate the use and reproduction of the data, we have provided more detailed information (e.g., size, chromosomal localization, etc.) of oxLDL-associated DE lncRNAs in HUVECs. In our study, several lncRNAs were observed to be differentially regulated, which has not been stated before. Additional studies on novel genes reported in our study will offer first-hand cues regarding the mechanisms of CVD development by oxLDL. We conceive that our recent investigation further adds to the current understanding of the molecular mechanism of oxLDL-mediated endothelial cell dysfunction and apoptosis, and may provide targets for future therapeutic interventions against different CVDs including atherosclerosis.

Acknowledgements This work was supported by in part by grants from the Canadian Institutes of Health Research and Heart and Stroke Foundation of Canada to S. Verma. S. Verma is the Canada Research Chair in Atherosclerosis at the University of Toronto.

Authors' contributions KKS and SV designed the studies and the experiments. KKS, PNM, YP, AQ, and VG conducted the experiments. KKS drafted the manuscript. KKS, AQ, HT, MAO, and SV interpreted the data and critically edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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